EARLY LIFE ORIGINS OF THE INSULIN RESISTANCE SYNDROME IN THE AGED GUINEA PIG

A thesis submitted for the degree of
DOCTOR OF PHILOSOPHY

by

Prema Thavaneswaran B.Sc. (Hons)

Discipline of Physiology
School of Molecular and Biomedical Science
University of Adelaide
Australia

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TABLE OF CONTENTS

ABSTRACT

In human populations, perturbed growth in early life and ageing have been identified as risk factors for the development of the 'Insulin Resistance Syndrome' (IRS). The consequences of restricted prenatal growth on postnatal function have been investigated using numerous experimental models of intrauterine growth retardation, mainly in the rat. These studies have shown that some, but not all aspects of postnatal function that are programmed in humans, are also programmed in the rat. In addition, few experimental studies have investigated the effect of perturbed postnatal growth on adult function, or whether ageing amplifies the effects of events in early life. Therefore this study was designed to determine firstly, whether the IRS develops with increasing age in the guinea pig as it does in the human, and secondly whether the development of this syndrome is more pronounced in aged offspring which have undergone spontaneous fetal growth restriction and accelerated growth in the neonatal period.

Whole body insulin sensitivity of glucose metabolism, subcutaneous adiposity and skeletal muscle mass were reduced, while visceral adiposity and fasting concentrations of plasma glucose, insulin, triglycerides and total cholesterol were increased, in aged (14 months) compared to young adult (4 months) guinea pigs. An increase in resting systolic, diastolic and mean arterial blood pressure and pulse pressure was also observed in offspring with increasing age.

Spontaneous fetal growth restriction in the guinea pig reduced size at birth, but increased the neonatal fractional growth rate for weight in male and female offspring. In aged female offspring, small size at birth was associated with decreased whole body insulin sensitivity of glucose metabolism, increased fasting concentrations of plasma glucose and insulin, impaired glucose tolerance and elevated resting systolic and mean arterial blood pressure, pulse pressure and heart rate. An increased neonatal fractional growth rate for weight was associated with elevated fasting concentrations of plasma glucose and triglycerides in aged females, while a low fractional growth rate for weight during the neonatal period increased resting systolic blood pressure. In aged male offspring, large size at birth was associated with decreased whole body insulin sensitivity of glucose metabolism and increased fasting concentrations of

plasma insulin, while disproportionate fetal growth, as indicated by a low ponderal index at birth, was associated with increased fasting concentrations of plasma total cholesterol. A low fractional growth rate for weight during the neonatal period decreased whole body insulin sensitivity of glucose metabolism and increased fasting concentrations of plasma free fatty acids in aged males, while an increased neonatal fractional growth rate for weight was associated with elevated fasting concentrations of plasma triglycerides and raised resting pulse pressure and heart rate.

In conclusion, the guinea pig appears to be a suitable animal model of ageing, displaying many of the metabolic, cardiovascular and anthropometric changes seen in humans. Furthermore, the effects of perturbed prenatal and early postnatal growth on the development of the IRS in the aged guinea pig exhibit a sexually dimorphic pattern, however the mechanisms responsible for the emergence of this syndrome in a gender-specific manner remain to be determined.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available in all forms of media, now or hereafter known.

Prema Thavaneswaran

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RELATED PUBLICATIONS

JA Owens **P Thavaneswaran**, MJ De Blasio, IC McMillen, JS Robinson and KL Gatford. Sex-specific effects of placental restriction on components of the metabolic syndrome in young adult sheep. *American Journal of Physiology – Endocrinology and Metabolism* Feb 27; 2007 Epub ahead of print.

KL Gatford, MJ De Blasio, **P Thavaneswaran**, JS Robinson, IC McMillen, and JA Owens. Postnatal ontogeny of glucose homeostasis and insulin action in the sheep. *American Journal of Physiology – Endocrinology and Metabolism* 286: E1050-E1059, 2004.

COMMONLY USED ABBREVIATIONS

ABMI	Adult body mass index
AdjSSGIR	Adjusted steady state glucose infusion rate
AGR_{10-30}	Absolute growth rate for weight from 10 to 30 days of age
ANOVA	Analysis of variance
ANRL	Adult nose to rump length
AUGC	Area under the glucose curve
AUIC	Area under the insulin curve
AUIC:AUGC	Ratio of area under the insulin curve to area under the glucose curve
AW	Adult weight
BAC	Birth abdominal circumference
BHL	Birth head length
BHW	Birth head width
BHW:BAC	Birth head width to abdominal circumference ratio
BNRL	Birth nose to rump length
BPI	Birth ponderal index
BWT	Birth weight
BWT:BNRL	Birth weight to length ratio
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
EDL	Extensor digitorum longus
FGR_{10-30}	Fractional growth rate for weight from 10 to 30 days of age
FPCHOL	Fasting plasma total cholesterol
FPFFA	Fasting plasma free fatty acids
FPG	Fasting plasma glucose
FPI	Fasting plasma insulin
FPTRIG	Fasting plasma triglycerides

HDL	High density lipoprotein
HEC	Hyperinsulinaemic euglycaemic clamp
HPAA	Hypothalamo-pituitary adrenal axis
HR	Heart rate
I:G	Fasting plasma insulin to glucose ratio
IMVS	Institute of Medical and Veterinary Science
IRS	Insulin Resistance Syndrome
IUGR	Intrauterine growth retardation
IVGTT	Intravenous glucose tolerance test
K_G	Glucose tolerance index
LDL	Low density lipoprotein
MAP	Mean arterial blood pressure
MCR_i	Post-hepatic metabolic clearance rate of human insulin
mmHg	Millimetres of mercury
NIDDM	Non-insulin dependent diabetes mellitus
PP	Pulse pressure
pc	Partial correlation
SBP	Systolic blood pressure
SEM	Standard error of the mean
SGA	Small for gestational age
SPSS	Statistical Package for Social Sciences
SSGIR	Steady state glucose infusion rate
SSPHI	Steady state plasma human insulin

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION

Cardiovascular disease (CVD) is a major cause of mortality in Australia, and in 2004 accounted for 38% of deaths, ahead of all other diseases, including cancer (Australian Institute of Health and Welfare, 2004). A number of metabolic and haemodynamic disorders, including insulin resistance, hyperinsulinaemia, impaired glucose tolerance, non-insulin dependent diabetes mellitus (NIDDM), dyslipidaemia and hypertension have been identified as major risk factors for CVD, particularly when they occur simultaneously (Reaven 1988). This cluster of abnormalities, the prevalence of which has been shown to increase with ageing (Ford *et al.*, 2002), has been termed the 'Insulin Resistance Syndrome' (IRS). This is because insulin resistance has been thought to be the underlying defect, to which all other abnormalities are secondary (Haffner *et al.*, 1992), although this has been questioned. Until recently, CVD and its risk factors were thought to arise primarily due to a combination of environmental factors such as cigarette smoking, physical inactivity, a high dietary fat intake and obesity, as well as a genetically determined susceptibility (Facchini *et al.*, 1992; Godsland *et al.*, 1998). In the last twenty years however, epidemiological studies have demonstrated that individuals who were small in size at birth as a result of growth restriction *in utero*, had an increased risk of developing (Forsen *et al.*, 1997; Rich-Edwards *et al.*, 1997; Frankel *et al.*, 1996; Stein *et al.*, 1996) and dying from CVD (Koupilova *et al.*, 1996; Martyn *et al.*, 1996; Osmond *et al.*, 1993; Barker *et al.*, 1993; Barker *et al.*, 1989). This association was found to be independent of possible confounding variables such as cigarette smoking and obesity; although these factors did exacerbate the effects of events *in utero* (Osmond *et al.* 1993). These findings led to the development of the 'fetal origins of adult disease' hypothesis, which proposes that 'undernutrition *in utero* permanently programmes the body's structure, physiology and metabolism, leading to CVD in adult life' (Barker 1998).

As the IRS is itself a major risk factor for and precursor to the development of CVD, it is possible that an individual's susceptibility to the development of this syndrome may also be influenced by events *in utero*, and in fact this has been demonstrated in epidemiological studies of several ethnic groups (Bavdekar *et al.*, 1999; Li *et al.*, 2001; Barker *et al.*, 1993; Mi *et al.*, 2000; Valdez *et al.*, 1994; Yarbrough *et al.*, 1998). Around 80% of individuals who are growth restricted *in utero* undergo some degree of accelerated growth in the first year of life, known as 'catch-up' growth (Karlberg *et al.*, 1995). Epidemiological studies have shown that catch-up growth is an independent risk factor for several of the adult-onset diseases that comprise the IRS (Eriksson *et al.*, 1999; Forsen *et al.*, 1999; Parker *et al.*, 2003; Crowther *et al.*, 1998; Forsen *et al.*, 2000). A number of experimental models of intrauterine growth retardation (IUGR) have been developed in an attempt to gain a more detailed understanding of the mechanisms underlying the relationship between perturbed growth in early life and the increased risk of certain metabolic and cardiovascular disorders in adulthood.

This review will begin by summarising the factors that regulate intrauterine and postnatal growth, the known risk factors for suboptimal intrauterine growth in humans, and the short and long-term consequences of IUGR and catch-up growth, followed by a description of several experimental models of IUGR and their outcomes. The metabolic and vascular actions of insulin and the aetiology of the IRS will also be addressed. Finally, the outcomes to date from epidemiological and experimental studies that have investigated the association between perturbed prenatal and early postnatal growth, ageing, and increased risk of the IRS and its individual components will be discussed, followed by the specific aims and hypotheses of this thesis. Overall, these are to define the consequences of prenatal restriction and accelerated postnatal growth for development of major elements of the IRS with ageing, in a species that resembles the human in developmental profile and initial programming outcomes for metabolic and cardiovascular homeostasis.

1.2 INTRAUTERINE GROWTH RETARDATION

A variety of anthropometric variables including birth weight, length, abdominal circumference and head width or circumference, as well as body proportions such as

the birth weight to length ratio and ponderal index (weight (g) x 100/length $(cm)^3$), have been used to characterise fetal growth (Robinson *et al.*, 1996). These measures of size at birth reflect growth of different systems within the body, each of which varies in their sensitivity to the factors that are responsible for growth restriction *in utero* (Robinson *et al.*, 1996). Birth weight is primarily an indicator of skeletal muscle and adipose tissue growth, and to a lesser degree growth of the visceral tissues, while body length reflects growth of both axial and appendicular skeletons (Robinson *et al.*, 1996). Head width or circumference is an indicator of brain growth, while the abdominal circumference reflects growth of the liver and gut. The ponderal index is a way of characterising the relationship of height to mass for an individual, reflecting growth of soft tissues relative to that of skeletal tissues (Robinson *et al.*, 1996). IUGR, which is also commonly referred to as being small for gestational age (SGA), is evident as a reduced weight, length and/or increased thinness at birth for a given gestational age (Kramer 1987). IUGR infants have been prevented from reaching their genetic potential for growth, typically by factors extrinsic to themselves (Robinson *et al.*, 1996). IUGR is usually defined as a birth weight below the tenth percentile, or two standard deviations (2-SD) below the mean, for a given gestational age (Kramer 1987) and can be classified as either symmetric or asymmetric, based on two different patterns or phenotypes of fetal growth restriction (Robinson *et al.*, 1996). Asymmetric or disproportionate IUGR, which accounts for the majority of IUGR in humans, is characterised by a small abdominal circumference, relative preservation of head circumference and body length and a reduced body weight, resulting in a low ponderal index (Kramer 1987). Symmetric IUGR on the other hand is characterised by a reduced weight, length and abdominal and head circumference, but a normal ponderal index (Kramer 1987). The pattern of fetal growth restriction is dependent on the nature and timing of factors responsible for growth restriction *in utero*, and may fall anywhere on the continuum from pure symmetric to pure asymmetric (Bakketeig 1998). In the developed world, the incidence of IUGR is approximately 2% of live births, however in many developing countries this figure is up to 6 times higher (de Onis *et al.*, 1998). Each year, approximately 14 million IUGR babies are born in the developing world, representing 11% of all newborns in these countries (de Onis *et al.*, 1998). The risk of being born IUGR is highest in South-central Asia (12.3%), followed by

Oceania (9.8%) (excluding Australia and New Zealand) and Latin America and the Caribbean (6.5%) (de Onis *et al.*, 1998).

1.2.1 Risk factors for IUGR in humans

While there are many factors that are associated with human IUGR, its aetiologies can generally be divided into factors extrinsic to the developing fetus, and intrinsic factors which originate from within the fetus (Robinson *et al.*, 1996). Extrinsic causes of IUGR include placental factors, which tend to involve abnormally implanted or formed placentas, and maternal factors such as nutrition, infection, uterine perfusion of the placenta, various medical conditions, substance abuse and certain demographic variables (Bernstein *et al.*, 1997). Intrinsic causes of IUGR are primarily fetal factors such as genetic conditions and congenital abnormalities (Bernstein *et al.*, 1997). In many cases the aetiology of the observed IUGR cannot be identified and the cause is termed 'idiopathic' (Kramer 1987). Evidence suggests however that abnormal or defective placentation is likely to be an important factor in the pathogenesis of idiopathic IUGR (Fox 1988).

Intrinsic fetal factors such as karyotypic abnormalities, including trisomy 21 or 18, Turner syndrome and other chromosomal abnormalities, slow fetal growth, and contribute to up to 5% of IUGR cases (Neerhof 1995). Other intrinsic fetal factors which slow growth include malformation, as well as fetal infections, such as cytomegalovirus and toxoplasmosis (Robinson *et al.*, 1996). Multiple gestation is another fetal factor that can contribute to IUGR, which is particularly severe in those with syndromes associated with shared fetal circulation (Bernstein *et al.*, 1997).

Extrinsic placental factors associated with IUGR include placental hemangiomas, bilobate placenta, velamentous umbilical cord insertion and single umbilical artery (Bjoro 1983). In addition, IUGR is more common in pregnancies complicated by placenta previa (Bernstein *et al.*, 1997). A number of extrinsic maternal factors are associated with slow fetal growth. Maternal undernutrition has been shown to reduce birth weight, although the impact of this perturbation is dependent on several factors, including the specific nutrients which are limiting as well as the timing and duration of the nutritional insult (Robinson *et al.*, 1996). During the Dutch Winter famine, which occurred at the end of World War II, severe undernutrition in previously well-nourished pregnant women reduced birth weight only if the nutritional insult

occurred during the third trimester and even then, only to a modest extent (Stein *et al.*, 1995). In addition, women who are underweight or exhibit poor weight gain during pregnancy have a greater risk of delivering an IUGR infant (Abrams *et al.*, 1986). Cigarette smoking is a common but preventable cause of IUGR. The average reduction in birth weight in infants whose mothers smoked heavily during pregnancy was 458 grams, while in those whose mothers were exposed to passive smoke during pregnancy the deficit was 192 grams (Roquer *et al.*, 1995). In addition, the use of alcohol, cocaine, opiates and other drugs during pregnancy are also thought to contribute to the incidence of IUGR (Manning 1995). A number of maternal infectious diseases are known to slow fetal growth and account for between 5 and 10% of IUGR cases (Creasy *et al.*, 1994). Viral infections that have been associated with IUGR include rubella and cytomegalovirus and more recently, varicella-zoster and human immunodeficiency virus (Klein *et al.*, 1995). Several protozoan infections, including malaria, toxoplasmosis and trypanosomiasis have also been associated with IUGR (Creasy *et al.*, 1994). Various maternal environmental and demographic variables are associated with an increased risk of delivering an IUGR infant. High altitude has been identified as a risk factor for poor fetal growth, with babies born at high altitude demonstrating a lower birth weight and an increased incidence of IUGR compared with those born at low altitude (Keyes *et al.*, 2003). Maternal age, height and weight are important determinants of IUGR risk, with extremes of maternal age increasing the risk of delivering an IUGR infant, while women who are smaller in terms of both weight and height have been observed to deliver smaller babies (Bernstein *et al.*, 1997). Both nulliparity and grand multiparity have also been associated with an increased risk of IUGR (Vorherr 1982).

In the developing world, a low pre-pregnancy BMI and gestational weight gain, due primarily to chronic maternal undernutrition, as well as short maternal stature due to undernutrition and infection in childhood, are major determinants of IUGR (Bakketeig 1998). In addition, respiratory infections, gastroenteritis, malaria and intestinal parasitosis, all of which are highly prevalent in many developing countries also contribute to the incidence of IUGR. More recently, cigarette smoking has emerged as an increasingly important aetiological determinant of IUGR in some developing countries (Bakketeig 1998). In the developed world, cigarette smoking, and to a lesser extent a low pre-pregnancy BMI and gestational weight gain, due in

part to maternal eating disorders, are responsible for much of the clinical IUGR observed (Abraham *et al.*, 1994). Within developed countries socioeconomic disparities in IUGR are due largely to socioeconomic gradients in maternal stature, weight gain and smoking, while the high prevalence of illicit drug use in many poor urban areas may also be an important determinant of IUGR (Bakketeig 1998).

1.2.2 Regulation of intrauterine growth

Intrauterine life can be divided into the embryonic and fetal phases of development. The embryonic phase of development spans the first 8 weeks of gestation and growth during this period is characterised by cellular hyperplasia (Kuller *et al.*, 1996). The fertilised ovum or zygote begins to divide mitotically, forming a solid ball of cells called the morula, which then continues to proliferate and differentiate into a blastocyst capable of implantation (Kuller *et al.*, 1996). The blastocyst is composed of an inner cell mass that will eventually develop into the fetus, a thin outer layer or trophoblast which develops into the fetal portion of the placenta, and a fluid-filled cavity or blastocoele which will become the amniotic sac (Kuller *et al.*, 1996). Within a week of fertilisation, the blastocyst implants in the endometrium through the action of its trophoblastic enzymes, and begins to grow. By the end of this phase of development, the embryo is approximately 3 cm long and weighs about 6 grams, but has already developed several thousand structures (Luke 1994). The fetal phase of development, which spans the third through to the ninth month of gestation, is characterised by cellular hyperplasia and hypertrophy as well as concomitant apoptosis, migration and differentiation of cells to ensure appropriate tissue remodelling (Garnica *et al.*, 1996). During the first half of gestation there are rapid increases in fetal length with peak length growth velocity before 20 weeks, while the rate of increase in fetal weight does not peak until 30 weeks (Falkner *et al.*, 1994). The rate of placental growth is highest during early gestation however significant remodelling occurs during the second half of pregnancy, in order to improve its functional capacity in response to the increased fetal demand for oxygen and nutrients (Robinson *et al.*, 1996).

The rate of fetal growth is determined by several factors, including the fetal genome, the mother and her environment, as well as the placenta (Owens *et al.*, 1989). While both the maternal genotype and environment, and the placenta are major influences

on fetal growth, determining the availability of essential substrates, genetic factors also play a key role, accounting for approximately 38% of the variation in birth weight in humans (Styne 1998). The major substrates that are essential for fetal growth include oxygen, glucose, lactate, amino acids, ketone bodies and lipids, as well as several key micronutrients (Owens *et al.*, 1989). These substrates are delivered from the mother to the fetus by the placenta, which also removes fetal metabolic waste products (Milner *et al.*, 1996). A variety of factors determine the efficiency of substrate transfer by the placenta, including the permeability, vascularity and surface area of the placenta itself, placental production of these substrates, the rate of uterine and umbilical blood flow, as well as the activity of various substrate transport mechanisms (Milner *et al.*, 1996). The supply of oxygen and nutrients from the placenta regulates the production of fetal hormones, which in turn facilitate the utilisation of these substrates for fetal growth and metabolism (Milner *et al.*, 1996; Robinson *et al.*, 1985). The major hormones that regulate intrauterine growth include insulin, insulin-like growth factors (IGFs), thyroid hormones and cortisol (Fowden *et al.*, 2001). The IGFs (IGF-I and IGF-II) promote growth throughout intrauterine life, stimulating hypertrophy and hyperplasia of fetal cells and influencing differentiation in a wide variety of cell types (Fowden *et al.*, 2001). While both IGF-I and IGF-II are present in fetal tissues from early gestation, IGF-II is the main driver of embryonic growth, while IGF-I is the dominant growth factor during late gestation (Gluckman *et al.*, 2003). IGF-I, in conjunction with several other local growth factors, is thought to influence the growth of skeletal muscle *in utero*, by mediating the differentiation and proliferation of satellite cells, while IGF-I secreted by growing adipocytes has been shown to induce the proliferation of preadipocytes (Bell 1992). Another important regulator of fetal growth is insulin, which stimulates fetal tissue accretion, but has little impact on the differentiation or maturation of fetal tissues (Fowden 1995). Insulin may also influence fetal growth indirectly, by altering the concentration of other growth-promoting factors, including IGF-I of which it is the primary regulator (Fowden 1995). The prepartum rise in cortisol helps to trigger birth and also plays a key role in the maturation of a number of fetal systems (Fowden 1995). Cortisol acts on the fetal liver during late gestation, up-regulating gluconeogenic enzyme activity, as well as switching from IGF-II to IGF-I gene expression in order to initiate the transition from the prenatal to the postnatal mode of growth regulation (Fowden 1995). In

addition, cortisol promotes lung maturation during fetal life by increasing compliance and surfactant release (Fowden 1995). Thyroid hormones (tetraiodothyronine, T_4 and triiodothyronine, T_3) play an important role in fetal growth and development, influencing both tissue accretion and differentiation (Fowden 1995). The prepartum rise in cortisol also increases the production of T_3, which like cortisol plays an important role in fetal lung maturation (Milner *et al.*, 1996), while T_4 plays a key role in controlling the stimulation of oxygen utilisation by fetal tissues (Fowden 1995). Thyroid hormones also play an important role in brain development *in utero* (Yen 2001) and promote maturation of the nervous system during the perinatal period (Kilby *et al.*, 2000).

1.2.2.1 Mechanisms underlying IUGR

Intrinsic factors, including genetic conditions and congenital abnormalities, restrict fetal growth by impairing the ability of fetal cells and tissues to utilise essential substrates, even when the supply of such substrates is adequate (Robinson *et al.*, 1996). Other intrinsic factors such as fetal infections may restrict fetal growth by interfering with cell replication (Robinson *et al.*, 1996). The majority of human IUGR cases however are due to extrinsic factors, including maternal or placental limitations (Owens *et al.*, 1987). Most extrinsic factors reduce fetal growth by reducing the delivery of essential substrates such as oxygen and glucose to the fetus, for example by reducing the availability of maternal substrates or the maternal capacity to deliver substrates to the placenta, or by impairing placental growth and development (Robinson *et al.*, 1996). Restriction of fetal growth as a result of adverse influences acting in early pregnancy, during the phase of cellular hyperplasia, will result in an undersized fetus with fewer cells, but normal cell size, causing symmetric IUGR (Robinson *et al.*, 1996). Symmetrical growth retardation is characterised by an equal reduction in brain and body size and normal skinfold thickness. In contrast, fetal growth restriction as a result of an insult acting in mid to late gestation, at the time of rapid increase in fetal weight but after the peak in fetal length growth velocity, will result in a decrease of cell size and fetal weight with less effect on total cell number and fetal length and head circumference, causing asymmetric or disproportionate IUGR (Kramer 1987). Asymmetrically growth retarded infants tend to have relatively large heads, a modest reduction in length relative to the reduction in weight, and thinner skinfolds, with the greatest reduction

in weight observed in organs such as the liver and thymus and the least in brain weight (Kramer 1987).

As a consequence of the reduced supply of oxygen and nutrients, the IUGR fetus undergoes a number of metabolic changes and is hypoxic, hypoglycaemic and has reduced concentrations of branched chain amino acids, but increased triglyceride concentrations (Robinson *et al.*, 1996). In response to these metabolic alterations, fetal neuroendocrine systems reduce the production of anabolic hormones which are essential for growth, such as insulin, IGFs and thyroid hormone, and increase the production of catabolic hormones such as cortisol and catecholamines (Fowden *et al.*, 2001). These endocrine responses to metabolic stress are adaptations which slow fetal growth, enabling the fetus to survive in a substrate-deprived environment, while ensuring the growth of vital organs (Robinson *et al.*, 1996). The rise in catecholamines in particular, causes a loss of muscle mass and fat and glycogen stores, as well as an increase in blood flow to essential organs such as the brain, heart and adrenal glands (Robinson *et al.*, 1996).

1.2.3 Catch-up growth

Following birth, the majority of IUGR infants undergo accelerated rates of growth in terms of weight or length, termed 'catch-up growth' (Karlberg *et al.*, 1995). Catch-up growth has been defined as a 'growth velocity in terms of height and/or length above the statistical limits of normality for age and/or maturity during a defined period of time following a transient period of growth inhibition' (Prader *et al.*, 1963). This period of rapid growth enables the IUGR infant to catch-up to its normal or genetically predetermined growth curve (Boersma *et al.*, 1997). A US study of IUGR infants at the Mount Sinai Hospital demonstrated that catch-up growth following IUGR occurred during the first 6 months of postnatal life and that by two years of age, 71% of infants had caught up to above the 5th centile for both length and weight (Fitzhardinge *et al.*, 1989). A larger study of IUGR infants in Sweden showed that 90% of low birth weight and 87% of low birth length infants had caught up to within 2-SD of average length by the age of 2 years, with the average final height in those that underwent catch-up growth still 0.7-SD below normal, while that of the non-catch-up group was 1.7-SD below normal (Albertsson-Wikland *et al.*, 1994). A subsequent study of Swedish and Hong Kong Chinese infants examined

the timing of early postnatal catch up growth after IUGR (Karlberg *et al.*, 1997). Seventy two percent of Swedish IUGR infants were within 2-SD of the average length and weight at 2 months of age (Karlberg *et al.*, 1997). In Hong Kong, IUGR infants showed evidence of catch-up growth as early as 2 weeks postnatally, and by 5 months of age 65% were within 1-SD of their normal length (Karlberg *et al.*, 1997). Up to 15% of IUGR infants do not undergo catch-up growth in the first 6 to 12 months of life and of these approximately 50% have persistent short stature in adulthood (Karlberg *et al.*, 1995).

1.2.4 Regulation of postnatal growth

The normal postnatal growth pattern in humans consists of three distinct phases. The high rates of growth observed in fetal life are maintained in the period immediately after birth and during early infancy until the age of about 2 years, after which there is a rapid deceleration in growth until the age of about 4 years (Tanaka 1996). This deceleration in the rate of growth is then followed by a period of steady growth during childhood up to the beginning of adolescence (Tanaka 1996). At the onset of adolescence there is a marked acceleration of growth, known as the pubertal growth spurt, which continues into the late teens, followed by a deceleration until growth ceases in early adulthood (Tanaka 1996). Postnatal growth involves lengthening of the long bones, as well as increases in the size and number of cells in the soft tissues, and is dependent upon the influence of genetic, nutritional and endocrine factors. While an individual's maximum growth potential is genetically predetermined, postnatal nutrition is also an important determinant of postnatal growth and adult size (Jackson 1996; Tanaka 1996), with infant undernutrition associated with a decreased postnatal growth rate, delayed bone age and onset of puberty and reduced adult stature (Tanaka 1996). In addition to these genetic and nutritional influences, normal postnatal growth is dependent upon the influence of a number of hormones, with each phase of growth corresponding to specific changes in circulating levels and tissue responsiveness to particular hormones. Early postnatal growth is dependent upon the actions of anabolic hormones such as insulin, the IGFs, growth hormone and thyroid hormones, as well as the catabolic hormone cortisol (Tanaka 1996). During childhood, growth is dependent largely on the influence of growth hormone, while the sex steroids are the major hormonal influences on pubertal growth (Tanaka 1996).

During early postnatal development the IGFs, which are produced by a number of tissues including the liver, stimulate cellular hypertrophy and hyperplasia and influence differentiation in a variety of cell types. In postnatal life the production of IGF-I is dependent on the influence of growth hormone and the presence of specific hepatic growth hormone receptors (Forhead *et al.*, 2002), as well as several other factors including adequate nutrition and thyroid stimulating hormone (TSH), which stimulates IGF-I production by thyroid follicular cells (Daughaday *et al.*, 1989). IGFs play a major role in skeletal muscle growth and development, stimulating glucose and amino uptake and protein synthesis in this tissue (Davis *et al.*, 2002), and are also able to stimulate preadipocyte proliferation and early differentiation (Stewart *et al.*, 1999). In addition, the IGFs are able to stimulate both proliferation and differentiation of myoblasts (Florini *et al.*, 1996), and stimulate bone growth by increasing the proliferation and hypertrophy of chondrocytes (Wang *et al.*, 1999).

Insulin is a major growth promoting hormone in neonatal life. The rise in blood glucose and amino acid concentrations that occurs following a meal triggers an increase in insulin secretion by the β cells of the pancreas. Insulin then stimulates an increase in glucose and amino acid uptake and glycogen and protein synthesis in skeletal muscle (Davis *et al.*, 1998). This anabolic hormone also increases triglyceride synthesis and storage in adipose tissue (Davis *et al.*, 1998), and promotes chondrocyte proliferation and hypertrophy in long bones (Henson *et al.*, 1997). Insulin also exerts its effects on early postnatal growth indirectly, by increasing the number of growth hormone receptors, which in turn increases growth hormone sensitivity and thus IGF-I secretion (Tanaka 1996).

Thyroid hormones play a key role in neonatal growth and development; in particular they have a major influence on the maturation of the cardiovascular and nervous systems during the perinatal period (Kilby *et al.*, 2000), and are important regulators of the basal metabolic rate (Fowden *et al.*, 1995). In addition, thyroid hormones regulate the production of IGF-I and enhance skeletal muscle growth and maturation, in part through their interactions with growth hormone and IGF-I (White *et al.*, 2001; Williams *et al.*, 1998). Thyroid hormones also play a significant role in the growth of long bones, and T_3 has been identified as an important regulator of endochondral bone formation in the epiphyseal growth plate of bones (Tanaka 1996).

Glucocorticoids such as cortisol inhibit postnatal growth, in part by antagonising the actions of insulin and down-regulating IGF gene expression (Fowden *et al.*, 1995; Li et *al.*, 1999). Cortisol inhibits glucose uptake in skeletal muscle and stimulates gluconeogenesis in the liver, increasing blood glucose concentrations. Additionally, cortisol stimulates protein breakdown in skeletal muscle and lipolysis in adipose tissue, increasing circulating concentrations of amino acids and free fatty acids in the blood. While cortisol's exact role in the regulation of neonatal growth in the human is still unclear, studies in children have shown that excessive production of cortisol, as occurs in Cushing Syndrome, inhibits the action of growth factors at the growth plate of bones, reducing the rate of growth of long bones (Tanaka 1996).

1.2.4.1 Mechanisms underlying catch-up growth

Catch-up growth following IUGR may occur in response to several factors including an increased nutrient intake or efficiency of nutrient utilisation, or changes in the activity of one or more of the major endocrine axes which regulate postnatal growth. To date, the rate of intake or efficiency of utilisation of nutrients in neonates undergoing catch-up growth following IUGR has not been well investigated. However in IUGR infants, breastfeeding has been associated with higher rates of growth in terms of weight, length and head length when compared to feeding with a standard infant formula, suggesting that variations in the type of nutrients may explain at least in part, catch-up growth and its variability (Lucas *et al.*, 1997). A number of studies have shown that catch-up growth following IUGR occurs in the presence of reduced, or at best, normal serum IGF-I concentrations during the first year of life (Bennett *et al.*, 1983; Giudice *et al.*, 1995; Ogilvy-Stuart *et al.*, 1998; Toumba *et al*, 2005). Plasma IGF-I concentrations 5 days after birth are lower in IUGR infants compared to controls, however by 9 months of age IGF-I levels are comparable in IUGR and control infants (Thieriot Prevost *et al.*, 1988). Interestingly, at 3 months of age, serum IGF-I and IGFBP-3 concentrations were similar in IUGR infants who underwent catch-up growth and those who did not experience catch-up growth (Garcia *et al.*, 1996). In addition, serum IGF-II concentrations were lower in IUGR children of short stature compared to controls (de Waal *et al.*, 1994), but were higher in IUGR infants that underwent catch-up growth compared to those infants that did not experience catch-up growth, at 3 months of age (Garcia *et al.*, 1996). During the first 2 days after birth, SGA infants

appear to be more insulin sensitive than their appropriate for gestational age (AGA) peers (Bazaes *et al.*, 2003). Similarly, infants with the lowest birth weights and highest weight gain velocity were the most insulin sensitive during the first 2 months of life (Gray *et al.*, 2002).

Therefore it appears that in the human IUGR infant, catch-up growth occurs in the presence of reduced or normal concentrations of the anabolic hormones that regulate neonatal growth, specifically the IGFs. Results from the few studies that have examined whether altered sensitivity to these hormones plays a role in catch-up growth following IUGR, suggest that individuals exposed to growth restriction *in utero* are transiently more sensitive to the actions of anabolic hormones, particularly insulin, as neonates, and that this increased sensitivity is associated with catch-up growth. The endocrine basis of catch-up growth has also been examined using experimental models of IUGR in several species, and the results of these studies will be discussed later in this chapter.

1.2.5 Short and long-term consequences of IUGR and catch-up growth

IUGR in humans has many serious immediate and short-term consequences. The risk of perinatal morbidity and mortality is higher in IUGR infants compared to those born AGA (Buehler *et al.*, 1987; Platt *et al.*, 1995) and these risks have been shown to increase with increasing severity of IUGR (Kramer *et al.*, 1990). IUGR infants have an increased risk of stillbirth, fetal distress, in-hospital mortality, and adverse metabolic and asphyxic neonatal outcomes (Kramer *et al.*, 1990). An increased incidence of hypoglycaemia has been observed in infants born with IUGR, with many demonstrating a significant reduction in hepatic gluconeogenesis and glycogenolysis (Hay 1984). Many IUGR infants suffer from hypothermia due to diminished subcutaneous fat and an elevated surface to volume ratio, as well as asphyxia and acidosis, which in turn have a number of adverse consequences including a high frequency of intrapartum death, low Apgar scores, meconium staining and aspiration syndromes (Kramer *et al.*, 1990). Asphyxia elicits a stress response, increasing serum glucagon levels, which in turn stimulate calcitonin excretion, resulting in a fall in serum calcium levels, which may explain the hypocalcaemia observed in many IUGR infants. Other short-term consequences of IUGR include delayed eruption of teeth and enamel hypoplasia (Wedgwood *et al.*,

1968), as well as an increased incidence of postnatal infections, which may be to delayed humoral and cellular immunity (Ahsworth 1998; Barros *et al.*, 1992). It appears that the preferential shunting of blood to the brain that occurs in response to an unfavorable intrauterine environment is unable to maintain normal cognitive development, with IUGR infants demonstrating an increased incidence of low IQ, learning and behavioral disorders and neurologic handicaps (Newnham 1998; Gaffney *et al.*, 1994; Newman *et al.*, 1997). Up to 10% of IUGR infants are born with a physical handicap (Gaffney *et al.*, 1994) and many demonstrate delayed neurodevelopment (Newnham 1998), performing more poorly on tests of hearing and speech compared to their AGA peers at 4 months of age (Newman *et al.*, 1997). The long-term neurologic outcome in IUGR infants is dependent on the type and severity of IUGR, as well as the presence of a concomitant asphyxial insult (Low *et al.*, 1978; Winer *et al.*, 1982).

Over twenty years ago, analysis of epidemiological data across 212 districts in England and Wales revealed a strong correlation between infant mortality rates between 1921 and 1925 and death rates from coronary heart disease (CHD) and stroke 70 years later, suggesting that a poor environment in early life may increase an individual's risk of disease in adult life (Barker *et al.*, 1986). These findings led to the development of the 'fetal origins of adult disease' hypothesis, which proposes that 'undernutrition *in utero* permanently programmes the body's structure, physiology and metabolism, leading to CVD in adult life' (Barker 1998). During life *in utero*, there are periods of rapid cell division known as 'critical periods' during which the tissues of the body grow and maturation must be achieved (Lucas 1994). 'Programming' describes the process whereby a stimulus or insult that occurs during a critical period of development exerts lasting or lifelong effects (Lucas 1994). Factors such as maternal undernutrition and placental insufficiency result in an inadequate supply of essential substrates to the fetus and cause fetal growth restriction. This restriction of fetal growth is achieved by a slowing in the rate of cell division, especially in those tissues undergoing critical periods of growth and development at the time of the substrate deprivation. Permanent changes in organ size and structure and alterations in cell type are mechanisms by which some adult-onset degenerative diseases are thought to be programmed (Lucas 1994). More recently, studies in a number of different communities around the world have shown that IUGR is associated with an increased risk of several adult onset diseases,

including insulin resistance (Leger *et al.*, 1997; Flanagan *et al.*, 2000; Jacquet *et al.*, 2000; Lithell *et al.*, 1996; Phillips *et al.*, 1994; McKeigue *et al.*, 1998), impaired glucose tolerance and NIDDM (Hales *et al.*, 1991; Phipps *et al.*, 1993; Lithell *et al.*, 1996; Ravelli *et al.*, 1998), hypertension (Barker *et al.*, 1993; Huxley *et al.*, 2000; Roseboom *et al.*, 1999), dyslipidaemia (Barker *et al.*, 1993; Fall *et al.*, 1995; Moore *et al.*, 1997) and obesity (Barker *et al.*, 1997; Law *et al.*, 1992; Valdez *et al.*, 1994; Ravelli *et al.*, 1979). These associations will be discussed in more detail later in this chapter.

Catch-up growth following IUGR has been shown to have a number of short and longer term benefits (Victora *et al.*, 2001; Karlberg *et al.*, 1995; Hokken-Koelega *et al.*, 1995; Lundgren *et al.*, 2001). A study of Brazilian children has shown that SGA infants who demonstrated accelerated growth between birth and 20 months of age had fewer hospital admissions and a lower rate of mortality to 5 years of age compared to SGA infants who did not undergo catch-up growth (Victora *et al.*, 2001). In IUGR infants, both size at birth and the extent of catch-up growth are strong predictors of final adult height, with those individuals failing to undergo catch-up growth demonstrating an increased risk of persistent short stature (Karlberg *et al.*, 1995; Hokken-Koelega *et al.*, 1995). In addition, individuals born SGA who underwent catch-up growth have been shown to have higher intellectual performance scores as adults compared to SGA individuals who did not undergo catch-up growth (Lundgren *et al.*, 2001).

Recently, studies in several populations worldwide have shown that catch-up growth following IUGR independently increases the risk of some chronic adult onset diseases, including insulin resistance (Eriksson *et al.*, 2002), impaired glucose tolerance and NIDDM (Forsen *et al.*, 2000), hypertension (Huxley *et al.*, 2000; Law *et al.*, 2002; Eriksson *et al.*, 2000) and obesity (Parsons *et al.*, 2001; Ravelli *et al.*, 1976). These associations will be discussed in more detail later in this chapter. Therefore, it appears that while catch-up growth may have some beneficial effects for IUGR infants in the short term, it may also increase their risk of disease in adult life.

1.2.6 Experimental models of IUGR and catch-up growth

While epidemiological studies have demonstrated associations between small size at birth, accelerated postnatal growth and an increased risk of various adult-onset disorders, they cannot prove that a causal relationship exists. Hence the need for studies employing appropriate models in non-human species, where fetal growth can be restricted experimentally, and various indices of postnatal function then measured in the growth restricted offspring as adults. While maternal undernutrition has been shown to restrict fetal growth *in utero*, epidemiological studies of women delivering babies during periods of famine have revealed that despite the extremely severe dietary deprivation, the effect on birth weight was slight (Bernstein *et al.*, 1997). In addition, IUGR in children born in developed countries where maternal nutrition is generally good is predominantly caused by substrate limitation due to placental insufficiency. The levels of nutrients in maternal blood perfusing the placenta are obviously important for fetal growth, but even when they are adequate, growth of the fetus may be retarded if uteroplacental blood flow is reduced. In all mammalian species examined to date, variations in placental size can account for much, if not most of the variation in fetal size in late gestation and at term (Owens *et al.*, 1989). Therefore there remains a need for an experimental model of IUGR employing a species that is similar to the human in terms of its developmental physiology, in which growth restriction *in utero* is due primarily to placental rather than maternal factors.

1.2.6.1 Guinea pig model of spontaneous fetal growth restriction

The guinea pig is a precocial species that possesses key developmental, physiological and endocrine similarities to the human (Sohlstrom *et al.*, 1998). The haemochorial placenta of the guinea pig is similar to that of the human, with trophoblast invasion of the maternal uterine vessels. Like the human, the guinea pig lays down both brown and white adipose tissue before birth (Kind *et al.*, 2005), and in spite of its relative glucocorticoid resistance (Keightley *et al.*, 1996), the qualitative response of the guinea pig hypothalamic pituitary adrenal axis (HPAA) to stress is the same as other species. As guinea pigs are more precocious at birth than many other species, including humans, pups can be established on a diet that is independent of maternal lactation soon after birth. In addition, its relatively short gestational length of

approximately 70 days and rapid rate of postnatal development, make it a useful species for examining adult consequences of perturbations before birth.

Guinea pigs produce young with a natural birth weight range of between 60 and 130 grams, as a result of the spontaneous fetal growth restriction that occurs in association with large litter size. Similar to IUGR in humans, spontaneous fetal growth restriction in the guinea pig has been associated with a smaller placenta, reduced placental blood flow and reduced transfer of essential amino acids to the fetus (Saintonge *et al.*, 1981), presumably because of the increased metabolic demand on the mother and increased competition for uterine blood flow and delivery of essential substrates to each developing placenta and fetus. It seems likely that the fetal guinea pig from a large litter will be hypoxic, hypoglycaemic and will have reduced circulating levels of amino acids *in utero*, compared to offspring from small litters; however this is yet to be established. In addition, it is hypothesised that similar to fetal growth restriction in other species, spontaneous fetal growth restriction in the guinea pig will be associated with increased levels of catabolic hormones such as cortisol, catecholamines and glucagon and reduced levels of anabolic hormones such as insulin, insulin-like growth factors and thyroid hormone.

Recently, a study in our laboratory has demonstrated that spontaneous fetal growth restriction in the guinea pig reduced weight, nose to rump length, abdominal circumference and head length and width at birth, but increased fractional growth rates in terms of weight and nose to rump length during the first 36 days of life (Campbell 2006). In addition, this study showed that accelerated growth during the first month of life was associated with increased insulin and IGF-1 sensitivity of glucose metabolism and thyroid hormone activation, but reduced circulating concentrations of IGF-I and II (Campbell 2006). These findings suggest that in the guinea pig, neonatal catch-up growth following spontaneous fetal growth restriction may be driven by an enhanced action of insulin, IGF-I and II and thyroid hormones.

1.2.6.2 Other experimental models of IUGR and catch-up growth

To date, a large number of experimental models of IUGR have been studied, including those which have employed either interference with placental function (Robinson *et al.*, 1979; Owens *et al.*, 1994; Robinson *et al.*, 1996; De Blasio *et al.*, 2007a; De Blasio *et al.*, 2007b; De Blasio *et al.*, 2006; MJ De Blasio *et al.*,

unpublished observations) or limitation of maternal energy intake (Woodall *et al.*, 1996; Sohlstrom *et al.*, 1998; Kind *et al.*, 2002) to restrict fetal growth in several species, including rats, pigs, guinea pigs and sheep. In addition, models employing naturally occurring IUGR as a result of variations in litter size have been studied (Schoknecht *et al.*, 1997; Bauer *et al.*, 1998; Finch *et al.*, 2000; Ritacco *et al.*, 1997; Poore *et al.*, 2003; Poore *et al.*, 2004).

In the rat, the effect of severe maternal feed restriction (30% *ad libitum*) throughout pregnancy on the postnatal growth and endocrine and metabolic function of offspring has been examined (Woodall *et al.*, 1996). Control dams had *ad libitum* access to food throughout pregnancy, while restricted dams were fed 30% of the *ad libitum* intake, determined by the amount of food consumed by the control dams on the previous day (Woodall *et al.*, 1996). Following birth, all pups were cross-fostered to *ad libitum* fed mothers. Maternal undernutrition did not affect litter size however late gestation fetal body weights and placental weights were significantly reduced in fetuses from restricted dams (Woodall *et al.*, 1996). The postnatal body weights of pups born to restricted dams were significantly lower compared to control offspring from birth to 90 days of age. Similarly, nose-rump length was reduced in pups from restricted dams from late gestation until weaning. Significant reductions in plasma IGF-I levels were observed in pups born to restricted mothers from late gestation to postnatal day 9, but not subsequently, while plasma IGFBP-1 and 2 levels increased significantly in restricted pups during late gestation and remained higher at postnatal day 9 (Woodall *et al.*, 1996). Circulating plasma insulin levels were significantly reduced in restricted pups at birth, but not at subsequent time points. Therefore pups exposed to severe maternal undernutrition *in utero* do not undergo catch-up following birth, demonstrating persistent postnatal growth failure (Woodall *et al.*, 1996). A guinea pig model of mild maternal undernutrition (85% *ad libitum*) has also been established (Sohlstrom *et al.*, 1998). Mild maternal feed restriction from 4 weeks before and throughout pregnancy reduced fetal weight, length, and abdominal circumference in late gestation, but did not alter ponderal index (Sohlstrom *et al.*, 1998). In addition, placental weight relative to fetal weight was increased in guinea pigs exposed to maternal undernutrition *in utero* (Sohlstrom *et al.*, 1998). Birth weight, abdominal circumference and head width and the birth weight to length ratio were reduced, and the ratio of head width to abdominal circumference at birth was increased, in male offspring of feed-restricted mothers (Kind *et al.*, 2002). Maternal

feed restriction did not alter the absolute or fractional growth rates for weight from birth to weaning in male or female offspring, although in males body weight was lower from postnatal days 21 to 98 in offspring of feed restricted mothers (Kind *et al.*, 2002). Therefore, like the rat, guinea pigs exposed to maternal undernutrition in fetal life do not undergo catch-up growth after birth and remain smaller than control offspring as adults.

In the pig, birth weight varies 2 to 3 fold amongst littermates providing a naturally occurring form of IUGR (Bauer *et al.*, 1998), which occurs in response to a reduced supply of essential substrates as a result of reduced placental size and blood flow (Finch *et al.*, 2000). Low birth weight pigs are thin and display an increased brain to liver ratio at birth, indicative of asymmetric growth restriction (Bauer *et al.*, 1998). In addition, despite undergoing accelerated rates of growth in fractional terms for weight during the first postnatal month, low birth weight pigs remained smaller than their high birth weight counterparts at 3 months of age (Poore *et al.*, 2003). Interestingly however, by 12 months of age low birth weight pigs had caught up to their high birth weight counterparts in terms of body weight, due to a second phase of accelerated growth between 3 and 12 months of age (Poore *et al.*, 2003). The catch-up growth observed in low birth weight pigs following IUGR occurs with reduced or at best normal levels of anabolic hormones, including IGF-I (Schoknecht *et al.*, 1997; Ritacco *et al.*, 1997). Low birth weight pigs have demonstrated reduced plasma levels of IGF-I, as well as reduced hepatic IGF-I mRNA expression (Schoknecht *et al.*, 1997; Ritacco *et al.*, 1997), however an exogenous infusion of IGF-I between 3 and 10 days of age increased protein and fat accretion in low but not high birth weight pigs (Schoknecht *et al.*, 1997). Further to this, low birth weight pigs that demonstrated high fractional growth rates for weight during the first postnatal month were more insulin sensitive at 3 months of age, as measured by an insulin tolerance test (Poore *et al.*, 2004). These findings suggest that the catch-up growth observed in pigs following IUGR may be driven by an increased sensitivity to the actions of insulin and IGF-I, rather than by increased circulating levels of these hormones.

In the sheep, restriction of placental, and thus fetal growth, can be achieved by the removal of placental attachment sites from the non-pregnant ewe prior to mating. In the cotyledonary placenta of the sheep, there are multiple, discrete areas of

attachment known as cotyledons, which are formed by the interaction of areas of the allantochorion with the endometrium (Robinson *et al.*, 1979). These cotyledons attach to specialised areas of the uterus known as endometrial caruncles, forming complexes known as placentomes. The sheep placenta consists of between 40 and 90 placentomes. Surgical removal of the majority of endometrial caruncles prior to pregnancy restricts total placental size by limiting the number of placentomes in the subsequent pregnancy (Robinson *et al.*, 1979). Placental restriction in the sheep has been shown to exert similar effects on fetal growth and metabolic and endocrine function as human IUGR (Owens *et al.*, 1994). Specifically, placental restriction reduces the supply of oxygen and essential substrates such as glucose to the fetus, reducing fetal growth (Owens *et al.*, 1994). In addition, fetuses exposed to placental restriction display reduced plasma concentrations of several hormones essential for fetal growth, including insulin, IGF-I and IGF-II, T_4 and T_3, as well as increased plasma concentrations of the catabolic hormones cortisol, adrenaline and noradrenaline (Robinson *et al.*, 1979). Like the majority of human IUGR infants, placentally restricted lambs are asymmetrically growth retarded, with the most severe growth restriction observed in organs such as the spleen, thymus, liver and gut, while organs such as the kidney and heart grow in proportion to body weight and growth of the brain is relatively spared, such that it becomes larger relative to body weight (Robinson *et al.*, 1996). More recently, studies in our laboratory have shown that placental restriction of fetal growth in the sheep reduces size at birth and induces catch-up growth postnatally (De Blasio *et al.*, 2007a). Placental restriction reduced size at birth in terms of soft tissues to a greater extent than skeletal tissues, while it increased fractional growth rates for body weight, tibia and metatarsal lengths and hind limb and abdominal circumferences, during the first 45 days of life (De Blasio *et al.*, 2007a). Neonatal catch-up growth following placental restriction was associated with an increased sensitivity to both insulin (De Blasio *et al.*, 2007b) and IGF-I (MJ De Blasio *et al.*, unpublished observations) and increased circulating concentrations of insulin (De Blasio *et al.*, 2007b) and thyroid hormone (De Blasio *et al.*, 2006). These findings suggest that in the sheep, neonatal catch-up growth following placental restriction of fetal growth may be driven by an enhanced action of insulin, IGF-I and thyroid hormone.

1.3 INSULIN RESISTANCE SYNDROME

The IRS is a cluster of metabolic and haemodynamic abnormalities including insulin resistance (an impaired physiological response to endogenous or exogenous insulin), hyperinsulinaemia, impaired glucose tolerance, NIDDM, dyslipidaemia (elevated triglycerides and low HDL-cholesterol) and hypertension (Reaven 1988), all of which are major risk factors for the development of CVD (Reaven 1992). The abnormalities that characterise the IRS are thought to arise due to a combination of environmental influences such as obesity, cigarette smoking and stress, as well as a genetically determined susceptibility (Reaven 1988). More recently, a second tier of associated features has also begun to emerge, involving haematologic and renal abnormalities, including hyperfibrinogenaemia, increased plasma plasminogen activator inhibitor-1 (Festa *et al.*, 1999), low tissue plasminogen activator, neuropathy, microalbuminuria, and hyperuricemia or elevated uric acid levels (Rantala *et al.*, 1999). Disturbances in sex hormones, including increased androgens in women and reduced androgens in men, have also been shown to contribute to the pathophysiology of the IRS in some patients. In particular, these sex hormone disturbances have been well documented in those with polycystic ovary syndrome (Fulghesu *et al.*, 1999).

A recent study in the UK examined differences in the prevalence of the IRS and its associations with prevalent CHD according to ethnicity and sex (Tillin *et al.*, 2005). The prevalence of the IRS, defined according to criteria recommended by the WHO and the National Cholesterol Education Programme (NCEP), was highest in South Asian men (WHO, 46%; NCEP, 29%) and lowest in European women (WHO, 9%; NCEP, 14%) and was associated with prevalent CHD in European and South Asian men, however the associations with CHD were weaker in African-Caribbeans and were inconsistent among European women (Tillin *et al.*, 2005). Similar ethnic and sex-specific variations in the prevalence of the IRS have also been observed in the three largest ethnic groups in the US, namely Caucasians, Hispanics and African-Americans (Ford *et al.*, 2003; Anand *et al.*, 2003). These findings suggest that current definitions of the IRS do not provide a consistent picture of CVD risk when applied to different ethnic groups. Therefore, if the IRS is to be used as a practical indicator of CVD risk, prospective studies are required to refine its definition by

validating readily measurable components and their cutoff values against CVD morbidity and mortality in different ethnic groups.

As the prevalence of the IRS varies across populations and depends heavily on defining characteristics and criteria for diagnosis, it is difficult to accurately define its prevalence worldwide; however it may be possible to derive an estimate based on the known prevalence of its various components. For example, in 1997, 124 million people worldwide were estimated to have NIDDM, an end-point of the IRS, and it has been estimated that by the year 2010 this number will reach 221 million, with the greatest increases in Asia and Africa (Alberti *et al.*, 1998). As the number of people with this syndrome worldwide is likely to be two or three fold higher than this, it is possible that the worldwide prevalence of the IRS by the year 2010 could be as high as 600 million (Hansen 1999).

1.3.1 Metabolic and vascular actions of insulin

The human insulin molecule is a protein with a molecular weight of approximately 6000 Daltons, and is composed of two polypeptide chains (Derewenda *et al.*, 1989). The A chain contains 21 amino acids (A1-A21), while the B chain is longer with 30 amino acids (B1-B30). The chains are held together by two inter-chain disulphide bonds (between A7 and B7, as well as A20 and A19), while an additional disulphide connects A6 and A11 within the A chain (Derewenda *et al.*, 1989). Insulin, which is produced by the β cells of the islets of Langerhans in the pancreas, is the most potent anabolic hormone known and is critical for normal cellular growth and development as well as metabolic function.

For most organisms, carbohydrates, particularly glucose, are an important source of energy. A constant supply of glucose is required by tissues such as the brain, and low blood glucose concentrations have the potential to cause seizures, loss of consciousness, and death. Conversely, prolonged elevations of blood glucose, as occurs in poorly controlled diabetes, can cause blindness, renal failure, cardiac and peripheral vascular disease, and neuropathy (Shepherd *et al.*, 1999). Thus, blood glucose concentrations need to be maintained within narrow limits. In the fasting state, the majority of glucose in the blood is supplied by the liver and is utilised by the brain independently of insulin. The rate at which glucose is normalized after a carbohydrate load is determined by the amount of insulin secreted in response to the

load, as well as the sensitivity of tissues to the secreted hormone. The rise in blood glucose levels that occurs after a meal rapidly stimulates insulin secretion by the β cells of the pancreas, which within minutes results in an increased rate of glucose uptake and glycogen synthesis, primarily in skeletal muscle and adipose tissue (Pessin *et al.*, 2000). Insulin also regulates glucose homeostasis in the liver, reducing hepatic glucose output via decreased gluconeogenesis and glycogenolysis (Pessin *et al.*, 2000). As well as its effects on glucose homeostasis, insulin also stimulates the uptake of amino acids and fatty acids into cells, and promotes the synthesis and storage of proteins and lipids by increasing the activity of enzymes that catalyse protein and lipid synthesis, while inhibiting the activity of those that catalyse degradation (Saltiel *et al.*, 2001). Specifically, insulin increases lipogenesis in liver and fat cells, and attenuates fatty acid release from triglycerides in fat and muscle (Pessin *et al.*, 2000), as well as increasing protein synthesis and inhibiting protein breakdown in muscle. In addition to its primary effects on carbohydrate, protein and lipid metabolism, insulin also promotes a number of other cellular events including the regulation of ion transport, DNA and RNA synthesis, as well as the transcription of specific genes (Cheatham *et al.*, 1995).

Insulin initiates its pleiotropic effects on cellular growth and metabolism by binding to and activating its specific cell-surface receptor (Cheatham *et al.*, 1995). The human insulin receptor is a 400 kDa heterotetrameric membrane glycoprotein, composed of two hormone-binding α subunits (120 kDa) and two signaling β subunits (80 kDa) containing tyrosine kinase activity, which are linked together by disulfide bonds (Kahn *et al.*, 1988). Insulin binds to the extracellular α-subunits of its heterotetrameric receptor, bringing them closer together. This conformational change enables ATP binding to the β subunit's intracellular tyrosine kinase domain, which in turn triggers a series of intramolecular transphosphorylation reactions in which one β subunit phosphorylates its adjacent partner on specific tyrosine residues (Pessin *et al.*, 2000). Insulin receptor autophosphorylation is followed by subsequent tyrosine phosphorylation of critical intracellular signaling intermediates such as the insulin-receptor substrates 1 and 2 (IRS-1 and 2), Shc and Gab1 (Cheatham *et al.*, 1995). Upon tyrosine phosphorylation, these activated intermediates then bind to and activate other signaling molecules, including the adapter proteins Grb2 and Nck, the tyrosine phosphatase Syp, and phosphoinositide-3 kinase (PI 3-kinase), amplifying and diversifying the original signal generated by insulin binding to its

receptor (Cheatham *et al.*, 1995). A multitude of protein intermediates are subsequently activated, including the *ras* (Grb2-mSOS-Ras)-mitogen-activated protein kinase pathway, pp70 kinase, PKB/Akt (protein kinase B), and probably many other unidentified pathways, which in turn coordinate insulin's well characterised effects, including stimulation of cellular glucose and amino acid uptake, glycogen synthesis, lipogenesis, and mitogenesis (Tritos *et al.*, 1998).

Insulin's primary function is the regulation of glucose uptake into skeletal muscle and adipose tissue, which is performed by specific glucose transporter proteins. GLUT-4 is the most abundant glucose transporter in fat and muscle cells, and is located in vesicles that continuously cycle from intracellular stores to the plasma membrane (Pessin *et al.*, 1999). These intracellular storage vesicles also contain proteins such as insulin-responsive aminopeptidase, synaptobrevin (also known as vesicle-associated membrane protein-2, or v-SNARE), and the small guanosine triphosphate-binding protein Rab-4 (Shepherd *et al.*, 1999). Insulin increases glucose transport by increasing the rate of GLUT-4 vesicle exocytosis, and by slightly decreasing the rate of internalisation (Pessin *et al.*, 1999). The insulin signaling pathway that regulates glucose uptake in muscle and fat cells is triggered by binding of insulin to its receptor, which in turn stimulates insulin receptor autophosphorylation, leading to phosphorylation of IRS-1 (Shepherd *et al.*, 1999). IRS-1 then forms complexes with the docking protein PI 3-kinase, which in turn activates phosphoinositide-dependent kinases that participate in the activation of protein kinase B (or Akt) and atypical forms of protein kinase C (Shepherd *et al.*, 1999). Expression of the active forms of protein kinase B and C stimulate the translocation of vesicles containing GLUT-4 from intracellular sites to the plasma membrane, where they dock, forming complexes involving syntaxin-4 (also known as target synaptosome-associated protein receptor, or t-SNARE) and synaptobrevin (Shepherd *et al.*, 1999). The vesicles then fuse with the plasma membrane, allowing extracellular exposure of the GLUT-4 protein and glucose transport into the cell (Shepherd *et al.*, 1999). Once insulin levels in the blood decrease and insulin receptors are no longer occupied, glucose transporters are internalised from the plasma membrane and are repackaged into intracellular storage vesicles.

In addition to its effects on growth and metabolism, recent studies have demonstrated that insulin also plays an important role in the normal functioning of the vasculature

(Verma *et al.*, 1999). Insulin acts as a vasodilatory hormone both in the peripheral and cardiac vasculature, increasing myocardial blood flow and decreasing coronary vascular resistance in a dose-dependent manner in healthy subjects (Laine *et al.*, 2000; Sundell *et al.*, 2002). The vasodilatory effects of insulin are mediated predominantly by nitric oxide (NO), which plays a key role in overall vascular health (Scherrer *et al.*, 1994). In response to insulin stimulation, the vascular wall produces NO, which is beneficial both in the short term for vasomotion and antithrombosis, and in the long term for inhibition of smooth muscle cell proliferation and migration (Cooke *et al.*, 1997). Interestingly, there is a link between the metabolic and vascular effects of insulin at the molecular level. Approximately half of the insulin-induced NO release can be blocked by wortmannin, an inhibitor of PI 3-kinase (Zeng *et al*,. 1996). As PI 3-kinase is a docking protein that is necessary for insulin-stimulated glucose uptake, these findings suggest that insulin-induced NO release and glucose transport share common signaling pathways in the endothelium. Insulin has been shown to induce vasodilation via a number of endothelium-dependent mechanisms. The most important mediator of insulin-induced vasodilation is the L-arginine NO pathway (Scherrer *et al.*, 1994). In peripheral vasculature insulin has been found to rapidly and dose-dependently stimulate NO production in human endothelial cells (Zeng *et al*,. 1996). Once generated, NO diffuses to nearby smooth muscle cells, activating soluble guanylate cyclase in a dose-dependent manner, which in turn increases cGMP concentrations, leading to decreasing intracellular concentrations of calcium and the relaxation of smooth muscle (Collins *et al.*, 1986). The sodium potassium ATPase pump is another important mechanism of insulin-mediated vasodilation. In vascular smooth muscle cells, insulin-induced stimulation of the sodium potassium ATPase pump causes hyperpolarisation, which decreases intracellular calcium concentrations, stimulating endothelial synthesis and release of NO (Dinerman *et al.*, 1993). Lastly, increased sympathetic nervous system (SNS) activity has also been shown to play a role in the regulation of insulin-induced vasodilation (Scherrer *et al.*, 1994).

1.3.2 Aetiology of the Insulin Resistance Syndrome

Resistance to insulin-mediated glucose disposal has been proposed as the underlying defect of the IRS (Reaven 1988; DeFronzo *et al.*, 1991). Both genetic and acquired abnormalities in any of the insulin signaling pathway molecules, from the insulin

receptor to the final effectors, could potentially be implicated in the pathogenesis of insulin resistance (Tritos *et al.*, 1998). Genetic defects in the insulin receptor that influence expression, ligand binding, and tyrosine kinase activity, have been identified (Pessin *et al.*, 2000). These defects are relatively rare, but represent the most severe forms of insulin resistance and are exemplified by leprechaunism, the Rabson Mendenhall Syndrome, and the type A syndrome of insulin resistance (Taylor *et al.*, 1998). The insulin resistance associated with NIDDM is characterised by defects at many levels, including decreases in receptor concentration and kinase activity, the concentration and phosphorylation of IRS-1 and IRS-2, PI 3-kinase activity, glucose transporter translocation, and the activity of intracellular enzymes (Pessin *et al.*, 2000). Reduced insulin-stimulation of glycogen synthesis, which is the primary metabolic defect in NIDDM, appears to be due largely to impaired glucose transport in skeletal muscle (Cline *et al.*, 1999). This occurs because of reductions in proximal insulin signaling, specifically decreased insulin stimulation of the association of PI 3-kinase with IRS-1 (Cusi *et al.*, 2000), which reduces translocation of GLUT-4 to the sarcolemma and thus glucose entry.

In humans, provided β cell function is normal, insulin resistance will result in compensatory hyperinsulinaemia, which in turn will help maintain relatively normal glucose metabolism. The major evidence for this mechanism comes from prospective studies, which have demonstrated that in various populations worldwide, insulin resistance and hyperinsulinaemia exist in the prediabetic state at a time when glucose tolerance is normal, well before the onset of frank NIDDM (Warram *et al.*, 1990; Saad *et al.*, 1988; Lillioja *et al.*, 1993). Approximately 25% of normal, healthy subjects are actually insulin resistant to the same extent as those with NIDDM, but are able to compensate adequately with hyperinsulinaemia, at least initially (Reaven 1995). However, while compensatory hyperinsulinaemia may be beneficial in the short term, maintaining glucose tolerance and delaying the onset of NIDDM, it does have serious long-term adverse effects. One of the main pathophysiological consequences of compensatory hyperinsulinaemia is hypertension (Reaven 1988). Several mechanisms have been suggested to explain the link between high plasma insulin concentrations and raised blood pressure, including increased activation of the SNS, altered peripheral resistance, increased renal sodium retention, altered transmembrane cation transport, growth-promoting effects on vascular smooth muscle cells and vascular hyperreactivity (DeFronzo *et*

al., 1991; Hopkins *et al.*, 1996; Scherrer *et al.*, 1997; Hunter *et al.*, 1998; Sowers *et al.*, 1994). As insulin secretory compensation for skeletal muscle resistance to insulin-mediated glucose disposal becomes inadequate, metabolic homeostasis deteriorates, resulting in an elevation of blood glucose and free fatty acid concentrations (Reaven 1995). There is now substantial evidence to suggest that chronic hyperglycaemia itself can further impair insulin action in peripheral tissues, as well as contribute to β cell desensitisation, impairing insulin secretory function (Yki-Jarvinen 1992). These harmful metabolic effects of chronic hyperglycaemia have been termed 'glucotoxicity' (Yki-Jarvinen 1992). Similar to the glucotoxic effect, increased circulating concentrations of free fatty acids may also further impair insulin secretion and action by 'lipotoxicity' (Reaven 1995). Chronic elevations of plasma free fatty acid concentrations have been shown to decrease insulin-stimulated glucose uptake into peripheral tissues (Boden 1997; Roden *et al.*, 1996), as well as exert lipotoxic effects on the β cell, impairing glucose-stimulated insulin secretion (Sako *et al.*, 1990). Finally, hyperinsulinaemia alone and in combination with elevated free fatty acids will stimulate hepatic production of triglycerides, leading to dyslipidaemia (Reaven 1988).

A subpopulation of individuals with compensated insulin resistance will eventually go on to develop NIDDM, and at least three pathophysiological changes have been observed during this transition from the compensated state to frank NIDDM. The first is a marked fall in β cell function and insulin secretion, although whether this is due to underlying genetic abnormalities in β cell function, acquired defects such as glucotoxicity and lipotoxicity, or both, remains to be determined. Patients with impaired glucose tolerance have normal basal rates of hepatic glucose output, while those with fasting hyperglycaemia have increased hepatic glucose output (Kolterman *et al.*, 1981). Thus increased glucose production by the liver is the second metabolic change that contributes to the pathogenesis of NIDDM. Lastly, there is an acquired worsening of the underlying insulin resistance that occurs during the transition from impaired glucose tolerance to NIDDM (Olefsky 1989). In combination, insulin resistance, hyperinsulinaemia, impaired glucose tolerance, NIDDM, dyslipidaemia and hypertension form the IRS and predispose to the development of CVD (Reaven 1988).

1.3.3 Ageing and the Insulin Resistance Syndrome

1.3.3.1 Human studies

In addition to environmental and genetic influences, ageing, or more precisely senescence, defined as the slow, progressive, irreversible loss of function that occurs in all members of a species with the passage of time (Hornick *et al.*, 1997), has also been identified as a major risk factor for the development of the IRS (Ford *et al.*, 2002). A study of US adults aged 20 years or older who participated in the Third National Health and Nutrition Examination Survey (1988-1994) demonstrated that the IRS is in fact highly prevalent, with approximately 22% of adults having the syndrome. Importantly, prevalence increased with age, from 6.7% among subjects aged 20 through 29 years to 43.5% and 42% for subjects aged 60 through 69 years and 70 years or older, respectively (Ford *et al.*, 2002).

Age-related changes in individual components of the IRS have been well documented. An age-related decline in glucose tolerance in human populations has been demonstrated in a number of studies, employing either oral or intravenous glucose tolerance tests (Davidson 1979; Maneatis *et al.*, 1982; Chen *et al.*, 1987; Shimokata *et al.*, 1991). This progressive decline in glucose tolerance with advancing age has in turn been associated with an increased prevalence of NIDDM in the elderly population (Wingard *et al.*, 1990). As insulin is the primary regulator of glucose homeostasis, several studies have examined insulin action and secretion at different ages to determine whether changes in one or both of these variables can account for the age-related deterioration of glucose tolerance.

The relationship between ageing and insulin resistance in humans is now well established (Fink *et al.*, 1983; Rowe *et al.*, 1983). Fasting plasma insulin levels are increased in elderly individuals (Reaven *et al.*, 1985; Davidson 1979), which is indicative of insulin resistance in either peripheral tissues or the liver. Studies employing the hyperinsulinaemic euglycaemic clamp (HEC) technique have demonstrated that peripheral glucose utilisation (DeFronzo 1979; Pagano *et al.*, 1996; Rowe *et al.*, 1983; Jackson *et al.*, 1988; Gumbiner *et al.*, 1992) and suppression of hepatic glucose production (HGP) by insulin (Jackson *et al.*, 1988; Fink *et al.*, 1983) are impaired with ageing while basal HGP remains relatively normal (DeFronzo 1979; Barzilai *et al.*, 1989; Gumbiner *et al.*, 1992). The effect of

ageing on β-cell function is less clear, with several studies demonstrating an age-related decrease in insulin secretion in response to a glucose challenge (Chen *et al.*, 1985; Gumbiner *et al.*, 1989; Muller *et al.*, 1996), while other studies have demonstrated no change (DeFronzo, 1979; Bourey *et al.*, 1993; Elahi *et al.*, 1993).

Several longitudinal studies in western populations have demonstrated an age-related increase in systolic blood pressure, which is evident well into the ninth decade of life (Folkow *et al.*, 1993). Diastolic blood pressure also increases with increasing age (Bengtsson *et al.*, 1973), reaching a maximum around the sixth decade of life, following which it plateaus or decreases (Landahl *et al.*, 1986; Pearson *et al.*, 1997; Svardsudd *et al.*, 1980). Together, these changes in systolic and diastolic blood pressure lead to a rise in pulse pressure (Franklin *et al.*, 1997). Similarly, the mean arterial pressure has been shown to plateau or decrease in later life (Master *et al.*, 1958; Staessen *et al.*, 1990), while the resting heart rate has also been reported to decrease in aged humans (Fleg *et al.*, 1982).

Ageing is also associated with a number of changes in body composition. Humans lose approximately 20-30% of their skeletal muscle mass between the ages of 20 and 80 years (Carmeli *et al.*, 2002). In addition to this decrease in lean body mass, ageing is also associated with a redistribution of body fat, with an increase in intra-abdominal or visceral adiposity, and a decrease in subcutaneous fat on the limbs (Barbieri *et al.*, 2001). A high visceral fat mass has been associated with many of the metabolic abnormalities that characterise the IRS, and in fact the clustering of these abnormalities is more marked in obese subjects with high levels of visceral fat than in those with a lower visceral fat mass (Lemieux 2001).

1.3.3.2 Animal studies

As a consequence of the advances in our knowledge of human ageing, there has been an increase in the demand for suitable animal models in which genetic as well as environmental influences can be strictly controlled. The most commonly used animal model of ageing has been the rat.

Previous studies in this species have demonstrated that ageing is associated with increased fasting plasma insulin levels (Brancho-Romero *et al.*, 1977). When measured directly, insulin-stimulated glucose uptake has been shown to decrease

(Reaven *et al.*, 1983; Narimiya *et al.*, 1984; Nishimura *et al.*, 1988), or remain unaltered (Barzilai *et al.*, 1995) with ageing. Similarly, the results from studies investigating the effect of ageing on glucose-stimulated insulin secretion in this species have been inconsistent, showing both a decreased (Reaven *et al.*, 1983) and unchanged (Starnes *et al.*, 1991; Ruhe *et al.*, 1992) β-cell response.

The effects of ageing on cardiovascular parameters in the rat have also been inconsistent, with both an increase (Yu *et al.*, 1985), as well as no change (Franchini *et al.*, 1996; McCarty 1985; Werner *et al.*, 1995) in systolic and diastolic blood pressure observed between adulthood and old age. Similarly, resting values for both heart rate and mean arterial pressure have been shown to decrease (Friberg *et al.*, 1985), as well as remain unchanged (Tanabe *et al.*, 1989; Werner *et al.*, 1995; Irigoyen *et al.*, 2000) in rats with ageing.

1.3.3.3 Significance of the current study

Some of the obvious inconsistencies between the rat and human models of ageing may be due to differences in the strain of rat used, as well as the ages at which the animals were studied. Ageing studies in the rat have employed a number of different strains, including Sprague Dawley, Wistar, Fischer 344, Ivanos and Long Evans, each of which may differ in terms of their longevity and thus age-dependent changes in cardiovascular and metabolic function. In a number of ageing studies, juvenile or immature animals have been compared with a young adult or aged population, investigating the effects of growth or maturation, rather than ageing. Unlike humans, in whom skeletal growth is largely complete by adolescence, rats demonstrate growth of the body and skeleton throughout life, highlighting differences in the action of growth-promoting hormones between the two species, which makes it difficult to relate chronological age to true ageing (Folkow *et al.*, 1993). There are also significant differences in the way cholesterol is transported and metabolised in the rat when compared to the human, with blood cholesterol being transported largely in high-density lipoprotein (HDL) (Chapman 1986), while rats are relatively resistant to the development of hypercholesterolaemia and atherogenesis, in contrast to humans (Folkow *et al.*, 1993). The guinea pig exhibits a lipoprotein profile similar to that of the human, with the majority of blood cholesterol being transported in low-density lipoprotein (LDL). In addition, this species responds to dietary fat

and cholesterol in a similar manner to that of the human (Chapman 1986; Lin *et al.*, 1992; Spady *et al.*, 1993). Consequently, the guinea pig is suggested as an ideal candidate for an alternative animal model of ageing.

The aims of the studies described in Chapter 2 were therefore to: (1) determine whether the IRS develops between young (4 months) and aged (14 months) adulthood in the guinea pig, as it does in the human; and (2) to determine whether alterations in body composition, specifically, a loss of skeletal muscle mass and changes in the distribution of adipose tissue, accompany the development of this syndrome with advancing age. The influence of sex on the development of the IRS with increasing age was also assessed.

1.3.4 Early life origins of the Insulin Resistance Syndrome

Epidemiological studies in the UK, North America, India and China, have identified impaired fetal growth, as indicated by a low weight or thinness at birth, as a risk factor for the development of the IRS in childhood (Bavdekar *et al.*, 1999; Li *et al.*, 2001) and adult life (Barker *et al.*, 1993; Phillips *et al.*, 1994; Mi *et al.*, 2000; Valdez *et al.*, 1994; Yarbrough *et al.*, 1998).

The prevalence of the IRS, as indicated by the simultaneous presence of glucose intolerance, NIDDM, hypertension and hypertriglyceridaemia, among 64 year old men in Hertfordshire in the UK, fell progressively from 30% in men who weighed 2.5 kg or less at birth, to 6% in those who weighed 4.3 kg or more at birth (Barker *et al.*, 1993). Similar findings were obtained from another UK study of 50 year old men and women in Preston, where individuals with a low birth weight, or a small head circumference or low ponderal index at birth, demonstrated an increased prevalence of the IRS (Phillips *et al.*, 1994). In both of these studies, the prevalence of the IRS was independent of the influence of adult lifestyle factors such as cigarette smoking and alcohol consumption, as well as social class.

A US study of postmenopausal Caucasian women aged between 50 and 84 years reported that women in the lowest birth weight tertile demonstrated an increased prevalence of the IRS when compared to those in the highest birth weight tertile (Yarbrough *et al.*, 1998). In addition, women who were in the lowest birth weight tertile, who were also in the highest tertile for body mass index (BMI) or waist circumference as adults, exhibited the highest prevalence of the IRS. These findings

reinforce the importance of the interaction between prenatal influences and postnatal lifestyle factors such as obesity, in determining an individual's susceptibility to the development of the IRS.

The association between size at birth and the risk of developing the IRS in adult life has been investigated in a biethnic population in the US (Valdez *et al.*, 1994). In a group of 30 year old Mexican-American and Non-Hispanic white men and women, low birth weight was found to be a major risk factor for the IRS, with the odds of developing the syndrome increasing 1.72 times for each tertile decrease in birth weight (Valdez *et al.*, 1994). These findings were independent of sex, ethnicity, and current levels of socioeconomic status or obesity (Valdez *et al.*, 1994).

To date, the majority of epidemiological studies investigating the link between small size at birth and increased risk of the IRS in adult life have focused on populations in the developed world (Barker *et al.*, 1993; Phillips *et al.*, 1994; Valdez *et al.*, 1994; Yarbrough *et al.*, 1998). The prevalence of a number of disorders including NIDDM and CVD has been shown to increase in developing countries undergoing the transition from chronic malnutrition to adequate nutrition, where improvements in prenatal nutrition often lag behind those of postnatal nutrition (Zimmet *et al.*, 1997; Ramachandran *et al.*, 1997). As the IRS is a precursor to many of these disorders, it has been suggested that there is an increasing prevalence of this syndrome amongst adults in the developing world, who experienced suboptimal fetal nutrition. A study in China has examined the prevalence of the IRS in 45 year old men and women who were born during a period when much of the country's population was chronically malnourished, and who have lived though a period of nutritional transition (Mi *et al.*, 2000). This study reported that as in the developed world, low birth weights infants in China demonstrated an increased risk of the IRS in adult life. Interestingly, although the participants in this study were relatively short in stature, their BMI as adults was similar to that of adults in the UK (Gregory *et al.*, 1990), suggesting that the growing prevalence of the IRS in developing countries may be due in part to a combination of poor fetal nutrition and an increasing BMI in the adult population.

A study of young adults in France failed to demonstrate an association between small size at birth and an increased risk of developing the IRS (Leger *et al.*, 1997). Unlike previous studies which focused on populations of mature adults ranging in age from 30 to 67 years, this study investigated a population of young adults aged 20 years in

order to determine whether the IRS is apparent at this early age (Leger *et al.*, 1997). Interestingly, individuals who were born SGA, as indicated by a low weight or reduced length at birth, were hyperinsulinaemic when compared to control subjects, but demonstrated none of the other abnormalities that characterise the IRS (Leger *et al.*, 1997). As hyperinsulinaemia is a common response by the pancreas to impaired insulin sensitivity, these findings suggest that small size at birth is associated with insulin resistance in young adults, which may lead to the development of other components of the IRS later in life. In contrast, a study of children in India has demonstrated that after adjustment for current weight, age and sex, low birth weight was associated with all of the components of the IRS at 8 years of age (Bavdekar *et al.*, 1999). In addition, the prevalence of the IRS was highest in children with a low birth weight, but high fat mass at 8 years (Bavdekar *et al.*, 1999). Similarly, a US study of Caucasian and African-American children aged between 4 and 14 years, reported an increased risk of the IRS in children of low birth weight, a relationship that was more pronounced in African-American children (Li *et al.*, 2001). These findings suggest that the effects of perturbed growth in early life on the development of the IRS may differ between different ethnic groups.

It has been shown that low birth weight Indian babies have a smaller abdominal circumference, a reduced mid-upper arm circumference and preserve subcutaneous fat, when compared with white European babies (Yajnik *et al.*, 2002a), suggesting that South Asians develop a greater fat mass relative to lean body mass during early development compared with European offspring. This influence of early development on the accumulation and distribution of fat may play an important role in the pathogenesis of the IRS in this population, and may explain why Indian children of low birth weight display many components of the IRS, while their European counterparts do not. Therefore, developing a better understanding of the factors that contribute to body composition within population subgroups is critical, particularly with regard to the ratio of lean body mass to fat mass, as the programming of body composition in different ethnic groups may differentially influence the development of the IRS within individual populations.

1.3.4.1 Altered body composition

1.3.4.1.1 Human studies

Studies in several populations worldwide have demonstrated that low birth weight is associated with an increased risk of obesity in children and adults, in particular, an increased central or truncal fat distribution (Gale *et al.*, 2001; Barker *et al.*, 1997; Law *et al.*, 1992; Rogers 2003; Valdez *et al.*, 1994). In addition, individuals who were small in size at birth tend to have a higher body fat content in childhood and adult life compared to those who were larger at birth, despite having a lower BMI (Fall *et al.*, 1995; Law *et al.*, 1992; Loos *et al.*, 2001; Loos *et al.*, 2002; Malina *et al.*, 1996; Okosun *et al.*, 2000; Singhal *et al.*, 2003). Birth weight was positively associated with the waist to hip ratio, an indicator of abdominal obesity, in men aged 50 years who were born in Hertfordshire or Preston in the UK (Law *et al.*, 1992). Interestingly, a similar UK study of 70 to 75 year old men and women born in Sheffield showed that birth weight was positively associated with adult fat mass as measured by dual energy x-ray absorptiometry (DXA), although simultaneous analysis with body weight revealed a tendency for increasing adiposity with decreasing birth weight (Gale *et al.*, 2001). Exposure to poor nutrition during early gestation has also been shown to increase the risk of obesity in adult life (Ravelli *et al.*, 1999; Ravelli *et al.*, 1976). Increases in body weight, BMI and waist circumference were observed in 19 year old men and 50 year old women who were exposed to the Dutch Winter Famine of 1944-1945 during the first trimester of pregnancy, however no differences were observed in 50 year old men (Ravelli *et al.*, 1999; Ravelli *et al.*, 1976).

The catch-up growth that occurs during the first few months of life following IUGR has also been identified as a risk factor for obesity in childhood and adult life (Ong *et al.*, 2000; Yajnik 2000; Ravelli *et al.*, 1976; Parsons *et al.*, 2001). Individuals who are small in size at birth and undergo catch-up growth during the first two years of life have been shown to be taller, heavier and fatter at 5 years of age compared to those who did not catch-up (Ong *et al.*, 2000). A study of 8 year old children in Pune, India demonstrated that poor growth *in utero* was associated with increased central adiposity in affluent urban children, however this relationship was not observed in poorer rural children (Yajnik 2000). Further to this, 19 year old men exposed to the Dutch Winter famine during the last trimester of pregnancy and during the first few

months of postnatal life, had a reduced risk of obesity, in contrast to those who were exposed to famine during early gestation (Ravelli *et al.*, 1976). The findings from both these studies suggest that poor nutrition in infancy slows the rate of growth during this period and thus prevents compensatory catch-up growth, reducing the risk of obesity in those exposed to suboptimal growth *in utero* (Yajnik 2000; Ravelli *et al.*, 1976). One study has demonstrated that men who were most at risk of becoming obese as adults were those who were light or thin at birth and who experienced a period of rapid childhood growth, achieving a greater proportion of their eventual adult height by age 7 (Parsons *et al.*, 2001).

In addition to an increased risk of obesity in childhood and adult life, individuals who were small in size at birth have also been shown to have deficits in muscle mass, with several studies reporting a direct relationship between birth weight and the relative proportion of lean body mass in later life (Gale *et al.*, 2001; Li *et al.*, 2003; Rogers 2003; Singhal *et al.*, 2003; Phillips *et al.*, 1995; Hediger *et al.*, 1998). Studies in India have shown that babies born small demonstrate increased adiposity and deficits in skeletal muscle at birth (Yajnik *et al.*, 2002a; Yajnik *et al.*, 2004; Yajnik *et al.*, 2003; Yajnik *et al.*, 2002b), a pattern that persists into adult life (Banerji *et al.*, 1999; Yajnik *et al.*, 2002b; Yajnik *et al.*, 2004). Similarly, SGA children aged between 2 and 48 months have been shown to remain lighter throughout childhood compared to their AGA or large for gestational age (LGA) peers, due primarily to a reduced lean body mass (Hediger *et al.*, 1998), while another study has demonstrated that low birth weight is associated with a reduced fat free mass in both children and adolescents (Singhal *et al.*, 2003). In men and women aged 50 years who were born in Preston in the UK, low birth weight was associated with low adult weight, due mainly to a reduced muscle mass (Phillips *et al.*, 1995), while a similar UK study of 70 to 75 year old men and women born in Sheffield used DXA scans to show that low birth weight was associated with a reduced lean muscle mass (Gale *et al.*, 2001).

1.3.4.1.2 Animal studies

A number of experimental studies have examined the relationship between impaired growth in early life and altered body composition in adulthood. In the rat, maternal feed restriction (30% *ad libitum*) throughout gestation reduced the body weight of

offspring throughout postnatal life independently of the level of postnatal nutrition (Vickers *et al.*, 2000). Interestingly, despite their lower body weight, the offspring of feed restricted mothers had an increased relative retroperitoneal fat mass at 100 days of age. This increased adiposity may have been due to the increased food intake observed in these undernourished animals during early post life, which increased with age and was further amplified by a hypercaloric postnatal diet (Vickers *et al.*, 2000). In the same species, maternal feed restriction (50% *ad libitum*) during the first two trimesters lead to increased body weight gains and hyperphagia, but no changes in adiposity, in male offspring maintained on a low fat, high carbohydrate, high protein postnatal diet (Jones *et al.*, 1984). When feed-restricted offspring were challenged with a high-fat diet from 111 days of age however, they developed an increased proportion of body fat as well as increased adipocyte cell size in the epididymal and retroperitoneal fat pads, compared to offspring maintained on a low fat diet (Jones *et al.*, 1984). A more recent study has shown that rat offspring that were exposed to maternal feed restriction (50% *ad libitum)* throughout mid and late gestation and then cross-fostered to *ad libitum* fed mothers after birth, grew faster, were heavier at 3 weeks and 9 months of age, and were fatter at 9 months of age, compared to offspring who suckled on feed-restricted mothers (Desai *et al.*, 2005). The findings from these studies suggest that in the rat, offspring exposed to undernutrition *in utero* may become obese later in life, depending on the degree and timing of maternal feed restriction, as well as the composition of the postnatal diet.

In the rat model of uteroplacental insufficiency, offspring that were exposed to bilateral uterine artery ligation (BUAL) at 19 days gestation and cross-fostered to non-operated mothers after birth were significantly lighter than sham-operated control offspring until 7 weeks of age (Simmons *et al.*, 2001). Between 7 and 10 weeks of age, the intrauterine growth restricted offspring underwent accelerated growth and by 26 weeks of age these animals were obese, demonstrating significant increases in epididymal, mesenteric and perinephric fat pad mass compared to sham-operated controls (Simmons *et al.*, 2001). More recently, placental restriction of fetal growth has been shown to increase relative visceral adiposity in the young lamb (De Blasio *et al.*, 2007a). This increase in visceral adiposity may have been due to the increased feeding activity observed in placentally restricted offspring (De Blasio *et al.*, 2007a).

In the guinea pig, maternal feed restriction (40% *ad libitum*) throughout gestation has been shown to reduce muscle fibre number in the neonate by 20% (Dwyer *et al.*, 1992). Similarly, in the rat, maternal protein restriction throughout gestation reduced skeletal muscle mass at 21 days of age and 11 months of age (Desai *et al.*, 1996). Low birth weight lambs have been shown to have a reduced skeletal muscle mass when compared to their high birth weight counterparts, with persistently lower rates of gain in several skeletal muscles, including the semitendinosus, (Greenwood *et al.*, 1998).

1.3.4.1.3 Significance of the current study

Studies in human populations have demonstrated age-related increases in body weight and body mass index, which peak around the sixth decade (Hornick *et al.*, 1997). In addition, ageing is associated with an increase in visceral adiposity, which is accompanied by a loss of subcutaneous fat (Borkan *et al.*, 1977; Durnin *et al.*, 1974) and skeletal muscle mass (Novak 1972). Only a small number of experimental studies have examined whether the deleterious effects of perturbed prenatal growth on postnatal size and body composition are exacerbated with increasing age. Furthermore, very few studies in animals have investigated the influence of early postnatal growth on adult size and body composition. Previously we have shown that moderate maternal feed restriction (70% *ad libitum* intake) increases the relative weights of the interscapular and retroperitoneal fat depots and reduces the relative weight of the biceps muscle, in the late-gestation fetal guinea pig (Kind *et al.*, 2005). Moderate maternal feed restriction also increased food intake and the relative weight of the retroperitoneal fat depot, and reduced the relative weight of the biceps muscle, in young adult male guinea pigs (Kind *et al.*, 2003). In addition, male and female offspring that experienced accelerated growth during the neonatal period following spontaneous fetal growth restriction, demonstrated increased adiposity and reduced skeletal muscle mass as young adults (DM Horton *et al.*, unpublished observations).

The aims of the studies described in Chapter 3 were therefore to determine the effects of (1) spontaneous fetal growth restriction and (2) accelerated neonatal growth, on body size and composition, in the aged guinea pig. Specifically, the effects of perturbed growth in early life on adult adiposity, skeletal muscle mass and organ and gland weights were investigated. The influence of sex on the impact of

altered perinatal growth on body size and composition in the aged adult was also assessed.

1.3.4.2 Insulin resistance and dyslipidaemia

1.3.4.2.1 Human studies

Epidemiological studies in Europe, India and Australia have shown that poor fetal growth, indicated by a low weight or thinness at birth, is associated with insulin resistance in children (Hofman *et al.*, 1997; Law *et al.*, 1995; Yajnik *et al.*, 1995; Bavdekar *et al.*, 1999), young adults (Leger *et al.*, 1997; Flanagan *et al.*, 2000; Jacquet *et al.*, 2000) and older adults (Lithell *et al.*, 1996; Phillips *et al.*, 1994; McKeigue *et al.*, 1998).

A study of 52-year-old men and women in the UK demonstrated an inverse relationship between insulin resistance and ponderal index at birth (Phillips *et al.*, 1994). In this study a 19% increase in the rate of fall in blood glucose following a bolus of insulin was observed in individuals with a ponderal index of greater than 25 kg m^{-3}, compared to individuals whose ponderal index was less than or equal to 20.6 kg m^{-3} (Phillips *et al.*, 1994). An inverse relationship between ponderal index at birth and adult insulin resistance was also observed in elderly Swedish men following an intravenous glucose tolerance test (IVGTT) (Lithell *et al.*, 1996) or HEC (McKeigue *et al.*, 1998).

A study of 20 year old adults in France reported that individuals with birth weights below the third percentile had higher fasting levels of insulin, higher levels of glucose and insulin following an oral glucose tolerance test (OGTT) (Leger *et al.*, 1997) and a lower insulin-stimulated glucose uptake during a HEC (Jacquet *et al.*, 2000), when compared to normal birth weight controls. Similarly, a low weight or ponderal index at birth was associated with insulin resistance, as measured during a frequently sampled IVGTT, in Australian men aged 20 years (Flanagan *et al.*, 2000).

In India, low birth weight was associated with increased glucose and insulin levels following an IVGTT in 4 year old children (Yajnik *et al.*, 1995) and increased fasting insulin levels in 8 year old children (Bavdekar *et al.*, 1999). A study of short, prepubertal IUGR children has demonstrated that they were more insulin resistant than their short, normal birth weight peers, as measured using a frequently sampled

IVGTT (Hofman *et al.*, 1997), while a study of seven year old children in Salisbury, England reported an inverse relationship between fasting insulin levels and ponderal index at birth (Law *et al.*, 1995).

The results from these studies have since been confirmed in a systematic review of the published literature, which examined the relationship between birth weight and insulin resistance after one year of age (Newsome *et al.*, 2003). Of the 22 studies reviewed, the majority reported an inverse relationship between birth weight and various measures of insulin resistance (Newsome *et al.*, 2003).

Indirect measures of skeletal and non-skeletal catch-up growth during childhood have also been associated with an increased risk of insulin resistance. A study of Finnish men and women aged 69 years has demonstrated that rapid growth in terms of weight and height between the ages of 7 and 15 years was associated with several indices of insulin resistance (Eriksson *et al.*, 2002). The findings from a study of British children support the suggestion that insulin sensitivity may be impaired in children of low birth weight who grow more rapidly postnatally, with the highest fasting plasma insulin concentrations reported in children who were small at birth but in the highest quintile for ponderal index at 10 years of age (Whincup *et al.*, 1997). A study in Indian children has demonstrated that measures of insulin resistance were highest in subjects with low birth weight and greatest current fat mass or tallest height at 8 years (Bavdekar *et al.*, 1999). Similarly, a higher incidence of insulin resistance, measured using homeostasis model assessment (HOMA), has been observed in black South African children of low birth weight who then caught up to a weight above the mean at 7 years of age, compared with those whose weights remained below the mean (Crowther *et al.*, 1998).

There is a strong relationship between insulin resistance, raised plasma triglyceride levels and low HDL-cholesterol levels in the general population (Stern *et al.*, 1986). In addition, the hepatic production of triglycerides is dependent on the supply of non-esterified free fatty acids (NEFA) from lipolysis in adipocytes. In light of the well established relationship between low birth weight and insulin resistance postnatally, it seems plausible that impaired growth *in utero* may also predict the development of an atherogenic lipid profile in later life, specifically, elevated levels of plasma triglycerides and free fatty acids, and reduced levels of HDL-cholesterol.

Several studies have examined the relationship between small size at birth and cholesterol homeostasis in childhood (Forrester *et al.*, 1996; Moore *et al.*, 1997) and adult life (Barker *et al.*, 1993; Clausen *et al.*, 1997; Fall *et al.*, 1995; Kolacek *et al.*, 1993). In children, a reduced length (Forrester *et al.*, 1996) or thinness (Moore *et al.*, 1997) at birth was associated with raised serum levels of total and LDL-cholesterol. In adults however, the relationship was less clear, with small size at birth associated with both an increase (Barker *et al.*, 1993; Fall *et al.*, 1995; Moore *et al.*, 1997) and no change (Clausen *et al.*, 1997; Kolacek *et al.*, 1993) in serum levels of total, LDL and HDL-cholesterol. An association between raised serum triglyceride levels and low birth weight has been demonstrated in children aged between 7 and 11 years (Donker *et al.*, 1997), however no association was observed in adults aged 50 years (Phillips *et al.*, 1995). Similarly, no relationship between birth weight or any other birth measurements and fasting NEFA levels was reported in 50 year old men and women (Phillips *et al.*, 1995).

1.3.4.2.2 Animal studies

Several animal models of IUGR, predominantly in rodents, have examined the long-term consequences for insulin sensitivity, of perturbed growth in early life. Maternal feed restriction (50% of *ad libitum* intake) in the rat during pregnancy and lactation was associated with impaired insulin sensitivity in young adult female offspring, as indicated by a decreased glucose infusion rate during the HEC (Holemans *et al.*, 1996). In contrast to human studies however, where insulin resistance occurs primarily in peripheral tissues, these animals displayed a decreased responsiveness of the liver to the actions of insulin, as indicated by a dampened suppression of glucose production during hyperinsulinaemia (Holemans *et al.*, 1996). More severe maternal feed restriction (30% *ad libitum* intake) throughout pregnancy was associated with fasting hyperinsulinaemia in adult male offspring, which was amplified by postnatal exposure to a high-fat diet (Vickers *et al.*, 2000).

In the same species, young adult offspring born to protein-restricted dams displayed increased insulin-stimulated glucose uptake when compared to controls (Ozanne *et al.*, 1997). Radiolabeled glucose uptake was higher in isolated muscle strips from low-protein animals compared to those from controls, and this enhanced insulin sensitivity was coupled with a twofold increase in insulin receptors in muscle

membranes from low-protein offspring compared with the control group (Ozanne *et al.*, 1997). By mature adulthood however, animals exposed to the low protein diet *in utero* demonstrated a decrease in the insulin sensitivity of glucose uptake in skeletal muscle; however this impairment of insulin action was not associated with altered expression of the insulin receptor or GLUT-4, suggesting that a defect down stream of the insulin receptor was most likely responsible for the observed insulin resistance (Ozanne *et al.*, 2003).

BUAL in the rat has been used to study the effect of placental insufficiency on postnatal insulin sensitivity, measured using a sequential insulin tolerance test (Simmons *et al.*, 2001). Following an intraperitoneal injection of insulin, the rate of fall in blood glucose was higher in control rats when compared with BUAL offspring at 1, 7 and 15 weeks of age; indicating impaired insulin sensitivity in BUAL animals (Simmons *et al.*, 2001). More recently, a study in pigs has demonstrated that thinness at birth, as a result of natural variations in litter size, and rapid catch up growth during the first month of life were associated with increased insulin sensitivity in juvenile males (3 months of age), measured using an insulin tolerance test (Poore *et al.*, 2004). However by adulthood (12 months of age), catch up growth in the first month of life was associated with insulin resistance in both male and female offspring (Poore *et al.*, 2004).

Few experimental studies have examined the effect of perturbed growth in early life on adult lipid metabolism. In rats, maternal protein restriction throughout pregnancy and lactation was associated with reduced levels of plasma total and HDL-cholesterol and triacylglycerol in adult offspring at 6 months of age, and this was seen primarily in males, indicating that impaired growth in early life may permanently alter adult lipid metabolism in the rat, but in the opposite fashion to that observed in human studies (Lucas *et al.*, 1996). However, uteroplacental insufficiency in the same species, achieved by BUAL, was associated with elevated serum triglyceride levels in male offspring at 4 months of age (Lane *et al.*, 2001).

1.3.4.2.3 Significance of the current study

In humans, the age-related decline in insulin sensitivity is now well-established (Fink *et al.*, 1983; Rowe *et al.*, 1983). The changes in body composition that occur with increasing age, namely the increase in visceral adiposity (Barbieri *et al.*, 2001) and

decline in skeletal muscle mass (Carmeli *et al.*, 2002), are thought to contribute to the insulin resistance of ageing (Lemieux 2001). To date, few experimental studies have examined whether the effects of perturbed prenatal growth on postnatal insulin action and lipid metabolism, are amplified with increasing age. Furthermore, only a small number of animal studies have investigated the influence of early postnatal growth on adult insulin sensitivity and circulating lipid concentrations. We have previously shown that in the guinea pig, spontaneous fetal growth restriction and accelerated neonatal growth are associated with impaired whole body insulin sensitivity of glucose metabolism, as measured during the HEC, as well as unaltered fasting free fatty acid concentrations, in young adult male and female offspring (DM Horton *et al.*, unpublished observations). In addition, young adult male guinea pigs of low birth weight demonstrated higher fasting total and LDL cholesterol concentrations when compared to their high birth weight counterparts, in response to a 6-week cholesterol supplemented diet (Kind *et al.*, 1999).

The aims of the study described in Chapter 4 were therefore to determine the effects of (1) spontaneous fetal growth restriction, due to natural variations in litter size, and (2) accelerated neonatal growth, on whole body insulin sensitivity of glucose metabolism and fasting plasma concentrations of total cholesterol, triglycerides and free fatty acids, in the aged guinea pig. The influence of sex on the impact of altered perinatal growth on whole body insulin sensitivity and circulating lipid concentrations in the aged adult was also assessed, as were the relationships between adult size and body composition and whole body insulin sensitivity and circulating lipid profile.

1.3.4.3 Impaired glucose tolerance and NIDDM

1.3.4.3.1 Human studies

Population studies have demonstrated that small size at birth is associated with an increased risk of impaired glucose tolerance and NIDDM in adult life (Hales *et al.*, 1991; Phipps *et al.*, 1993; Lithell *et al.*, 1996; Ravelli *et al.*, 1998). In addition to these studies in older adults, studies in children (Yajnik *et al.*, 1995; Law *et al.*, 1995) and young adults (Robinson *et al.*, 1992) have shown that plasma glucose levels in the basal state and after an oral glucose load are inversely related to birth weight.

A UK study of 64 year old men in Hertfordshire demonstrated that individuals with a low birth weight had an increased risk of impaired glucose tolerance and NIDDM as adults (Hales *et al.*, 1991). Similarly, a study of men and women in Preston showed that the incidence of glucose intolerance and NIDDM increased from 6% in those who weighed greater than 3.4 kg at birth to 27% in those who weighed less than 2.5 kg at birth (Phipps *et al.*, 1993). Thinness at birth, as indicated by a low ponderal index, has also been associated with adult glucose intolerance and NIDDM, with a study of 60 year old Swedish men reporting that the prevalence of NIDDM was three times higher in the lowest quintile of ponderal index than in the other four quintiles, an association that was independent of adult BMI (Lithell *et al.*, 1996).

A follow-up study of 50 year old men and women born to mothers exposed to the Dutch Winter famine in 1944-45 has demonstrated that the rate of glucose intolerance was higher in those exposed to famine during mid and late gestation (Ravelli *et al.*, 1998). Fasting proinsulin and 2-h plasma glucose and insulin levels after oral glucose administration were higher in individuals exposed to the famine *in utero* compared to a non-exposed population (Ravelli *et al.*, 1998).

The relationship between small size at birth and altered glucose regulation has also been reported in young adults, with a study of 21 year old British men demonstrating that low birth weight was associated with increased glucose levels 30 minutes after an oral glucose load (Robinson *et al.*, 1992). Similar relationships have been observed in 4 year old children in India (Yajnik *et al.*, 1995) and 7 year old children in the UK (Law *et al.*, 1995), where 30 minute glucose levels were inversely related to birth weight and ponderal index at birth respectively.

The majority of studies that have examined the association between low birth weight and impaired postnatal insulin secretion have failed to report positive findings (Bavdekar et al., 1999; Lithell *et al.*, 1996; Choi *et al.*, 2000; Cook *et al.*, 1993; Phillips *et al.*, 1994). This suggests that a defect in pancreatic function may not be the primary cause of glucose intolerance in individuals who were growth restricted *in utero*. Studies in short prepubertal children who were growth retarded at birth (Hofman *et al.*, 1997) and in young adults of small size at birth (Flanagan *et al.*, 2000; Jacquet *et al.*, 2000) have shown that despite impaired insulin sensitivity, normal glucose tolerance was been maintained, due primarily to an increased first-

phase acute insulin response, but also to increased glucose effectiveness, which is the ability of glucose per se to enhance glucose disposal and suppress endogenous glucose production (Flanagan *et al.*, 2000).

These findings have since been confirmed in a systematic review of the published literature which examined the relationship between birth weight and measures of glucose and insulin metabolism, including the prevalence of NIDDM, after one year of age (Newsome *et al.*, 2003). The majority of studies reviewed reported an inverse relationship between birth weight and fasting plasma glucose and insulin concentrations, plasma glucose concentrations 2 hours after a glucose load, and the prevalence of NIDDM (Newsome *et al.*, 2003). The relationship between birth weight and insulin secretion however, was less clear (Newsome *et al.*, 2003).

Indirect measures of skeletal and non-skeletal postnatal catch-up growth have been associated with an increased risk of developing impaired glucose tolerance and NIDDM in childhood (Crowther *et al.*, 1998) and adult life (Forsen *et al.*, 2000). Low birth weight in combination with rapid weight gain during childhood has been shown to impair glucose tolerance in 7 year old black South African children, increasing their susceptibility to NIDDM later in life (Crowther *et al.*, 1998). In this population, suboptimal growth *in utero* followed by accelerated growth between birth and 7 years, has been shown to reduce the capacity of the pancreas to process proinsulin into insulin, leading to glucose intolerance (Crowther *et al.*, 1998). Similarly, a study of 69 year old Finnish men and women has demonstrated that the risk of impaired glucose tolerance and NIDDM in adulthood was higher in those who were light, short or thin at birth, but had caught up in terms of weight and height between birth and 7 years of age, and continued to grow at an accelerated rate to 15 years (Forsen *et al.*, 2000).

1.3.4.3.2 Animal studies

Numerous experimental models of IUGR have been employed to investigate the relationship between suboptimal growth *in utero* and the development of impaired glucose tolerance and NIDDM in adult life. In the rat, maternal feed restriction (50% *ad libitum* intake) during early pregnancy did not alter glucose tolerance or insulin secretion in young adult offspring (Portha *et al.*, 1995), while the same level of restriction employed from day 15 of pregnancy until weaning was associated with an

age-dependent loss of glucose tolerance, which was evident in male offspring at 12 months of age (Garofano *et al.*, 1999). One study has examined the combined effects of severe maternal feed restriction (30% *ad libitum* intake) during pregnancy, and post-weaning overnutrition (30% hypercaloric diet) on adult glucose tolerance (Vickers *et al.*, 2001). Offspring that were exposed to maternal feed restriction *in utero* and a hypercaloric diet post-weaning, demonstrated elevated fasting levels of glucose and insulin at 100 days of age (Vickers *et al.*, 2001).

Maternal protein restriction in the rat during pregnancy and lactation was associated with unaltered (Langley-Evans *et al.*, 1994) or improved (Langley-Evans *et al.*, 1994; Dahri *et al.*, 1991; Shepherd *et al.*, 1997; Hales *et al.*, 1996) glucose tolerance in young adult offspring. By mature adulthood however, low-protein offspring displayed impaired glucose tolerance (Hales *et al.*, 1996), and frank NIDDM (Petry *et al.*, 2001). In the same species, maternal iron restriction has been employed to investigate the long-term consequences for glucose tolerance and insulin secretion, of what is a common micronutrient deficiency in human populations (Lewis *et al.*, 2001). The young adult offspring of iron-restricted dams displayed improved glucose tolerance when compared to controls; however fasting insulin levels were not significantly different between the two groups (Lewis *et al.*, 2001).

In the rat, the effect of uterine artery ligation on adult glucose tolerance and insulin secretion varies depending on the timing and nature (unilateral or bilateral) of the ligation (Jansson *et al.*, 1999; Simmons *et al.*, 2001). One study demonstrated that unilateral uterine artery ligation on day 18 of gestation resulted in elevated fasting blood glucose levels, and impaired glucose tolerance and reduced insulin secretion in response to a glucose load, in female, but not male offspring at 3 months of age (Jansson *et al.*, 1999). BUAL on day 19 of gestation resulted in elevated fasting levels of glucose and insulin between 7 and 10 weeks of age, which progressively worsened by 26 weeks, in both male and female offspring (Simmons *et al.*, 2001). In addition, in response to an intraperitoneal injection of glucose, the acute, the first phase insulin response of male and female BUAL animals was reduced by 50% at 1 week of age and did not occur at all at 26 weeks of age, when compared to sham operated controls (Simmons *et al.*, 2001). Assay results explained the cause of this insulin secretory defect, showing that compared to sham operated controls, BUAL

rats had lost 50% of their β cell mass at 15 weeks, and two thirds of their β cell mass by 26 weeks (Simmons *et al.*, 2001).

The effect of natural variations in birth weight on glucose tolerance in juvenile and adult pigs has been investigated (Poore *et al.*, 2002). Low birth weight offspring demonstrated unaltered glucose tolerance as juveniles; however as adults these animals displayed impaired glucose tolerance and an increased insulin response to glucose administration (Poore *et al.*, 2002). Additionally, impaired adult glucose tolerance was associated with a low BMI and disproportionate size at birth, while catch up growth during the first month of life was associated with reduced fasting insulin levels in adulthood (Poore *et al.*, 2002).

1.3.4.3.3 Significance of the current study

The age-related deterioration in glucose tolerance and subsequent rise in the prevalence of NIDDM in human populations is well-documented (Davison *et al.*, 1979; Shimokata *et al.*, 1991; Harris *et al.*, 1987). These alterations in glucose metabolism with increasing age are thought to occur primarily due to a defect in insulin action (DeFronzo 1979; Chen *et al.*, 1985). Only a small number of animal studies have investigated the effect of suboptimal growth *in utero* on postnatal glucose tolerance and insulin secretion in aged offspring. Further to this, few experimental studies have examined the influence of early postnatal growth on adult glucose homeostasis. Previously we have shown that moderate maternal feed restriction in the guinea pig (85% *ad libitum* intake) causes fasting hyperinsulinaemia and an increased insulin response to an IVGTT, in young adult male offspring (Kind *et al.*, 2003). Additionally, male offspring that experienced accelerated growth during the neonatal period, demonstrated elevated fasting insulin concentrations as young adults (Kind *et al.*, 2003).

The aims of the studies described in Chapter 5 were therefore to determine the effects of (1) spontaneous fetal growth restriction, due to natural variations in litter size, and (2) accelerated neonatal growth, on glucose tolerance and insulin secretion, in the aged guinea pig. The influence of sex on the impact of altered perinatal growth on glucose tolerance and insulin secretion in the aged adult was also assessed, as were the relationships between adult size and body composition and glucose tolerance and insulin secretion.

1.3.4.4 Hypertension

1.3.4.4.1 Human studies

Studies in human populations have shown that low birth weight, as a result of suboptimal intrauterine growth, is associated with an increased risk of hypertension in adult life (Barker *et al.*, 1989). The relationship between birth weight and systolic blood pressure has been investigated in a systematic review of 80 studies, involving male and female subjects aged between 0 and 84 years, from a wide variety of ethnic backgrounds (Huxley *et al.*, 2000). This review demonstrated that in both males and females, and in all ages and races, blood pressure fell by approximately 2 mmHg with each kilogram increase in birth weight, and this negative relationship was amplified with increasing age in adult life (Huxley *et al.*, 2000). In addition, disproportionate growth *in utero*, as indicated by a low ponderal index or reduced head circumference at birth, was also associated with raised blood pressure in adult life (Barker *et al.*, 1993), with a 0.5 mmHg fall in systolic blood pressure observed for each centimetre increase in head circumference at birth (Huxley *et al.*, 2000).

Interestingly, men and women who were exposed *in utero* to the Dutch Winter famine of 1944-1945, did not display increased blood pressure as adults, when compared to a non-exposed population (Roseboom *et al.*, 1999). In this population, birth weight was negatively associated with adult blood pressure, suggesting that prenatal growth was a more important determinant of adult blood pressure than maternal famine exposure (Roseboom *et al.*, 1999).

In addition to impaired growth *in utero*, accelerated postnatal growth of skeletal and soft tissues has also been associated with raised blood pressure in adult life (Huxley *et al.*, 2000). In a UK study of 22 year old men and women, rapid weight gain during the first year of life did not influence adult systolic blood pressure (Law *et al.*, 2002). Low birth weight and rapid weight gain between the ages of 1 and 5 years however, were independently associated with a higher systolic blood pressure, such that the highest adult blood pressures were observed in individuals who were the lightest at birth, but gained the most weight during early childhood (Law *et al.*, 2002). Similarly, a study of elderly Finnish men and women demonstrated that individuals who were small at birth, but who subsequently caught up to average

weight and height at 7 years of age, and were heavier and fatter than average at 15 years, had a higher risk of becoming hypertensive as adults (Eriksson *et al.*, 2000).

1.3.4.4.2 Animal studies

The relationship between impaired growth in early life and elevated blood pressure in adulthood has been investigated in a number of experimental models of IUGR. In the rat, the effect of varying degrees of maternal feed restriction on the blood pressure of adult offspring has been investigated (Holemans *et al.*, 1999; Ozaki *et al.*, 2001; Vickers *et al.*, 2000; Woodall *et al.*, 1996). Severe maternal feed restriction (30% *ad libitum* intake) throughout pregnancy was associated with elevated systolic blood pressure in offspring at 14 weeks of age (Vickers *et al.*, 2000), with blood pressure remaining elevated in at 30 weeks (Woodall *et al.*, 1996). The restriction of maternal feed intake to 50% of *ad libitum* intake throughout the second half of pregnancy did not alter systolic blood pressure or heart rate in young adult offspring (Holemans *et al.*, 1999), however a milder restriction throughout gestation (70% *ad libitum* intake) was associated with a rise in adult systolic blood pressure (Ozaki *et al.*, 2001). In the sheep, mild maternal undernutrition during early gestation has been shown to increase mean arterial blood pressure in chronically catheterised lambs (Hawkins *et al.*, 2000), however these effects appear to be transient, as systolic blood pressure was subsequently reduced in adult offspring (Itoh *et al.*, 2000).

Varying degrees of maternal protein restriction in the rat, before conception and throughout pregnancy, was associated with elevated blood pressure in offspring by 9 weeks of age, with blood pressure remaining elevated in protein-restricted offspring at 21 weeks (Langley *et al.*, 1994). Similarly, maternal protein restriction during fixed periods in early, mid or late gestation was associated with raised blood pressure in the offspring by weaning, however protein restriction throughout the entire gestational period elicited a greater increase in blood pressure than discrete periods of protein restriction alone (Langley-Evans *et al.*, 1996).

In addition to isocaloric maternal protein restriction, the effect of deficiencies in other micronutrients in the maternal diet, including iron (Crowe *et al.*, 1995; Lewis *et al.*, 2001) has been investigated. At 3 weeks of age, offspring born to iron-restricted dams displayed decreased systolic blood pressure when compared to controls, however by 6 weeks systolic blood pressure was significantly higher in iron-

restricted pups than controls (Crowe *et al.*, 1995), a relationship that was still evident at 12 weeks of age (Lewis *et al.*, 2001).

The majority of experimental studies that have examined the effect of perturbed growth in early life on blood pressure in adulthood have employed either vascular catheters acutely implanted under anaesthesia, or tail-cuff plethysmography, a technique that requires restraint and elevated temperature, both of which are potential sources of stress for the animal. The effect of maternal protein restriction before conception and throughout pregnancy, on blood pressure in young adult rat offspring, has been measured under nonstress conditions using 24-hour radiotelemetric measurements in conscious, unrestrained animals (Tonkiss *et al.*, 1998). Adult offspring born to protein-restricted dams displayed small increases in diastolic blood pressure and heart rate; however systolic blood pressure was not significantly different between control and protein-restricted pups (Tonkiss *et al.*, 1998). These results are in contrast to previous studies in the same species in which similar or less severe levels of maternal protein restriction have yielded large elevations in systolic blood pressure in adult offspring, when measured by tail-cuff plethysmography. This suggests that stress may have contributed to the significant differences in blood pressure seen in these earlier investigations, highlighting the need for a chronically catheterised experimental model of IUGR.

In the guinea pig, severe fetal growth restriction, caused by unilateral uterine artery ligation, has been associated with increased mean arterial blood pressure and heart rate in chronically catheterised young adult offspring (Persson *et al.*, 1992). More recently, the effect low birth weight, as a result of natural variations within litters, on basal cardiovascular function, has been investigated in chronically catheterised juvenile pigs (Poore *et al.*, 2002). Basal mean arterial pressure was negatively associated with birth weight, and positively associated with disproportionate size at birth, as indicated by an increased head length to birth weight ratio, in pigs at 12 weeks of age (Poore *et al.*, 2002).

1.3.4.4.3 Significance of the current study

Longitudinal studies in urban human populations have demonstrated an age-related rise in systolic blood pressure at least into the ninth decade, which is accompanied by an increase in diastolic blood pressure that peaks in the sixth decade, resulting in a

widening of the pulse pressure in elderly individuals (Landahl *et al.*, 1986; Pearson *et al.*, 1997; Svardsudd *et al.*, 1980). These age-related alterations in blood pressure are thought to occur in response to changes in the structure and function of the vasculature (Lakatta *et al.*, 1987), as well as the influence of anthropometric (Sutton-Tyrrell *et al.*, 2001) and metabolic (Taquet *et al.*, 1993; Salomaa *et al.*, 1995) factors. A study of children and young adults in Australia has shown that the amplification of blood pressure between 8 and 20 years of age was greatest in those with the lowest birth weights (Moore *et al.*, 1996), however very few studies in animals have examined whether the effects of perturbed prenatal growth on postnatal blood pressure are amplified with increasing age. Additionally, only a small number of experimental studies have investigated the influence of early postnatal growth on adult blood pressure. We have previously shown that moderate maternal feed restriction (85% *ad libitum* intake) in the guinea pig increases systolic blood pressure in chronically catheterised young adult male offspring (Kind *et al.*, 2002). A reduced head width at birth was associated with raised systolic and mean arterial blood pressure in young adult offspring of *ad libitum* fed and feed-restricted mothers, however other parameters of basal cardiovascular function, including heart rate and pulse pressure were unaltered (Kind *et al.*, 2002). In addition, male offspring of *ad libitum* fed mothers that experienced accelerated growth during the neonatal period, demonstrated elevated systolic blood pressure as young adults (Kind *et al.*, 2002).

The aims of the studies described in Chapter 6 were therefore to determine the effects of (1) spontaneous fetal growth restriction and (2) accelerated neonatal growth, on resting systolic, diastolic and mean arterial blood pressure, pulse pressure and heart rate, in the aged guinea pig. The influence of sex on the impact of altered perinatal growth on resting blood pressure and heart rate in the aged adult was also assessed, as were the relationships between adult size and body composition and resting blood pressure and heart rate.

1.4 AIMS AND HYPOTHESES

It is clear from the reviewed literature that in human populations, both perturbed growth in early life and ageing are associated with an increased risk of developing many of the adult-onset degenerative diseases which comprise the IRS. Several

experimental models of IUGR, predominantly in the rat, have investigated the relationship between suboptimal growth *in utero* and the risk of developing metabolic and cardiovascular disorders in adulthood, however the results from a number of these studies have failed to support the findings of epidemiological studies. The inconsistent nature of the results from these animal studies may be due in part to species differences and methodological limitations. For example, the rat is relatively immature at birth when compared to the human and only reaches a comparable maturity at weaning. Therefore many of the critical developmental events that take place during fetal life in humans occur postnatally in the rat. In addition, many of these studies have used animals that were anaesthetised immediately before and during experimentation, to allow for the implantation of vascular catheters. This process places the animal under considerable stress, which in turn has effects on its metabolic and cardiovascular homeostasis that may obscure or amplify the outcomes under investigation. Furthermore, few experimental studies have examined the effect of accelerated postnatal growth on adult function, or whether the effects of perturbed prenatal and early postnatal growth are exacerbated with increasing age.

Guinea pigs reach sexual maturity by 2 months of age, and previous studies in our laboratory have demonstrated that in this species, which is more precocious at birth than the rat, spontaneous fetal growth restriction due to large litter size, and neonatal catch-up growth predict insulin resistance and some, but not all of its related abnormalities in young adult offspring at 4 months of age (DM Horton *et al.*, unpublished observations; Kind *et al.*, 1999; Kind *et al.*, 2002; Kind *et al.*, 2003). It was therefore hypothesised that by 14 months of age, guinea pigs that underwent spontaneous fetal growth restriction will have developed most, if not all of the metabolic and cardiovascular abnormalities that characterise the IRS, and could thus be classed as 'aged'. Therefore in this study, the effects of spontaneous fetal growth restriction, accelerated neonatal growth, and ageing on the development of the IRS in the chronically catheterised guinea pig were investigated. Additionally, the effect of these factors on body size and composition were also examined. Several indices of fetal growth have been used in human studies with the most easily measured being weight, head width reflecting brain growth which is relatively spared, and longitudinal growth or length, which is also less affected, and their ratios to weight. To facilitate comparison with the outcomes of studies in humans and other species,

these indices were used in the studies described in this thesis. The results from this study will help determine if altered growth in early life and ageing play a role in the aetiology of the IRS in this animal model, and this knowledge may in turn assist in the design and testing of interventions to prevent or restrain the onset and progression of the IRS in humans, either following perinatal perturbations or more generally.

1.4.1 General hypothesis

In the guinea pig, spontaneous fetal growth restriction and accelerated neonatal growth exert deleterious effects on postnatal metabolic and cardiovascular function, that are then amplified by the process of ageing, resulting in offspring which as aged adults display all of the abnormalities that characterise the IRS.

1.4.2 Specific hypotheses

1.4.2.1 Chapter 2

In the guinea pig, ageing will decrease whole body insulin sensitivity of glucose metabolism, glucose tolerance and insulin secretion in response to an IVGTT, combined skeletal muscle mass and subcutaneous adiposity, and will increase fasting plasma glucose, insulin, free fatty acid, triglyceride and total cholesterol concentrations, resting systolic, diastolic and mean arterial blood pressure, pulse pressure, body weight, BMI, and combined and visceral adiposity, in male and female offspring.

1.4.2.2 Chapter 3

Spontaneous fetal growth restriction in the guinea pig, will decrease size at birth and adult size and increase the neonatal fractional growth rate for weight, in male and female offspring.

Spontaneous fetal growth restriction and accelerated neonatal fractional growth rate for weight in the guinea pig will decrease combined skeletal muscle mass and increase combined and visceral adiposity, in aged male and female offspring.

1.4.2.3 Chapter 4

Spontaneous fetal growth restriction and accelerated neonatal fractional growth rate for weight in the guinea pig will decrease whole body insulin sensitivity of glucose metabolism and increase fasting plasma free fatty acid, triglyceride and total cholesterol concentrations, in aged male and female offspring.

1.4.2.4 Chapter 5

Spontaneous fetal growth restriction and accelerated neonatal fractional growth rate for weight in the guinea pig will increase fasting plasma glucose and insulin concentrations and decrease glucose tolerance and insulin secretion in response to an IVGTT, in aged male and female offspring.

1.4.2.5 Chapter 6

Spontaneous fetal growth restriction and accelerated neonatal fractional growth rate for weight in the guinea pig will increase resting systolic, diastolic and mean arterial blood pressure, pulse pressure and heart rate, in aged male and female offspring.

1.4.3 Aims

1.4.3.1 Chapter 2

To determine the effects of age and gender on the development of the IRS in the guinea pig, by measuring:

- whole body insulin sensitivity of glucose metabolism
- fasting plasma free fatty acid, triglyceride and total cholesterol concentrations
- fasting plasma glucose and insulin concentrations
- glucose tolerance and insulin secretion in response to an IVGTT
- resting systolic, diastolic, and mean arterial blood pressure, pulse pressure and heart rate
- body weight, nose to rump length and BMI
- combined, type 2 and mixed skeletal muscle mass
- combined, subcutaneous and visceral adiposity

in young adult (4 months) and aged (14 months) male and female offspring.

1.4.3.2 Chapter 3

To determine the effect of spontaneous fetal growth restriction in the guinea pig on:

- size at birth
- neonatal growth rate for weight

in male and female offspring.

To determine the effects of spontaneous fetal growth restriction and accelerated neonatal fractional growth rate for weight in the guinea pig on:

- body weight, nose to rump length and BMI
- combined, type 2 and mixed skeletal muscle mass
- combined, subcutaneous and visceral adiposity
- organ and gland weights

in aged (14 months) male and female offspring.

1.4.3.3 Chapter 4

To determine the effects of spontaneous fetal growth restriction and accelerated neonatal fractional growth rate for weight in the guinea pig on:

- whole body insulin sensitivity of glucose metabolism
- fasting plasma free fatty acid concentrations
- fasting plasma triglyceride concentrations
- fasting plasma total cholesterol concentrations

in aged (14 months) male and female offspring.

1.4.3.4 Chapter 5

To determine the effects of spontaneous fetal growth restriction and accelerated neonatal fractional growth rate for weight in the guinea pig on:

- fasting plasma glucose concentrations
- fasting plasma insulin concentrations
- glucose tolerance in response to an IVGTT
- insulin secretion in response to an IVGTT

in aged (14 months) male and female offspring.

1.4.3.5 Chapter 6

To determine the effects of spontaneous fetal growth restriction and accelerated neonatal fractional growth rate for weight in the guinea pig on:

- resting systolic blood pressure
- resting diastolic blood pressure
- resting mean arterial blood pressure
- resting pulse pressure
- resting heart rate

in aged (14 months) male and female offspring.

CHAPTER 2

EFFECT OF AGEING ON THE METABOLIC AND CARDIOVASCULAR HEALTH OF THE GUINEA PIG

2.1 INTRODUCTION

Ageing has been shown to play an important role in the development of the IRS in humans, with the prevalence of this syndrome increasing with age, at least into the seventies (Ford *et al.*, 2000). The IRS is a cluster of metabolic and haemodynamic abnormalities including insulin resistance, hyperinsulinaemia, impaired glucose tolerance, NIDDM, dyslipidaemia and hypertension (Reaven 1988), all of which are major risk factors for the development of CVD (DeFronzo *et al.*, 1991; Reaven 1988). An increased prevalence of impaired glucose tolerance and NIDDM has been reported in elderly populations (Davidson 1979; Maneatis *et al.*, 1982; Chen *et al.*, 1987; Shimokata *et al.*, 1991; Wingard *et al.*, 1990), due primarily to an age-related decline in insulin sensitivity (Fink *et al.*, 1983; Rowe *et al.*, 1983). Similarly, systolic blood pressure has been shown to increase with advancing age, and this is evident well into the ninth decade of life (Folkow *et al.*, 1993). Age-related alterations in body composition, including an increase in visceral adiposity (Barbieri *et al.*, 2001) and a decline in skeletal muscle mass (Carmeli *et al.*, 2002), are thought to contribute to the deterioration in metabolic and cardiovascular function observed with increasing age (Lemieux 2001).

Various non-human species, predominantly the rat, have been utilised in an attempt to determine the mechanisms underlying the age-related changes in metabolic (Brancho-Romero *et al.*, 1977; Nishimura *et al.*, 1988; Barzilai *et al.*, 1995; Reaven *et al.*, 1983; Ruhe *et al.*, 1992) and cardiovascular function (Yu *et al.*, 1985; Franchini *et al.*, 1996; Friberg *et al.*, 1985; Irigoyen *et al.*, 2000) seen in human populations. While informative, the outcomes of such studies have revealed differences in some consequences of ageing for the rat and human, which may reflect amongst other things, known species differences in lipid metabolism and in the actions of growth-promoting hormones (Chapman 1986; Folkow *et al.*, 1993). These include some of the responses of circulating lipids and vascular health to dietary fat,

which are similar in the guinea pig and human, which share a more comparable circulating lipid profile and homeostatic capacity in relation to atherogenic lipids (Chapman, 1986; Lin *et al.*, 1992; Spady *et al.*, 1993). A number of environmental factors, including a high dietary fat intake, may interact with ageing, to influence and increase an individual's risk of developing the IRS. Hence the guinea pig may be a species with the potential to allow gaps in the study of ageing and its related metabolic and cardiovascular consequences to be addressed.

The aims of the studies described in Chapter 2 were therefore to: (1) determine whether the IRS develops between young (4 months) and aged (14 months) adulthood in the guinea pig, as it does in the human; and (2) to determine whether alterations in body composition, specifically, a loss of skeletal muscle mass and changes in the distribution of adipose tissue, accompany the development of this syndrome with advancing age. The influence of sex on the development of the IRS with increasing age was also assessed. We hypothesised that in the guinea pig ageing will decrease whole body insulin sensitivity of glucose metabolism, glucose tolerance and insulin secretion in response to an IVGTT, as well as combined skeletal muscle mass and subcutaneous adiposity, in males and females. It was also hypothesised that in male and female guinea pigs, ageing will increase fasting plasma glucose, insulin, free fatty acid, triglyceride and total cholesterol concentrations, as well as resting systolic, diastolic and mean arterial blood pressure, pulse pressure, body weight, BMI, and combined and visceral adiposity.

2.2 MATERIALS AND METHODS

2.2.1 Animals

Nulliparous, 3 to 4 month old female guinea pigs (IMVS Tri-coloured) were obtained from the Gilles Plains Animal Resource Centre (Gilles Plains, SA, Australia), and were housed individually in wire-bottomed cages and subjected to a standard 12:12 hour light:dark cycle. Animals had *ad libitum* access to a commercial guinea pig and rabbit chow (Ridley Agri Products, Murray Bridge, SA, Australia) supplemented with an increased content of Vitamin E (200 mg kg^{-1}), and to tap water supplemented with Vitamin C (400 mg L^{-1} ascorbic acid, Ace Chemical Company, Camden Park, SA, Australia). Body weight was monitored three times weekly and animals were checked for oestrous daily. Following a two-week acclimatisation period, females in oestrous were pair mated with male guinea pigs (IMVS Tri-coloured) overnight. Females were presumed to be pregnant upon the finding of a vaginal copulatory plug the following morning, and pregnancy was then confirmed upon the failure to return to oestrous in the subsequent cycle. At 60 days gestation (term 70 days), pregnant animals were transferred to plastic tubs containing paper bedding, where they gave birth (range at term 67-71 days, mean term 69.6 ± 0.2 days). All mothers and their litters were then transferred to plastic tubs bedded with lucerne, and were allowed *ad libitum* access to guinea pig chow. At 30 days of age pups were transferred to individual wire-bottomed cages where they were weaned onto normal guinea pig chow *ad libitum*. Postnatal food intakes were not able to be measured for logistic reasons. This was a cross-sectional study, and animals from each litter were allocated to be studied either as weanlings (in a separate project), young adults at 4 months of age, or aged adults at 14 months of age, starting with the random selection of one from each litter. Animals were then allocated subsequently on the basis of weight at birth and sex to achieve a balance across size at birth and sex. A total of 172 pups born to 84 mothers were randomly assigned for study as young (79 pups) or aged adults (93 pups). A description of the total number of litters used and their size is summarised in Appendix A.

Vascular catheters were inserted into the right carotid artery (Polyvinyl, 0.40 mm inner diameter, 0.80 mm outer diameter) and jugular vein (Silastic, 0.51 mm inner diameter, 0.94 mm outer diameter) under general anaesthesia induced by a combination of Ketamine hydrochloride (75 mg kg^{-1} body weight, intraperitoneal)

and Xylazine hydrochloride (6 mg kg^{-1} body weight, intramuscular). Atropine sulphate was administered before surgery (0.05 mg kg^{-1} body weight, subcutaneous). Both catheters were tunnelled under the skin and exteriorised through the skin at the back of the neck. Catheter patency was maintained by daily flushing with 800 µl of heparinized saline (250 IU ml^{-1}, Multiparin, Fisons Pharmaceuticals, NSW, Australia). Animals were allowed at least 5 days postoperative recovery before the commencement of *in vivo* studies and at least 2 days recovery between *in vivo* studies, which were performed in awake, unrestrained, chronically catheterised guinea pigs. Extension lines were attached to the catheters and passed through the top of the cage prior to the start of *in vivo* studies, to allow for intravenous infusions and the sampling of arterial blood with minimal disturbance to the animal. It was not possible to obtain all data from all animals due to human resource limitations, and the number of observations for each experimental data set is indicated in each table or figure. Fasting plasma free fatty acid, triglyceride and total cholesterol concentrations and measures of body composition were obtained from a significantly higher number of aged adults compared with young adults, however the size at birth and neonatal growth rate characteristics of animals in both these groups were comparable. All procedures in this study were reviewed and approved by the University of Adelaide Animal Ethics Committee.

2.2.2 Hyperinsulinaemic euglycaemic clamp

A HEC was performed to determine the insulin sensitivity of net whole-body glucose uptake, following a 16-hour overnight fast. Arterial blood was sampled (800 µl) at 20, 15, 10, 5 and 0 minutes prior to the start of the HEC for the immediate determination of fasting blood glucose concentrations, and the subsequent determination of fasting plasma free fatty acid, triglyceride and total cholesterol concentrations. Human insulin (Actrapid, 100 IU ml^{-1}, Novo Nordisk, Copenhagen, Denmark) was then infused intravenously at a rate of 7.5 mU/kg/min over the 2-hour duration of the clamp. Arterial blood was sampled (20 µl) at 5-minute intervals throughout the HEC, and blood glucose was rapidly analysed immediately following sample collection using a glucometer (HemoCue AB, Angelholm, Sweden), for the continuous monitoring of the blood glucose concentration. Fifteen minutes after the commencement of the insulin infusion, an intravenous infusion of glucose (10% w/v

dextrose, Baxter HealthCare, NSW, Australia) was commenced at an initial rate of 2 mg/kg/min, which was then adjusted every 5 minutes in order to restore and maintain euglycaemia (modified from DeFronzo *et al.*, 1979). The mean blood glucose concentration achieved during the last 60 minutes of the HEC was 6.69 mmol/l for young adults and 9.05 mmol/l for aged adults, with coefficients of variation of 5.9% and 4.7% respectively. Insulin and glucose were infused using sp220i syringe pumps (World Precision Instruments, USA). The glucose infusion rate increased during the first hour of insulin infusion but reached a plateau within 60-80 minutes. Plateau rates of glucose infusion that remained stable during the second hour of the clamp were termed steady-state glucose infusion rates (SSGIR). The mean glucose infusion rate during the last 60 minutes of the HEC was 7.89 mg/min/kg for young adults and 5.17 mg/min/kg for aged adults, with coefficients of variation of 23% and 26% respectively. Arterial blood was sampled (800 µl) at 60, 75, 90, 105 and 120 minutes after the commencement of the insulin infusion for the determination of steady state plateau plasma human insulin concentrations. All blood samples were placed on ice, and at the end of the experiment were centrifuged at 3,000 rpm for 15 minutes at 4°C, and the plasma removed and stored at -20°C for subsequent analyses.

The insulin sensitivity of net whole body glucose uptake was calculated as the SSGIR needed to maintain euglycaemia (averaged across the second hour of the HEC), corrected for the steady state plateau plasma human insulin concentration, and was termed the adjusted SSGIR. The post-hepatic metabolic clearance rate of human insulin (MCR_i) was calculated as the ratio of the exogenous insulin infusion rate to the steady state plateau plasma human insulin concentration.

2.2.3 Intravenous glucose tolerance test

An IVGTT was performed to determine glucose tolerance and insulin secretion, following a 16-hour overnight fast. Arterial blood was sampled (500 µl) at 10, 5 and 0 minutes prior to the commencement of the IVGTT for the determination of fasting plasma glucose and insulin concentrations. A dextrose solution (50% w/v, Baxter HealthCare, NSW, Australia) was diluted in a 0.9% NaCl solution (Baxter HealthCare, NSW, Australia) to give a dose of 0.5 g dextrose/kg body weight in a total volume of 2.5 ml. The bolus of dextrose was then injected intravenously over a 2 minute period, immediately followed by 2 ml of 0.9% NaCl. Arterial blood was

then sampled (500 µl) at 2, 5, 10, 20, 30, 40, 60, 80, 120, 150, 180 and 210 minutes after the administration of dextrose, and saline (500 µl) was administered following each blood sample. All blood samples were placed on ice, and at the end of the experiment were centrifuged at 3,000 rpm for 15 minutes at 4°C, and the plasma removed and stored at -20°C for subsequent analyses of glucose and insulin concentrations.

Glucose tolerance was measured as the area under the glucose concentration curve (AUGC). Absolute insulin secretion was measured as the area under the insulin concentration curve (AUIC) and relative insulin secretion was calculated as AUIC divided by AUGC. Plasma insulin and glucose concentrations throughout the IVGTT were plotted using SigmaPlot (Jandel Scientific Software, CA, USA). Areas under the glucose (AUGC) or insulin (AUIC) curves were calculated as follows. The mean plasma glucose or insulin concentrations prior to dextrose administration were used as the baseline and the area under the plasma glucose or insulin profile determined using SigmaScan Pro image analysis software (Jandel Scientific Software, CA, USA). The glucose tolerance index (K_G), which provides an estimate of the rate of glucose elimination after glucose dose was also calculated. The slope of the regression line obtained from the plot of the natural logarithm-transformed plasma glucose concentrations between 2 and 60 minutes was expressed as a percentage per minute (Kind *et al.*, 2003).

2.2.4 Blood pressure

Resting systolic, diastolic and mean arterial blood pressure and heart rate were measured directly from the carotid artery catheter. The catheter was filled with saline and connected to a MacLab 1050 displacement transducer (ADInstruments, Castle Hill, NSW, Australia), which had been previously calibrated using a water filled manometer. The transducer was connected to a MacLab data acquisition system via a quad bridge amplifier (ADInstruments, Castle Hill, NSW, Australia). Arterial blood pressure and heart rate were measured using MacLab chart software on a Power Macintosh computer. Pulse pressure was calculated as the difference between the systolic and diastolic blood pressures. Resting arterial blood pressure and heart rate were recorded continuously for a period of 2 hours between 1000h and 1200h.

2.2.5 Plasma metabolite and hormone analyses

Human insulin concentrations in plasma were analysed in duplicate by radioimmunoassay using a commercially available kit in which guinea pig insulin did not cross react (Insulin-CT, CIS bio international, France), with an intra-assay coefficient of variation of 4.5% and an interassay coefficient of variation of 5.2%. Guinea pig insulin concentrations in plasma were analysed in duplicate by radioimmunoassay using guinea pig insulin standards, as described previously (Gorray *et al.*, 1980; Kind *et al.*, 2003), with an intra-assay coefficient of variation of 8.4% and an interassay coefficient of variation of 10.9%. Plasma glucose, total cholesterol, triglyceride and free fatty acid concentrations were measured in duplicate by enzymatic colorimetric analysis on a COBAS Mira automated centrifugal analyser using commercially available kits and control sera, with intra and interassay coefficients of variation below 5%. Plasma glucose was measured using a Unimate GLUT HK kit, which employed the hexokinase method (Roche Diagnostic Systems, Basel, Switzerland). Plasma total cholesterol was measured using a Unimate CHOL kit, which employed the cholesterol oxidase method (Roche Diagnostic Systems, Basel, Switzerland). Plasma triglycerides were measured using a Unimate TRIG kit, which employed the glycerol phosphate dehydrogenase method (Roche Diagnostic Systems, Basel, Switzerland). Plasma free fatty acids were measured using a NEFA C kit, which employed the acyl-coA synthetase-acyl-coA oxidase method (Wako Pure Chemical Industries, Osaka, Japan).

2.2.6 Body composition

Animals were sacrificed by an intravenous overdose of sodium pentobarbitone (Virbac, NSW, Australia) following a 20-hour overnight fast, and a post mortem was performed between 1400h and 1600h. Body weight and nose to rump length were measured and the body mass index was calculated (weight/nose to rump length^2). Selected skeletal muscles (plantaris, gastrocnemius, tibialis cranialis, semitendinosus, biceps femoris, vastus lateralis, extensor digitorum longus (EDL), biceps brachii and diaphragm) and adipose depots (interscapular, shoulder, neck, parametrial, gastrointestinal, retroperitoneal, perirenal, epididymal and groin) were dissected out and weighed immediately, and the left and right sides summed for total weight. The gastrocnemius, semitendinosus, biceps femoris, tibialis cranialis, vastus lateralis and

diaphragm weights were summed and expressed as a percentage of body weight at post mortem, to give an index of the percentage of body weight composed of mixed muscle. The plantaris, biceps brachii and EDL weights were summed and expressed as a percentage of body weight at post mortem, to give an index of the percentage of body weight composed of type 2 muscle. The gastrointestinal, retroperitoneal and perirenal adipose depot weights were summed and expressed as a percentage of body weight at post mortem, to give an index of the percentage of body weight composed of visceral adipose tissue. The interscapular, shoulder, neck and groin adipose depots were summed and expressed as a percentage of body weight at post mortem, to give an index of the percentage of body weight composed of subcutaneous adipose tissue. In addition, individual skeletal muscle and adipose depot weights were summed and expressed as a percentage of body weight at post mortem, to give an index of the percentage of body weight composed of skeletal muscle (combined muscle) and adipose tissue (combined adiposity) respectively.

2.2.7 Statistical analysis

All statistical analyses were carried out using SPSS for Windows (Version 13.0, SPSS Inc., Chicago, IL, USA). The effect of age on individual components of the IRS and on measures of adult size and body composition was assessed by a single between factor analysis of variance (ANOVA) in all animals combined, and in males and females separately. The effects of age, sex and their interaction, on individual components of the IRS and on measures of adult size and body composition were examined by a two between factor ANOVA. Specific comparisons were carried out by Bonferroni post hoc tests. For all statistical tests, significance was accepted at $P<0.05$. All data are presented as mean ± standard error of the mean (S.E.M).

2.3 RESULTS

2.3.1 Whole body insulin sensitivity and circulating lipid profile

Ageing reduced whole body insulin sensitivity as indicated by the non-adjusted (-34%) ($p<0.001$) and adjusted (-30%) ($p=0.004$) steady-state glucose infusion rates achieved during the HEC (Table 2.1). Plateau plasma human insulin concentrations and the metabolic clearance rate of insulin during the HEC were not different between aged and young adult guinea pigs (Table 2.1). In males, the unadjusted steady-state glucose infusion rate achieved during the HEC was reduced in aged adults (-32%) ($p=0.005$) (Figure 2.1a) however other parameters of whole body insulin sensitivity did not vary with age (data shown for adjusted steady state glucose infusion rate only, Figure 2.1b). In females, ageing reduced the non-adjusted (-37%) ($p<0.001$) (Figure 2.1a) and adjusted (-37%) ($p=0.008$) (Figure 2.1b) steady-state glucose infusion rates achieved during the HEC. Females exhibited a higher metabolic clearance rate of insulin during the HEC when compared to males ($p=0.046$) in both the young (+21%) and aged adult (+18%) (data not shown).

Fasting plasma free fatty acid concentrations did not differ between young and aged adult guinea pigs, however ageing increased fasting plasma triglyceride (+181%) ($p<0.001$) and total cholesterol (+55%) ($p<0.001$) concentrations (Table 2.1). In males, ageing increased fasting plasma triglyceride (+173%) ($p=0.006$) (Figure 2.2a) and total cholesterol (+70%) ($p<0.001$) (Figure 2.2b) concentrations, but did not alter fasting plasma free fatty acid concentrations (data not shown). In females, ageing increased fasting plasma triglyceride (+186%) ($p=0.007$) (Figure 2.2a) and total cholesterol (+42%) ($p=0.003$) (Figure 2.2b) concentrations, but did not alter fasting plasma free fatty acid concentrations (data not shown).

2.3.2 Glucose tolerance and insulin secretion

Ageing increased fasting plasma glucose (+20%) ($p<0.001$) and insulin (+67%) ($p=0.012$) concentrations, however the fasting plasma insulin to glucose ratio was not different between aged and young adult guinea pigs (Table 2.2). Ageing reduced the area under the glucose curve (-22%) ($p=0.042$) and increased the glucose

Table 2.1 Effect of age on whole body insulin sensitivity and circulating lipid profile in the guinea pig

	Age group		
	Young adults	Aged adults	ANOVA *P*-value
SSGIR ($mg.min^{-1}.kg^{-1}$)	7.89 ± 0.51 (36)	5.17 ± 0.31 (31)	<0.001
Adjusted SSGIR ($mg.min^{-1}.kg^{-1}$ $\mu U.ml^{-1}$)	0.037 ± 0.003 (36)	0.026 ± 0.003 (31)	0.004
Steady state plasma human insulin ($\mu U\ ml^{-1}$)	253 ± 17 (36)	234 ± 20 (31)	NS
Metabolic clearance rate of insulin ($ml.min^{-1}.kg^{-1}$)	35.1 ± 2.6 (36)	38.4 ± 2.7 (31)	NS
Fasting plasma free fatty acids ($meq\ l^{-1}$)	2.34 ± 0.09 (36)	2.50 ± 0.09 (57)	NS
Fasting plasma triglycerides ($mmol\ l^{-1}$)	0.57 ± 0.03 (25)	1.60 ± 0.18 (57)	<0.001
Fasting plasma total cholesterol ($mmol\ l^{-1}$)	0.96 ± 0.05 (33)	1.49 ± 0.08 (57)	<0.001

Data are presented as means ± S.E.M. Numbers in parentheses represent the number of animals. SSGIR, steady state glucose infusion rate. Statistical significance was assumed at $P < 0.05$. NS, not significant.

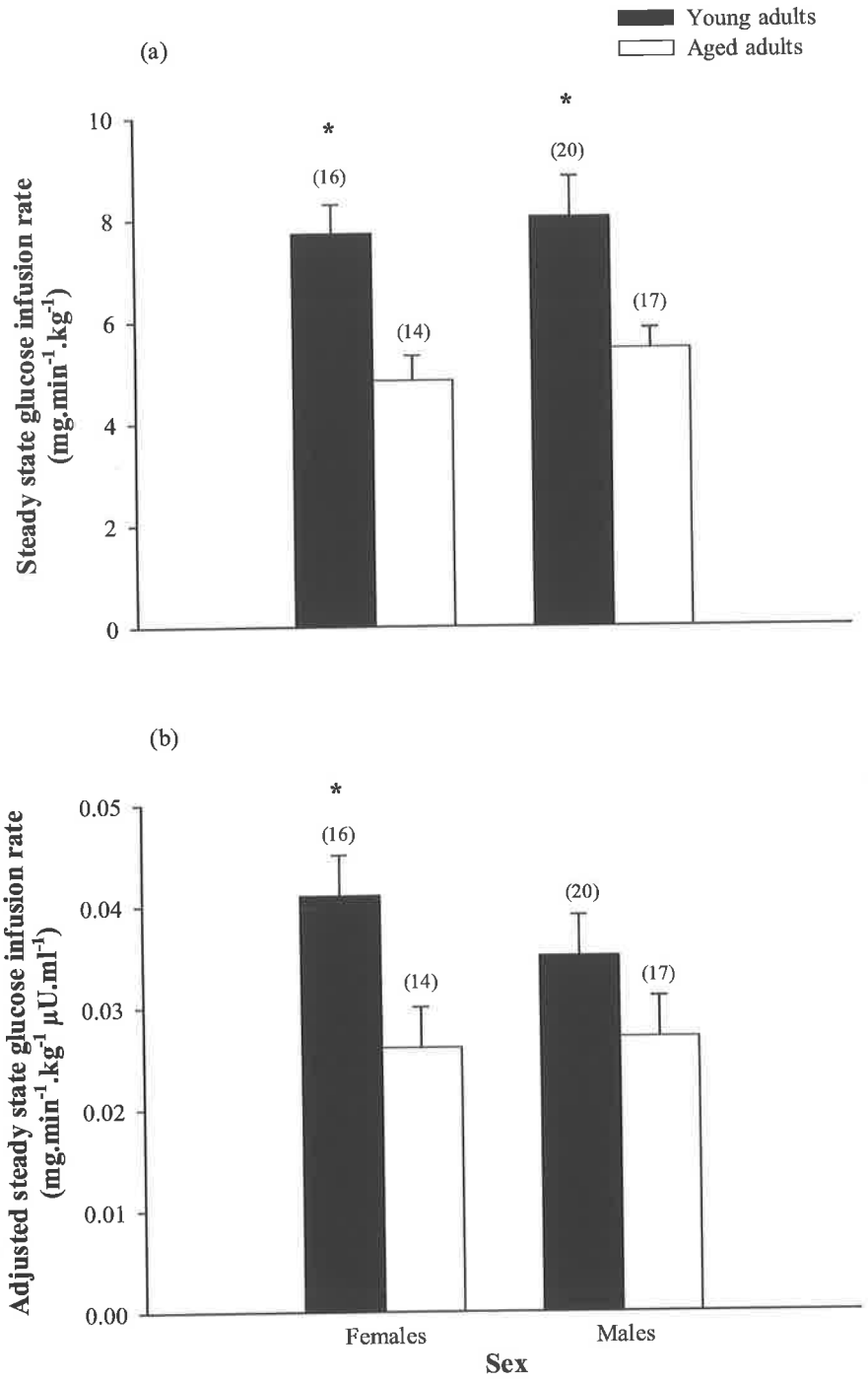

Figure 2.1 The effect of age and sex on whole body insulin sensitivity of glucose metabolism in the guinea pig

Unadjusted (a) and adjusted (b) steady state glucose infusion rate during the HEC in young and aged adult guinea pigs. Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. *$P < 0.05$ compared with aged adult offspring of the same sex.

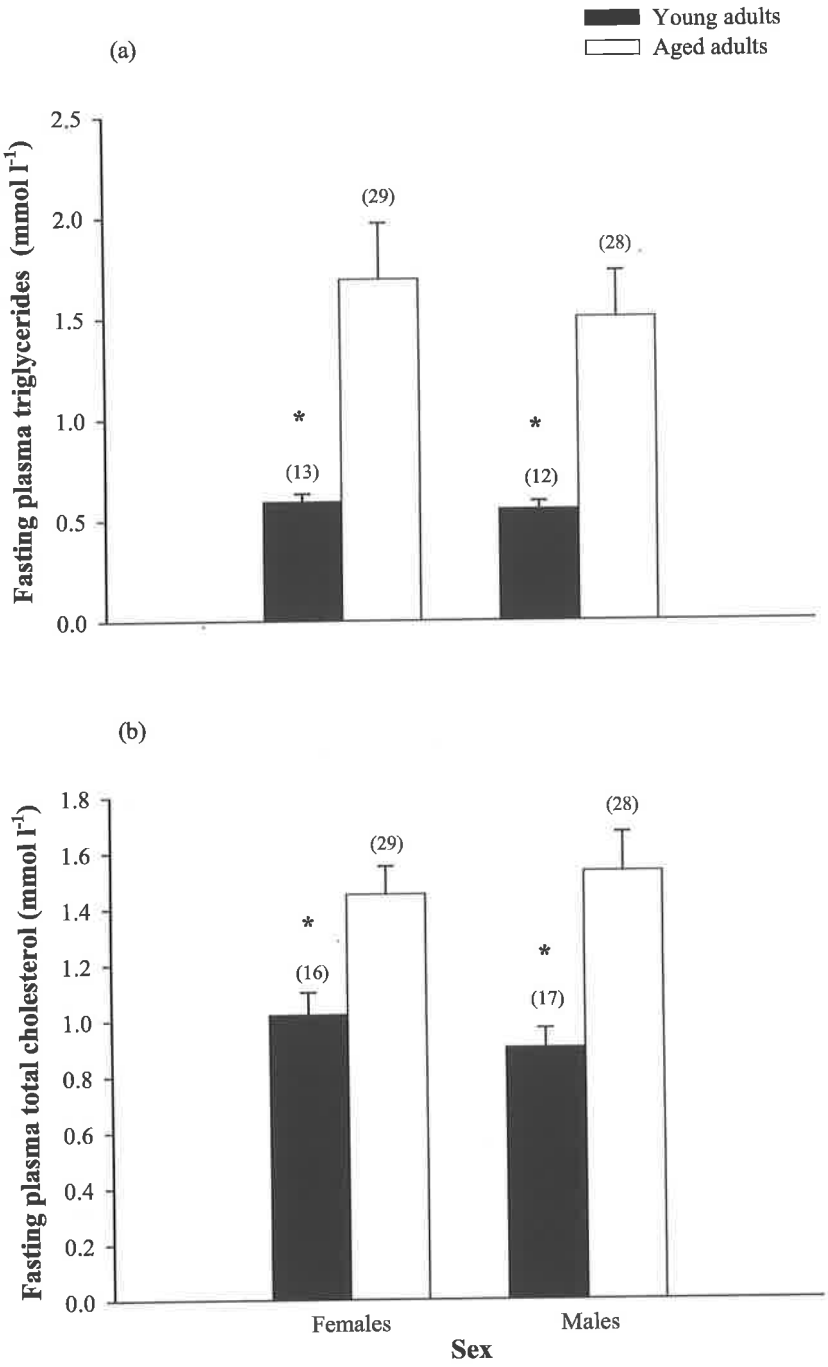

Figure 2.2 The effect of age and sex on fasting plasma triglyceride and total cholesterol concentrations in the guinea pig

Fasting plasma triglyceride (a) and total cholesterol (b) concentrations in young and aged adult guinea pigs. Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. *$P < 0.05$ compared with aged adult offspring of the same sex.

Table 2.2 Effect of age on glucose tolerance and insulin secretion in the guinea pig

	Age group		
	Young adults	Aged adults	ANOVA *P*-value
Fasting plasma glucose (mmol l^{-1})	7.41 ± 0.15 (51)	8.89 ± 0.23 (57)	<0.001
Fasting plasma insulin (ng ml^{-1})	8.23 ± 0.74 (15)	13.77 ± 1.70 (27)	0.012
Fasting plasma insulin to glucose ratio	1.04 ± 0.09 (15)	1.42 ± 0.16 (27)	NS
Area under the glucose curve (mmol l^{-1} min^{-1})	824 ± 104 (15)	642 ± 51 (27)	0.042
Area under the insulin curve (ng ml^{-1} min^{-1})	973 ± 139 (15)	2974 ± 403 (27)	<0.001
AUIC:AUGC	1.18 ± 0.12 (15)	4.83 ± 0.60 (27)	<0.001
Glucose tolerance index, K_G (% min^{-1})	0.83 ± 0.08 (15)	1.02 ± 0.06 (27)	0.043

Data are presented as means ± S.E.M. Numbers in parentheses represent the number of animals. AUIC:AUGC, ratio of area under the insulin curve to area under the glucose curve. Statistical significance was assumed at $P < 0.05$. NS, not significant.

tolerance index (+23%) (p=0.043) (Table 2.2). Insulin secretion increased with age in both absolute (+206%) (p<0.001) and relative (+309%) (p<0.001) terms (Table 2.2). In males, ageing increased fasting plasma glucose concentrations (+19%) (p<0.001) (Figure 2.3a), insulin secretion in both absolute (+184%) (p=0.013) (Figure 2.4a) and relative (+310%) terms (p=0.01) (Figure 2.4b), and the glucose tolerance index (+42%) (p=0.025) (Figure 2.5), however other parameters of glucose tolerance and insulin secretion did not vary with age (data shown for fasting plasma insulin concentrations only, Figure 2.3b). In females, ageing increased fasting plasma glucose (+21%) (p<0.001) (Figure 2.3a) and insulin (+68%) (p=0.041) (Figure 2.3b) concentrations and insulin secretion in both absolute (+224%) (p=0.009) (Figure 2.4a) and relative (+307%) terms (p<0.001) (Figure 2.4b), however other parameters of glucose tolerance and insulin secretion did not vary with age (data shown for the glucose tolerance index only, Figure 2.5).

2.3.3 Blood pressure

Basal heart rate did not differ between aged and young adult guinea pigs, however ageing increased basal systolic blood pressure (+17%) (p<0.001), diastolic blood pressure (+18%) (p<0.001), mean arterial pressure (+10%) (p<0.001) and pulse pressure (+16%) (p=0.04) (Table 2.3). In males, ageing increased basal systolic blood pressure (+19%) (p<0.001) (Figure 2.6a), diastolic blood pressure (+18%) (p<0.001) (Figure 2.6b), mean arterial pressure (+10%) (p=0.006) (Figure 2.6c) and pulse pressure (+19%) (p=0.036) (Figure 2.6d), however basal heart rate did not vary with age (data not shown). In females, ageing increased basal systolic blood pressure (+15%) (p=0.001) (Figure 2.6a), diastolic blood pressure (+17%) (p=0.005) (Figure 2.6b), and mean arterial pressure (+10%) (p=0.012) (Figure 2.6c), but did not affect basal pulse pressure and heart rate (data shown for basal pulse pressure only, Figure 2.6d). Males demonstrated a higher basal heart rate when compared to females (p=0.018) in both the young (+8%) and aged adult (+7%) (data not shown).

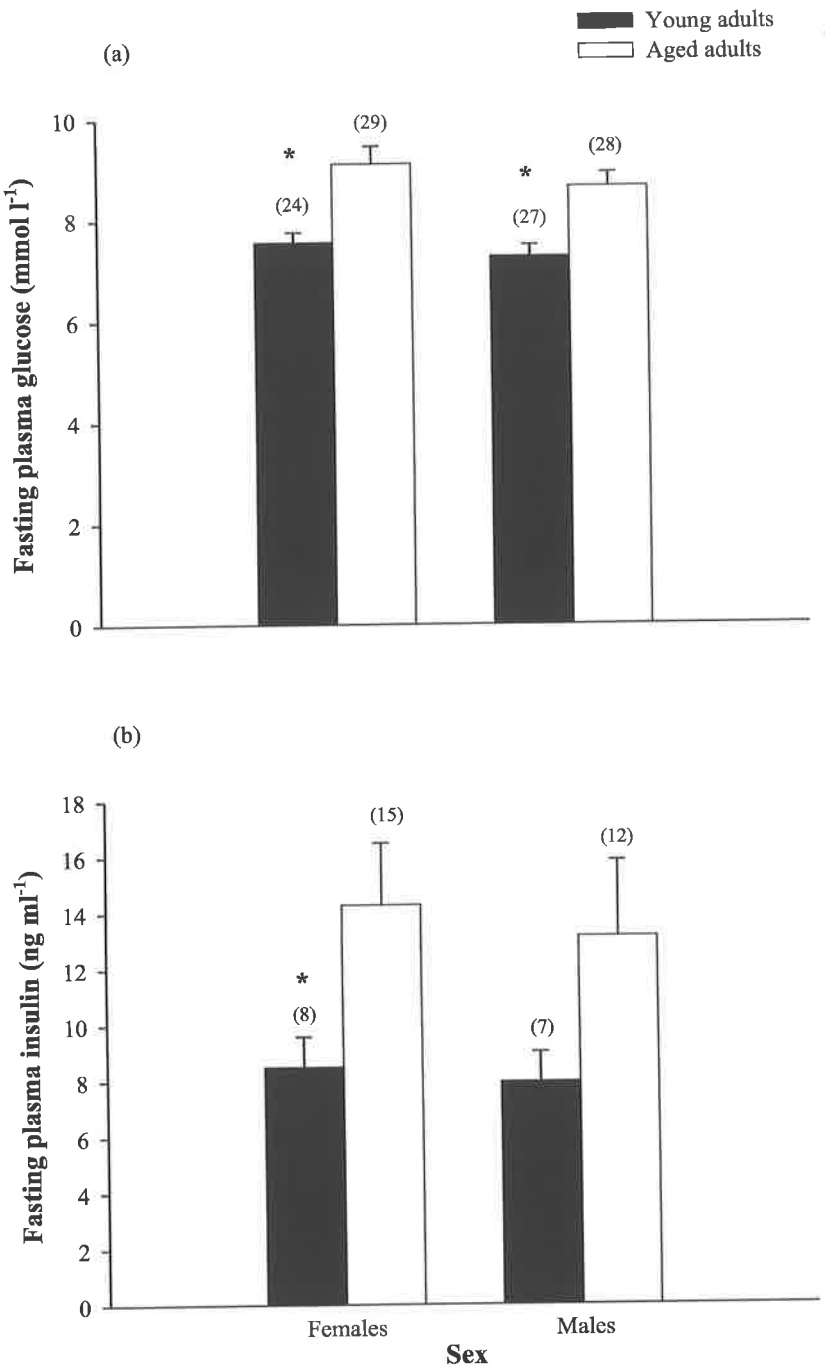

Figure 2.3 The effect of age and sex on fasting plasma glucose and insulin concentrations in the guinea pig

Fasting plasma glucose (a) and insulin (b) concentrations in young and aged adult guinea pigs. Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. *$P < 0.05$ compared with aged adult offspring of the same sex.

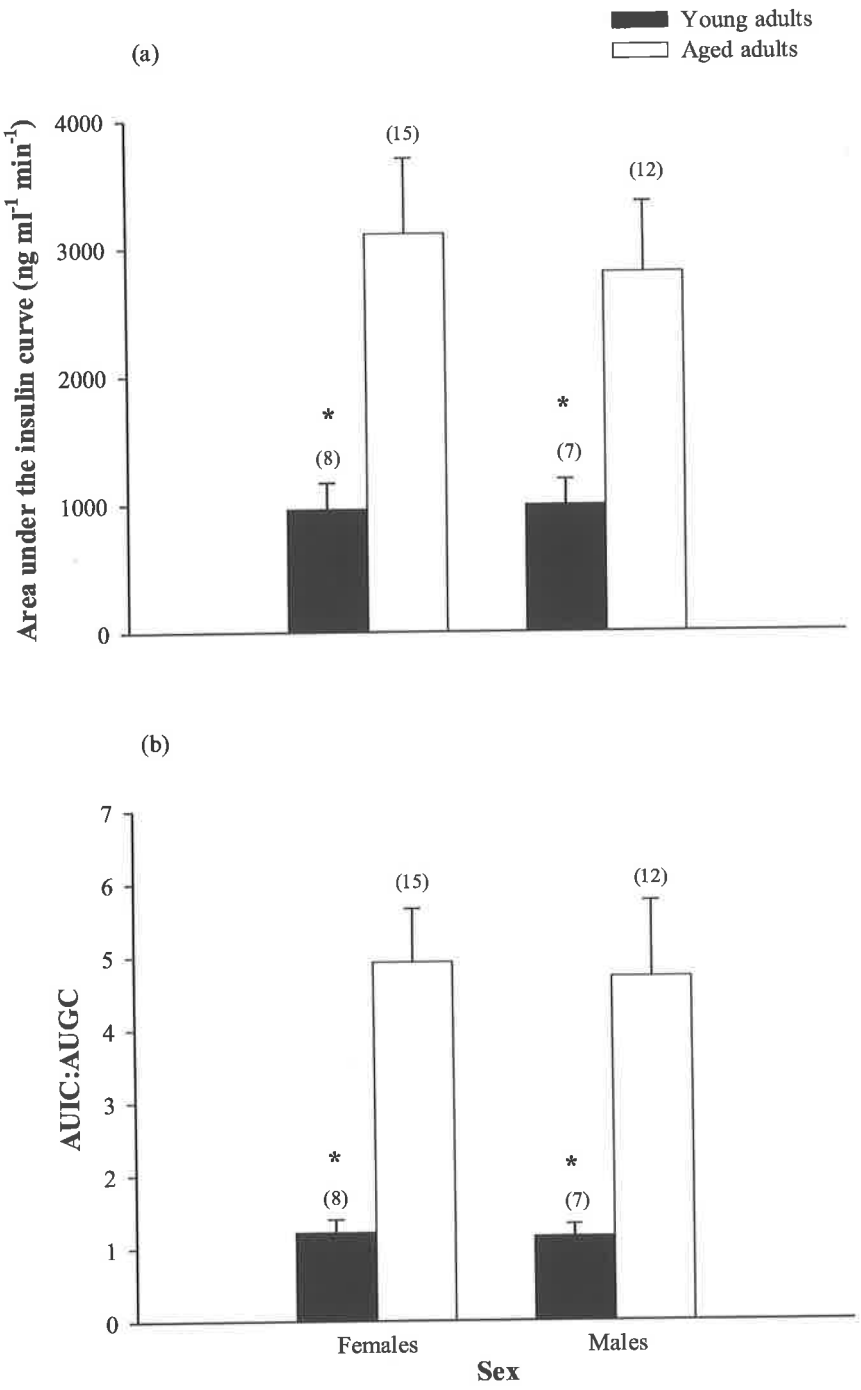

Figure 2.4 The effect of age and sex on absolute and relative insulin secretion in the guinea pig

The area under the insulin curve (a) and the ratio of the area under the insulin curve to the area under the glucose curve (AUIC:AUGC) (b) during the IVGTT in young and aged adult guinea pigs. Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. *$P < 0.05$ compared with aged adult offspring of the same sex.

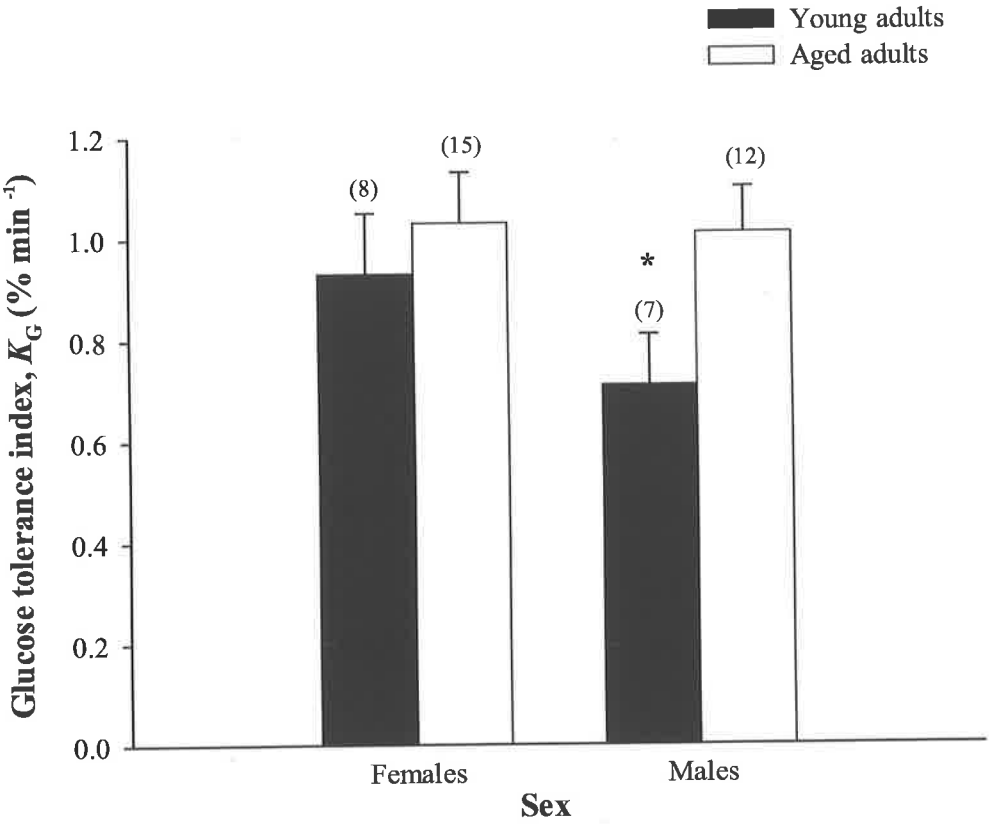

Figure 2.5 The effect of age and sex on the glucose tolerance index in the guinea pig

The glucose tolerance index (K_G) during the IVGTT in young and aged adult guinea pigs. Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. *$P < 0.05$ compared with aged adult offspring of the same sex.

Table 2.3 Effect of age on blood pressure in the guinea pig

	Age group		
	Young adults	Aged adults	ANOVA *P*-value
Systolic blood pressure (mmHg)	73.5 ± 1.3 (22)	86.1 ± 1.7 (24)	<0.001
Diastolic blood pressure (mmHg)	51.1 ± 1.4 (22)	60.2 ± 1.1 (24)	<0.001
Mean arterial pressure (mmHg)	62.5 ± 1.2 (22)	68.9 ± 1.1 (24)	<0.001
Pulse pressure (mmHg)	22.4 ± 1.1 (22)	25.9 ± 1.6 (24)	0.040
Heart rate (beats min^{-1})	252 ± 6 (22)	260 ± 6 (24)	NS

Data are presented as means ± S.E.M. Numbers in parentheses represent the number of animals. Statistical significance was assumed at $P < 0.05$. NS, not significant.

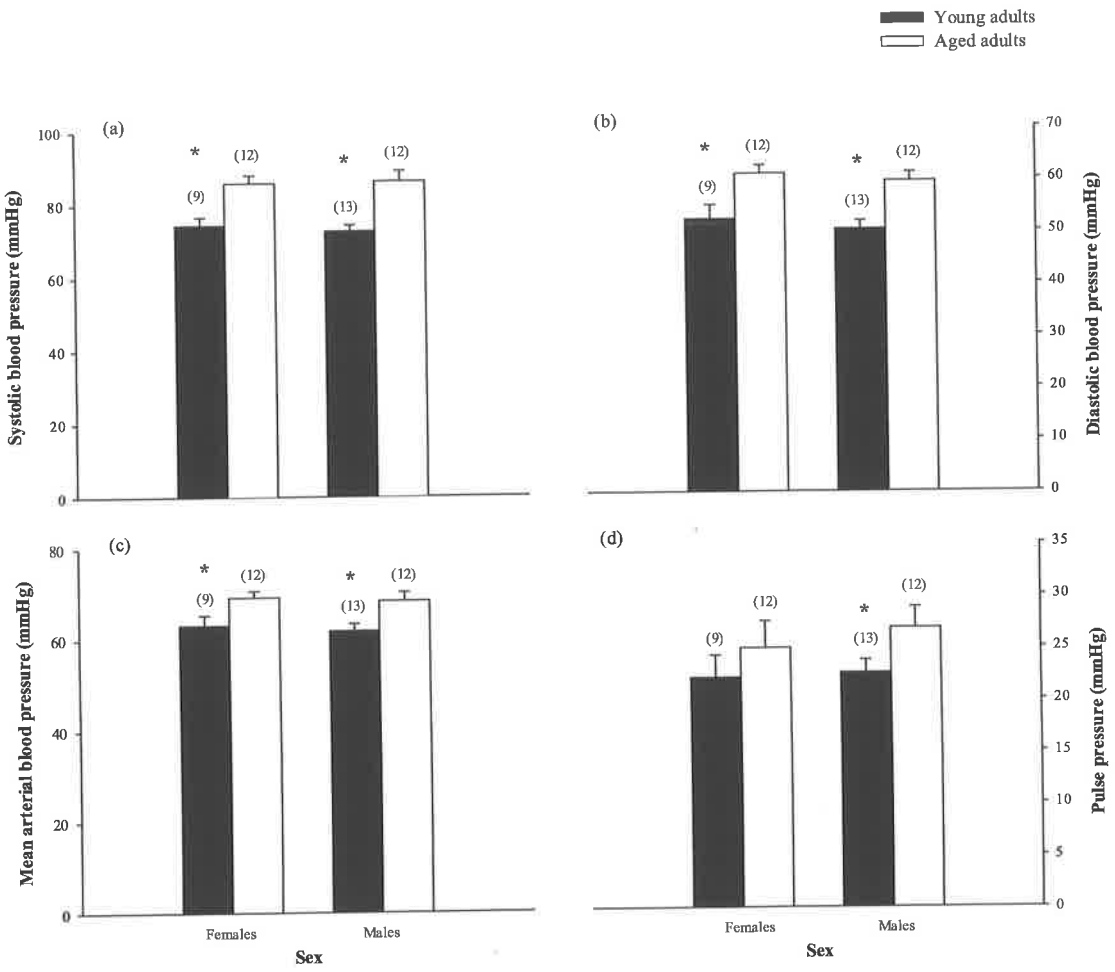

Figure 2.6 The effect of age and sex on systolic, diastolic and mean arterial blood pressure and pulse pressure in the guinea pig

Systolic (a) diastolic (b) and mean arterial (c) blood pressure and pulse pressure (d) in young and aged adult guinea pigs. Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. $*P < 0.05$ compared with aged adult offspring of the same sex.

2.3.4 Body size and composition

Ageing increased body weight (+17%) ($p<0.001$) and body mass index (+16%) ($p<0.001$), but did not alter nose to rump length (Table 2.4). In males, ageing increased body weight (+15%) ($p<0.001$) and body mass index (+14%) ($p<0.001$), however nose to rump length was unaffected (Table 2.5). In females, ageing increased body weight (+22%) ($p<0.001$) and body mass index (+18%) ($p<0.001$), but did not alter nose to rump length (Table 2.6). Males were heavier ($p<0.001$) and longer ($p<0.001$) than females as both young (+17% and +7% respectively) and aged adults (+11% and 5% respectively) (data not shown).

Combined adiposity did not differ between aged and young adult guinea pigs in absolute or relative terms, however ageing increased visceral adiposity in absolute (+30%) ($p<0.001$) and relative (+11%) ($p=0.007$) terms and decreased subcutaneous adiposity in absolute (-17%) ($p=0.002$) and relative (-29%) ($p<0.001$) terms (Table 2.4). In males, ageing decreased combined adiposity in relative terms (-14%) ($p=0.01$) and subcutaneous adiposity in absolute (-21%) ($p=0.006$) and relative (-32%) ($p<0.001$) terms, but increased visceral adiposity in absolute (+24%) ($p=0.009$) but not relative terms (Table 2.5). In females, ageing increased combined adiposity in absolute terms (+23%) ($p=0.011$) and visceral adiposity in absolute (+41%) ($p<0.001$) and relative (+16%) ($p=0.007$) terms, but decreased subcutaneous adiposity in relative terms (-25%) ($p<0.001$) (Table 2.6). Males demonstrated a higher subcutaneous adiposity in absolute terms ($p=0.002$) when compared to females in both the young (+29%) and aged adult (+13%) (data not shown).

Ageing increased combined (+7%) ($p=0.006$) and mixed muscles (+8%) ($p=0.003$) in absolute terms, but decreased combined (-7%) ($p<0.001$) and mixed muscles (-7%) ($p<0.001$) in relative terms, and type 2 muscles in absolute (-7%) ($p=0.011$) and relative (-18%) ($p<0.001$) terms (Table 2.4). In males, ageing increased combined (+6%) ($p=0.045$) and mixed muscles (+7%) ($p=0.029$) in absolute terms, but decreased combined (-7%) ($p=0.001$) and mixed muscles (-6%) ($p=0.004$) in relative terms, and type 2 muscles in absolute (-6%) ($p=0.043$) and relative (-14%) ($p<0.001$) terms (Table 2.5). In females, ageing increased combined (+11%) ($p=0.009$) and mixed muscles (+12%) ($p=0.004$) in absolute terms, but decreased combined (-9%)

Table 2.4 Effect of age on body size and composition in the guinea pig

	Age group		
	Young adults	Aged adults	ANOVA *P*-value
Body weight (g)	754 ± 16 (42)	880 ± 15 (79)	<0.001
Nose to rump length (mm)	333 ± 3 (40)	331 ± 2 (69)	NS
Body mass index (g mm^{-2})	0.0068 ± 0.0001 (40)	0.0079 ± 0.0001 (69)	<0.001
Combined adiposity (g)	73.3 ± 3.3 (36)	78.2 ± 2.8 (72)	NS
(% body weight)	9.35 ± 0.28 (36)	8.74 ± 0.24 (72)	NS
Visceral adiposity (g)	30.6 ± 1.3 (41)	39.9 ± 1.4 (76)	<0.001
(% body weight)	4.01 ± 0.11 (41)	4.45 ± 0.11 (76)	0.007
Subcutaneous adiposity (g)	34.8 ± 1.8 (40)	28.9 ± 1.1 (75)	0.002
(% body weight)	4.53 ± 0.15 (40)	3.22 ± 0.09 (75)	<0.001
Combined muscle (g)	25.9 ± 0.6 (40)	27.8 ± 0.5 (78)	0.006
(% body weight)	3.43 ± 0.05 (40)	3.18 ± 0.03 (78)	<0.001
Mixed muscle (g)	24.2 ± 0.6 (40)	26.3 ± 0.4 (79)	0.003
(% body weight)	3.21 ± 0.05 (40)	3.00 ± 0.03 (79)	<0.001
Type 2 muscle (g)	1.68 ± 0.05 (41)	1.57 ± 0.02 (78)	0.011
(% body weight)	0.22 ± 0.01 (41)	0.18 ± 0.002 (78)	<0.001

Data are presented as means ± S.E.M. Numbers in parentheses represent the number of animals. Statistical significance was assumed at $P < 0.05$. NS, not significant.

Table 2.5 Effect of age on body size and composition in male guinea pigs

	Age group		
	Young adults	Aged adults	ANOVA *P*-value
Body weight (g)	801 ± 19 (25)	924 ± 23 (41)	<0.001
Nose to rump length (mm)	342 ± 3 (24)	338 ± 3 (36)	NS
Body mass index (g mm^{-2})	0.0069 ± 0.0002 (24)	0.0079 ± 0.0002 (36)	<0.001
Combined adiposity (g)	78.7 ± 4.3 (21)	78.4 ± 4.5 (36)	NS
(% body weight)	9.49 ± 0.35 (21)	8.20 ± 0.35 (36)	0.01
Visceral adiposity (g)	32.5 ± 1.6 (24)	40.3± 2.3 (38)	0.009
(% body weight)	4.00 ± 0.13 (24)	4.22 ± 0.17 (38)	NS
Subcutaneous adiposity (g)	38.4 ± 2.5 (23)	30.5 ± 1.8 (39)	0.006
(% body weight)	4.69 ± 0.21 (23)	3.21 ± 0.15 (39)	<0.001
Combined muscle (g)	27.4 ± 0.7 (23)	29.0 ± 0.6 (41)	0.045
(% body weight)	3.38 ± 0.06 (23)	3.16 ± 0.04 (41)	0.001
Mixed muscle (g)	25.6 ± 0.7 (23)	27.4 ± 0.6 (41)	0.029
(% body weight)	3.17 ± 0.06 (23)	2.99 ± 0.04 (41)	0.004
Type 2 muscle (g)	1.70 ± 0.05 (24)	1.60 ± 0.03 (41)	0.043
(% body weight)	0.21 ± 0.004 (24)	0.18 ± 0.003 (41)	<0.001

Data are presented as means ± S.E.M. Numbers in parentheses represent the number of animals. Statistical significance was assumed at $P < 0.05$. NS, not significant.

Table 2.6 Effect of age on body size and composition in female guinea pigs

	Age group		
	Young adults	Aged adults	ANOVA *P*-value
Body weight (g)	684 ± 17 (17)	833 ± 16 (38)	<0.001
Nose to rump length (mm)	321 ± 3 (16)	323 ± 3 (33)	NS
Body mass index (g mm^{-2})	0.0067 ± 0.0002 (16)	0.0079 ± 0.0002 (33)	<0.001
Combined adiposity (g)	63.4 ± 4.4 (15)	78.0 ± 3.5 (36)	0.011
(% body weight)	9.17 ± 0.45 (15)	9.28 ± 0.30 (36)	NS
Visceral adiposity (g)	28.0 ± 2.0 (17)	39.5 ± 1.7 (38)	<0.001
(% body weight)	4.03 ± 0.21 (17)	4.68 ± 0.14 (38)	0.007
Subcutaneous adiposity (g)	29.9 ± 1.9 (17)	27.1 ± 1.2 (36)	NS
(% body weight)	4.32 ± 0.20 (17)	3.23 ± 0.10 (36)	<0.001
Combined muscle (g)	23.9 ± 0.8 (17)	26.5 ± 0.6 (37)	0.009
(% body weight)	3.50 ± 0.10 (17)	3.20 ± 0.06 (37)	0.004
Mixed muscle (g)	22.3 ± 0.8 (17)	25.0 ± 0.6 (38)	0.004
(% body weight)	3.26 ± 0.10 (17)	3.01 ± 0.05 (38)	0.011
Type 2 muscle (g)	1.66 ± 0.10 (17)	1.54 ± 0.03 (37)	NS
(% body weight)	0.24 ± 0.01 (17)	0.19 ± 0.003 (37)	<0.001

Data are presented as means ± S.E.M. Numbers in parentheses represent the number of animals. Statistical significance was assumed at $P < 0.05$. NS, not significant.

($p=0.004$) and mixed muscles (-8%) ($p=0.011$) in relative terms, and type 2 muscles in relative terms (-21%) ($p<0.001$) (Table 2.6). Males demonstrated higher combined ($p<0.001$) and mixed muscles ($p<0.001$) in absolute terms when compared to females in both the young (+14% and +15% respectively) and aged adult (+10% for both), and females demonstrated higher type 2 muscle in relative terms ($p<0.001$) when compared to males in both the young (+14%) and aged adult (+6%) (data not shown).

2.4 DISCUSSION

The current study has demonstrated for the first time that in the guinea pig, ageing is associated with the development of the IRS and is accompanied by alterations in body composition, specifically, a loss of skeletal muscle mass, an increase in visceral adiposity and a decrease in subcutaneous adiposity. Aged adult guinea pigs exhibited reduced whole body insulin sensitivity of glucose metabolism and increased fasting blood plasma levels of glucose, insulin, triglycerides and total cholesterol when compared to their young adult counterparts, however fasting free fatty acid levels remained unchanged with increasing age. In addition, ageing was associated with an increase in resting systolic, diastolic and mean arterial blood pressure and pulse pressure, while no age-related change in resting heart rate was observed. These findings are consistent with most of the observations in human studies of ageing, making the guinea pig a suitable non-human species in which to investigate further the mechanisms underlying the metabolic and cardiovascular changes that occur with increasing age in human populations.

In the current study, guinea pigs demonstrated an age-related increase in body weight, body mass index and visceral adiposity, while subcutaneous adiposity and skeletal muscle mass decreased with increasing age. These results are consistent with the findings in human populations where average body weight peaks around the age of 60 years, with a 25% increase in men and an 18% increase in women over young adult levels (Hornick *et al.*, 1997). The distribution of fat is also altered with age, with an increase in visceral adiposity coinciding with a loss of subcutaneous fat in the arm and leg (Borkan *et al.*, 1977; Durnin *et al.*, 1974), a pattern that is associated with an increased risk for CVD and NIDDM (Chumlea *et al.*, 1992). In contrast, lean body mass, comprised predominantly of skeletal muscle, has been shown to decrease by as much as 19% in men and 12% in women by the mid-sixties (Novak 1972). Interestingly, in the current study, nose to rump length was unaltered with increasing age, indicating that growth of the skeleton in guinea pigs like humans is largely complete by young adulthood, in contrast with rats which demonstrate skeletal growth throughout life (Folkow *et al.*, 1993).

The development of impaired glucose tolerance is an established part of the ageing process in humans (Davison *et al.*, 1979; Maneatis *et al.*, 1982; Chen *et al.*, 1987;

Shimokata *et al.*, 1991), with a dramatic rise in the prevalence of NIDDM observed with increasing age (Palumbo *et al.*, 1976; Harris *et al.*, 1987). This change in glucose metabolism has been shown to be due primarily to an increasing defect in peripheral insulin sensitivity (DeFronzo 1979; Fink *et al.*, 1983; Chen *et al.*, 1985) and to a lesser degree, growing impairment of β cell function (Chen *et al.*, 1985; Kahn *et al.*, 1992). In the current study, ageing decreased whole body insulin sensitivity of glucose metabolism, but increased the fasting plasma insulin concentration and the insulin response to an IVGTT in the guinea pig. The age-related defect in insulin action was still adequately compensated for by the pancreas, as indicated by the slight improvement in glucose tolerance, although fasting plasma glucose concentrations did increase. Furthermore, when male and female guinea pigs were considered separately, glucose tolerance did not deteriorate with ageing. Thus the guinea pig develops impaired fasting glycaemia by aged adulthood, but has yet to exhibit impaired glucose tolerance, despite substantial insulin resistance compared to the young adult. The anthropometric changes that occur with increasing age, namely the increase in visceral fat and decrease in lean body mass, have been implicated as major causes of age-related insulin resistance in humans, which may also be the case in the guinea pig (Barbieri *et al.*, 2001).

Whole body insulin sensitivity of glucose metabolism decreased with ageing in the female but not in the male guinea pig in the current study, while only females demonstrated an age-related increase in relative visceral adiposity. These findings suggest that in the female guinea pig, like the human, an increase in visceral adiposity may contribute in part to the insulin resistance of ageing, although in the present study no significant association between whole body insulin sensitivity of glucose metabolism and relative visceral adiposity was observed in aged male or female guinea pigs (see Chapter 4). While the mechanisms explaining the link between insulin resistance and visceral fat accumulation are yet to be fully defined, it has been suggested that the high lipolytic response of visceral adipose depots to catecholamines (Lonnqvist *et al.*, 1995; Fried *et al.*, 1993; Hoffstedt *et al.*, 1997; Mauriege *et al.*, 1999) results in elevated free fatty acid concentrations, which in turn are known to induce insulin resistance (Boden 1997; Paolisso *et al.*, 1995). Interestingly, fasting plasma free fatty acid concentrations were unaltered with increasing age in both male and female guinea pigs in this study.

Another adipocyte-derived factor, the cytokine tumour necrosis factor-α (TNF-α), has also been implicated in the development of insulin resistance (Feingold *et al.*, 1992; Hotamisligil *et al.*, 1994). It is now well established that TNF-α can impair insulin receptor signalling in skeletal muscle and adipose tissue, resulting in decreased insulin-mediated glucose uptake in these tissues (Del Aguila *et al.*, 1999; Del Aguila *et al.*, 2000; Feinstein *et al.*, 1993; Hotamisligil *et al.*, 1994; Hotamisligil *et al.*, 1996; Ling *et al.*, 1994). In addition, studies in mice (Chorinchath *et al.*, 1996; Han *et al.*, 1995), rats (Mondon *et al.*, 1992) and humans (Paolisso *et al.*, 1998) have shown that plasma TNF-α concentrations increase with advancing age, suggesting that this cytokine could play a role in the development of age-related insulin resistance. The expression of TNF-α has been shown to be higher in visceral adipose tissue compared to subcutaneous adipose depots (Katsuki *et al.*, 1998). In addition, larger fat cells contain more TNF-α than smaller adipocytes obtained from the same individual, while enlarged adipocytes from obese animals and humans have been shown to overexpress this cytokine (Peraldi *et al.*, 1998). Adipose tissue also expresses and secretes adiponectin, an insulin sensitising adipocytokine whose expression is higher in visceral, compared to subcutaneous adipose depots (Altomonte *et al.*, 2003). In obese individuals however, adiponectin expression in visceral adipose tissue is suppressed and correlates positively with insulin sensitivity (Altomonte *et al.*, 2003). Characterisation of the expression and circulating levels of these adipocytokines in the ageing guinea pig would help assess their contribution to age-related insulin resistance in this species.

As skeletal muscle is the major site of insulin-mediated glucose uptake, the age-related loss of skeletal muscle mass and function, which is sometimes referred to as the 'sarcopenia of old age' (Carmeli *et al.*, 2002), leads to a simultaneous decrease in glucose disposal. In the current study, both male and female guinea pigs demonstrated an age-related decrease in relative combined, mixed and type 2 muscle mass. This age-related reduction in skeletal muscle mass may contribute to the impaired insulin sensitivity observed in female guinea pigs with increasing age. In the current study however, whole body insulin sensitivity of glucose metabolism was not related to any measure of relative skeletal muscle mass in aged male or female guinea pigs (see Chapter 4). A number of studies have suggested that the insulin resistance of ageing is due largely to a postreceptor defect in skeletal muscle (Chen *et al.*, 1985; DeFronzo *et al.*, 1979; Fink *et al.*, 1983; Jackson *et al.*, 1990; Rowe *et*

al., 1983; Fink *et al.*, 1992), and as transport is the rate-limiting step in insulin-stimulated glucose metabolism, the glucose transport effector system has been put forward as a likely candidate (Fink *et al.*, 1992; Yki-Jarvinen *et al.*, 1987). In skeletal muscle, the protein responsible for glucose transport is GLUT-4, which translocates from an intracellular pool to the skeletal muscle cell membrane following insulin stimulation (Friedman *et al.*, 1991; Klip *et al.*, 1990). Studies in rats have demonstrated a fall in skeletal muscle GLUT-4 protein concentration during growth and development; however the findings regarding changes in the concentration of this transporter with ageing are inconsistent (Cartee *et al.*, 1993; Ezaki *et al.*, 1992; Gulve *et al.*, 1993; Kern *et al.*, 1992; Lin *et al.*, 1991; Barnard *et al.*, 1992). In humans, one study has demonstrated that the GLUT-4 protein concentration in the vastus lateralis is negatively associated with chronological age and positively associated with whole body insulin sensitivity in both men and women (Houmard *et al.*, 1995). These observations are consistent with the suggestion that a decrease in the concentration of this skeletal muscle glucose transporter may contribute in part to the insulin resistance of ageing, and could explain the remainder of the age-related decrement in insulin sensitivity in the female guinea pig.

An age-related increase in resting systolic, diastolic, mean arterial blood pressure and pulse pressure was observed in the current study, while the resting heart rate was unchanged with increasing age in the guinea pig. These findings are in agreement with longitudinal studies in urban human populations which have demonstrated that systolic blood pressure rises steadily with increasing age at least into the ninth decade, accompanied by an increase in diastolic blood pressure which peaks around the sixth decade, leading to an increase in pulse pressure in the elderly (Landahl *et al.*, 1986; Pearson *et al.*, 1997; Svardsudd *et al.*, 1980). The relationship of different blood pressure indices to CHD risk is heavily influenced by the ageing process (Franklin *et al.*, 2001). Diastolic blood pressure is a stronger predictor of CHD risk than systolic blood pressure or pulse pressure in individuals under the age of 50 years, indicating that an increased peripheral resistance is the main haemodynamic determinant of risk in this age group (Franklin *et al.*, 2001). In individuals aged between 50 and 59 years, systolic and diastolic blood pressure and pulse pressure are all equally predictive of CHD risk, suggesting a balance between small vessel resistance and large artery stiffness. Pulse pressure and systolic blood pressure are the strongest predictors of CHD risk in individuals aged 60 years or older, indicating

that in this group of patients large artery stiffness is the dominant measure of risk (Franklin *et al.*, 2001). In the current study, male and female guinea pigs demonstrated increased systolic and diastolic blood pressure as aged adults, while pulse pressure was increased in aged males but not females, suggesting that at this age CHD risk may be higher in male guinea pigs. While the aetiology of these age-related alterations in blood pressure remains unclear, a number of putative mechanisms have been suggested, including changes in the structure and function of the vasculature, as well as the influence of anthropometric, metabolic and hormonal factors.

A combination of factors contributes to the increased peripheral resistance and arterial stiffening which form the pathophysiological basis of the age-related changes in blood pressure observed in human populations. Hypertension seen in the elderly population may be secondary to chronically elevated peripheral resistance caused by atherosclerosis, impaired smooth muscle relaxation associated with decreased β2 receptor sensitivity (Folkow 1993) or increased circulating noradrenaline levels (Meeks 2002), all of which are known to occur with increasing age. In the current study, the age-related increase in fasting plasma concentrations of triglycerides and total cholesterol may have contributed to the rise in blood pressure in the ageing guinea pig, as these lipids, together with collagen and mineral deposits, have been shown to accumulate with age, leading to a decreased distensibility of the peripheral arterial system (Lakatta *et al.*, 1999; Lakatta *et al.*, 1993; Robert 1999). Ageing is also associated with a number of structural changes in the aorta and large arteries, including fragmentation and degeneration of elastin, an increase in collagen and a thickening of the smooth muscle layer in the media (Lakatta *et al.*, 1987), all of which result in stiffer arteries. In addition, ageing is associated with changes in arterial function that in turn reduce compliance, including impaired vasodilation of the large arteries due to a decline in nitric oxide release by the endothelium (Meeks 2002). The aortic pulse wave velocity (aPWV), which is the speed of the systolic pressure wave as it travels down the aorta, is often used as a measure of arterial stiffening, with a faster aPWV indicative of a stiffer aorta (Sutton-Tyrrell *et al.*, 2001).

Several of the age-related metabolic, hormonal and anthropometric changes observed in the current study, namely impaired whole body insulin sensitivity of glucose

metabolism, elevated fasting blood plasma concentrations of glucose and insulin and increased visceral adiposity, may also have contributed to the rise in resting blood pressure with ageing. A number of studies have suggested that insulin resistance, hyperglycaemia and hyperinsulinaemia may promote arterial stiffening (Toto-Moukouo *et al.*, 1986; Taquet *et al.*, 1993; Amar *et al.*, 1995; Salomaa *et al.*, 1995; Kupari *et al.*, 1994). In addition to increasing heart rate and blood pressure via sympathetic nervous system stimulation, elevated insulin levels have also been shown to cause hypertrophy of the vascular wall, resulting in the proliferation of smooth muscle cells, an increase in the number and size of monocytes, as well as increases in collagen (DeFronzo *et al.*, 1991; King *et al.*, 1999). In the current study, fasting plasma insulin levels were positively associated with heart rate in aged female guinea pigs, however insulin secretion both in the fasting state and following an intravenous glucose load, was not associated with any measure of blood pressure in aged male guinea pigs (data not shown). Hyperglycaemia has been shown to stimulate collagen synthesis and cause glycation of proteins in the arterial wall, which in turn leads to cross linking between protein fibres, causing vascular damage (Feener *et al.*, 1997), however in the present study, fasting plasma glucose concentrations were not predictive of any measure of blood pressure in aged male and female guinea pigs (data not shown). Increased visceral adiposity has also been associated with greater aortic stiffness in older individuals, as indicated by an elevated aPWV (Sutton-Tyrrell *et al.*, 2001). In the present study, relative visceral adiposity was positively associated with systolic blood pressure and pulse pressure in aged female guinea pigs, but was not predictive of any measure of blood pressure in aged male guinea pigs (see Chapter 6). This relationship between high levels of visceral fat and stiffer arteries is thought to be mediated in part by elevated plasma free fatty acid concentrations, which promote vascular stiffness by increasing vascular tone, α-adrenergic reactivity and blood pressure (Egan *et al.*, 1999). As discussed above however, fasting plasma free fatty acid concentrations were unaltered with increasing age in this study, although two other adipocyte-derived factors, the proinflammatory cytokines interleukin-6 (IL-6) and TNF-α, have been identified as potential causes of arterial stiffening and remain as possible mediators of this relationship. Both IL-6 and TNF-α are thought to cause a low-grade systemic inflammation in individuals with excess visceral fat (Visser *et al.*, 1999), in whom

various measures of inflammation have been shown to be positively associated with aPWV (Selzer *et al.*, 2001).

In summary, the guinea pig appears to be a species which displays many of the age-related metabolic, cardiovascular and anthropometric changes seen in humans. We have demonstrated that in female guinea pigs, ageing is associated with impaired insulin action, due possibly to an age-related increase in visceral adiposity or decline in skeletal muscle mass. Fasting hyperinsulinaemia also developed in female guinea pigs with increasing age, while age-related increases in fasting plasma glucose, triglyceride and total cholesterol concentrations occurred in both the male and female guinea pig. Resting systolic, diastolic and mean arterial blood pressure increased in the ageing male and female guinea pig, however an age-related rise in pulse pressure occurred only in males. Furthermore, a number of the age-related metabolic, hormonal and anthropometric changes reported here in male and female guinea pigs could have contributed to these age-related increases in blood pressure. In addition to ageing, altered prenatal and early postnatal growth have also been identified as risk factors for the development of the IRS in adult life, however the majority of experimental studies to date have examined the consequences of impaired growth in early life in young adult, rather than aged offspring, and have employed rats, which are relatively immature at birth when compared to humans. The guinea pig, which is more precocious at birth than the rat, may be a more informative species in which to investigate whether the effects of perturbed growth in early life on metabolic and cardiovascular function, particularly the potential links via lipid homeostasis, are amplified with increasing age.

CHAPTER 3

EARLY LIFE INFLUENCES ON BODY SIZE AND COMPOSITION IN THE AGED GUINEA PIG

3.1 INTRODUCTION

Low birth weight has been associated with increased central or truncal fat distribution in children and adults in a number of populations (Barker *et al.*, 1997; Law *et al.*, 1992; Rogers 2003; Valdez *et al.*, 1994). Further to this, rapid postnatal weight gain has also been shown to increase adiposity in childhood (Ong *et al.*, 2000) and adult life (Parsons *et al.*, 2001). In individuals who were growth restricted *in utero*, reductions in skeletal muscle mass have been shown to accompany increases in relative adiposity, with several studies reporting a positive association between proportional lean body mass and size at birth (Gale *et al.*, 2001; Li *et al.*, 2003; Rogers 2003; Singhal *et al.*, 2003).

Experimental studies in a number of species have demonstrated that maternal feed or protein restriction during pregnancy increases adiposity (Anguita *et al.*, 1993; Jones *et al.*, 1982; Jones *et al.*, 1984; Kind *et al.*, 2003; Vickers *et al.*, 2000) and reduces skeletal muscle mass (Dwyer *et al.*, 1992; Desai *et al.*, 1996) in offspring postnatally.

Previous studies in our laboratory have shown that moderate maternal feed restriction (70% *ad libitum* intake) in the guinea pig, which restricts fetal growth, increases the relative weights of the interscapular and retroperitoneal fat depots and reduces the relative weight of the biceps muscle, in the late-gestation fetus (Kind *et al.*, 2005). Moderate maternal feed restriction in the guinea pig, which reduces birth weight, also increases food intake and the relative weight of the retroperitoneal fat depot, and reduces the relative weight of the biceps muscle, in male offspring as young adults (Kind *et al.*, 2003). However, very few studies in non-human species have investigated whether the deleterious effects of perturbed prenatal growth on postnatal size and body composition are exacerbated with increasing age. Similarly, few studies have examined the relationship between early postnatal growth after prenatal restriction and adult size and body composition in the aged adult. Male and female

guinea pigs that experienced accelerated neonatal growth demonstrated increased adiposity and reduced skeletal muscle mass as young adults (DM Horton *et al.*, unpublished observations), but whether this persists or is exacerbated with ageing has not been examined.

The aims of the studies described in Chapter 3 were therefore to determine the effects of (1) spontaneous fetal growth restriction and (2) accelerated neonatal growth, on body size and composition, in the aged guinea pig. Specifically, the effects of perturbed growth in early life on adult adiposity, skeletal muscle mass and organ and gland weights were investigated. The influence of sex on the impact of altered perinatal growth on body size and composition in the aged adult was also assessed. We hypothesised that spontaneous fetal growth restriction and accelerated neonatal fractional growth rate for weight will predict decreased combined skeletal muscle mass and increased combined and visceral adiposity, in the aged male and female guinea pig. In addition, we hypothesised that spontaneous fetal growth restriction and accelerated neonatal fractional growth rate for weight would be associated with alterations in key glucoregulatory and cardiovascular effector organs and tissues and endocrine glands, consistent with previously reported perinatally induced modifications of metabolic and cardiovascular function.

3.2 MATERIALS AND METHODS

3.2.1 Animals

Nulliparous, 3 to 4 month old female guinea pigs (IMVS Tri-coloured) were obtained from the Gilles Plains Animal Resource Centre (Gilles Plains, SA, Australia), and following a two-week acclimatisation period, females in oestrous were pair mated with male guinea pigs (IMVS Tri-coloured) overnight (as described in Section 2.2.1). At 60 days gestation, pregnant animals were transferred to plastic tubs containing paper bedding, where they gave birth. Offspring were weighed at birth, and the nose to rump length, abdominal circumference, and head length and head width of each pup was measured. A total of 79 pups born to 46 mothers were randomly assigned to this study of aged guinea pigs (38 male and 41 female), with the remaining offspring allocated to other studies (as described in Section 2.2.1). A description of the total number of litters used and their size is summarised in Appendix A. Of the 79 pups assigned to this study, 55 had at least one other littermate in the study while 24 did not. All pups were weighed daily from birth to 30 days of age, and the neonatal absolute growth rate for weight ($AGR_{10\text{-}30}$) (g day^{-1}) was calculated from the slope of the growth curve from 10 days of age to weaning at 30 days of age. The neonatal fractional growth rate for weight ($FGR_{10\text{-}30}$) (g day^{-1} g^{-1}) was calculated as the $AGR_{10\text{-}30}$ divided by weight at 10 days of age for each animal. The absolute and fractional neonatal growth rates were calculated from 10 to 30 days, rather than from birth to 30 days, so as to minimise the problem of collinearity, as a significant negative correlation between birth weight and growth rates in early postnatal life has been demonstrated in both humans and in non-human species. The term 'neonatal' has been used as a convenience to encompass the period of infancy to childhood. At 30 days of age, pups were transferred to individual wire-bottomed cages where they were weaned onto normal guinea pig chow *ad libitum*. Postnatal food intakes were not able to be measured for logistic reasons. It was not possible to obtain all data from all animals due to human resource limitations, and the number of observations for each experimental data set is indicated in each table or figure. All procedures in this study were reviewed and approved by the University of Adelaide Animal Ethics Committee.

3.2.2 Body composition

At 430 ± 2 days of age, and following a 20 hour-overnight fast, animals were sacrificed by an intravenous overdose of sodium pentobarbitone (Virbac, NSW, Australia), and a post mortem was performed between 1400h and 1600h. Body weight and nose to rump length were measured and the body mass index was calculated (weight/nose to rump length^2). Selected skeletal muscles and adipose depots were dissected out, weighed immediately, summed, and expressed as a percentage of body weight at post mortem, to give an index of the percentage of body weight composed of mixed and type 2 muscle and visceral and subcutaneous adipose tissue (as described in Section 2.2.6). In addition, all individual skeletal muscle and adipose depot weights were summed and expressed as a percentage of body weight at post mortem, to give an index of the percentage of body weight composed of skeletal muscle (combined muscle) and adipose tissue (combined adiposity) respectively. The following organs and glands were also dissected out and weighed: adrenals, kidneys, brain, pancreas and liver. The heart was dissected out, weighed and then separated into the right and left ventricles, which were weighed individually. All adipose depot, skeletal muscle, organ and gland weights were expressed in absolute terms as well as in relative terms as a percentage of body weight at post mortem.

3.2.3 Statistical analysis

All statistical analyses were carried out using SPSS for Windows (Version 13.0, SPSS Inc., Chicago, IL, USA). The effect of litter size on birth weight was assessed by a single between factor ANOVA. The effects of birth weight class, sex and their interaction, on size at birth and neonatal growth rates were examined by a two between factor ANOVA. To examine the effect of size at birth on adult size and body composition, offspring were classed into two groups, those with birth weights greater than (high birth weight) or less than (low birth weight) the median birth weight for the cohort of guinea pigs described in this thesis, which was 95.55 grams. Males and females were also analysed separately, classing each as high or low birth weight, using the median birth weight for each sex (94.8 grams for males and 99.58 grams for females). The definition of fetal growth restriction in experimental studies in non-human species varies, and this approach of below versus above the median

size at birth has been commonly used in these studies, as well as in some human studies. The effect of birth weight class on adult size and body composition was assessed by a single between factor ANOVA in all animals combined, and in males and females separately. The effects of birth weight class, sex and their interaction, on adult size and body composition were examined by a two between factor ANOVA. Specific comparisons were carried out by Bonferroni post hoc tests.

To examine the effect of neonatal fractional growth rate on adult size and body composition, offspring were classed into two groups as either 'high growers' or 'low growers' using the following approach. In order to classify animals according to growth, males and females were separated, and the neonatal fractional growth rate was plotted against birth weight for each sex separately. Animals above and below the regression line were classified as 'high growers' and 'low growers' respectively. The effect of growth rate class on adult size and body composition was assessed by a single between factor ANOVA in all animals combined, and in males and females separately. A two between factor ANOVA was used to determine the effect of growth rate class, sex and their interaction, on adult size and body composition. Specific comparisons were carried out by Bonferroni post hoc tests.

Relationships between adult size and body composition and size at birth and neonatal growth rates were examined using simple correlation and multiple linear regression analyses, in all animals combined and in male and female offspring separately. One sided p-values were used to test *a priori* hypotheses regarding the relationships between adult size and body composition and size at birth and neonatal growth rates, based on similar relationships reported in humans.

For all statistical tests, significance was accepted at $P<0.05$. All data are presented as mean ± S.E.M.

3.3 RESULTS

3.3.1 Effect of litter size on birth weight

Offspring from larger litters had a lower birth weight compared to those from smaller litters, with guinea pigs born in a litter of one having an increased weight at birth when compared to offspring born in a litter of four or five ($p<0.05$ for both), and guinea pigs born in a litter of two having an increased weight at birth when compared to offspring born in a litter of four ($p<0.05$) (Figure 3.1).

3.3.2 Effect of birth weight class on size at birth, neonatal growth and survival to 400 days of age

Birth weight class did not influence ponderal index or the ratio of head width to abdominal circumference at birth, however all other parameters of size at birth were influenced by birth weight class (Table 3.1). Specifically, nose to rump length (-9%), weight to length ratio (-13%), abdominal circumference (-5%) and head length (-4%) and width (-3%) were reduced in low compared to high birth weight offspring ($p<0.05$ for all) (Table 3.1). The absolute neonatal growth rate was lower (-7%), and the fractional neonatal growth rate was higher (+10%), in low compared to high birth weight offspring ($p<0.05$ for both). Males demonstrated a higher absolute ($p<0.0001$) and fractional ($p<0.0001$) neonatal growth rate when compared to females in both the low birth weight (+11% and +5% respectively) and high birth weight (+23% for both) groups (Table 3.1).

Of the animals allocated for study as aged adults, ten percent failed to survive to 400 days of age, and of those that died before 400 days of age, 70% were males, however neither birth weight class nor neonatal fractional growth rate class influenced survival rate in male or female offspring (data not shown).

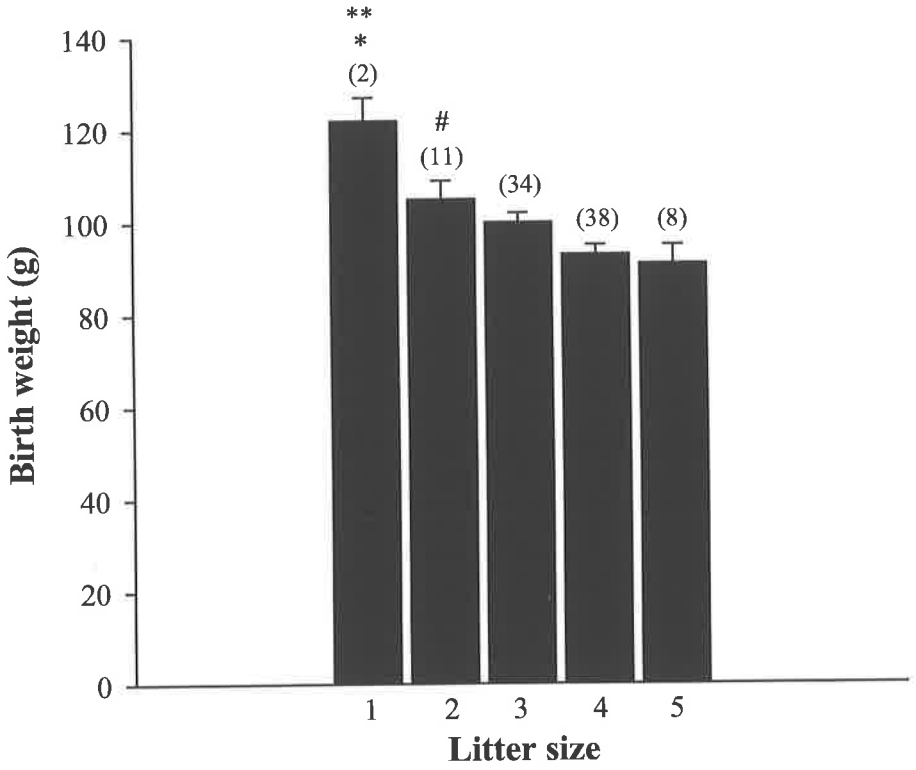

Figure 3.1 The effect of litter size on birth weight in the guinea pig

Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. $^{*}P < 0.05$ compared with litter size of four, $^{**}P < 0.05$ compared with litter size of five, $^{\#}P < 0.05$ compared with litter size of four.

Table 3.1 Effect of birth weight class and sex on size at birth and neonatal growth rate in the guinea pig

	Birth weight class						
	Low birth weight		High birth weight		ANOVA *P*-value		
	Males	Females	Males	Females	BW	S	BW x S
Size at birth							
Weight (g)	87.0 ± 1.2 (25)	85.6 ± 1.8 (21)	108.8 ± 2.1 (21)	107.4 ± 1.2 (26)	<0.0001	NS	NS
Nose to rump length (mm)	151 ± 2 (25)	148 ± 2 (21)	164 ± 2 (19)	163 ± 1 (25)	<0.0001	NS	NS
Weight:Length (g mm^{-1})	0.58 ± 0.008 (25)	0.58 ± 0.012 (21)	0.67 ± 0.01 (19)	0.66 ± 0.009 (25)	<0.0001	NS	NS
Ponderal index (g mm^{-3}) ($x10^3$)	0.025 ± 0.0008 (23)	0.027 ± 0.001 (20)	0.025 ± 0.001 (19)	0.025 ± 0.0007 (24)	NS	NS	NS
Abdominal cicumference (mm)	101 ± 2 (25)	97 ± 1 (21)	105 ± 2 (19)	105 ± 2 (25)	0.002	NS	NS
Head length (mm)	44.9 ± 0.5 (25)	44.2 ± 0.3 (21)	47.1 ± 0.5 (19)	46.0 ± 0.5 (25)	<0.0001	NS	NS
Head width (mm)	22.5 ± 0.3 (25)	22.3 ± 0.3 (21)	23.5 ± 0.4 (19)	23.0 ± 0.4 (25)	0.017	NS	NS
Head width:Abdominal circumference	0.22 ± 0.006 (25)	0.23 ± 0.004 (21)	0.22 ± 0.007 (19)	0.22 ± 0.008 (25)	NS	NS	NS
Neonatal growth rate (10-30 days)							
Absolute (g day^{-1}) (AGR_{10-30})	9.99 ± 0.20 (25)	9.03 ± 0.16 (21)	11.50 ± 0.21 (21)	9.32 ± 0.24 (26)	<0.0001	<0.0001	NS
Fractional (g day^{-1} g^{-1}) (FGR_{10-30})	0.065 ± 0.002 (25)	0.062 ± 0.002 (21)	0.065 ± 0.003 (21)	0.053 ± 0.002 (26)	0.012	<0.0001	NS

Data are presented as means ± S.E.M. Numbers in parentheses represent the number of animals. ANOVA: effect of birth weight class (BW), sex (S) and their interaction (BW x S). Statistical significance was accepted at $P < 0.05$. NS, not significant.

3.3.3 Effect of birth weight class on body size and composition in the aged guinea pig

3.3.3.1 Adult size

Birth weight class altered adult size in all male and female offspring combined, such that adult weight (-8%) ($p<0.005$) and nose to rump length (-4%) ($p<0.0001$) were reduced in low birth weight compared to high birth weight offspring (Table 3.2). Males demonstrated a higher adult weight ($p<0.0001$) and nose to rump length ($p<0.0001$) than females in both the low birth weight (+8% and +4% respectively) and high birth weight (+16% and +6% respectively) classes (Table 3.2). In male offspring only, adult weight (-12%) ($p<0.005$) and nose to rump length (-5%) ($p<0.003$) were reduced in low compared to high birth weight offspring (Figures 3.2a and b). In female offspring only, adult nose to rump length (-4%) ($p<0.02$) was reduced in low compared to high birth weight offspring (Figure 3.2b).

3.3.3.2 Adiposity

Birth weight class did not alter any parameter of adult adiposity in absolute or relative terms, in all male and female offspring combined (Table 3.2), or in male or female offspring only (data not shown). Females demonstrated higher relative combined ($p<0.020$) and visceral ($p<0.03$) adiposity when compared to males, in both the low birth weight (+15% for both) and high birth weight (+11% and +7% respectively) classes (Table 3.2).

3.3.3.3 Skeletal muscle mass

Birth weight class altered adult skeletal mass in all male and female offspring combined, such that combined (-11%) ($p<0.0001$), mixed (-10%) ($p<0.0001$) and type 2 (-11%) ($p<0.0001$) muscle mass in absolute terms were reduced, but were unchanged in relative terms, in low compared to high birth weight offspring (Table 3.2). Males demonstrated an increased adult combined ($p<0.0001$) and mixed ($p<0.0001$) muscle mass in absolute terms and a reduced relative type 2 muscle mass ($p<0.03$), when compared to females in the low (+10%, +11% and –11% respectively) and high birth weight (+11%, +11% and –5% respectively) classes (Table 3.2).

Table 3.2 Effect of birth weight class and sex on size, adiposity and skeletal muscle mass in the aged guinea pig

	Birth weight class				ANOVA *P*-value		
	Low birth weight		High birth weight		BW	S	BW x S
	Males	Females	Males	Females			
Body weight (g)	869 ± 34 (22)	807 ± 28 (17)	989 ± 25 (19)	854 ± 17 (21)	0.002	<0.0001	NS
Nose to rump length (mm)	329 ± 5 (19)	316 ± 3 (14)	348 ± 3 (17)	328 ± 4 (19)	<0.0001	<0.0001	NS
Body mass index (g mm^{-2})	0.0078 ± 0.0003 (19)	0.0079 ± 0.0003 (14)	0.0081 ± 0.0002 (17)	0.0079 ± 0.0002 (19)	NS	NS	NS
Combined adiposity (g)	73.2 ± 6.3 (18)	76.0 ± 6.0 (16)	83.7 ± 6.4 (18)	79.6 ± 4.2 (20)	NS	NS	NS
(% body weight)	8.05 ± 0.55 (18)	9.26 ± 0.46 (16)	8.34 ± 0.45 (18)	9.29 ± 0.39 (20)	NS	0.012	NS
Visceral adiposity (g)	37.0 ± 3.2 (19)	39.1 ± 2.9 (17)	43.6 ± 3.2 (19)	39.8 ± 1.9 (21)	NS	NS	NS
(% body weight)	4.12 ± 0.26 (19)	4.74 ± 0.23 (17)	4.32 ± 0.23 (19)	4.64 ± 0.18 (21)	NS	0.022	NS
Subcutaneous adiposity (g)	28.9 ± 2.6 (21)	26.6 ± 1.8 (16)	32.5 ± 2.6 (18)	27.5 ± 1.6 (20)	NS	NS	NS
(% body weight)	3.19 ± 0.23 (21)	3.27 ± 0.14 (16)	3.24 ± 0.19 (18)	3.19 ± 0.15 (20)	NS	NS	NS
Combined muscle (g)	27.4 ± 0.8 (22)	24.8 ± 0.8 (17)	31.0 ± 0.7 (19)	28.0 ± 0.7 (20)	<0.0001	<0.0001	NS
(% body weight)	3.18 ± 0.06 (22)	3.09 ± 0.09 (17)	3.14 ± 0.04 (19)	3.29 ± 0.07 (20)	NS	NS	NS
Mixed muscle (g)	25.9 ± 0.8 (22)	23.3 ± 0.8 (17)	29.2 ± 0.7 (19)	26.3 ± 0.7 (21)	<0.0001	<0.0001	NS
(% body weight)	3.01 ± 0.06 (22)	2.91 ± 0.09 (17)	2.97 ± 0.04 (19)	3.09 ± 0.06 (21)	NS	NS	NS
Type 2 muscle (g)	1.48 ± 0.04 (22)	1.48 ± 0.05 (17)	1.73 ± 0.03 (19)	1.59 ± 0.03 (20)	<0.0001	NS	NS
(% body weight)	0.17 ± 0.006 (22)	0.19 ± 0.006 (17)	0.18 ± 0.005 (19)	0.19 ± 0.003 (20)	NS	0.020	NS

Data are presented as means ± S.E.M. Numbers in parentheses represent the number of animals. ANOVA: effect of birth weight class (BW), sex (S) and their interaction (BW x S). Statistical significance was assumed at $P < 0.05$. NS, not significant.

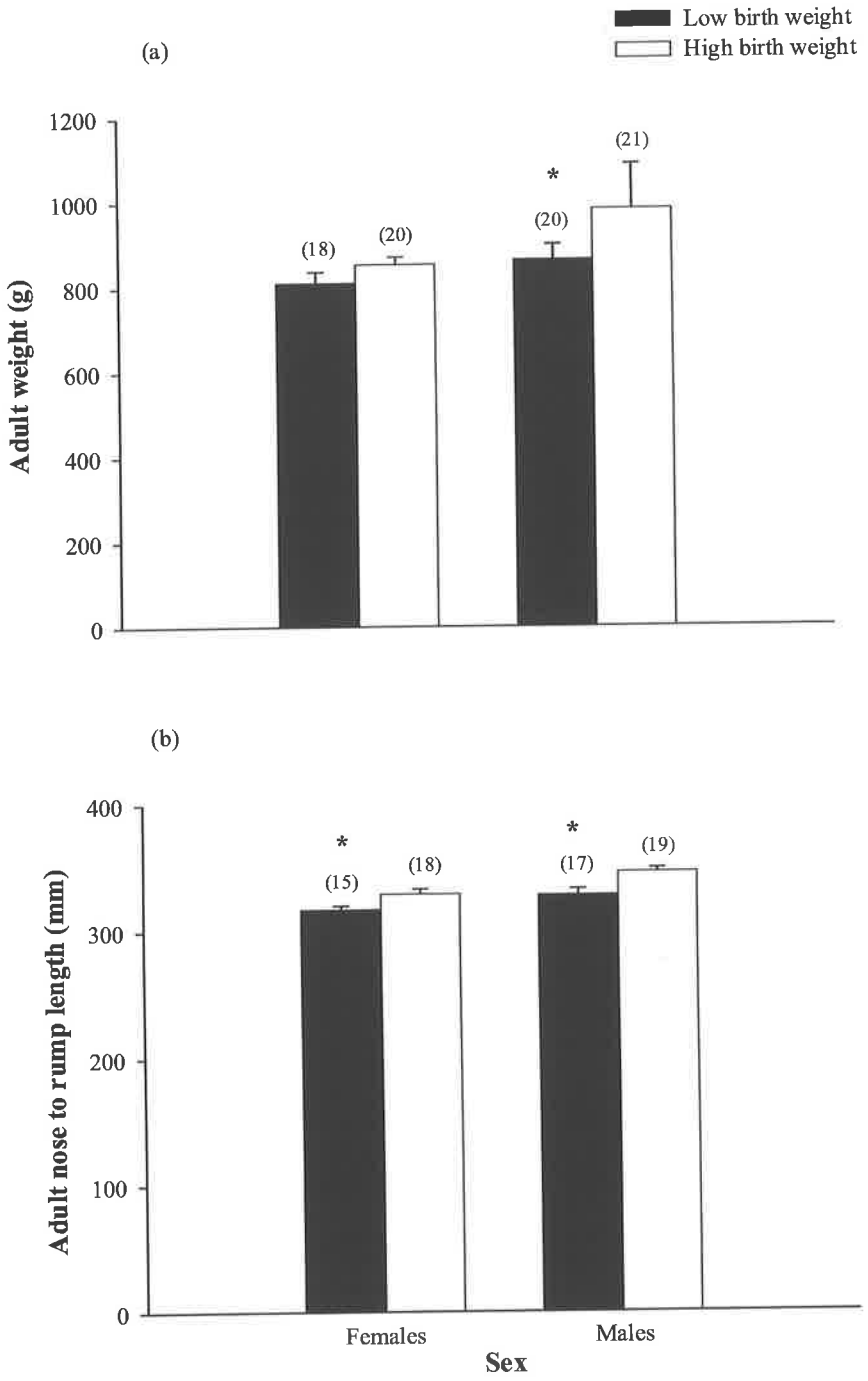

Figure 3.2 The effect of birth weight class and sex on weight and nose to rump length in the aged guinea pig

Weight (a) and nose to rump length (b) in aged adult guinea pigs of low and high birth weight. Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. *$P < 0.05$ compared with high birth weight offspring of the same sex.

In male offspring only, birth weight class altered adult skeletal muscle mass, such that combined (-12%) ($p<0.001$), mixed (-12%) ($p<0.001$) and type 2 (-16%) ($p<0.0001$) muscle mass in absolute terms were reduced, but were unchanged in relative terms in low compared to high birth weight offspring (Figures 3.3a, b and c). In female offspring only, birth weight class altered adult skeletal muscle mass, such that combined (-10%) ($p<0.02$) and mixed (-10%) ($p<0.02$) muscle mass in absolute terms were reduced but not changed in relative terms, in low compared to high birth weight offspring (Figures 3.3a and b).

3.3.3.4 Organ and gland weights

In all male and female offspring combined, birth weight class altered adult organ and gland weights, such that pancreas (-12%) ($p<0.03$), liver (-8%) ($p<0.04$), kidney (-10%) ($p<0.001$) and brain (-4%) ($p<0.02$) weights were decreased and relative adrenal (+8%) ($p<0.02$), heart (+5%) ($p<0.05$) and brain (+6%) ($p<0.05$) weights were increased, in low compared to high birth weight offspring (Table 3.3). Males demonstrated increased adrenal ($p<0.0001$), left ventricle ($p<0.03$), kidney ($p<0.0001$) and relative kidney ($p<0.03$) weight and reduced relative liver weight ($p<0.03$), when compared to females in both the low (+12%, +13%, +13%, +8% and –9% respectively) and high birth weight (+26%, +12%, +27%, +9% and –7% respectively) classes (Table 3.3).

In male offspring only, birth weight class altered adult organ weights, such that kidney (-15%) ($p<0.001$) and brain (-5%) ($p<0.04$) weights were reduced (Figures 3.4a and b) and relative heart weight was increased (+11%) ($p<0.03$) (Figure 3.5b), in low compared to high birth weight offspring. In female offspring only, adult relative adrenal weight was increased (+18%) ($p<0.02$) in low compared to high birth weight offspring (Figure 3.5a).

3.3.4 Effect of neonatal fractional growth rate class on body size and composition in the aged guinea pig

3.3.4.1 Adult size

Neonatal fractional growth rate class did not alter any parameter of adult size in all male and female offspring combined (Table 3.4), or in male or female offspring

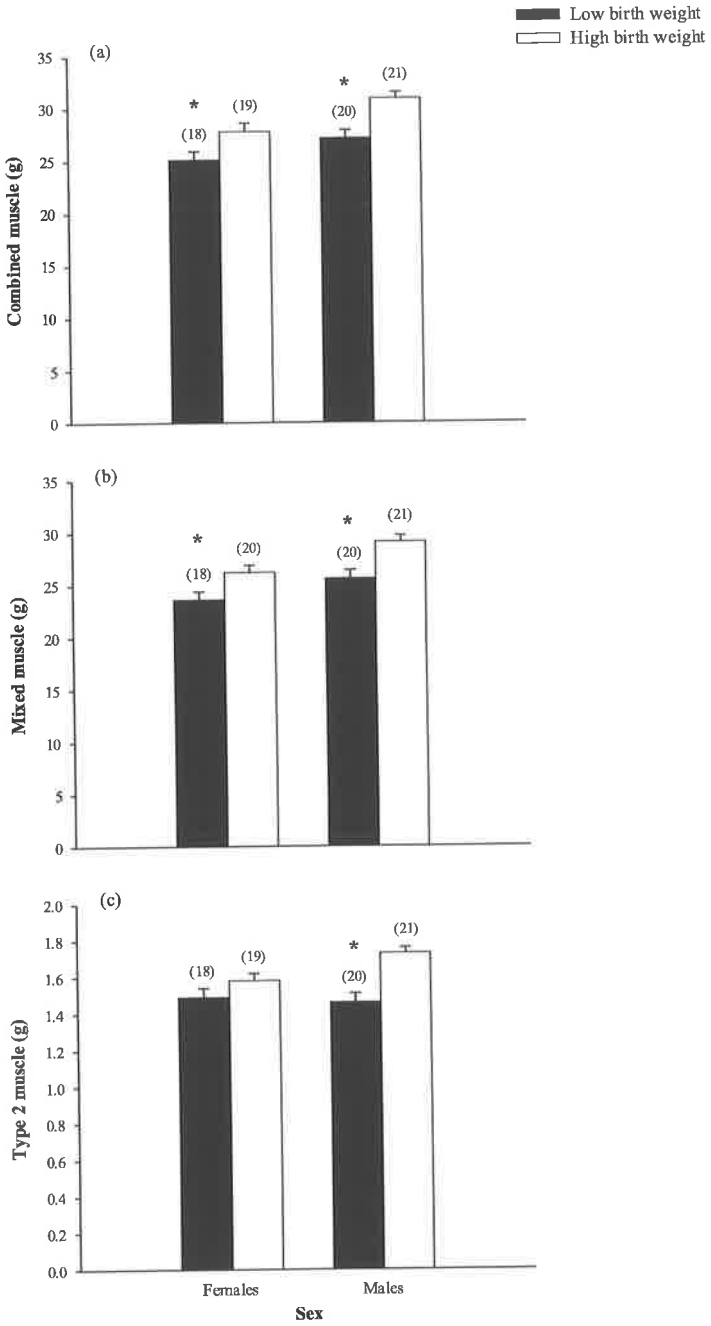

Figure 3.3 The effect of birth weight class and sex on combined, mixed and type 2 muscle mass in the aged guinea pig

Absolute combined (a) mixed (b) and type 2 (c) muscle mass in aged adult guinea pigs of low and high birth weight. Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. *$P < 0.05$ compared with high birth weight offspring of the same sex.

Table 3.3 Effect of birth weight class and sex on organ and gland weights in the aged guinea pig

	Birth weight class				ANOVA *P*-value		
	Low birth weight		High birth weight		BW	S	BW x S
	Males	Females	Males	Females			
Pancreas (g)	3.98 ± 0.24 (22)	3.74 ± 0.28 (17)	4.69 ± 0.29 (19)	4.14 ± 0.28 (21)	0.023	NS	NS
(% body weight)	0.45 ± 0.02 (22)	0.46 ± 0.03 (17)	0.47 ± 0.03 (19)	0.49 ± 0.03 (21)	NS	NS	NS
Liver (g)	33.5 ± 1.6 (22)	34.5 ± 2.0 (17)	38.4 ± 1.9 (19)	35.5 ± 0.9 (21)	0.039	NS	NS
(% body weight)	3.91 ± 0.19 (22)	4.28 ± 0.21 (17)	3.87 ± 0.15 (19)	4.18 ± 0.13 (21)	NS	0.028	NS
Adrenals (g)	1.15 ± 0.05 (22)	1.03 ±0.04 (17)	1.22 ± 0.04 (19)	0.97 ± 0.03 (21)	NS	<0.0001	NS
(% body weight)	0.13 ± 0.006 (22)	0.13 ± 0.005 (17)	0.12 ± 0.004 (19)	0.11 ± 0.004 (21)	0.013	NS	NS
Kidneys (g)	8.00 ± 0.22 (22)	7.08 ± 0.29 (17)	9.47 ± 0.32 (19)	7.48 ± 0.25 (21)	0.0005	<0.0001	NS
(% body weight)	0.95 ± 0.04 (22)	0.88 ± 0.03 (17)	0.96 ± 0.04 (19)	0.88 ± 0.03 (21)	NS	0.026	NS
Heart (g)	3.40 ± 0.22 (22)	3.17 ± 0.16 (17)	3.40 ± 0.10 (19)	3.29 ± 0.11 (21)	NS	NS	NS
(% body weight)	0.39 ± 0.02 (22)	0.39 ± 0.01 (17)	0.35 ± 0.01 (19)	0.39 ± 0.01 (21)	0.0445	NS	NS
Left Ventricle (g)	2.03 ± 0.15 (22)	1.80 ± 0.12 (17)	2.11 ± 0.09 (19)	1.88 ± 0.07 (21)	NS	0.024	NS
(% body weight)	0.23 ± 0.01 (22)	0.22 ± 0.01 (17)	0.21 ± 0.008 (19)	0.22 ± 0.009 (21)	NS	NS	NS
Right Ventricle (g)	0.92 ± 0.10 (22)	0.87 ± 0.07 (17)	0.86 ± 0.07 (19)	0.86 ± 0.07 (21)	NS	NS	NS
(% body weight)	0.11 ± 0.01 (22)	0.11 ± 0.009 (17)	0.09 ± 0.007 (19)	0.10 ± 0.008 (21)	NS	NS	NS
Brain (g)	4.13 ± 0.06 (21)	4.00 ± 0.07 (16)	4.32 ± 0.10 (14)	4.15 ± 0.09 (18)	0.017	NS	NS
(% body weight)	0.49 ± 0.02 (21)	0.51 ± 0.02 (16)	0.44 ± 0.02 (14)	0.49 ± 0.01 (18)	0.05	NS	NS

Data are presented as means ± S.E.M. Numbers in parentheses represent the number of animals. ANOVA: effect of birth weight class (BW), sex (S) and their interaction (BW x S). Statistical significance was assumed at $P < 0.05$. NS, not significant.

Table 3.4 Effect of neonatal fractional growth rate class and sex on size, adiposity and skeletal muscle mass in the aged guinea pig

	Neonatal fractional growth rate class				ANOVA *P*-value		
	Low growth rate		High growth rate		GR	S	GR x S
	Males	Females	Males	Females			
Body weight (g)	911 ± 31 (24)	840 ± 21 (19)	944 ± 25 (17)	826 ± 17 (19)	NS	0.001	NS
Nose to rump length (mm)	337 ± 5 (21)	323 ± 4 (16)	340 ± 3 (15)	323 ± 4 (17)	NS	0.0005	NS
Body mass index (g mm^{-2})	0.0080 ± 0.0002 (21)	0.0080 ± 0.0002 (16)	0.0079 ± 0.0003 (15)	0.0078 ± 0.0002 (17)	NS	NS	NS
Combined adiposity (g)	75.4 ± 6.4 (22)	81.8 ± 5.6 (18)	83.2 ± 6.0 (14)	74.3 ± 4.3 (18)	NS	NS	NS
(% body weight)	7.95 ± 0.47 (22)	9.65 ± 0.46 (18)	8.58 ± 0.53 (14)	8.90 ± 0.36 (18)	NS	0.016	NS
Visceral adiposity (g)	39.7 ± 3.2 (23)	41.6 ± 2.6 (19)	41.2 ± 3.3 (15)	37.4 ± 2.0 (19)	NS	NS	NS
(% body weight)	4.16 ± 0.23 (23)	4.89 ± 0.22 (19)	4.30 ± 0.27 (15)	4.47 ± 0.17 (19)	NS	0.026	NS
Subcutaneous adiposity (g)	28.6 ± 2.5 (23)	27.1 ± 1.8 (18)	33.3 ± 2.6 (16)	27.1 ± 1.5 (18)	NS	NS	NS
(% body weight)	3.04 ± 0.20 (23)	3.20 ± 0.15 (18)	3.46 ± 0.24 (16)	3.26 ± 0.14 (18)	NS	NS	NS
Combined muscle (g)	29.2 ± 0.9 (23)	26.3 ± 0.8 (19)	28.9 ± 0.8 (17)	26.7 ± 0.9 (18)	NS	0.0025	NS
(% body weight)	3.22 ± 0.04 (24)	3.14 ± 0.08 (19)	3.09 ± 0.06 (17)	3.26 ± 0.08 (18)	NS	NS	NS
Mixed muscle (g)	27.5 ± 0.8 (24)	24.7 ± 0.8 (19)	27.3 ± 0.7 (17)	25.2 ± 0.8 (19)	NS	0.002	NS
(% body weight)	3.04 ± 0.04 (24)	2.95 ± 0.08 (19)	2.92 ± 0.06 (17)	3.07 ± 0.07 (19)	NS	NS	NS
Type 2 muscle (g)	1.63 ± 0.04 (24)	1.54 ± 0.04 (19)	1.57 ± 0.06 (17)	1.53 ± 0.04 (18)	NS	NS	NS
(% body weight)	0.18 ± 0.01 (24)	0.19 ± 0.01 (19)	0.18 ± 0.01 (17)	0.19 ± 0.004 (18)	NS	0.010	NS

Data are presented as means ± S.E.M. Numbers in parentheses represent the number of animals. ANOVA: effect of neonatal fractional growth rate class (GR), sex (S) and their interaction (GR x S). Statistical significance was assumed at $P < 0.05$. NS, not significant.

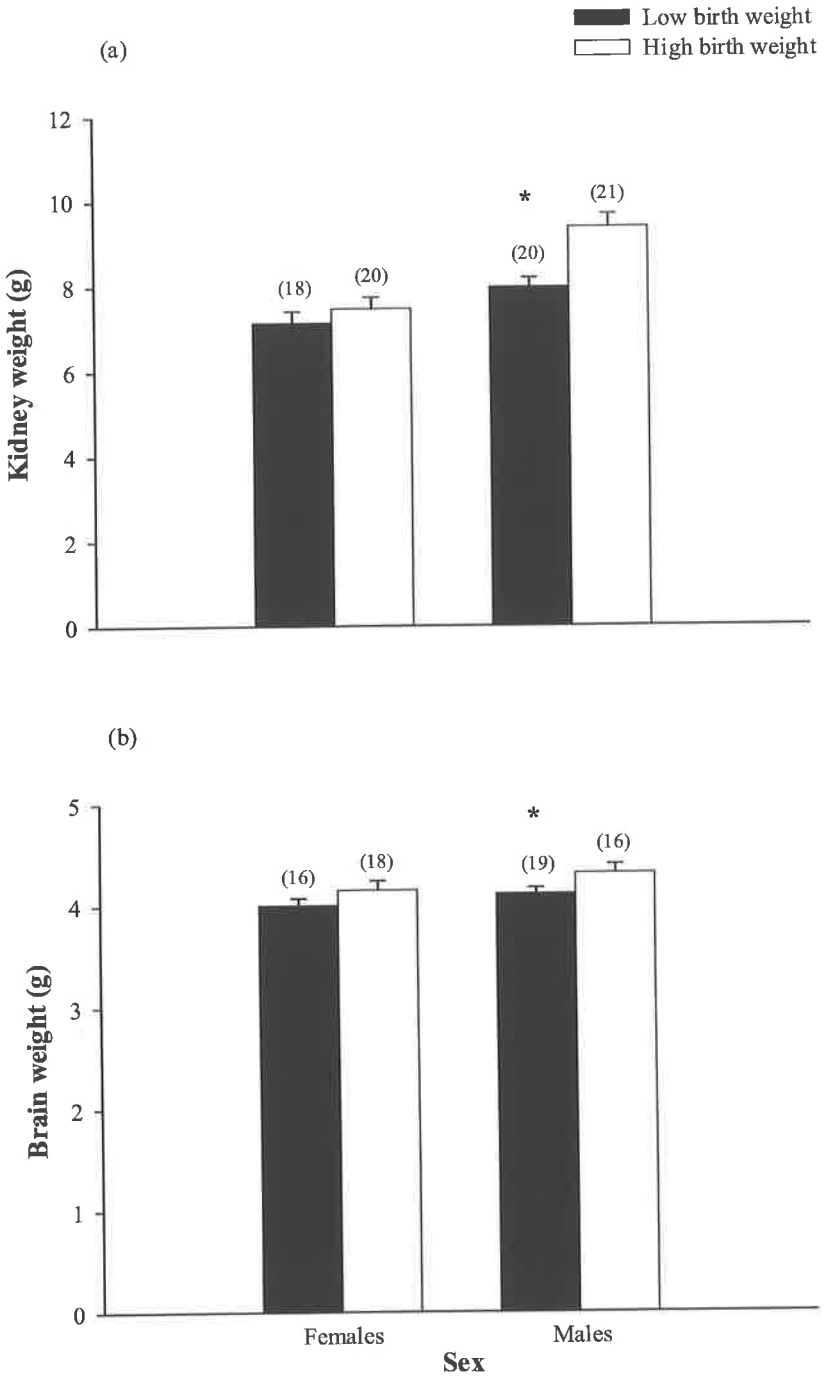

Figure 3.4 The effect of birth weight class and sex on absolute kidney and brain weight in the aged guinea pig

Absolute kidney (a) and brain (b) weight in aged adult guinea pigs of low and high birth weight. Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. *$P < 0.05$ compared with high birth weight offspring of the same sex.

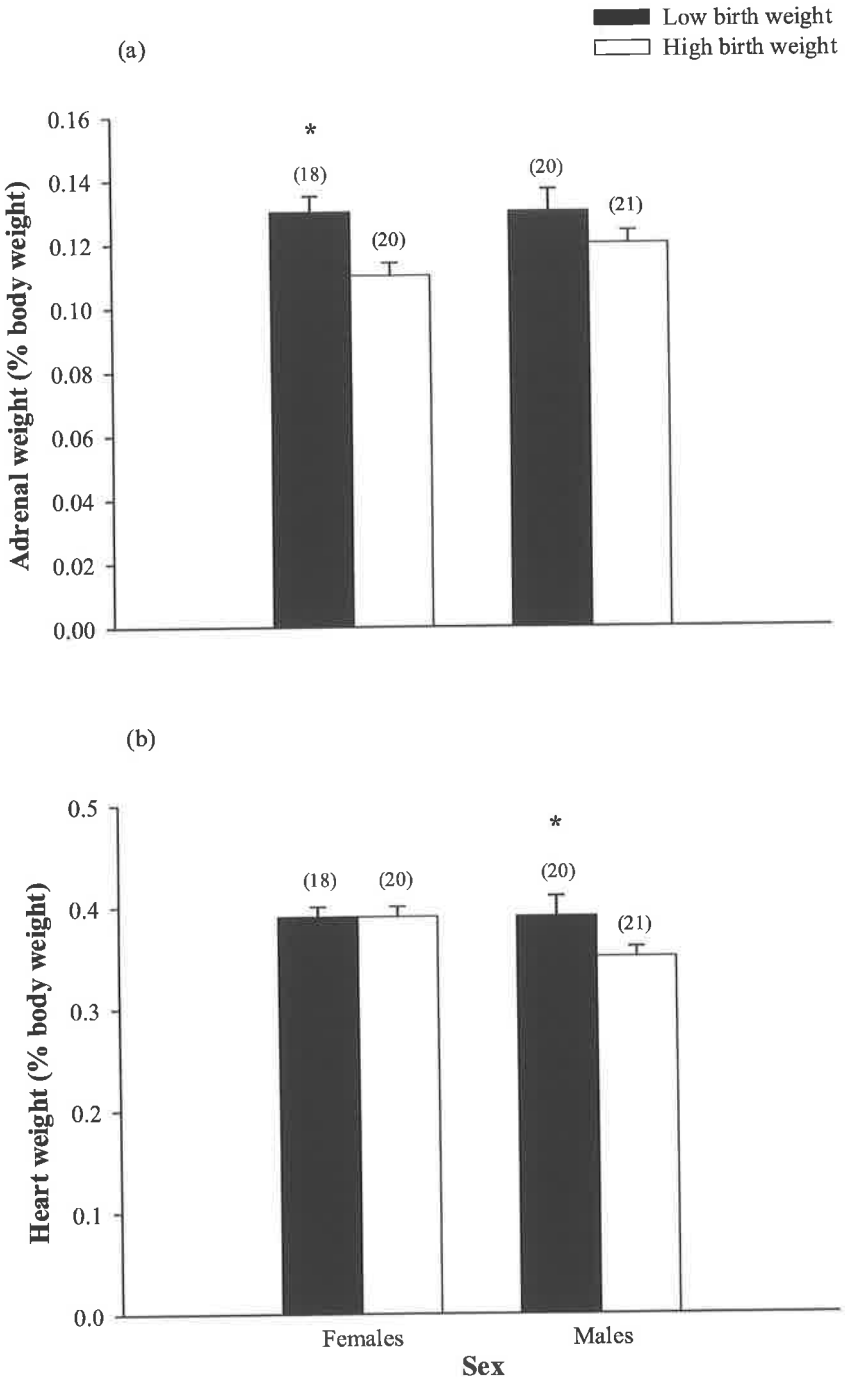

Figure 3.5 The effect of birth weight class and sex on relative adrenal and heart weight in the aged guinea pig

Relative adrenal (a) and heart (b) weight in old adult guinea pigs of low and high birth weight. Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. *$P < 0.05$ compared with high birth weight offspring of the same sex.

separately (data not shown). Inclusion of birth weight as a co-variate did not alter these outcomes.

3.3.4.2 *Adiposity*

Neonatal fractional growth rate class did not alter any parameter of adult adiposity in absolute or relative terms in all male and female offspring combined (Table 3.4), or in male or female offspring only (data not shown). Inclusion of birth weight as a co-variate did not alter these outcomes.

3.3.4.3 *Skeletal muscle mass*

Neonatal fractional growth rate class did not alter any parameter of adult skeletal muscle mass in absolute or relative terms in all male and female offspring combined (Table 3.4), or in female offspring only (data shown for relative combined and mixed muscle mass only, Figures 3.6a and b). In male offspring only, neonatal fractional growth rate class altered adult skeletal muscle mass, with fast growing males demonstrating reduced relative combined (-4%) ($p<0.05$) and mixed (-4%) ($p<0.05$) muscle mass when compared to their slow growing counterparts (Figures 3.6a and b). Inclusion of birth weight as a co-variate did not alter these outcomes.

3.3.4.3 *Organ and gland weights*

In all male and female offspring combined, neonatal fractional growth rate class altered adult organ weights, with fast growing animals demonstrating reduced liver (-8%) ($p<0.05$) and relative liver (-8%) ($p<0.02$) weight and increased kidney weight (+5%) ($p<0.05$) when compared to their slow growing counterparts (Table 3.5).

In male offspring only, neonatal fractional growth rate class altered adult organ and gland weights, with fast growing males demonstrating increased pancreas (+18%) ($p<0.04$) (Figure 3.7a), relative pancreas (+14%) ($p<0.03$) (Figure 3.7b), adrenal (+11%) ($p<0.04$) (Figure 3.8), heart (+16%) ($p<0.03$) (Figure 3.9a), relative heart (+11%) ($p<0.05$) (Figure 3.9b), left ventricle (+16%) ($p<0.04$) (Figure 3.9c), right ventricle (+32%) ($p<0.02$) (Figure 3.9e) and relative right ventricle (+22%) ($p<0.04$) (Figure 3.9f) weight when compared to their slow growing counterparts.

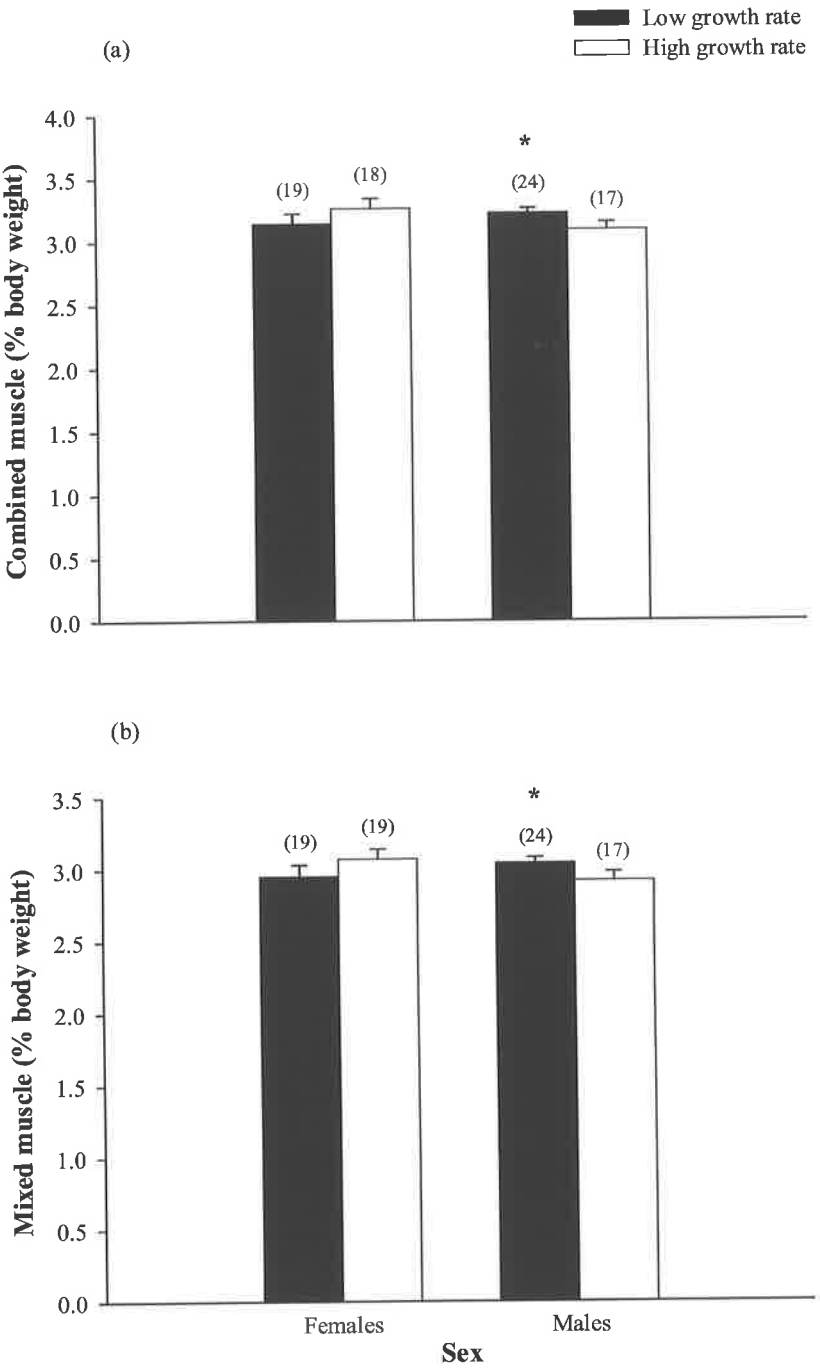

Figure 3.6 The effect of neonatal fractional growth rate class and sex on relative combined and mixed muscle mass in the aged guinea pig

Relative combined (a) and mixed (b) muscle mass in aged adult guinea pigs of low and high neonatal fractional growth rate. Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. *$P < 0.05$ compared with high growth rate offspring of the same sex.

Table 3.5 Effect of neonatal fractional growth rate class and sex on organ and gland weights in the aged guinea pig

	Neonatal fractional growth rate class				ANOVA *P*-value		
	Low growth rate		High growth rate		GR	S	GR x S
	Males	Females	Males	Females			
Pancreas (g)	4.01 ± 0.23 (24)	4.12 ± 0.36 (19)	4.73 ± 0.31 (17)	3.81 ± 0.18 (19)	NS	NS	0.032
(% body weight)	0.44 ± 0.02 (24)	0.49 ± 0.04 (19)	0.50 ± 0.03 (17)	0.46 ± 0.02 (19)	NS	NS	0.044
Liver (g)	36.2 ± 2.0 (24)	37.3 ± 1.5 (19)	35.1 ± 1.3 (17)	32.7 ± 1.2 (19)	0.044	NS	NS
(% body weight)	3.99 ± 0.19 (24)	4.47 ± 0.18 (19)	3.76 ± 0.13 (17)	3.98 ± 0.13 (19)	0.019	0.021	NS
Adrenals (g)	1.13 ± 0.05 (24)	1.00 ±0.03 (19)	1.25 ± 0.04 (17)	0.99 ± 0.03 (19)	NS	<0.0001	NS
(% body weight)	0.12 ± 0.004 (24)	0.12 ± 0.004 (19)	0.14 ± 0.007 (17)	0.12 ± 0.005 (19)	NS	NS	NS
Kidneys (g)	8.40 ± 0.27 (24)	7.15 ± 0.24 (19)	9.08 ± 0.35 (17)	7.45 ± 0.30 (19)	0.049	<0.0001	NS
(% body weight)	0.94 ± 0.03 (24)	0.85 ± 0.02 (19)	0.98 ± 0.04 (17)	0.91 ± 0.04 (19)	NS	0.022	NS
Heart (g)	3.19 ± 0.14 (24)	3.38 ± 0.14 (19)	3.69 ± 0.20 (17)	3.08 ± 0.11 (19)	NS	NS	0.005
(% body weight)	0.35 ± 0.01 (24)	0.40 ± 0.01 (19)	0.39 ± 0.02 (17)	0.37 ± 0.01 (19)	NS	NS	0.012
Left Ventricle (g)	1.94 ± 0.11 (24)	1.99 ± 0.09 (19)	2.26 ± 0.14 (17)	1.70 ± 0.09 (19)	NS	0.012	0.004
(% body weight)	0.21 ± 0.01 (24)	0.24 ± 0.009 (19)	0.24 ± 0.01 (17)	0.21 ± 0.009 (19)	NS	NS	0.005
Right Ventricle (g)	0.79 ± 0.06 (24)	0.89 ± 0.08 (19)	1.04 ± 0.10 (17)	0.84 ± 0.06 (19)	NS	NS	0.024
(% body weight)	0.09 ± 0.007 (24)	0.11 ± 0.01 (19)	0.11 ± 0.011 (17)	0.10 ± 0.007 (19)	NS	NS	NS
Brain (g)	4.25 ± 0.06 (20)	4.04 ± 0.08 (17)	4.14 ± 0.10 (15)	4.12 ± 0.09 (17)	NS	NS	NS
(% body weight)	0.48 ± 0.02 (20)	0.49 ± 0.02 (17)	0.45 ± 0.02 (15)	0.51 ± 0.02 (17)	NS	NS	NS

Data are presented as means ± S.E.M. Numbers in parentheses represent the number of animals. ANOVA: effect of neonatal fractional growth rate class (GR), sex (S) and their interaction (GR x S). Statistical significance was assumed at $P < 0.05$. NS, not significant.

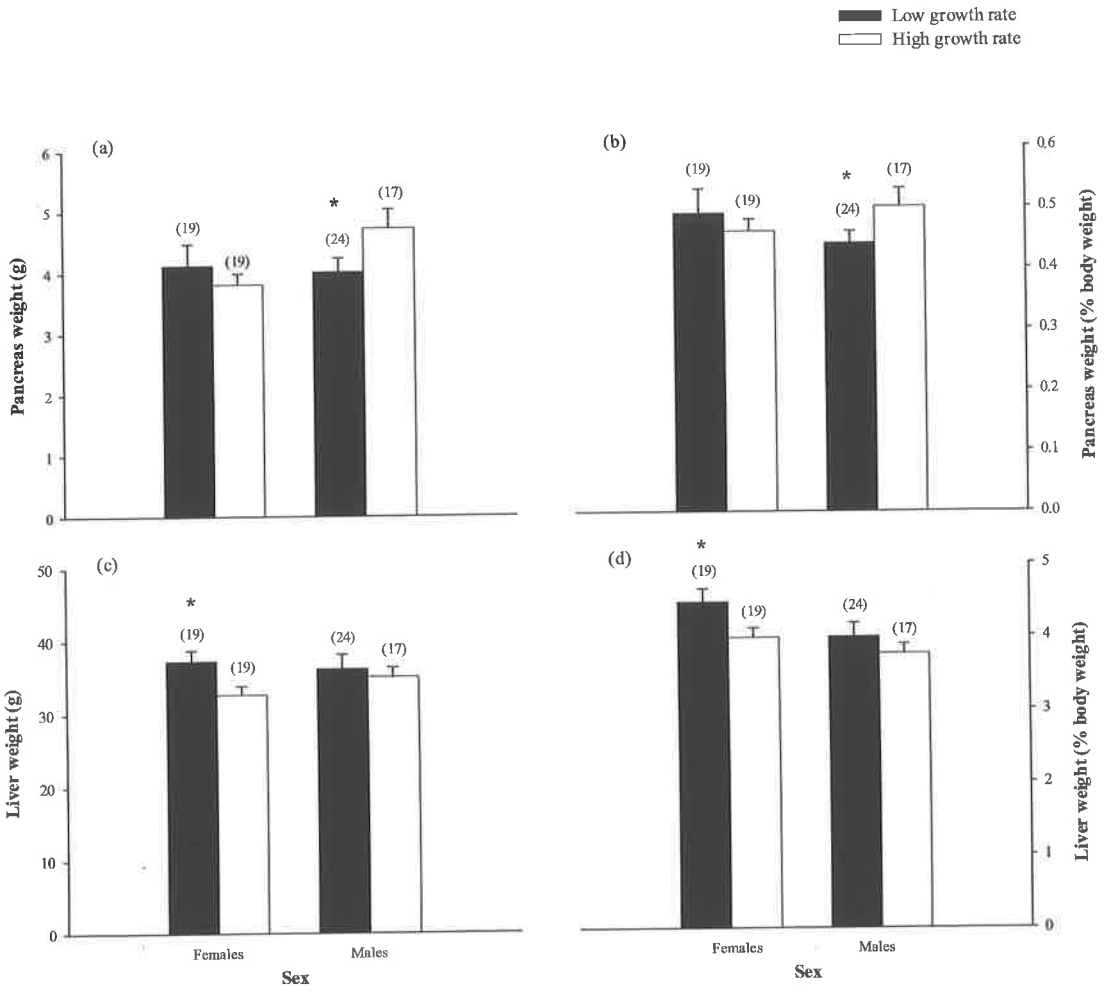

Figure 3.7 The effect of neonatal fractional growth rate class and sex on absolute and relative pancreas and liver weight in the aged guinea pig

Absolute and relative pancreas (a and b) and liver (c and d) weight in aged adult guinea pigs of low and high neonatal fractional growth rate. Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. *$P < 0.05$ compared with high growth rate offspring of the same sex.

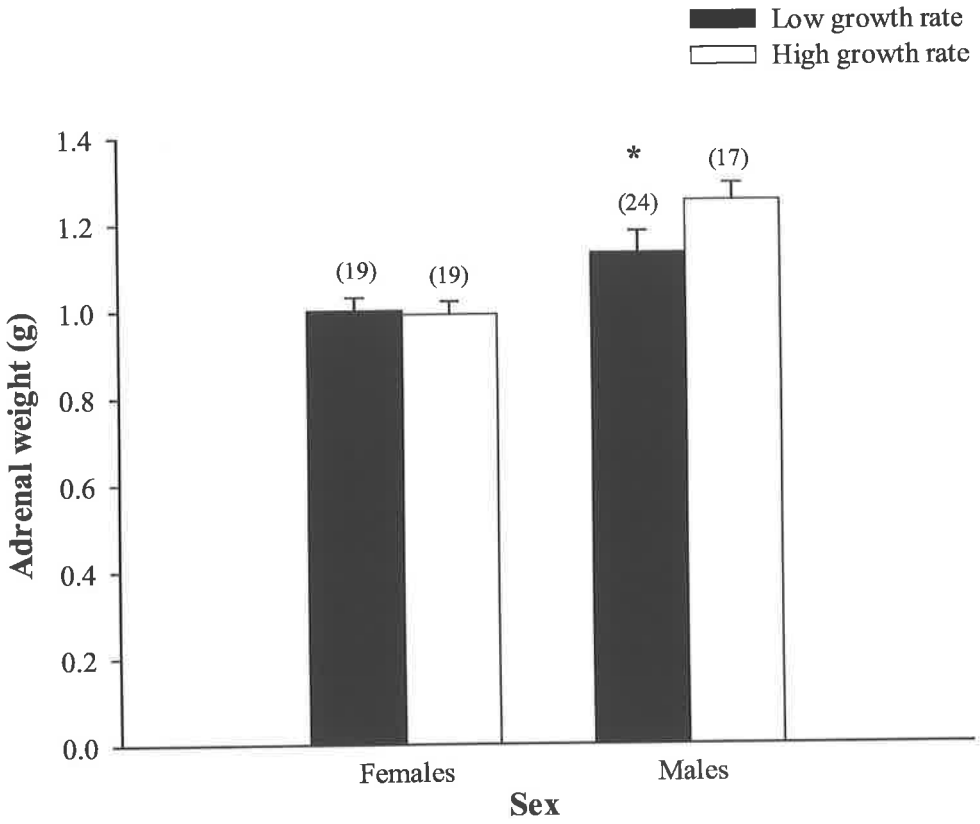

Figure 3.8 The effect of neonatal fractional growth rate class and sex on absolute adrenal weight in the aged guinea pig

Absolute adrenal weight in aged adult guinea pigs of low and high neonatal fractional growth rate. Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. *$P < 0.05$ compared with high growth rate offspring of the same sex.

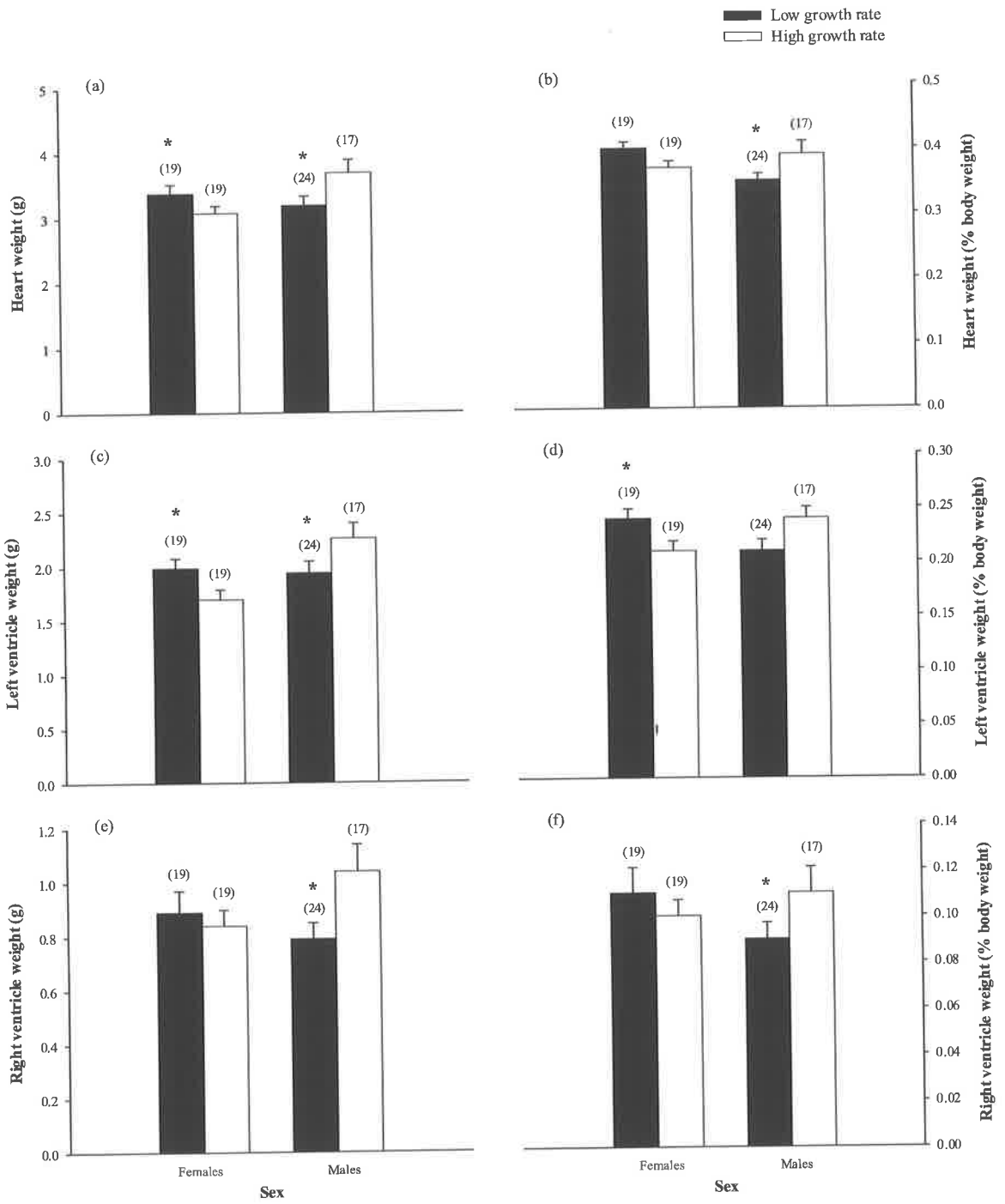

Figure 3.9 The effect of neonatal fractional growth rate class and sex on absolute and relative heart, and right and left ventricle weight in the aged guinea pig

Absolute and relative heart (a and b) left ventricle (c and d) and right ventricle (e and f) weight in aged adult guinea pigs of low and high neonatal fractional growth rate. Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. *$P < 0.05$ compared with high growth rate offspring of the same sex.

In female offspring only, neonatal fractional growth rate class altered adult organ weights, with fast growing females demonstrating reduced liver (-12%) ($p<0.02$) (Figure 3.7c), relative liver (-11%) ($p<0.02$) (Figure 3.7d), heart (-9%) ($p<0.05$) (Figure 3.9a), left ventricle (-15%) ($p<0.02$) (Figure 3.9c), and relative left ventricle (-13%) ($p<0.02$) (Figure 3.9d) weight when compared to their slow growing counterparts. Inclusion of birth weight as a co-variate did not alter these outcomes.

3.3.5 Relationship of adult size to size at birth and neonatal growth rate in the aged guinea pig

3.3.5.1 Size at birth

In all male and female offspring combined, adult weight and nose to rump length correlated positively with birth weight ($r=0.27$, $p=0.009$, $n=79$ and $r=0.33$, $p=0.003$, $n=69$, respectively) and the birth weight to length ratio ($r=0.25$, $p=0.013$, $n=76$ and $r=0.31$, $p=0.006$, $n=67$, respectively) (Table 3.6). In male offspring, adult weight and nose to rump length correlated positively with birth weight ($r=0.27$, $p=0.043$, $n=41$ and $r=0.30$, $p=0.041$, $n=36$, respectively) (Figures 3.10a and b) and the birth weight to length ratio ($r=0.31$, $p=0.027$, $n=39$ and $r=0.34$, $p=0.024$, $n=35$, respectively) (Figures 3.11a and b). In female offspring, adult weight and nose to rump length correlated positively with birth weight ($r=0.34$, $p=0.020$, $n=38$ and $r=0.45$, $p=0.004$, $n=33$, respectively) (Figures 3.10a and b) and the birth weight to length ratio ($r=0.28$, $p=0.049$, $n=37$ and $r=0.36$, $p=0.021$, $n=32$, respectively) (Figures 3.11a and b).

3.3.5.2 Neonatal growth rate

In all male and female offspring combined, adult weight and nose to rump length correlated positively with the neonatal absolute growth rate ($r=0.47$, $p<0.001$, $n=79$ and $r=0.47$, $p<0.001$, $n=69$, respectively) (Table 3.6). In male offspring, adult weight and nose to rump length correlated positively with the neonatal absolute growth rate ($r=0.42$, $p=0.003$, $n=41$ and $r=0.32$, $p=0.030$, $n=36$, respectively) (Figures 3.12a and b). In female offspring, adult nose to rump length correlated positively with the neonatal absolute growth rate ($r=0.30$, $p=0.045$, $n=33$) (Figure 3.12b).

Table 3.6 Relationship of size, adiposity and skeletal muscle mass to size at birth and neonatal growth rate in the aged guinea pig

	Correlation coefficients (*r*)									
	BWT	BNRL	BWT:BNRL	BPI	BAC	BHL	BHW	BHW:BAC	AGR_{10-30}	FGR_{10-30}
Body weight (g)	0.27 (79)**	0.16 (76)	0.25 (76)*	0.05 (76)	0.12 (76)	0.03 (76)	0.00 (76)	-0.06 (76)	0.47 (79)**	0.06 (79)
Nose to rump length (mm)	0.33 (69)**	0.14 (67)	0.31 (67)**	0.06 (67)	-0.04 (67)	0.12 (67)	0.17 (67)	0.10 (67)	0.47 (69)**	0.11 (69)
Body mass index (g mm^{-2})	0.08 (69)	0.04 (67)	0.08 (67)	0.01 (67)	0.20 (67)	-0.02 (67)	-0.19 (67)	-0.05 (67)	0.16 (69)	-0.09 (69)
Combined adiposity (g)	0.15 (72)	0.03 (69)	0.18 (69)	0.11 (69)	-0.04 (69)	-0.17 (69)	-0.08 (69)	0.02 (69)	0.18 (72)	-0.06 (72)
(% body weight)	0.05 (72)	-0.02 (69)	0.08 (69)	0.07 (69)	-0.12 (69)	-0.26 (69)*	-0.15 (69)	0.04 (69)	-0.06 (72)	-0.13 (72)
Visceral adiposity (g)	0.12 (76)	0.05 (73)	0.13 (73)	0.06 (73)	-0.05 (73)	-0.20 (73)	-0.10 (73)	0.03 (73)	0.16 (76)	-0.09 (76)
(% body weight)	-0.01 (76)	-0.01 (73)	-0.00 (73)	0.01 (73)	-0.15 (73)	-0.32 (73)**	-0.18 (73)	0.05 (73)	-0.07 (76)	-0.15 (76)
Subcutaneous adiposity (g)	0.10 (75)	0.03 (72)	0.11 (72)	0.06 (72)	-0.01 (72)	-0.12 (72)	-0.11 (72)	-0.03 (72)	0.14 (75)	0.09 (75)
(% body weight)	0.00 (75)	-0.02 (72)	0.02 (72)	0.04 (72)	-0.09 (72)	-0.18 (72)	-0.17 (72)	-0.01 (72)	0.16 (75)	0.08 (75)
Combined muscle (g)	0.37 (78)**	0.12 (75)	0.35 (75)**	0.07 (75)	0.07 (75)	0.17 (75)	0.10 (75)	0.03 (75)	0.19 (78)	0.01 (78)
(% body weight)	0.11 (78)	0.06 (75)	0.12 (75)	0.04 (75)	-0.09 (75)	0.19 (75)	0.15 (75)	0.16 (75)	-0.14 (78)	-0.06 (78)
Mixed muscle (g)	0.36 (79)**	0.13 (76)	0.34 (76)**	0.06 (76)	0.07 (76)	0.16 (76)	0.09 (76)	0.03 (76)	0.13 (79)	0.02 (79)
(% body weight)	0.11 (79)	0.06 (76)	0.11 (76)	0.03 (76)	-0.10 (76)	0.17 (76)	0.14 (76)	0.16 (76)	-0.13 (79)	-0.05 (79)
Type 2 muscle (g)	0.05 (78)	0.15 (75)	0.09 (75)	0.17 (75)	0.15 (75)	0.13 (75)	0.16 (75)	0.02 (75)	0.20 (78)	-0.10 (78)
(% body weight)	0.11 (78)	-0.07 (75)	0.19 (75)	0.06 (75)	-0.03 (75)	0.05 (75)	0.11 (75)	0.13 (75)	-0.19 (78)	-0.15 (78)

r represents the correlation coefficient between each variable of adult size, adiposity and skeletal muscle mass and each variable of size at birth and neonatal growth. Numbers in parentheses represent the number of animals. Partial correlations: all significant correlations highlighted in grey were independent of FGR_{10-30}. Statistical significance of correlation coefficients: $^{*}P < 0.05$ $^{**}P < 0.01$. BW, birth weight (g); BNRL, birth nose to rump length (mm); BW:BNRL, birth weight to length ratio (g mm^{-1}); BPI, birth ponderal index (g mm^{-3}); BAC, birth abdominal circumference (mm); BHL, birth head length (mm); BHW, birth head width (mm); BHW:BAC, birth head width to abdominal circumference ratio; AGR_{10-30}, absolute growth rate (10-30 days) (g day^{-1}); FGR_{10-30}, fractional growth rate (10-30 days) (g day^{-1} g^{-1}).

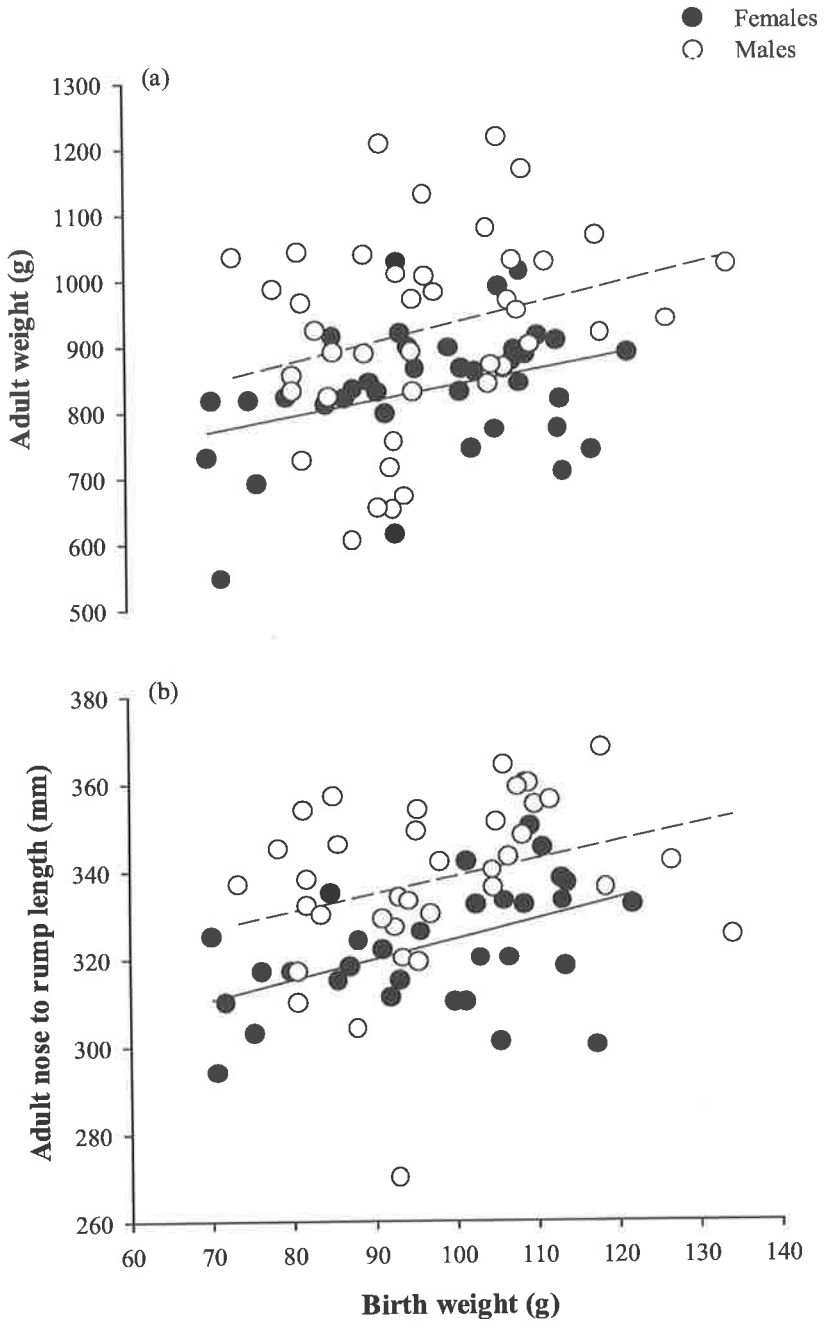

Figure 3.10 The relationship of weight and nose to rump length to birth weight in the aged guinea pig

Relationship of birth weight to (a) weight (females: $r=0.34$, $n=38$, $p<0.03$; males: $r=0.27$, $n=41$, $p<0.05$) and (b) nose to rump length (females: $r=0.45$, $n=33$, $p<0.005$; males: $r=0.30$, $n=36$, $p<0.05$) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

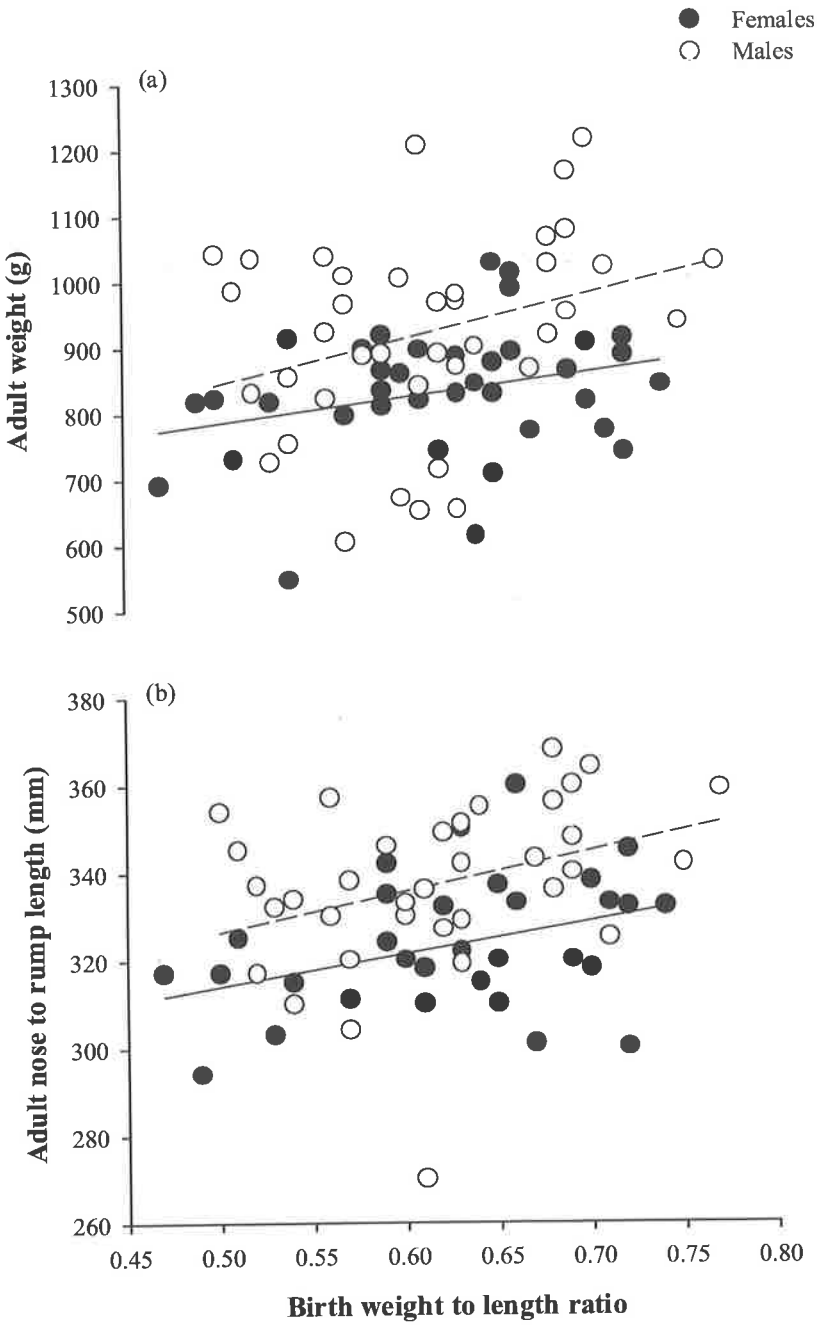

Figure 3.11 The relationship of weight and nose to rump length to birth weight to length ratio in the aged guinea pig

Relationship of birth weight to length ratio to (a) weight (females: r=0.28, n=37, p<0.05; males: r=0.31, n=39, p<0.03) and (b) nose to rump length (females: r=0.36, n=32, p<0.03; males: r=0.34, n=35, p<0.03) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

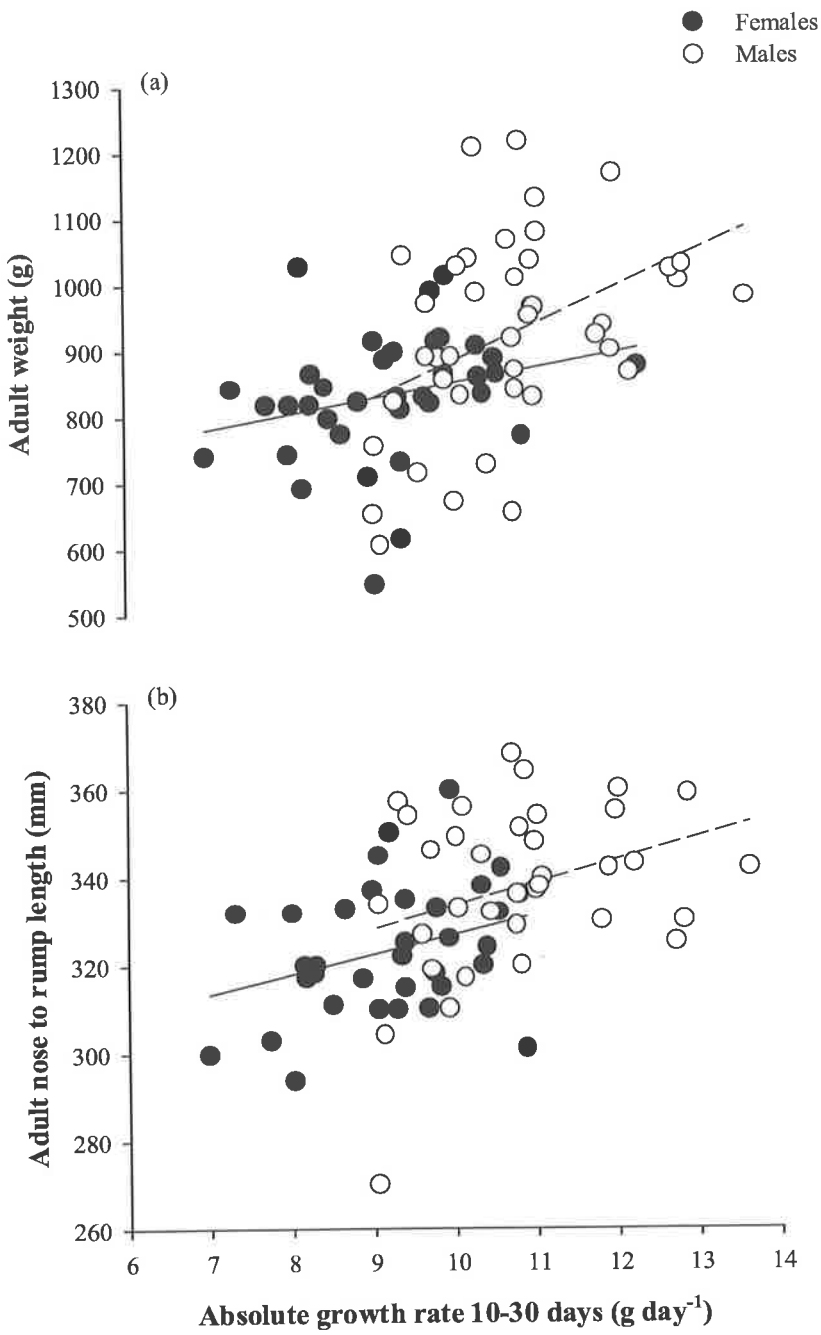

Figure 3.12 The relationship of weight and nose to rump length to neonatal absolute growth rate in the aged guinea pig

Relationship of neonatal absolute growth rate to (a) weight (females: r=0.26, n=38, ns; males: r=0.42, n=41, p<0.004) and (b) nose to rump length (females: r=0.30, n=33, p<0.05; males: r=0.32, n=36, p<0.04) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

3.3.5.3 *Partial correlations*

The positive associations between adult weight and nose to rump length and birth weight and the birth weight to length ratio in all offspring were independent of neonatal fractional growth rate (partial correlation (pc): r=0.31, p=0.003, r=0.41, p<0.001, r=0.29, p=0.006 and r=0.37, p=0.001, respectively).

In male offspring, the positive associations between adult weight and nose to rump length and birth weight and the birth weight to length ratio were independent of neonatal fractional growth rate (pc: r=0.27, p=0.045, r=0.31, p=0.034, r=0.31, p=0.029 and r=0.35, p=0.021, respectively).

In female offspring, the positive associations between adult weight and nose to rump length and birth weight were independent of neonatal fractional growth rate (pc: r=0.28, p=0.046 and r=0.45, p=0.005, respectively), as was the positive association between adult nose to rump length and the birth weight to length ratio (pc: r=0.32, p=0.042). The positive association between adult weight and the birth weight to length ratio in female offspring was not independent of neonatal fractional growth rate.

The positive associations between adult weight and nose to rump length and the neonatal absolute growth rate in all offspring were independent of birth weight (pc: r=0.43, p<0.001 and r=0.42, p<0.001, respectively). In male offspring, the positive association between adult weight and the neonatal absolute growth rate was independent of birth weight (pc: r=0.34, p=0.016), however the positive association between adult nose to rump length and the neonatal absolute growth rate was not. In female offspring, the positive association between adult nose to rump length and the neonatal absolute growth rate was also not independent of birth weight.

3.3.6 Relationship of adult adiposity to size at birth and neonatal growth rate in the aged guinea pig

3.3.6.1 *Size at birth*

In all male and female offspring combined, adult relative combined and visceral adiposity correlated negatively with head length at birth (r=-0.26, p=0.016, n=69 and r=-0.32, p=0.003, n=73, respectively) (Table 3.6). In male offspring, adult relative

combined, visceral and subcutaneous adiposity correlated negatively with head length at birth (r=-0.57, p<0.001, n=34, r=-0.57, p<0.001, n=36 and r=-0.49, p=0.001, n=37, respectively) (Figures 3.13a, b and c). In female offspring, parameters of adult adiposity were not associated with any measures of size at birth (data shown for selected associations only, Figures 3.13a, b and c).

3.3.6.2 Neonatal growth rate

Adult adiposity parameters were not associated with any measures of neonatal growth in all male and female offspring combined (Table 3.6), or in male or female offspring only (data not shown).

3.3.6.3 Partial correlations

The negative associations between adult relative combined and visceral adiposity and head length at birth in all offspring were independent of neonatal fractional growth rate (pc: r=-0.29, p=0.008 and r=-0.35, p=0.001, respectively). In male offspring, the negative associations between adult relative combined, visceral and subcutaneous adiposity and head length at birth were independent of neonatal fractional growth rate (pc: r=-0.36, p=0.021, r=-0.34, p=0.022 and r=-0.40, p=0.008, respectively).

3.3.7 Relationship of adult skeletal muscle mass to size at birth and neonatal growth rate in the aged guinea pig

3.3.7.1 Size at birth

In all male and female offspring combined, adult combined and mixed muscle mass correlated positively with birth weight (r=0.37, p<0.001, n=78 and r=0.36, p=0.001, n=79, respectively) and the birth weight to length ratio (r=0.35, p=0.001, n=75 and r=0.34, p=0.001, n=76, respectively) (Table 3.6). In male offspring, adult combined, mixed and type 2 muscle mass correlated positively with birth weight (r=0.38, p=0.007, r=0.36, p=0.010 and r=0.53, p<0.001, respectively, n=41) (Figures 3.14a, b and c). In female offspring, adult combined, mixed and type 2 muscle mass correlated positively with birth weight (r=0.41, p=0.006, n=37, r=0.41, p=0.006, n=38 and r=0.37, p=0.012, n=37, respectively) (Figures 3.14a, b and c).

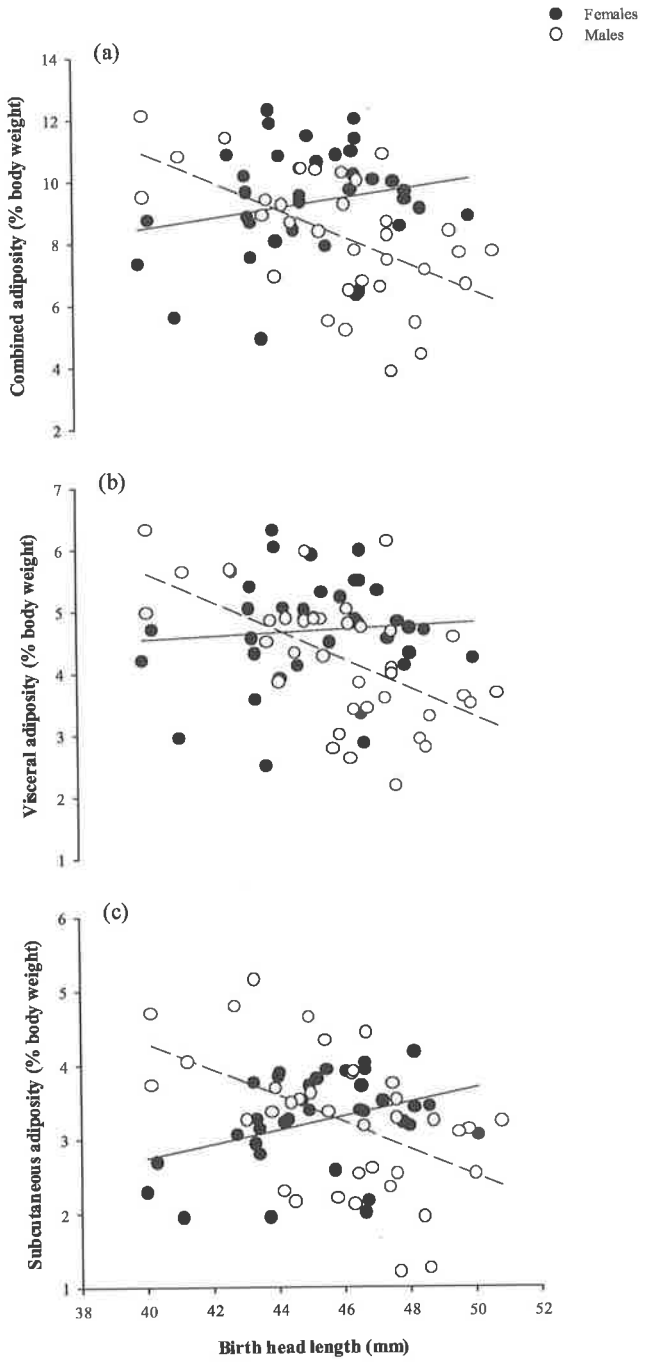

Figure 3.13 The relationship of relative combined, visceral and subcutaneous adiposity to head length at birth in the aged guinea pig

Relationship of head length at birth to relative (a) combined (females: r=0.21, n=35, ns; males: r=-0.57, n=34, p<0.001) (b) visceral (females: r=0.07, n=37, ns; males: r=-0.57, n=36, p<0.001) and (c) subcutaneous (females: r=0.29, n=35, ns; males: r=-0.49, n=37, p<0.002) adiposity in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

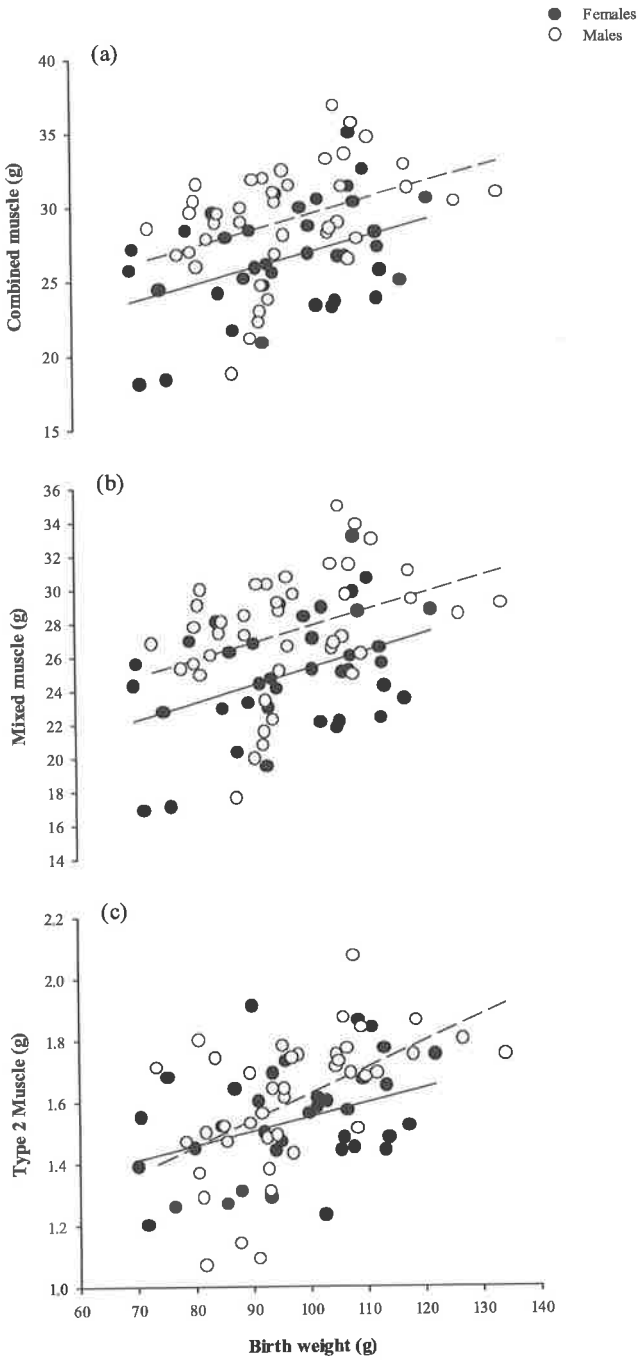

Figure 3.14 The relationship of combined, mixed and type 2 muscle mass to birth weight in the aged guinea pig

Relationship of birth weight to (a) combined (females: r=0.41, n=37, p<0.007; males: r=0.38, n=41, p<0.008) (b) mixed (females: r=0.41, n=38, p<0.007; males: r=0.36, n=41, p<0.02) and (c) type 2 (females: r=0.37, n=37, p<0.02; males: r=0.53, n=41, p<0.001) muscle mass in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

3.3.7.2 *Neonatal growth rate*

Adult skeletal muscle parameters were not associated with any measures of neonatal growth in all male and female offspring combined (Table 3.6), or in male or female offspring only (data not shown).

3.3.7.3 *Partial correlations*

The positive associations between adult combined and mixed muscle mass and birth weight and the birth weight to length ratio in all offspring were independent of neonatal fractional growth rate (pc: $r=0.41$, $p<0.001$, $r=0.40$, $p<0.001$, $r=0.38$, $p<0.001$ and $r=0.37$, $p=0.001$, respectively).

In male offspring, the positive associations between adult combined, mixed and type 2 muscle mass and birth weight were independent of neonatal fractional growth rate (pc: $r=0.37$, $p=0.010$, $r=0.35$, $p=0.013$ and $r=0.52$, $p<0.001$, respectively).

In female offspring, the positive associations between adult combined, mixed and type 2 muscle mass and birth weight were independent of neonatal fractional growth rate (pc: $r=0.44$, $p=0.003$, $r=0.44$, $p=0.003$ and $r=0.30$, $p=0.036$, respectively).

3.3.8 Relationship of adult organ and gland weights to size at birth and neonatal growth rate in the aged guinea pig

3.3.8.1 *Size at birth*

In all male and female offspring combined, adult absolute and relative pancreas weights correlated positively with abdominal circumference at birth ($r=0.39$, $p<0.001$ and $r=0.39$, $p<0.001$, respectively, $n=76$) (Table 3.7), while adult relative adrenal weight correlated negatively with birth weight and the birth weight to length ratio ($r=-0.24$, $p=0.015$, $n=79$ and $r=-0.20$, $p=0.039$, $n=76$, respectively) (Table 3.7). Adult absolute and relative heart and left ventricle weights correlated negatively with head length at birth ($r=-0.31$, $p=0.003$, $r=-0.40$, $p<0.001$, $r=-0.27$, $p=0.008$ and $r=-0.36$, $p=0.001$, respectively, $n=76$) and head width at birth ($r=-0.26$, $p=0.011$, $r=-0.31$, $p=0.003$, $r=-0.33$, $p=0.002$ and $r=-0.37$, $p=0.001$, respectively, $n=76$) (Table 3.7), in all male and female offspring combined.

Table 3.7 Relationship of organ and gland weights to size at birth and neonatal growth rate in the aged guinea pig

	Correlation coefficients (r)									
	BWT	BNRL	BWT:BNRL	BPI	BAC	BHL	BHW	BHW:BAC	AGR_{10-30}	FGR_{10-30}
Pancreas (g)	0.13 (79)	0.10 (76)	0.12 (76)	0.06 (76)	0.39 (76)**	-0.08 (76)	-0.02 (76)	-0.18 (76)	0.17 (79)	0.11 (79)
(% body weight)	0.11 (79)	0.03 (76)	0.11 (76)	0.04 (76)	0.39 (76)**	-0.15 (76)	-0.05 (76)	-0.16 (76)	-0.04 (79)	0.10 (79)
Liver (g)	0.17 (79)	0.09 (76)	0.17 (76)	0.08 (76)	0.02 (76)	-0.01 (76)	0.03 (76)	0.02 (76)	0.19 (79)	-0.19 (79)
(% body weight)	-0.01 (79)	-0.03 (76)	0.02 (76)	0.05 (76)	-0.07 (76)	-0.01 (76)	0.03 (76)	0.06 (76)	-0.16 (79)	-0.25 (79)*
Adrenals (g)	-0.02 (79)	-0.05 (76)	0.00 (76)	0.04 (76)	0.05 (76)	-0.15 (76)	-0.05 (76)	-0.08 (76)	0.14 (79)	0.17 (79)
(% body weight)	-0.24 (79)*	-0.17 (76)	-0.20 (76)*	0.04 (76)	-0.05 (76)	-0.16 (76)	-0.04 (76)	-0.03 (76)	0.07 (79)	0.26 (79)*
Kidneys (g)	0.19 (79)	0.11 (76)	0.17 (76)	0.07 (76)	0.18 (76)	0.15 (76)	0.10 (76)	-0.08 (76)	0.21 (79)	0.09 (79)
(% body weight)	-0.04 (79)	-0.04 (76)	-0.02 (76)	0.02 (76)	0.06 (76)	0.12 (76)	0.12 (76)	-0.01 (76)	0.18 (79)	0.12 (79)
Heart (g)	0.05 (79)	0.05 (76)	0.02 (76)	-0.05 (76)	0.14 (76)	-0.31 (76)**	-0.26 (76)*	-0.10 (76)	0.13 (79)	0.10 (79)
(% body weight)	-0.15 (79)	-0.08 (76)	-0.17 (76)	-0.07 (76)	0.18 (76)	-0.40 (76)**	-0.31 (76)**	-0.16 (76)	-0.14 (79)	0.08 (79)
Left Ventricle (g)	0.08 (79)	0.03 (76)	0.08 (76)	0.03 (76)	0.19 (76)	-0.27 (76)**	-0.33 (76)**	-0.19 (76)	0.09 (79)	0.15 (79)
(% body weight)	-0.07 (79)	-0.06 (76)	-0.07 (76)	0.00 (76)	0.10 (76)	-0.36 (76)**	-0.37 (76)**	-0.20 (76)	0.04 (79)	0.13 (79)
Right Ventricle (g)	-0.11 (79)	-0.10 (76)	-0.10 (76)	-0.01 (76)	0.16 (76)	-0.20 (76)	-0.18 (76)	-0.13 (76)	0.06 (79)	0.14 (79)
(% body weight)	-0.18 (79)	-0.17 (76)	-0.19 (76)	-0.01 (76)	0.18 (76)	-0.16 (76)	-0.10 (76)	-0.10 (76)	-0.11 (79)	0.13 (79)
Brain (g)	0.12 (69)	0.19 (69)	0.18 (69)	-0.19 (69)	-0.20 (69)	0.14 (69)	0.15 (69)	0.12 (69)	0.19 (69)	-0.08 (69)
(% body weight)	-0.15 (69)	-0.10 (69)	-0.13 (69)	-0.00 (69)	-0.17 (69)	0.17 (69)	0.13 (69)	0.11 (69)	-0.15 (69)	-0.04 (69)

r represents the correlation coefficient between each variable of adult organ and gland weights and each variable of size at birth and neonatal growth. Numbers in parentheses represent the number of animals. Partial correlations: all significant correlations highlighted in grey were independent of FGR_{10-30}. Statistical significance of correlation coefficients: $^{*}P < 0.05$ $^{**}P < 0.01$. BW, birth weight (g); BNRL, birth nose to rump length (mm); BW:BNRL, birth weight to length ratio (g mm^{-1}); BPI, birth ponderal index (g mm^{-3}); BAC, birth abdominal circumference (mm); BHL, birth head length (mm); BHW, birth head width (mm); BHW:BAC, birth head width to abdominal circumference ratio; AGR_{10-30}, absolute growth rate (10-30 days) (g day^{-1}); FGR_{10-30}, fractional growth rate (10-30 days) (g day^{-1} g^{-1}).

In male offspring, adult relative pancreas weight correlated positively with abdominal circumference at birth (r=0.41, p=0.005, n=39) (Figure 3.15), while adult absolute and relative heart and left ventricle weights correlated negatively with head length at birth (r=-0.43, p=0.003, r=-0.36, p=0.011, r=-0.36, p=0.012 and r=-0.32, p=0.023, respectively, n=39) (Figures 3.16a, b, c and d).

In female offspring, adult relative pancreas weight correlated positively with abdominal circumference at birth (r=0.38, p=0.010, n=37) (Figure 3.15), adult relative adrenal weight correlated negatively with birth weight (r=-0.38, p=0.010, n=38) (Figure 3.17) and adult relative heart and left ventricle weights correlated negatively with head length at birth (r=-0.43, p=0.004 and r=-0.44, p=0.003, respectively, n=37) (Figures 3.16b and d).

3.3.8.2 Neonatal growth rate

In all male and female offspring combined, adult relative liver weight correlated negatively (r=-0.25, p=0.013, n=79) and adult relative adrenal weight correlated positively (r=0.26, p=0.010, n=79) with the neonatal fractional growth rate (Table 3.7). In male offspring, adult relative heart and left ventricle weights correlated positively with the neonatal fractional growth rate (r=0.29, p=0.035 and r=0.40, p=0.005, respectively, n=41) (Figures 3.18a and b). In female offspring, adult relative liver weight correlated negatively with the neonatal fractional growth rate (r=-0.30, p=0.035, n=38) (Figure 3.19).

3.3.8.3 Partial correlations

The positive associations between adult absolute and relative pancreas weights and abdominal circumference at birth in all offspring were independent of the neonatal fractional growth rate (pc: r=0.42, p<0.001 and r=0.41, p<0.001, respectively), however the negative associations between adult relative adrenal weight and birth weight and the birth weight to length ratio, were not. In all male and female offspring combined, the negative associations between adult absolute and relative heart and left ventricle weights and head length at birth were independent of the neonatal fractional growth rate (pc: r=-0.30, p=0.005, r=-0.39, p<0.001, r=-0.25, p=0.014 and r=-0.34, p=0.001, respectively), as were the negative associations between adult absolute and relative heart and left ventricle weights and head width at

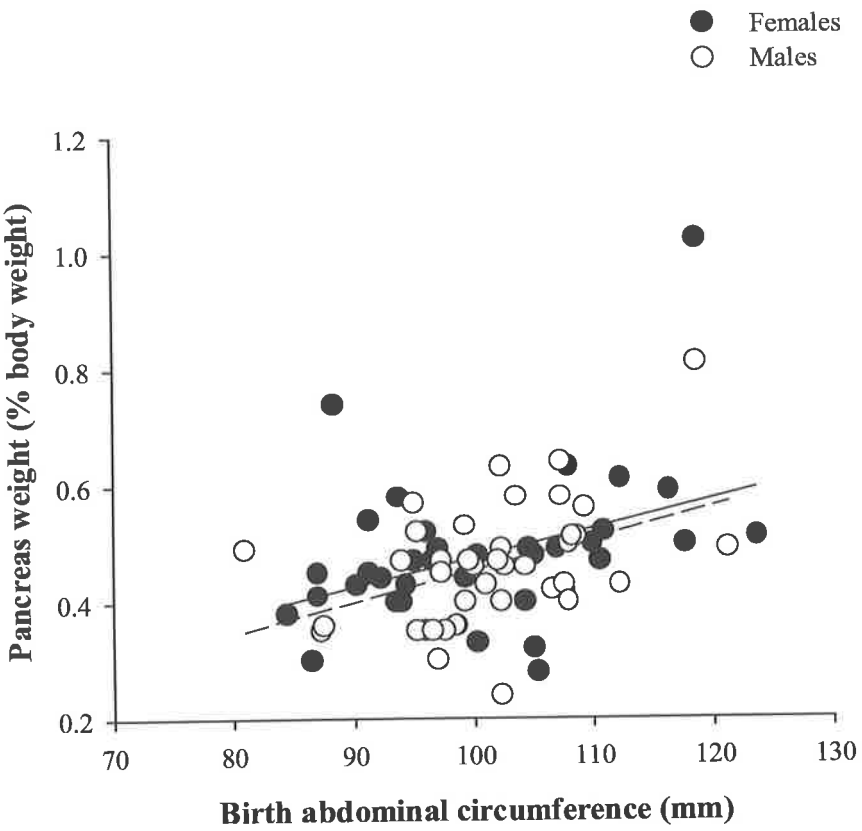

Figure 3.15 The relationship of relative pancreas weight to abdominal circumference at birth in the aged guinea pig

Relationship of abdominal circumference at birth to adult relative pancreas weight (females: $r=0.38$, $n=37$, $p<0.02$; males: $r=0.41$, $n=39$, $p<0.006$) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

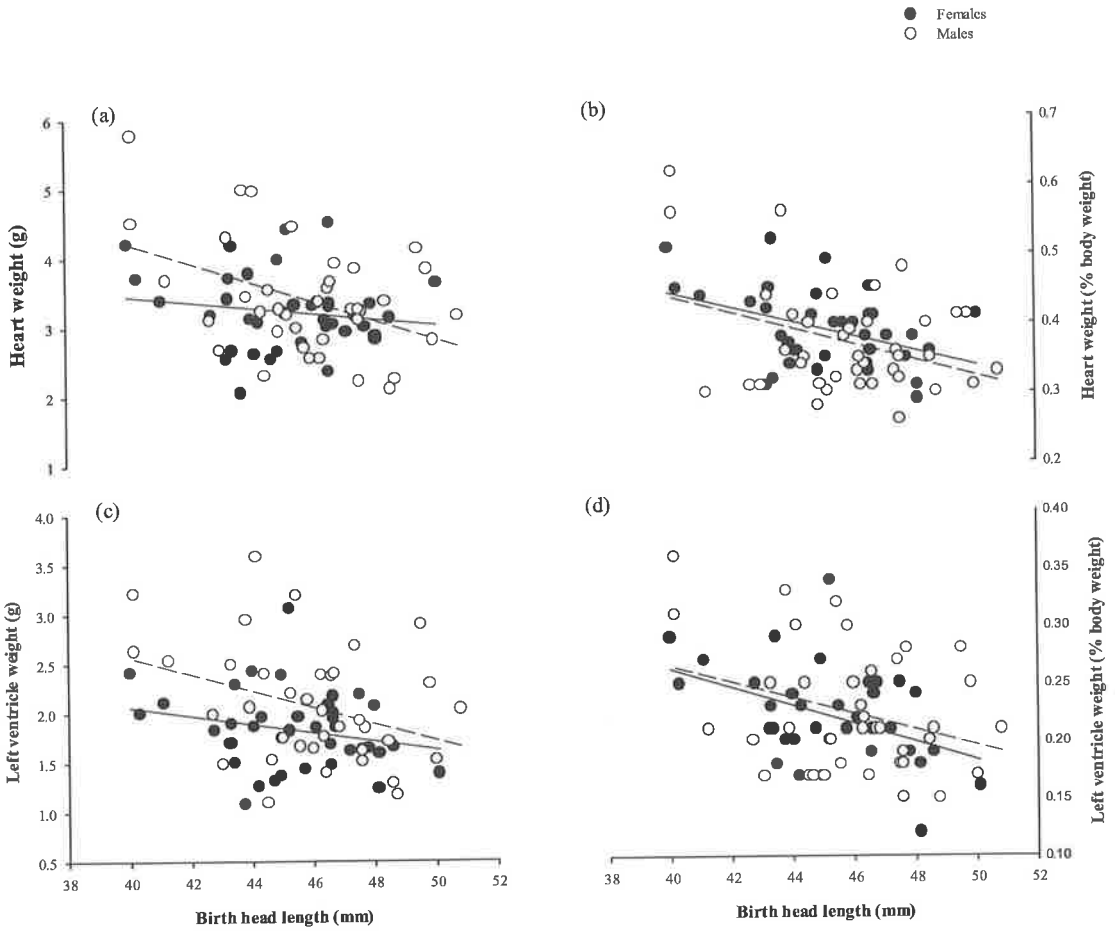

Figure 3.16 The relationship of absolute and relative heart and left ventricle weight to head length at birth in the aged guinea pig

Relationship of head length at birth to adult (a) absolute heart weight (females: r=-0.17, n=37, ns; males: r=-0.43, n=39, p<0.004) (b) relative heart weight (females: r=-0.43, n=37, p<0.005; males: r=-0.36, n=39, p<0.02) (c) absolute left ventricle weight (females: r=-0.25, n=37, ns; males: r=-0.36, n=39, p<0.02) and (d) relative left ventricle weight (females: r=-0.44, n=37, p<0.004; males: r=-0.32, n=39, p<0.03) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

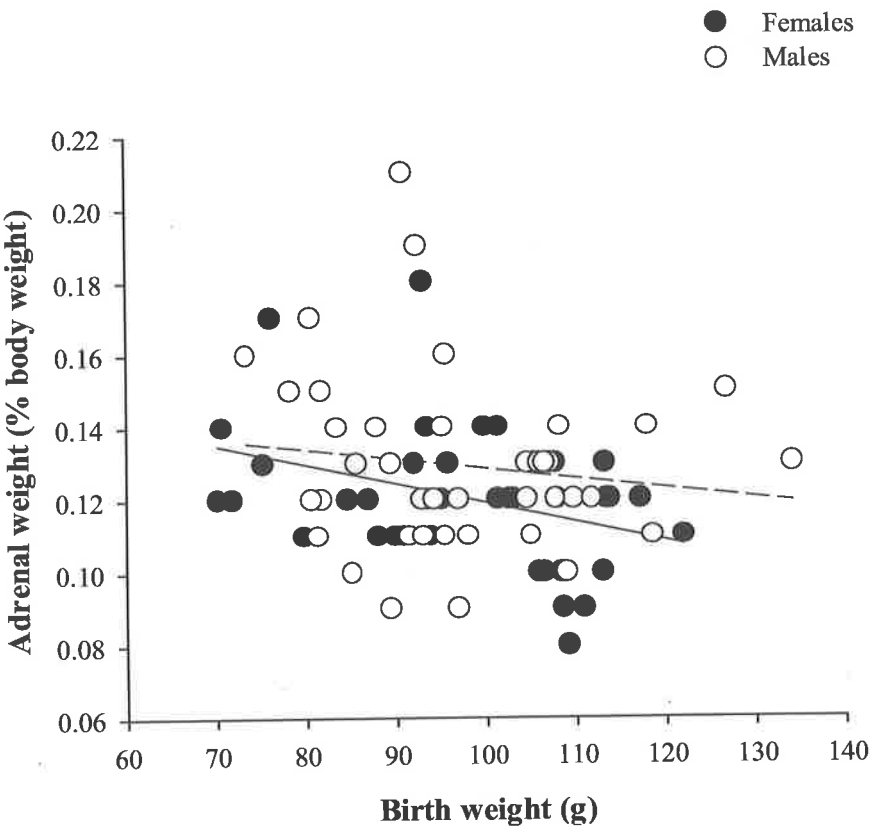

Figure 3.17 The relationship of relative adrenal weight to birth weight in the aged guinea pig

Relationship of birth weight to adult relative adrenal weight (females: r=-0.38, n=38, p<0.02; males: r=-0.15, n=41, ns) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

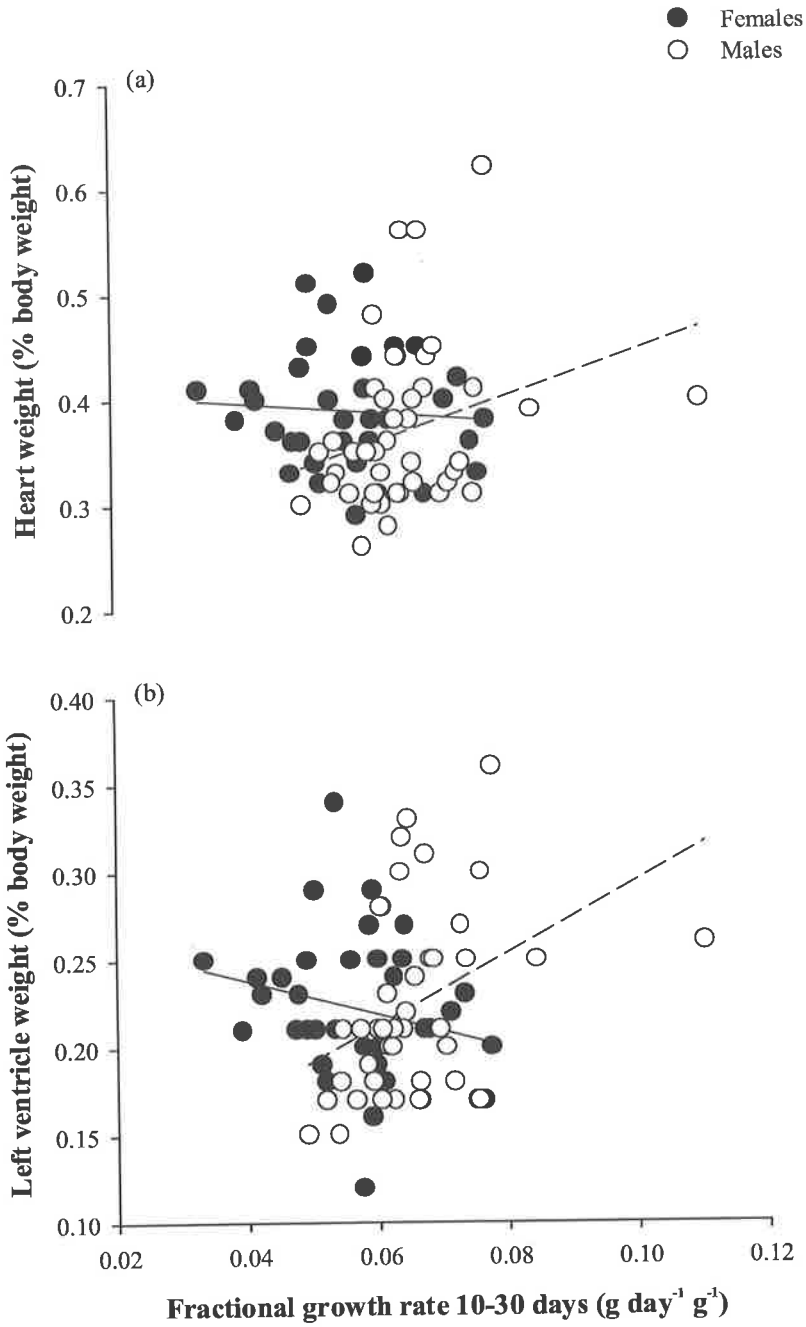

Figure 3.18 The relationship of relative heart and left ventricle weight to neonatal fractional growth rate in the aged guinea pig

Relationship of neonatal fractional growth rate to relative (a) heart (females: r=-0.08, n=38, ns; males: r=0.29, n=41, p<0.04) and (b) left ventricle (females: r=-0.24, n=38, ns; males: r=0.40, n=41, p<0.006) weights in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

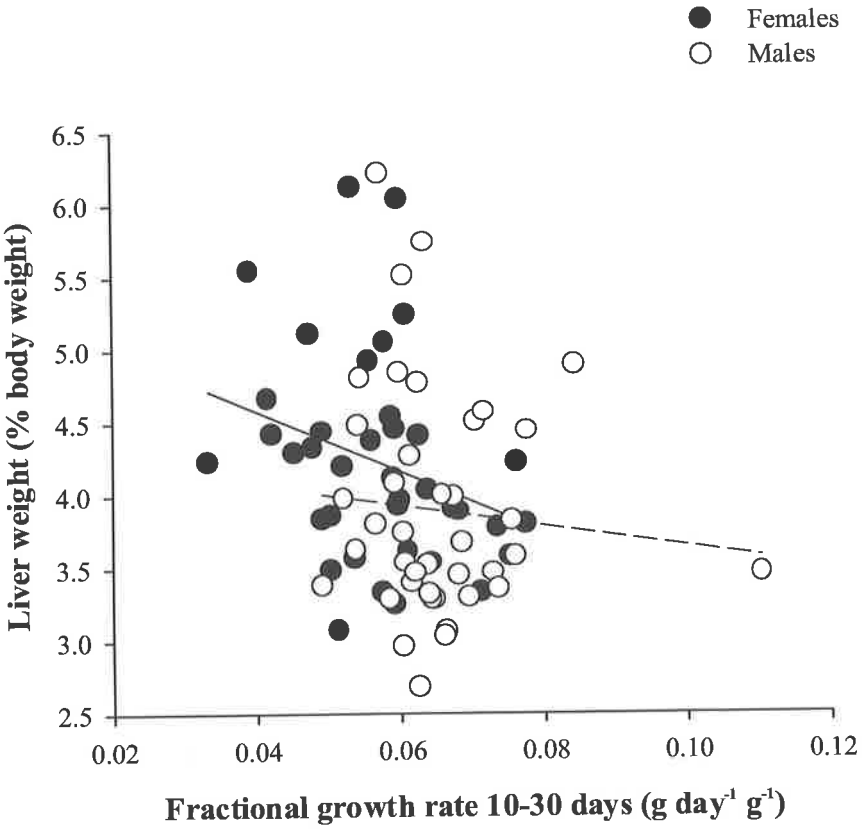

Figure 3.19 The relationship of relative liver weight to neonatal fractional growth rate in the aged guinea pig

Relationship of neonatal fractional growth rate to adult relative liver weight (females: r=-0.30, n=38, p<0.04; males: r=-0.09, n=41, ns) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

birth (pc: r=-0.25, p=0.014, r=-0.30, p=0.004, r=-0.32, p=0.003 and r=-0.36, p=0.001, respectively).

In male offspring, the positive association between adult relative pancreas weight and abdominal circumference at birth was independent of the neonatal fractional growth rate (pc: r=0.39, p=0.007), as were the negative associations between adult absolute and relative heart weights and relative left ventricle weights and head length at birth (pc: r=-0.39, p=0.008, r=-0.30, p=0.032 and r=-0.29, p=0.039, respectively), however the negative association between adult absolute left ventricle weight and head length at birth was not independent of the neonatal fractional growth rate.

In female offspring, the positive association between adult relative pancreas weight and abdominal circumference at birth was independent of the neonatal fractional growth rate (pc: r=0.43, p=0.004), as were the negative associations between adult relative adrenal weight and birth weight (pc: r=-0.37, p=0.013) and adult relative heart and left ventricle weights and head length at birth (pc: r=-0.46, p=0.002 and r=-0.51, p=0.001, respectively).

The negative association between adult relative liver weight and the neonatal fractional growth rate in all offspring was independent of birth weight (pc: r=-0.27, p=0.008), however the positive association between adult relative adrenal weight and the neonatal fractional growth rate was not. In male offspring, the positive association between adult relative left ventricle weight and the neonatal fractional growth rate was independent of birth weight (pc: r=0.39, p=0.007), however the positive association between adult relative adrenal weight and the neonatal fractional growth rate was not. In female offspring, the negative association between adult relative liver weight and the neonatal fractional growth rate was independent of birth weight (pc: r=-0.39, p=0.009).

3.4 DISCUSSION

The current study has demonstrated that in the guinea pig, spontaneous fetal growth restriction occurs due to natural variations in litter size and reduces the nose to rump length, weight to length ratio, abdominal circumference and head length and width of offspring at birth. In addition, compared to their high birth weight counterparts, low birth weight animals display increased fractional growth rates for weight, but decreased absolute growth rates for weight, during the neonatal period. Therefore, the newborn guinea pig that is exposed to growth restriction *in utero* resembles the human IUGR infant, which is generally characterised by a reduced weight, length or increased thinness for gestational age (Robinson *et al.*, 1994). Furthermore, like the human (Karlberg *et al.*, 1997) and other mammalian species, including the sheep (De Blasio *et al.*, 2006) and pig (Poore *et al.*, 2002), spontaneous fetal growth restriction in the guinea pig is followed by catch-up growth in the first month of life.

Small size at birth was predictive of reduced adult weight, length, and pancreas weight and increased adrenal, heart and left ventricle weight in the aged female guinea pig. In aged male guinea pigs, small size at birth was also predictive of reduced weight, length, and pancreas weight and increased heart and left ventricle weight and of increased combined, visceral, and subcutaneous adiposity. These effects of size at birth on body size and composition in aged male and female guinea pigs were largely independent of the influence of neonatal growth.

We have shown that in the guinea pig, both male and female offspring that are small in size at birth remain shorter and lighter as aged adults. These findings are consistent with epidemiological studies where low birth weight was positively associated with later body weight, height and to a lesser degree BMI, in children (Malina *et al.*, 1996; Binkin *et al.*, 1988), adolescents (Seidman *et al.*, 1991; Barker *et al.*, 1997; Paz *et al.*, 1993; Pharoah *et al.*, 1998), and adults (Curhan *et al.*, 1996; Allison *et al.*, 1995; Leon *et al.*, 1996; Sorensen *et al.*, 1999). Similarly, the results from studies in other non-human species also suggest that adult size is influenced by events in prenatal life. In the rat, following moderate maternal feed restriction during pregnancy alone (Holemans *et al.*, 1997; Woodall *et al.*, 1999), or during both pregnancy and lactation (Holemans *et al.*, 1997), adult weights were lower in the offspring of restricted dams compared to controls.

It is important to note that the definition of fetal growth restriction used in this study, namely a birth weight below the median, differs from clinical IUGR and SGA, and in fact variations in fetal growth and the programming phenomenon are typically on a continuum.

The mechanisms underlying the association between a suboptimal intrauterine environment and alterations in adult body composition remain unclear, however several putative mechanisms have been suggested. Undernutrition, as a consequence of a restricted supply of essential maternal substrates, particularly in the last trimester, a period which is characterised by marked fetal weight gain, may result in a slowing of both the metabolic rate and intrauterine growth of the fetus, which in turn increases the likelihood of fetal survival (Barker 1998). The growth restricted fetus may reduce its metabolic dependence on glucose and increase oxidation of other substrates, including amino acids, resulting in increased protein breakdown and decreased protein accretion. This may result not only in a reduced muscle mass, but may also affect muscle structure and impair insulin-regulated glucose metabolism. Alternatively, substrate deprivation may cause abnormalities intrinsic to the adipocyte, leading to an increased accumulation of fat (Proietto *et al.*, 1994).

Low birth weight has been associated with an increase in central or truncal fat distribution in both children and adults in a number of populations (Barker *et al.*, 1997; Law *et al.*, 1992; Rogers 2003; Valdez *et al.*, 1994; Gale *et al.*, 2001). In addition, individuals who were small in size at birth demonstrated this increase in adiposity as adolescents and adults, despite their lower BMI (Fall *et al.*, 1995; Law *et al.*, 1992; Loos *et al.*, 2001; Loos *et al.*, 2002; Malina *et al.*, 1996; Okosun *et al.*, 2000; Singhal *et al.*, 2003). Similarly, in the current study, spontaneous fetal growth restriction, as indicated by a reduced head width at birth, was associated with an increase in relative combined, visceral and subcutaneous adiposity in aged male guinea pigs, despite their reduced size in terms of weight and length.

In addition to possible changes in intrinsic cell or fibre characteristics, a number of other factors which normally contribute to the development of postnatal obesity, including physical inactivity and an increased energy intake, may also be programmed by events in early life. In the rat severe maternal undernutrition (30% of *ad libitum* intake) throughout pregnancy and a hypercaloric (30% fat) postnatal

diet, increased the relative retroperitoneal fat mass in adult offspring, despite a reduction in body weight (Vickers *et al.*, 2000). In addition, the offspring of undernourished rats demonstrated an increased food intake in early postnatal life, which was amplified with increasing age and by hypercaloric nutrition (Vickers *et al.*, 2000). These animals have subsequently been shown to be less active compared to control offspring, independent of postnatal nutrition, however sedentary behaviour in undernourished offspring was exacerbated by a hypercaloric postnatal diet (Vickers *et al.*, 2003). Further to this, the prenatal influence on locomotor activity may be permanent, as undernourished offspring were still less active compared with control animals as mature adults, even in the presence of a healthy diet throughout postnatal life (Vickers *et al.*, 2003).

In the guinea pig, spontaneous fetal growth restriction, as indicated by a reduced weight to length ratio at birth, has been associated with an increased relative food intake in young adult male offspring (DM Horton *et al.*, unpublished observations). However, despite these alterations in appetite, male offspring who were exposed to fetal growth restriction did not display increased adiposity as young adults (DM Horton *et al.*, unpublished observations), in contrast to offspring subjected to maternal feed restriction and probably greater challenges *in utero* (Kind *et al.*, 2003). This could also have been due to the relatively low fat content of their diet (3.3% fat), which may not have been enough to challenge these growth restricted animals, in spite of their observed hyperphagia. The observed subsequent increase in adiposity in the aged male guinea pigs described here may be due to a number of factors, including long-term increased food and caloric intake, as well as a reduction in locomotor activity.

The increase in relative adiposity in people who were small in size at birth may also be accompanied by deficits in lean body mass, with a number of studies reporting a direct association between relative muscle mass and size at birth (Gale *et al.*, 2001; Li *et al.*, 2003; Rogers 2003; Singhal *et al.*, 2003). Children born SGA have been shown to remain, as a group, slightly smaller than AGA peers, with their deficiency in size related largely to a reduced lean tissue mass (Hediger *et al.*, 1998). Similarly, studies in non-human species have shown that growth restriction *in utero* is associated with a reduction in skeletal muscle mass postnatally (Desai *et al.*, 1996: Greenwood *et al.*, 1998).

In the present study, spontaneous fetal growth restriction did not alter muscle mass in aged guinea pigs, however when animals were classed according to their neonatal fractional growth rate, fast growing male offspring demonstrated a reduced adult combined and mixed muscle mass, when compared to their slow growing counterparts. Similar observations in this species have been reported in young adult males, where an accelerated neonatal fractional growth rate for weight was associated with reduced muscle mass in adult life (DM Horton *et al.*, unpublished observations).

This study has demonstrated that in aged female guinea pigs, spontaneous fetal growth restriction, as indicated by a low birth weight, is associated with increased adrenal size, which may reflect an increased sensitivity of this gland to the trophic actions of adrenocorticotropic hormone (ACTH). Interestingly, the findings from other studies in the same species (Liu *et al.*, 2001) suggest that perturbed intrauterine growth programmes a sex-specific increase in the activity of, and responsiveness to the HPAA in adult offspring, with females demonstrating a greater susceptibility to programming of the HPAA than males.

Epidemiological studies have shown that suboptimal intrauterine growth is associated with raised cortisol concentrations in infants (Goland *et al.*, 1993), children (Clark *et al.*, 1996) and adults (Phillips *et al.*, 1998; Levitt *et al.*, 2000; Phillips *et al.*, 2000). Babies born SGA demonstrated raised cortisol concentrations in umbilical cord blood (Goland *et al.*, 1993) and raised urinary cortisol excretion in childhood (Clark *et al.*, 1996). A UK study of men aged 64 years reported that low birth weight was associated with increased 0900h plasma cortisol concentrations (Phillips *et al.*, 1998), and this finding has since been reported in 20 year old men and women in Australia, as well as in 50 year old men and women who were born in Preston in the UK (Phillips *et al.*, 2000). A study of young adults in South Africa has shown that individuals who were born SGA demonstrated both an increased area under the cortisol curve and 0900h plasma cortisol concentrations compared to their AGA peers (Levitt *et al.*, 2000). It has been suggested that the relationship between small size at birth and elevated fasting cortisol levels may represent a stress response, due to the combination of fasting and the novel clinic setting in which the blood samples were taken (Phillips *et al.*, 2006). More recently, sex differences in the HPAA response to acute psychological stress have been identified (Jones *et al.*,

2006). In a study of boys and girls aged between 7 and 9 years, birth weight was inversely related to salivary cortisol responses to psychological stress in boys, while in girls birth weight was inversely related to morning peak cortisol levels (Jones *et al.*, 2006). Additionally, studies in animals have shown that exposure of pregnant rats to a range of stressors, including low protein diets (Langley-Evans 1997), physical restraint (Barbazanges *et al.*, 1996), maternal infections (Reul *et al.*, 1994) and dexamethasone administration (Levitt *et al.*, 1996), increases basal and stress-induced corticosteroid secretion and glucocorticoid sensitivity of target tissues in adult offspring (Langley-Evans 1997). This increased activity of and sensitivity to the HPAA as a consequence of environmental perturbations in prenatal life can in turn impair adult metabolic (Nyirenda *et al.*, 1998) and cardiovascular function (Levitt *et al.*, 1996). Therefore, in the present study, the increased HPAA activity observed in aged adult female guinea pigs that were small in size at birth could impair their metabolic and cardiovascular function.

In the current study, we have shown that an accelerated neonatal fractional growth rate was associated with reduced liver weight in aged female guinea pigs. The liver plays a critical role in the maintenance of carbohydrate and lipid homeostasis, with many of the enzymes involved in these processes sectioned or zoned within specific areas of the liver (Lawrence *et al.*, 1986; Wimmer *et al.*, 1990; Jungermann *et al.*, 1989). The changes in hepatic size observed in the present study following perturbed postnatal growth in the guinea pig, could have consequences for glucose and lipid metabolism.

The endocrine pancreas also plays a major role in the regulation of glucose metabolism, and a number of studies in the rat have shown that fetal malnutrition is associated with a permanent impairment of pancreatic β cell development and function (Dahri *et al.*, 1991; Garofano *et al.*, 1997). As mentioned earlier, in the present study we have shown that small size at birth was associated with reduced relative size of the pancreas in the aged male and female guinea pig. Whether this is accompanied by a similar reduction in the size of the endocrine pancreas and β cell mass and other endocrine cells is unknown however. In addition, when offspring were classed according to their neonatal fractional growth rate, slow growing male offspring demonstrated a reduced relative pancreas weight as aged adults. These alterations in pancreatic size following perturbed perinatal growth in the guinea pig

could have long-term adverse consequences for insulin-regulated glucose homeostasis.

As previously mentioned, in the current study small size at birth was associated with increased heart and left ventricle weight in the aged male and female guinea pig. Additionally, an accelerated neonatal fractional growth rate predicted an increased heart and left and right ventricle weight in the aged male guinea pig, and a reduced left ventricle weight in the aged female. Alterations in the development of key organs involved in the control of cardiovascular function, including the heart, have been suggested as potential mechanisms through which perturbed prenatal and early postnatal growth may programme blood pressure in adult life. The changes in heart and left and right ventricle size observed in the present study following perturbed postnatal growth in the guinea pig, could exert negative long-term effects on basal cardiovascular function.

In summary, spontaneous fetal growth restriction in the guinea pig reduced size at birth and increased the neonatal fractional growth rate for weight in male and female offspring. Small size at birth predicted reduced adult weight and length and increased adult adrenal weight in female guinea pigs. In males, small size at birth was associated with reduced adult weight and length, and increased adiposity in adult life. In addition, male guinea pigs that experienced accelerated growth during the neonatal period demonstrated reduced adult muscle mass when compared to their slow growing counterparts. This study has also demonstrated that perturbed fetal and early postnatal growth alters the long-term size of several other key organs involved in neuroendocrine, endocrine and cardiovascular function, and the putative mechanisms explaining these relationships are discussed in subsequent chapters. Therefore alterations in adult size and body composition occur following perturbed growth in early life and may explain at least in part, perinatal programming of metabolic and cardiovascular dysfunction in the aged guinea pig.

CHAPTER 4

EARLY LIFE INFLUENCES ON WHOLE BODY INSULIN SENSITIVITY AND LIPID PROFILE IN THE AGED GUINEA PIG

4.1 INTRODUCTION

Studies in human populations have demonstrated that suboptimal growth *in utero*, as indicated by a low weight or thinness at birth, is associated with insulin resistance in adult life (Lithell *et al.*, 1996; Phillips *et al.*, 1994; McKeigue *et al.*, 1998; Leger *et al.*, 1997; Flanagan *et al.*, 2000; Jacquet *et al.*, 2000). In addition, indirect measures of skeletal and non-skeletal catch-up growth during childhood have been associated with an increased risk of adult insulin resistance (Eriksson *et al.*, 2002). Although increased adiposity and plasma non-esterified free fatty acid concentrations impair insulin sensitivity, whether they mediate part of the influence of a poor prenatal environment on insulin sensitivity has been little examined. In one study, no relationship was observed between size at birth and adult fasting plasma non-esterified free fatty acid levels (Phillips *et al.*, 1995).

The impact of poor fetal growth on circulating concentrations of other lipids in adult life is also unclear. Insulin resistance and its associated hyperinsulinaemia can contribute to elevated circulating triglycerides and cholesterol (Reaven 1998). Prenatally induced insulin resistance might be expected to contribute to such changes in adult life. Small size at birth in humans has been associated with either an increase (Barker *et al.*, 1993; Fall *et al.*, 1995; Moore *et al.*, 1997) or no change (Clausen *et al.*, 1997; Kolacek *et al.*, 1993) in adult serum levels of total, LDL and HDL-cholesterol however. Another study demonstrated no relationship between size at birth and adult fasting plasma triglyceride concentrations (Phillips *et al.*, 1995). More recently, a life course study has demonstrated that the relative contribution of early life influences, including a low birth weight, to the risk of developing the IRS, including dyslipidaemia (hypertriglyceridaemia and low HDL-cholesterol), in adulthood, is in fact similar to that of genetic and adult lifestyle factors (Parker *et al.*, 2003).

Experimental studies in rats have demonstrated that placental insufficiency (Simmons *et al.*, 2001) and maternal feed (Holemans *et al.*, 1996; Vickers *et al.*, 2000) or protein restriction (Ozanne *et al.*, 1997) are associated with impaired insulin sensitivity in adult offspring. In pigs, catch up growth during the first month of life has also been associated with impaired insulin action in adulthood (Poore *et al.*, 2004). The effects of perturbed growth in early life on adult lipid metabolism have not been examined as extensively in non-human species. In the rat however, maternal protein restriction throughout pregnancy and lactation has been shown to reduce plasma triglyceride and total and HDL-cholesterol levels in adult offspring (Lucas *et al.*, 1996), while placental insufficiency, as a result of BUAL during late gestation, increased adult plasma triglyceride levels (Lane *et al.*, 2001).

Previous studies in our laboratory have demonstrated that in the guinea pig, spontaneous fetal growth restriction and accelerated neonatal growth are associated with impaired whole body insulin sensitivity of glucose metabolism, but unaltered fasting plasma free fatty acid concentrations, in young adult male and female offspring (DM Horton *et al.*, unpublished observations). Further to this, young adult male guinea pigs of low birth weight demonstrated higher fasting plasma total and LDL cholesterol concentrations when compared to their high birth weight counterparts, in response to a 6-week cholesterol supplemented diet (Kind *et al.*, 1999). However few experimental studies have examined the effects of perturbed perinatal growth on postnatal insulin action and lipid metabolism in the aged adult.

The aims of the study described in Chapter 4 were therefore to determine the effects of (1) spontaneous fetal growth restriction, due to natural variations in litter size, and (2) accelerated neonatal growth, on whole body insulin sensitivity of glucose metabolism and fasting plasma concentrations of total cholesterol, triglycerides and free fatty acids, in the aged guinea pig. The influence of sex on the impact of altered perinatal growth on whole body insulin sensitivity and circulating lipid concentrations in the aged adult was also assessed, as were the relationships between adult size and body composition and whole body insulin sensitivity and circulating lipid profile. We hypothesised that spontaneous fetal growth restriction and accelerated neonatal fractional growth rate for weight in the guinea pig will decrease whole body insulin sensitivity of glucose metabolism and increase fasting plasma

free fatty acid, triglyceride and total cholesterol concentrations, in aged male and female guinea pigs.

4.2 MATERIALS AND METHODS

4.2.1 Animals

Nulliparous, 3 to 4 month old female guinea pigs (IMVS Tri-coloured) were obtained from the Gilles Plains Animal Resource Centre (Gilles Plains, SA, Australia), and following a two-week acclimatisation period, females in oestrous were pair mated with male guinea pigs (IMVS Tri-coloured) overnight (as described in Section 2.2.1). At 60 days gestation, pregnant animals were transferred to plastic tubs containing paper bedding, where they gave birth. Offspring were weighed at birth, and the nose to rump length, abdominal circumference, and head length and head width of each pup was measured. A total of 31 pups born to 19 mothers were randomly assigned to this study of aged guinea pigs (17 male and 14 female), with the remaining offspring allocated to other studies (as described in Section 2.2.1). A description of the total number of litters used and their size is summarised in Appendix A. Of the 31 pups assigned to this study, 21 had at least one other litter mate in the study while 10 did not. Fasting plasma free fatty acid, triglyceride and total cholesterol concentrations obtained from an additional 25 animals prior to the IVGTT (described in Chapter 5) were included in the analyses. The size at birth and neonatal growth rate characteristics of these additional animals were largely similar to those of animals assigned to this study. All pups were weighed daily from birth to 30 days of age, and the neonatal absolute ($AGR_{10\text{-}30}$) (g day^{-1}) and fractional ($FGR_{10\text{-}30}$) (g day^{-1} g^{-1}) growth rates for weight were calculated from 10 days of age to weaning at 30 days of age (as described in Section 3.2.1), following which pups were transferred to individual wire-bottomed cages and weaned onto normal guinea pig chow *ad libitum*. Postnatal food intakes were not able to be measured for logistic reasons.

At 424 ± 3 days of age catheters were inserted into the right carotid artery and jugular vein under general anaesthesia (as described in Section 2.2.1). Catheter patency was maintained by daily flushing with 800 µl of heparinized saline (250 IU ml^{-1}, Multiparin, Fisons Pharmaceuticals, NSW, Australia). Animals were allowed at least 5 days postoperative recovery before the commencement of *in vivo* studies. It was not possible to obtain all data from all animals due to human resource limitations, and the number of observations for each experimental data set is indicated in each table or figure. Only three of the guinea pigs studied had both a

HEC and IVGTT carried out, so direct within animal comparisons of insulin sensitivity and secretion with glucose homeostasis were not possible. All procedures in this study were reviewed and approved by the University of Adelaide Animal Ethics Committee.

4.2.2 Hyperinsulinaemic euglycaemic clamp

At 431 ± 3 days of age, and following a 16-hour overnight fast, a HEC was performed to determine the insulin sensitivity of net whole-body glucose uptake (as described in Section 2.2.2). The mean basal blood glucose concentration prior to the start of the HEC was 8.51 mmol/l. The mean blood glucose concentration achieved during the last 60 minutes of the HEC was 9.05 mmol/l, with a coefficient of variation of 4.7%. The mean glucose infusion rate during the last 60 minutes of the HEC was 5.17 mg/min/kg, with a coefficient of variation of 26%.

The insulin sensitivity of net whole body glucose uptake was calculated as the SSGIR needed to maintain euglycaemia (averaged across the second hour of the HEC), corrected for the steady state plateau plasma human insulin concentration, and was termed the adjusted SSGIR. The post-hepatic metabolic clearance rate of human insulin (MCR_i) was calculated as the ratio of the exogenous insulin infusion rate to the steady state plateau plasma human insulin concentration.

4.2.3 Plasma metabolite and hormone analyses

Human insulin concentrations in plasma were analysed in duplicate by radioimmunoassay using a commercially available kit in which guinea pig insulin did not cross react (Insulin-CT, CIS bio international, France), with an intra-assay coefficient of variation of 4.5% and an interassay coefficient of variation of 5.2%. Plasma total cholesterol, triglyceride and free fatty acid concentrations were measured in duplicate by enzymatic colorimetric analysis on a COBAS Mira automated centrifugal analyser using commercially available kits and control sera, with intra and interassay coefficients of variation below 5% (as described in Section 2.2.5).

4.2.4 Body composition

At 433 ± 3 days of age, and following a 20 hour-overnight fast, animals were sacrificed by an intravenous overdose of sodium pentobarbitone (Virbac, NSW, Australia), and a post mortem was performed between 1400h and 1600h. Body weight and nose to rump length were measured and the body mass index was calculated (weight/nose to rump length^2). Selected skeletal muscles and adipose depots were dissected out, weighed immediately, summed, and expressed as a percentage of body weight at post mortem, to give an index of the percentage of body weight composed of mixed and type 2 muscle and visceral and subcutaneous adipose tissue (as described in Section 2.2.6). In addition, all individual skeletal muscle and adipose depot weights were summed and expressed as a percentage of body weight at post mortem, to give an index of the percentage of body weight composed of skeletal muscle (combined muscle) and adipose tissue (combined adiposity) respectively. The liver was also dissected out and weighed. All adipose depot, skeletal muscle, and liver weights were expressed in absolute terms as well as in relative terms as a percentage of body weight at post mortem.

4.2.5 Statistical analysis

All statistical analyses were carried out using SPSS for Windows (Version 13.0, SPSS Inc., Chicago, IL, USA). To examine the effect of size at birth on adult whole body insulin sensitivity and circulating lipids, offspring were classed into two groups, those with birth weights greater than (high birth weight) or less than (low birth weight) the median birth weight for the cohort of guinea pigs described in this thesis, which was 95.55 grams. Males and females were also analysed separately, classing each as high or low birth weight, using the median birth weight for each sex (94.8 grams for males and 99.58 grams for females). The definition of fetal growth restriction in experimental studies in non-human species varies, and this approach of below versus above the median size at birth has been commonly used in these studies, as well as in some human studies. The effect of birth weight class on adult whole body insulin sensitivity and circulating lipids was assessed by a single between factor ANOVA in all animals combined, and in males and females separately. The effects of birth weight class, sex and their interaction, on adult whole

body insulin sensitivity and circulating lipids were examined by a two between factor ANOVA. Specific comparisons were carried out by Bonferroni post hoc tests.

To examine the effect of neonatal fractional growth rate on adult whole body insulin sensitivity and circulating lipids, offspring were classed into two groups as either 'high growers' or 'low growers' using the following approach. In order to classify animals according to growth, males and females were separated, and the neonatal fractional growth rate was plotted against birth weight for each sex separately. Animals above and below the regression line were classified as 'high growers' and 'low growers' respectively. The effect of growth rate class on adult whole body insulin sensitivity and circulating lipids was assessed by a single between factor ANOVA in all animals combined, and in males and females separately. A two between factor ANOVA was used to determine the effect of growth rate class, sex and their interaction, on adult whole body insulin sensitivity and circulating lipids. Specific comparisons were carried out by Bonferroni post hoc tests.

Relationships between adult whole body insulin sensitivity and circulating lipids and size at birth, neonatal growth rates and adult size and body composition were examined using simple correlation and multiple linear regression analyses, in all animals combined and in male and female offspring separately. One sided p-values were used to test *a priori* hypotheses regarding the relationships between adult whole body insulin sensitivity and circulating lipids and size at birth and neonatal growth rates, based on similar relationships reported in humans.

For all statistical tests, significance was accepted at $P<0.05$. All data are presented as mean ± S.E.M.

4.3 RESULTS

4.3.1 Effect of birth weight class on adult whole body insulin sensitivity and circulating lipid profile in the aged guinea pig

In all male and female offspring combined, low birth weight increased the steady state plasma human insulin concentration during the HEC (+28%) ($p<0.05$), but did not alter any other parameter of adult whole body insulin sensitivity (Table 4.1). Low birth weight altered a number of parameters of adult whole body insulin sensitivity differently with sex, such that the adjusted steady state glucose infusion rate and metabolic clearance rate of insulin tended to decrease with low birth weight in females, but increased in males ($p<0.03$ for both) (Table 4.1), while the steady state plasma human insulin concentration tended to increase with low birth weight in females, but decreased in males ($p<0.03$) (Table 4.1). In male offspring only, birth weight class did not influence any parameter of adult whole body insulin sensitivity (data shown for the adjusted steady state glucose infusion rate only, Figure 4.1). In female offspring only, the adjusted steady state glucose infusion rate was reduced in low birth weight compared to high birth weight offspring (-51%) ($p<0.01$) (Figure 4.1).

Birth weight class did not alter any parameter of the adult circulating lipid profile in all male and female offspring combined (Table 4.1), or in male or female offspring only (data not shown).

4.3.2 Effect of neonatal fractional growth rate class on whole body insulin sensitivity and circulating lipid profile in the aged guinea pig

In all male and female offspring combined, neonatal fractional growth rate class altered parameters of adult whole body insulin sensitivity, such that fast growing animals demonstrated a higher unadjusted (+31%) ($p<0.02$) and adjusted (+59%) ($p<0.01$) steady state glucose infusion rate than their slow growing counterparts (Table 4.2). In male offspring only, fast growing animals demonstrated a higher unadjusted (+33%) ($p<0.03$) and adjusted (+90%) ($p<0.01$) steady state glucose infusion rate than their slow growing counterparts (Figures 4.2a and b). In female offspring only, neonatal fractional growth rate class did not alter any parameter of

Table 4.1 Effect of birth weight class and sex on whole body insulin sensitivity and circulating lipid profile in the aged guinea pig

	Birth weight class				ANOVA *P*-value		
	Low birth weight		High birth weight		BW	S	BW x S
	Males (*n*=7)	Females (*n*=7)	Males (*n*=10)	Females (*n*=7)			
SSGIR ($mg.min^{-1}.kg^{-1}$)	5.59 ± 0.51	4.47 ± 0.64	5.35 ± 0.60	5.18 ± 0.76	NS	NS	NS
Adjusted SSGIR ($mg.min^{-1}.kg^{-1}$ $\mu U.ml^{-1}$)	0.028 ± 0.006	0.017 ± 0.002	0.026 ± 0.005	0.035 ± 0.006	NS	NS	0.025
Steady state plasma human insulin ($\mu U\ ml^{-1}$)	232 ± 31	301 ± 62	245 ± 29	153 ± 14	0.040	NS	0.021
Metabolic clearance rate of insulin ($ml.min^{-1}.kg^{-1}$)	36.9 ± 6.0	32.5 ± 6.5	34.6 ± 4.0	51.1 ± 4.2	NS	NS	0.027
	Males (*n*=13)	Females (*n*=12)	Males (*n*=15)	Females (*n*=17)			
Fasting plasma free fatty acids ($meq\ l^{-1}$)	2.35 ± 0.15	2.49 ± 0.21	2.54 ± 0.23	2.59 ± 0.16	NS	NS	NS
Fasting plasma triglycerides ($mmol\ l^{-1}$)	1.31 ± 0.20	1.45 ± 0.26	1.67 ± 0.41	1.85 ± 0.45	NS	NS	NS
Fasting plasma total cholesterol ($mmol\ l^{-1}$)	1.51 ± 0.17	1.27 ± 0.13	1.55 ± 0.21	1.58 ± 0.13	NS	NS	NS

Data are presented as means ± S.E.M. *n* refers to the number of animals. SSGIR, steady state glucose infusion rate. ANOVA: effect of birth weight class (BW), sex (S) and their interaction (BW x S). Statistical significance was assumed at $P < 0.05$. NS, not significant.

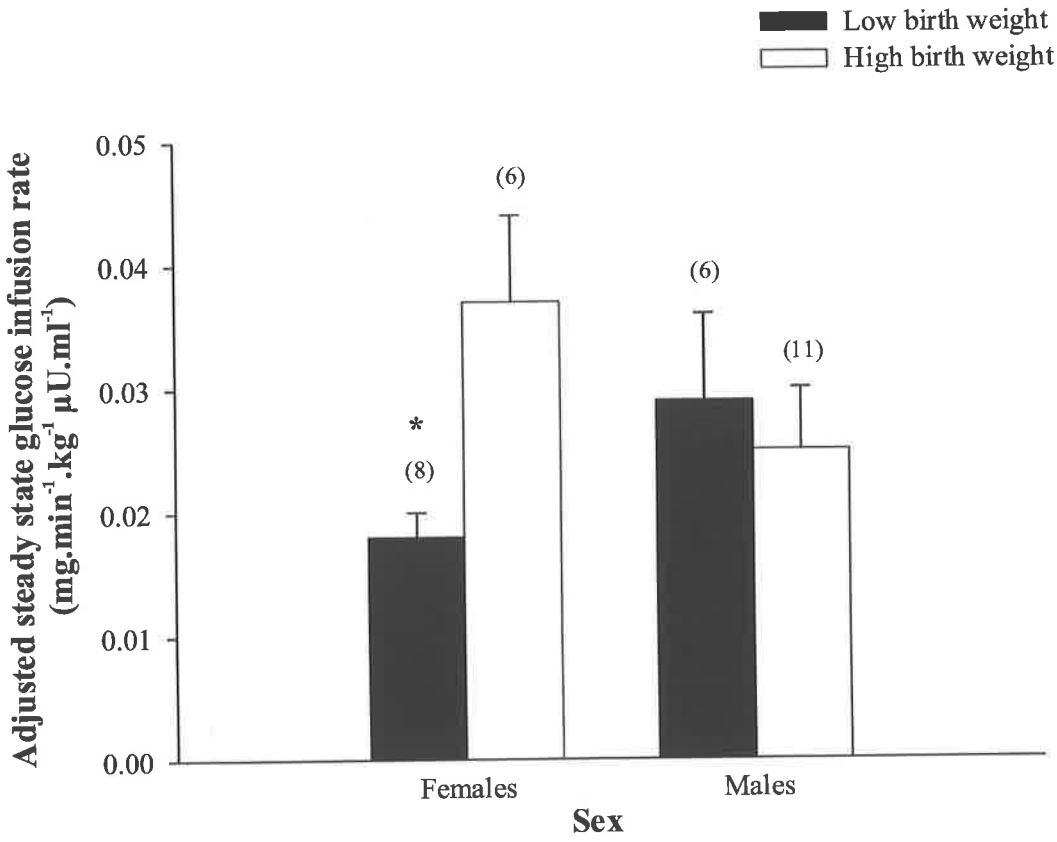

Figure 4.1 The effect of birth weight class and sex on whole body insulin sensitivity of glucose metabolism in the aged guinea pig

The adjusted steady state glucose infusion rate during the HEC in aged adult guinea pigs of low and high birth weight. Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. $*P < 0.05$ compared with high birth weight offspring of the same sex.

Table 4.2 Effect of neonatal fractional growth rate class and sex on whole body insulin sensitivity in the aged guinea pig

	Neonatal fractional growth rate class				ANOVA *P*-value		
	Low growth rate		High growth rate		GR	S	GR x S
	Males (*n*=11)	Females (*n*=9)	Males (*n*=6)	Females (*n*=5)			
SSGIR ($mg.min^{-1}.kg^{-1}$)	4.78 ± 0.49	4.39 ± 0.54	6.50 ± 0.47	5.60 ± 0.93	0.015	NS	NS
Adjusted SSGIR ($mg.min^{-1}.kg^{-1}$ $\mu U.ml^{-1}$)	0.020 ± 0.003	0.023 ± 0.005	0.038 ± 0.007	0.031 ± 0.008	0.012	NS	NS
Steady state plasma human insulin ($\mu U\ ml^{-1}$)	263 ± 25	223 ± 41	197 ± 33	235 ± 78	NS	NS	NS
Metabolic clearance rate of insulin ($ml.min^{-1}.kg^{-1}$)	31.7 ± 3.8	41.1 ± 5.4	42.5 ± 5.6	43.1 ± 9.1	NS	NS	NS
	Males (*n*=13)	Females (*n*=18)	Males (*n*=15)	Females (*n*=11)			
Fasting plasma free fatty acids ($meq\ l^{-1}$)	2.70 ± 0.25	2.68 ± 0.18	2.23 ± 0.13	2.33 ± 0.13	0.015	NS	NS
Fasting plasma triglycerides ($mmol\ l^{-1}$)	1.42 ± 0.20	1.39 ± 0.17	1.57 ± 0.41	2.18 ± 0.68	NS	NS	NS
Fasting plasma total cholesterol ($mmol\ l^{-1}$)	1.50 ± 0.18	1.36 ± 0.11	1.57 ± 0.21	1.59 ± 0.17	NS	NS	NS

Data are presented as means ± S.E.M. *n* refers to the number of animals. SSGIR, steady state glucose infusion rate. ANOVA: effect of neonatal fractional growth rate class (GR), sex (S) and their interaction (GR x S). Statistical significance was assumed at $P < 0.05$. NS, not significant.

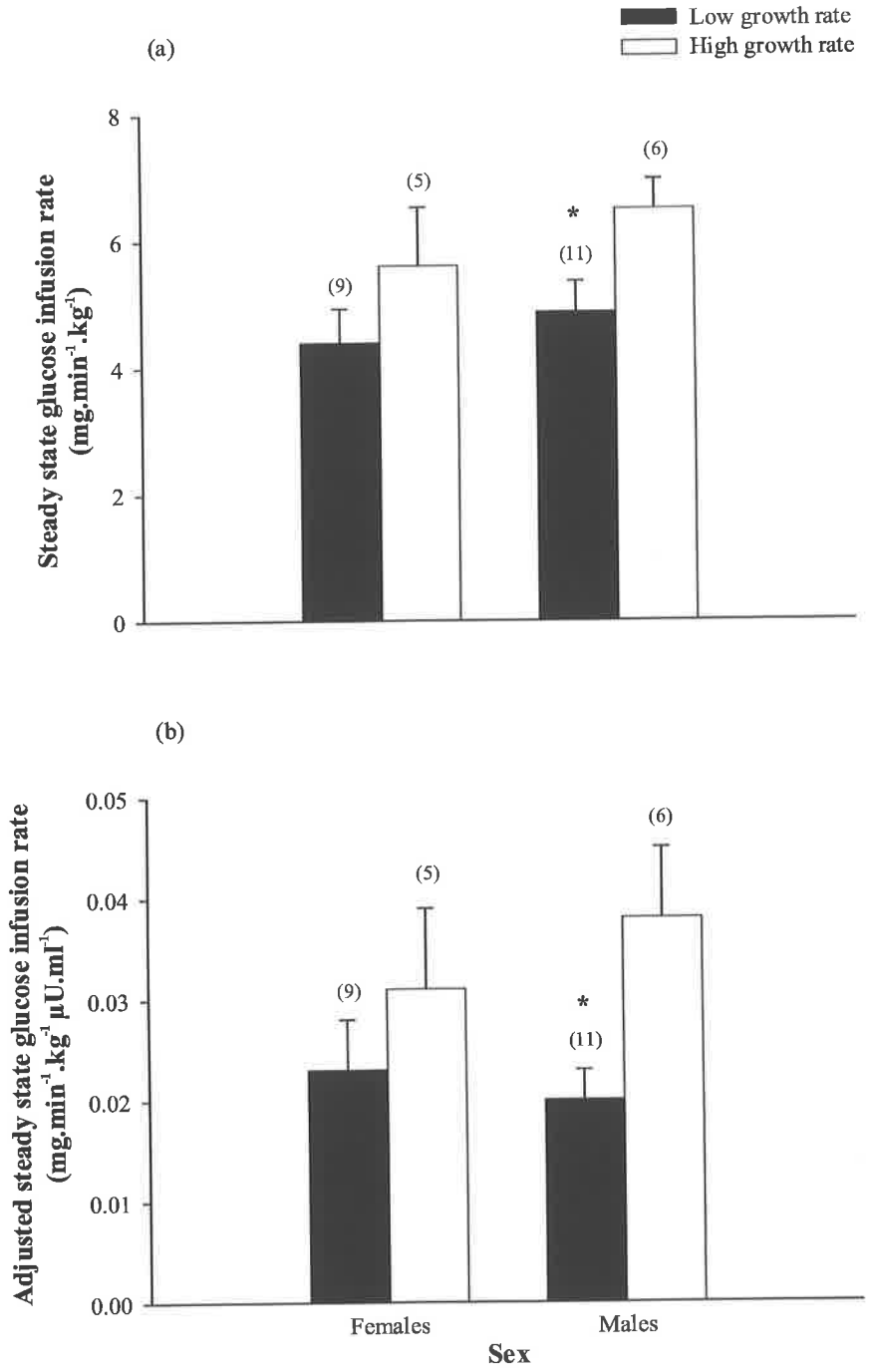

Figure 4.2 The effect of neonatal fractional growth rate class and sex on whole body insulin sensitivity in the aged guinea pig

Unadjusted (a) and adjusted (b) steady state glucose infusion rate during the HEC in aged adult guinea pigs of low and high neonatal fractional growth rate. Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. *$P < 0.05$ compared with high growth rate offspring of the same sex.

adult whole body insulin sensitivity (data shown for unadjusted and adjusted steady state glucose infusion rate only, Figures 4.2a and b). Inclusion of birth weight as a co-variate did not alter these outcomes.

In all male and female offspring combined, fast growing animals demonstrated lower adult fasting plasma free fatty acid concentrations (-16%) ($p<0.02$) than their slow growing counterparts (Table 4.2). In male offspring only, fast growing animals demonstrated lower adult fasting plasma free fatty acid concentrations (-17%) ($p<0.05$) than their slow growing counterparts (Figure 4.3). In female offspring only, neonatal fractional growth rate class did not alter any parameter of the adult circulating lipid profile (data shown for fasting plasma free fatty acids only, Figure 4.3). Inclusion of birth weight as a co-variate did not alter these outcomes.

4.3.3 Relationship of whole body insulin sensitivity and circulating lipid profile to size at birth, neonatal growth rate and adult size in the aged guinea pig

4.3.3.1 Size at birth

In all male and female offspring combined, the adjusted steady state glucose infusion rate and metabolic clearance rate of insulin correlated positively ($r=0.32$, $p=0.049$ and $r=0.40$, $p=0.018$, respectively, $n=28$) and the steady state plasma human insulin concentration was correlated negatively ($r=-0.43$, $p=0.011$, $n=28$) with birth nose to rump length (Table 4.3). The steady state plasma human insulin concentration correlated negatively with head length at birth ($r=-0.40$, $p=0.026$, $n=24$), and the adjusted steady state glucose infusion rate and metabolic clearance rate of insulin correlated negatively ($r=-0.38$, $p=0.024$ and $r=-0.44$, $p=0.009$, respectively, $n=28$) and the steady state plasma human insulin concentration correlated positively ($r=0.38$, $p=0.022$, $n=28$) with the ponderal index at birth in all male and female offspring combined (Table 4.3).

In male offspring, the adjusted steady state glucose infusion rate correlated negatively with birth weight ($r=-0.46$, $p=0.033$, $n=17$) (Figure 4.4a), and the adjusted steady state glucose infusion rate and metabolic clearance rate of insulin correlated negatively ($r=-0.61$, $p=0.008$ and $r=-0.51$, $p=0.012$, respectively, $n=15$) (Figures 4.5

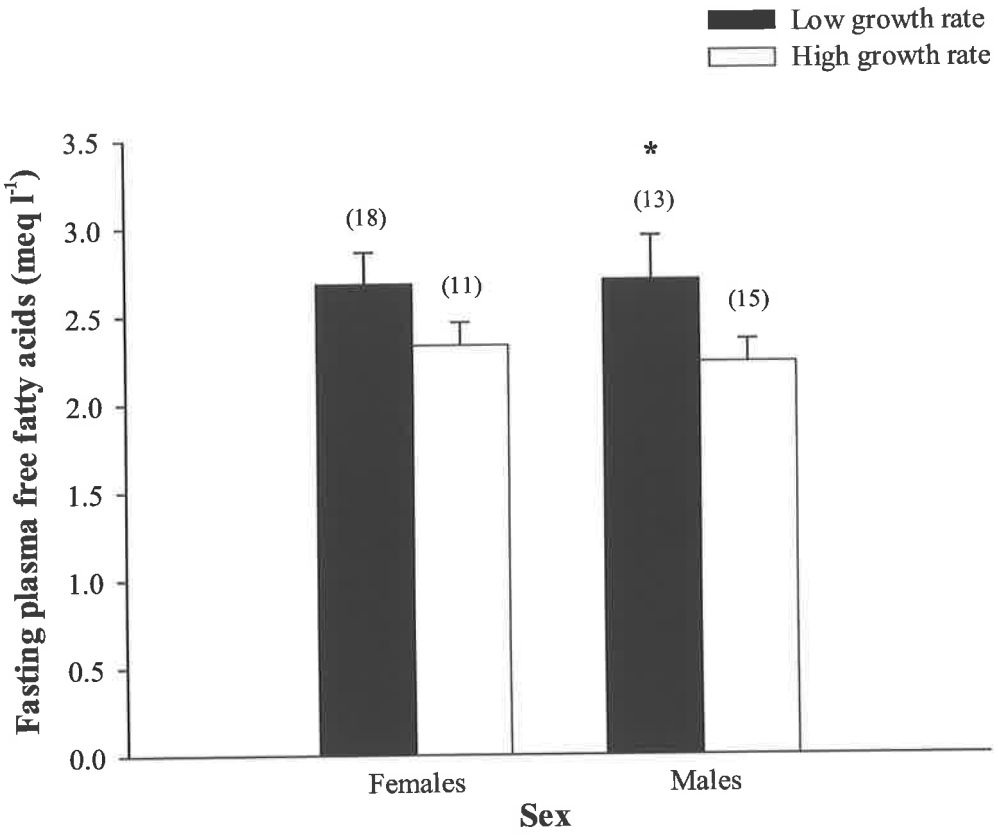

Figure 4.3 The effect of neonatal fractional growth rate class and sex on fasting plasma free fatty acid concentrations in the aged guinea pig

Fasting plasma free fatty acid concentrations in aged adult guinea pigs of low and high neonatal fractional growth rate. Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. $*P < 0.05$ compared with high growth rate offspring of the same sex.

Table 4.3 Relationship of whole body insulin sensitivity and circulating lipid profile to size at birth in the aged guinea pig

	Correlation coefficients (*r*)						
	SSGIR	AdjSSGIR	SSPHI	MCRi	FPFFA	FPTRIG	FPCHOL
BWT	-0.05 (31)	0.06 (31)	-0.19 (31)	0.06 (31)	0.23 (57)*	0.10 (57)	0.10 (57)
BNRL	0.05 (28)	0.32 (28)*	-0.43 (28)*	0.40 (28)*	0.18 (54)	0.15 (54)	0.32 (54)*
BWT:BNRL	-0.10 (28)	-0.15 (28)	0.06 (28)	-0.20 (28)	0.17 (54)	0.03 (54)	-0.05 (54)
BPI	-0.13 (28)	-0.38 (28)*	0.38 (28)*	-0.44 (28)**	-0.06 (54)	-0.12 (54)	-0.33 (54)**
BAC	0.02 (28)	0.08 (28)	-0.07 (28)	0.06 (28)	-0.20 (54)	0.17 (54)	-0.17 (54)
BHL	-0.15 (24)	0.14 (24)	-0.40 (24)*	0.30 (24)	0.13 (54)	0.09 (54)	0.21 (54)
BHW	-0.28 (28)	-0.18 (28)	-0.05 (28)	0.07 (28)	0.21 (54)	0.19 (54)	0.25 (54)*
BHW:BAC	-0.17 (28)	-0.15 (28)	0.02 (28)	-0.01 (28)	0.29 (54)*	-0.03 (54)	0.24 (54)*

r represents the correlation coefficient between each variable of adult whole body insulin sensitivity or circulating lipid profile and each variable of size at birth. Numbers in parentheses represent the number of animals. Partial correlations: all significant correlations highlighted in light grey were independent of $FGR_{10\text{-}30}$ and those highlighted in dark grey were independent of adult weight, all remaining significant correlations were independent of $FGR_{10\text{-}30}$ and adult weight. Statistical significance of correlation coefficients: $^{*}P < 0.05$, $^{**}P < 0.01$. SSGIR, steady state glucose infusion rate ($mg.min^{-1}.kg^{-1}$); AdjSSGIR, adjusted steady state glucose infusion rate ($mg.min^{-1}.kg^{-1}$ $\mu U.ml^{-1}$); SSPHI, steady state plasma human insulin, ($\mu U\ ml^{-1}$); MCRi, metabolic clearance rate of insulin; FPFFA, fasting plasma free fatty acids ($meq\ l^{-1}$); FPTRIG, fasting plasma triglycerides ($mmol\ l^{-1}$); FPCHOL, fasting plasma total cholesterol ($mmol\ l^{-1}$). BW, birth weight (g); BNRL, birth nose to rump length (mm); BW:BNRL, birth weight to nose to rump length ratio ($g\ mm^{-1}$); BPI, birth ponderal index ($g\ mm^{-3}$); BAC, birth abdominal circumference (mm); BHL, birth head length (mm); BHW, birth head width (mm); BHW:BAC, birth head width to abdominal circumference ratio.

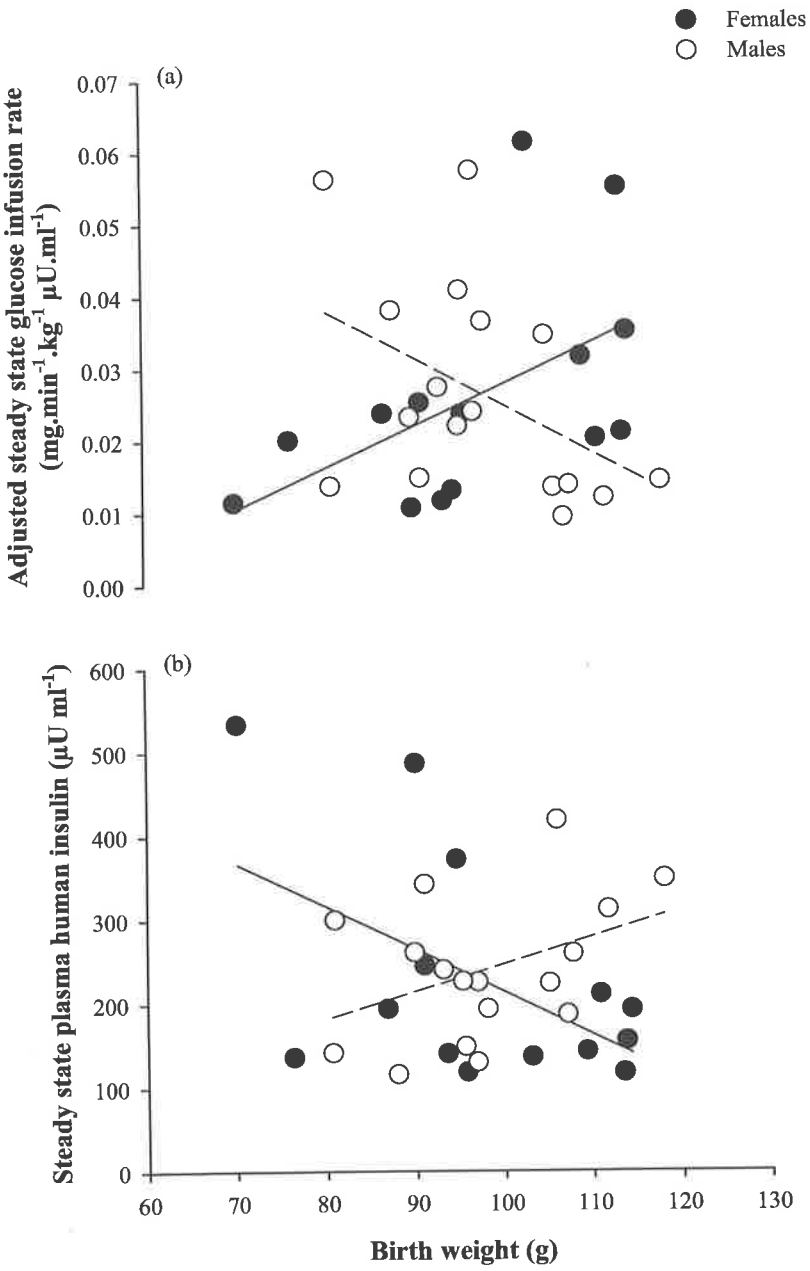

Figure 4.4 The relationship of whole body insulin sensitivity of glucose metabolism to birth weight in the aged guinea pig

Relationship of birth weight to (a) adjusted steady state glucose infusion rate (females: r=0.53, n=14, p<0.03; males: r=-0.46, n=17, p<0.04) and (b) steady state plasma human insulin concentration (females: r=-0.53, n=14, p<0.03; males: r=0.40, n=17, ns) during the HEC in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

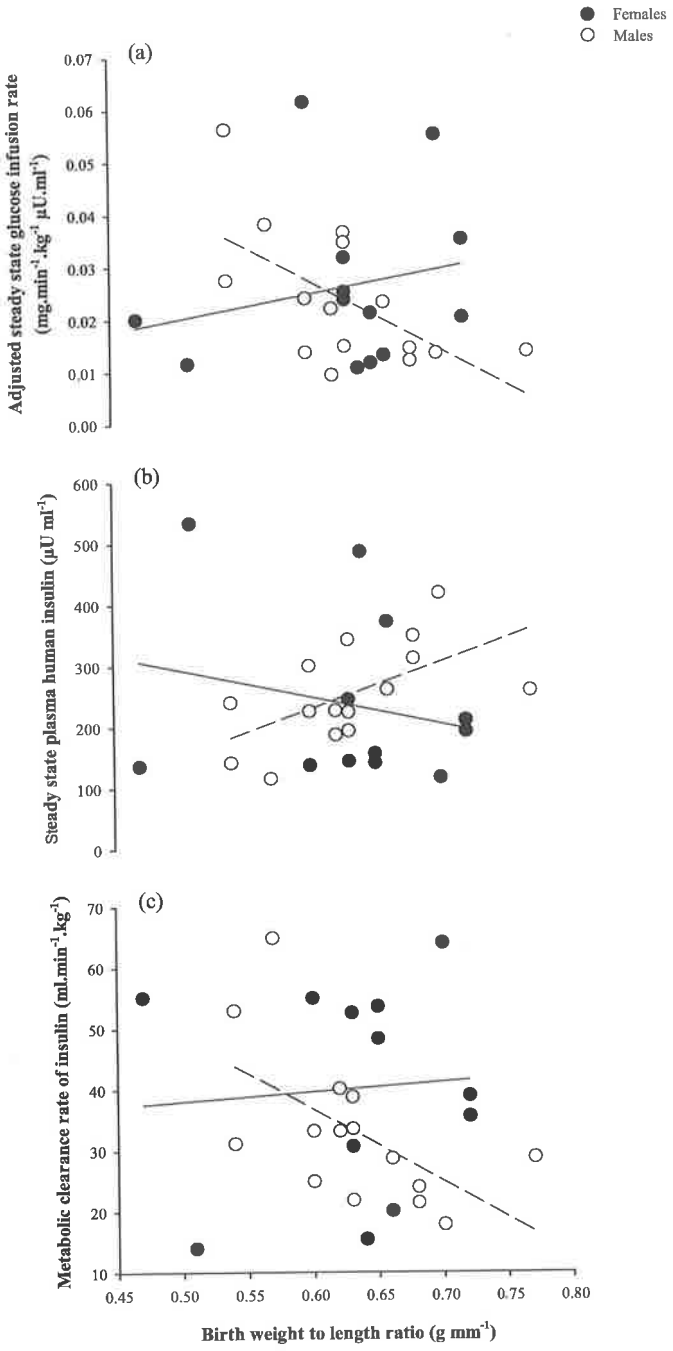

Figure 4.5 The relationship of whole body insulin sensitivity of glucose metabolism and the metabolic clearance rate of insulin to birth weight to length ratio in the aged guinea pig

Relationship of birth weight to length ratio to (a) adjusted steady state glucose infusion rate (females: r=0.22, n=13, ns; males: r=-0.61, n=15, p<0.01) (b) steady state plasma human insulin (females: r=-0.23, n=13, ns; males: r=0.58, n=15, p<0.02) and (c) metabolic clearance rate of insulin (females: r=0.07, n=13, ns; males: r=-0.51, n=15, p<0.02) during the HEC in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

a and c) and the steady state plasma human insulin concentration correlated positively (r=0.58, p=0.012, n=15) (Figure 4.5b) with birth weight to length ratio.

In female offspring, the adjusted steady state glucose infusion rate correlated positively (r=0.53, p=0.027, n=14) and the steady state plasma human insulin concentration correlated negatively (r=-0.53, p=0.027, n=14) with birth weight (Figures 4.4a and b) and the adjusted steady state glucose infusion rate and metabolic clearance rate of insulin correlated positively (r=0.63, p=0.011 and r=0.70, p=0.004, respectively, n=13) (Figures 4.6 a and c) and the steady state plasma human insulin concentration correlated negatively (r=-0.65, p=0.008, n=13) (Figure 4.6b) with birth nose to rump length. The metabolic clearance rate of insulin correlated negatively with ponderal index at birth (r=-0.56, p=0.023, n=13) (Figure 4.7), and the adjusted steady state glucose infusion rate and metabolic clearance rate of insulin correlated positively (r=0.71, p=0.007 and r=0.68, p=0.011, respectively, n=11) (Figures 4.8a and c) and the steady state plasma human insulin concentration correlated negatively (r=-0.65, p=0.016, n=11) (Figure 4.8b) with head length at birth in female offspring.

In all male and female offspring combined, fasting plasma free fatty acid concentrations correlated positively with birth weight (r=0.23, p=0.045, n=57) and head width to abdominal circumference ratio (r=0.29, p=0.018, n=54), and fasting plasma total cholesterol concentrations correlated positively with birth nose to rump length, head width and head width to abdominal circumference ratio (r=0.32, p=0.01; r=0.25, p=0.035 and r=0.24, p=0.038, respectively, n=54) and correlated negatively with ponderal index at birth (r=-0.33, p=0.007, n=54) (Table 4.3).

In male offspring, fasting plasma total cholesterol concentrations correlated positively with birth nose to rump length (r=0.34, p=0.043, n=26) (Figure 4.9) and negatively with ponderal index at birth (r=-0.46, p=0.009, n=26) (Figure 4.10).

In female offspring, parameters of the adult circulating lipid profile were not associated with any measures of size at birth (data shown for selected associations only, Figures 4.9 and 4.10).

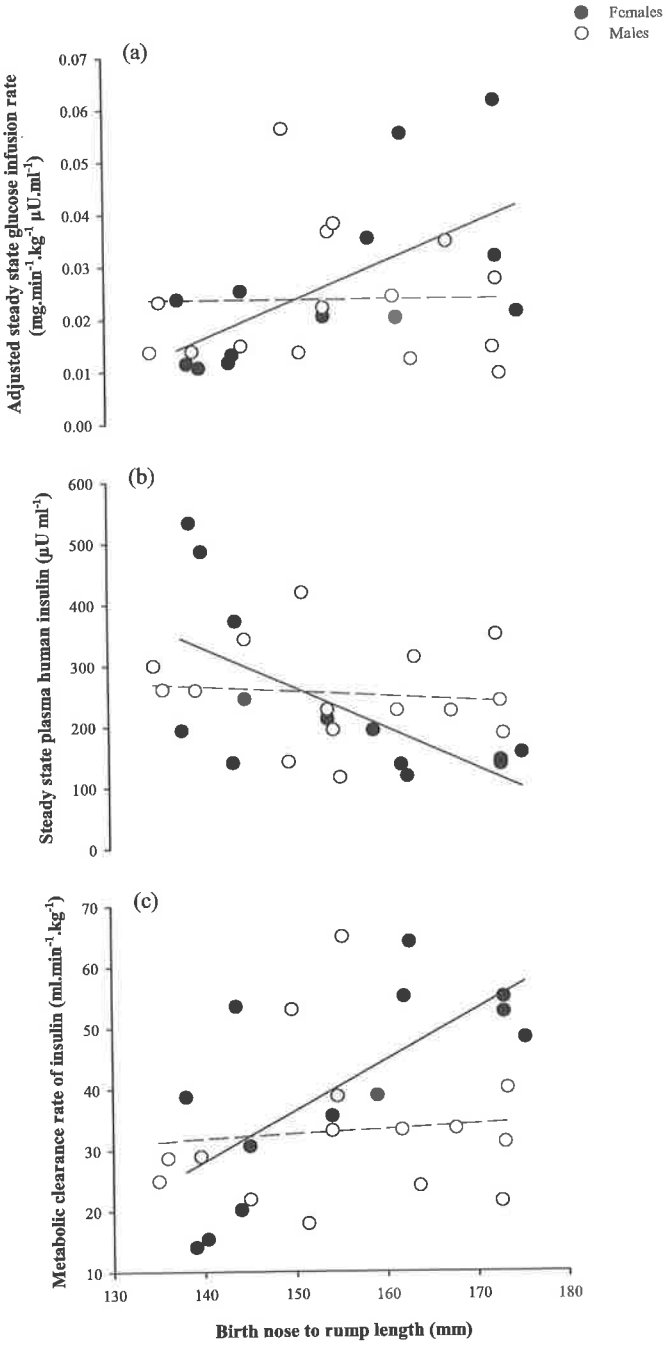

Figure 4.6 The relationship of whole body insulin sensitivity of glucose metabolism and the metabolic clearance rate of insulin to nose to rump length at birth in the aged guinea pig

Relationship of nose to rump length at birth to (a) adjusted steady state glucose infusion rate (females: $r=0.63$, $n=13$, $p<0.02$; males: $r=-0.01$, $n=15$, ns) (b) steady state plasma human insulin (females: $r=-0.65$, $n=13$, $p<0.01$; males: $r=-0.13$, $n=15$, ns) and (c) metabolic clearance rate of insulin (females: $r=0.70$, $n=13$, $p<0.01$; males: $r=0.09$, $n=15$, ns) during the HEC in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

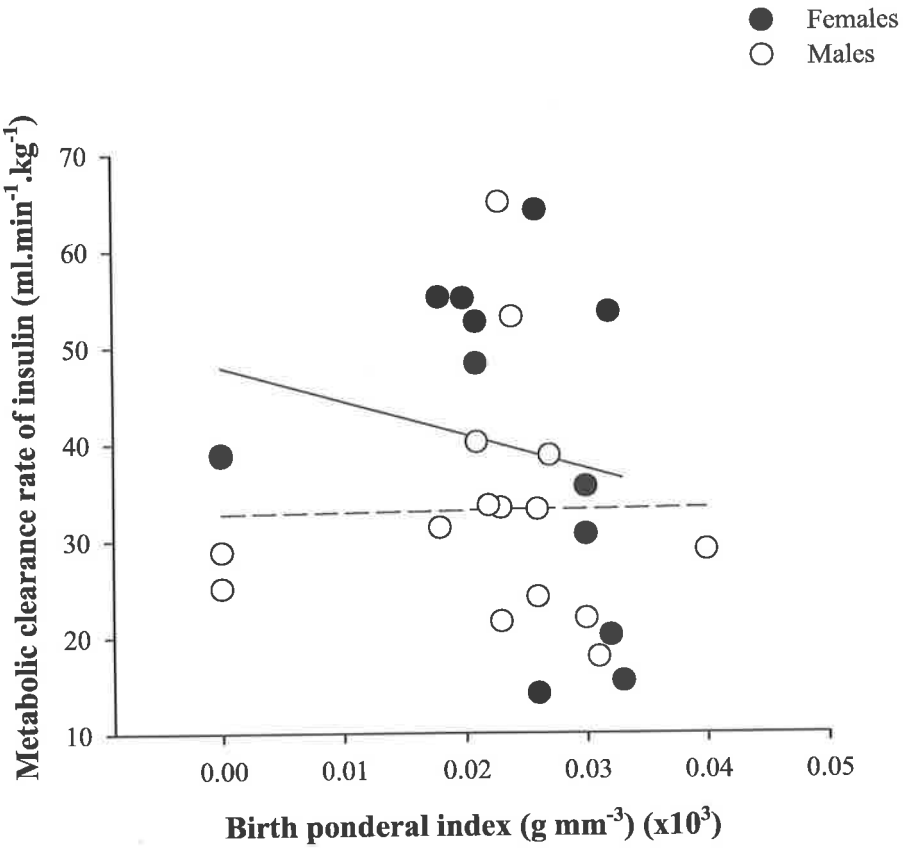

Figure 4.7 The relationship of the metabolic clearance rate of insulin to ponderal index at birth in the aged guinea pig

Relationship of ponderal index at birth to the metabolic clearance rate of insulin (females: r=-0.56, n=13, p<0.03; males: r=-0.38, n=15, ns) during the HEC in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

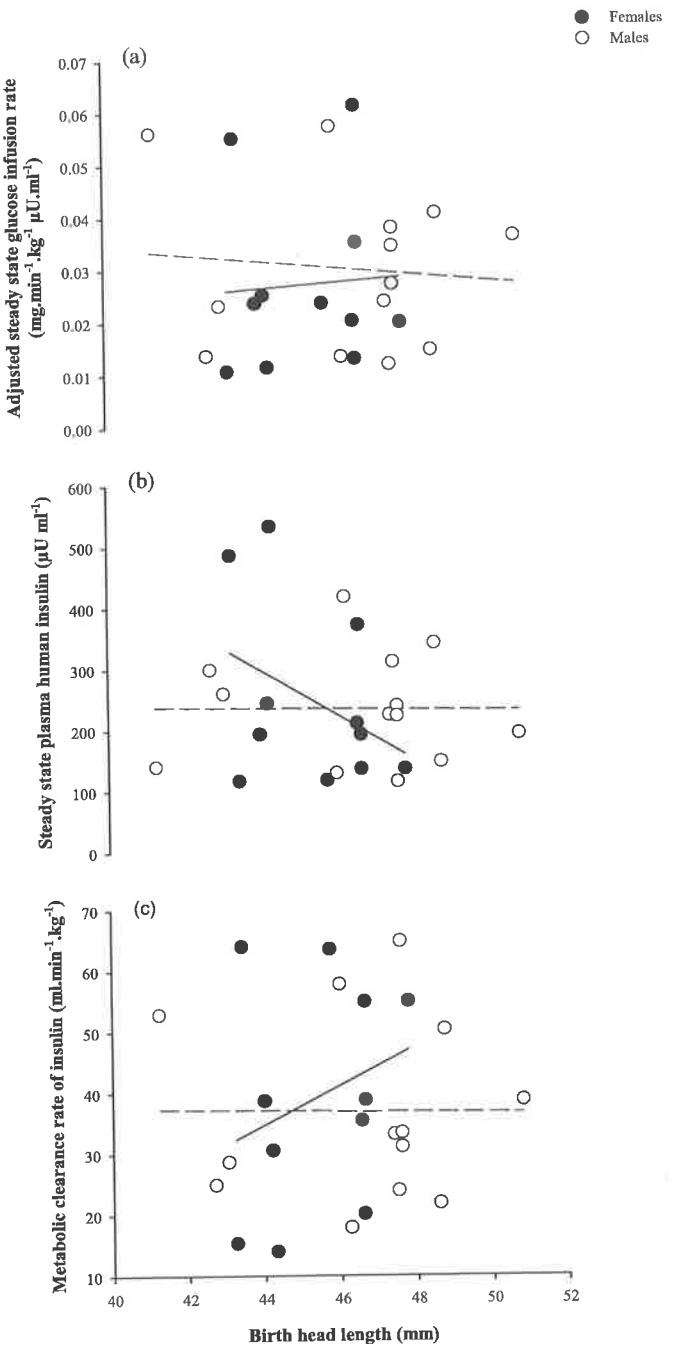

Figure 4.8 The relationship of whole body insulin sensitivity of glucose metabolism and the metabolic clearance rate of insulin to head length at birth in the aged guinea pig

Relationship of head length at birth to (a) adjusted steady state glucose infusion rate (females: r=0.71, n=11, p<0.01; males: r=-0.17, n=13, ns) (b) steady state plasma human insulin (females: r=-0.65, n=11, p<0.02; males: r=-0.35, n=13, ns) and (c) metabolic clearance rate of insulin (females: r=0.68, n=11, p<0.02; males: r=0.22, n=13, ns) during the HEC in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

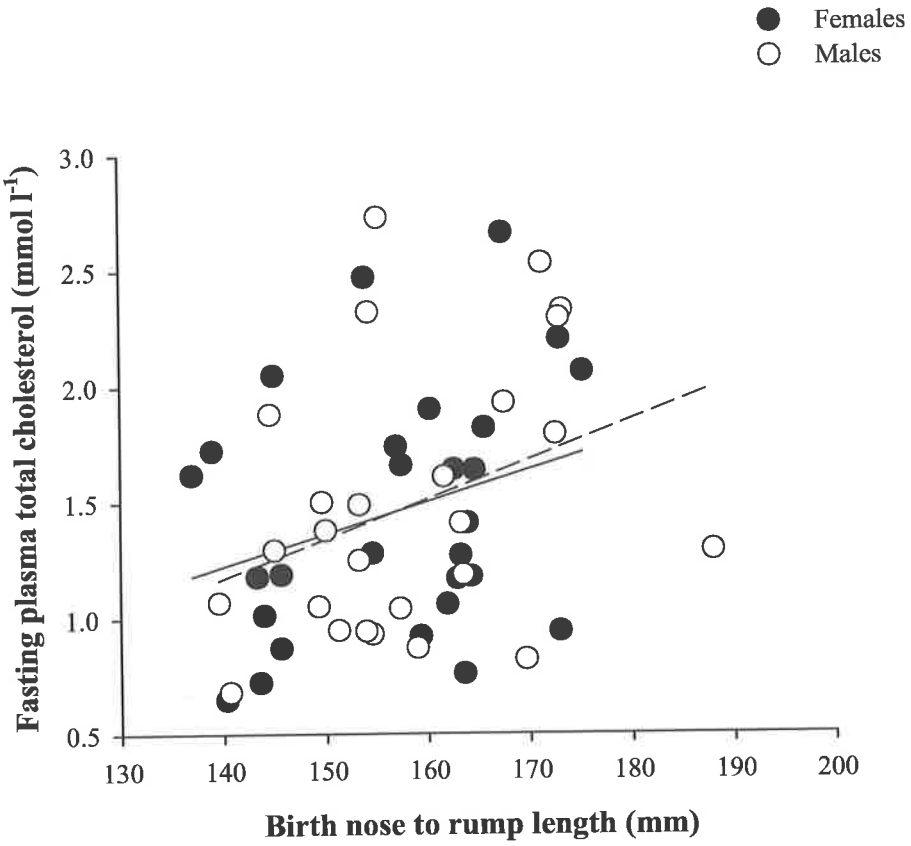

Figure 4.9 The relationship of fasting plasma total cholesterol concentrations to nose to rump length at birth in the aged guinea pig

Relationship of nose to rump length at birth to fasting plasma total cholesterol concentrations (females: r=0.29, n=28, ns; males: r=0.34, n=26, $p<0.05$) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

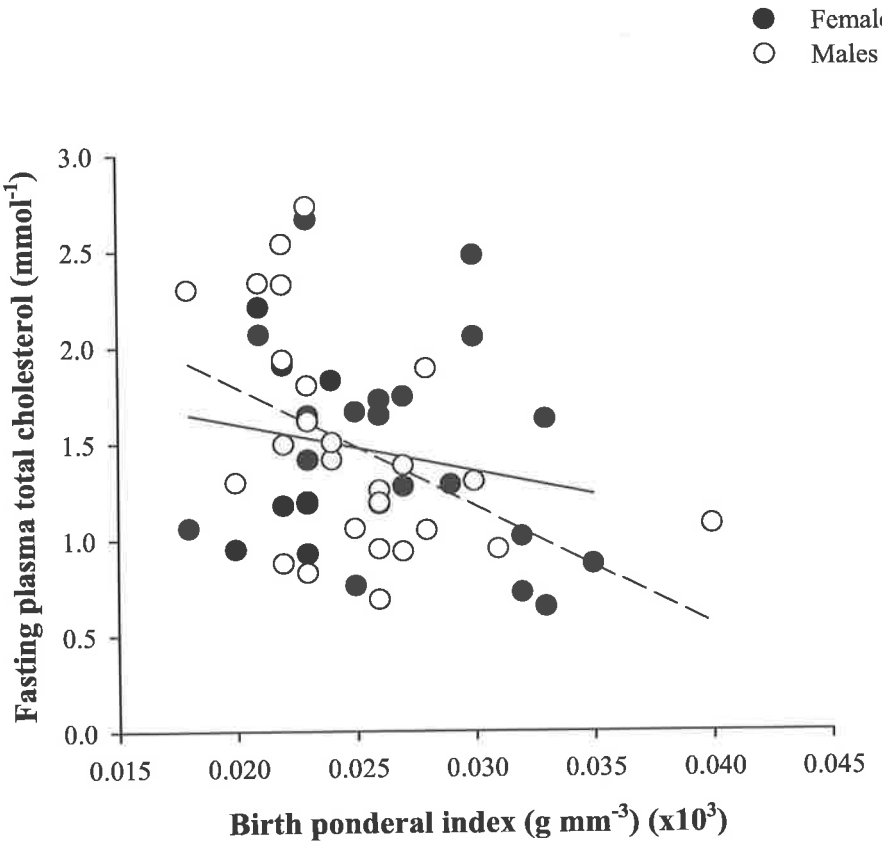

Figure 4.10 The relationship of fasting plasma total cholesterol concentrations to ponderal index at birth in the aged guinea pig

Relationship of ponderal index at birth to fasting plasma total cholesterol concentrations (females: r=-0.20, n=28, ns; males: r=-0.46, n=26, $p<0.01$) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

4.3.3.2 Neonatal growth rate

In all male and female offspring combined, the steady state glucose infusion rate correlated positively with neonatal fractional growth rate (r=0.34, p=0.03, n=31) (Table 4.4). In male offspring, parameters of adult whole body insulin sensitivity were not associated with any measure of neonatal growth (data shown for selected association only, Figure 4.11). In female offspring, the steady state plasma human insulin concentration correlated positively with neonatal fractional growth rate (r=0.52, p=0.029, n=14) (Figure 4.11).

In all male and female offspring combined, fasting plasma triglyceride concentrations correlated positively with neonatal fractional growth rate (r=0.38, p=0.002, n=57) (Table 4.4). In male offspring, fasting plasma triglyceride concentrations correlated positively with neonatal fractional growth rate (r=0.57, p=0.001, n=28) (Figure 4.12). In female offspring, fasting plasma triglyceride concentrations correlated positively with neonatal fractional growth rate (r=0.36, p=0.028, n=29) (Figure 4.12).

4.3.3.3 Adult size

In all male and female offspring combined, the adjusted steady state glucose infusion rate and metabolic clearance rate of insulin correlated negatively (r=-0.42, p=0.022 and r=-0.58, p=0.002, respectively, n=23), and the steady state plasma human insulin concentration correlated positively (r=0.41, p=0.025, n=23) with adult nose to rump length (Table 4.4).

In male offspring, the adjusted steady state glucose infusion rate and metabolic clearance rate of insulin correlated negatively (r=-0.62, p=0.012 and r=-0.70, p=0.004, respectively, n=13) (Figures 4.13 a and c), and the steady state plasma human insulin concentration correlated positively (r=0.63, p=0.01, n=13) (Figure 4.13b) with adult nose to rump length.

In female offspring, parameters of adult whole body insulin sensitivity were not associated with any measures of adult size (data shown for selected associations only, Figures 4.13a, b and c).

Table 4.4 Relationship of whole body insulin sensitivity and circulating lipid profile to neonatal growth rate and adult size in the aged guinea pig

	Correlation coefficients (r)						
	SSGIR	AdjSSGIR	SSPHI	MCRi	FPFFA	FPTRIG	FPCHOL
AGR_{10-30}	0.15 (31)	0.05 (31)	0.04 (31)	-0.12 (31)	-0.16 (57)	-0.01 (57)	0.04 (57)
FGR_{10-30}	0.34 (31)*	0.10 (31)	0.22 (31)	-0.18 (31)	-0.18 (57)	0.38 (57)†	0.16 (57)
AW	0.01 (26)	-0.10 (26)	0.12 (26)	-0.22 (26)	-0.04 (48)	-0.13 (48)	-0.14 (48)
ANRL	-0.05 (23)	-0.42 (23)*	0.41 (23)*	-0.58 (23)†	0.18 (39)	0.02 (39)	0.00 (39)
ABMI	0.01 (23)	0.05 (23)	-0.05 (23)	0.03 (23)	-0.14 (39)	-0.13 (39)	-0.48 (39)†

r represents the correlation coefficient between each variable of adult whole body insulin sensitivity or circulating lipid profile and each variable of neonatal growth rate or adult size. Numbers in parentheses represent the number of animals. Statistical significance of correlation coefficients: $^{*}P < 0.05$, $^{\dagger}P < 0.005$. SSGIR, steady state glucose infusion rate ($mg.min^{-1}.kg^{-1}$); AdjSSGIR, adjusted steady state glucose infusion rate ($mg.min^{-1}.kg^{-1}$ $\mu U.ml^{-1}$); SSPHI, steady state plasma human insulin, ($\mu U\ ml^{-1}$); MCRi, metabolic clearance rate of insulin; FPFFA, fasting plasma free fatty acids ($meq\ l^{-1}$); FPTRIG, fasting plasma triglycerides ($mmol\ l^{-1}$); FPCHOL, fasting plasma total cholesterol ($mmol\ l^{-1}$). AGR_{10-30}, absolute growth rate (10-30 days) ($g\ day^{-1}$); FGR_{10-30}, fractional growth rate (10-30 days) ($g\ day^{-1}\ g^{-1}$); AW, adult weight (g); ANRL, adult nose to rump length (mm); ABMI, adult body mass index ($g\ mm^{-2}$).

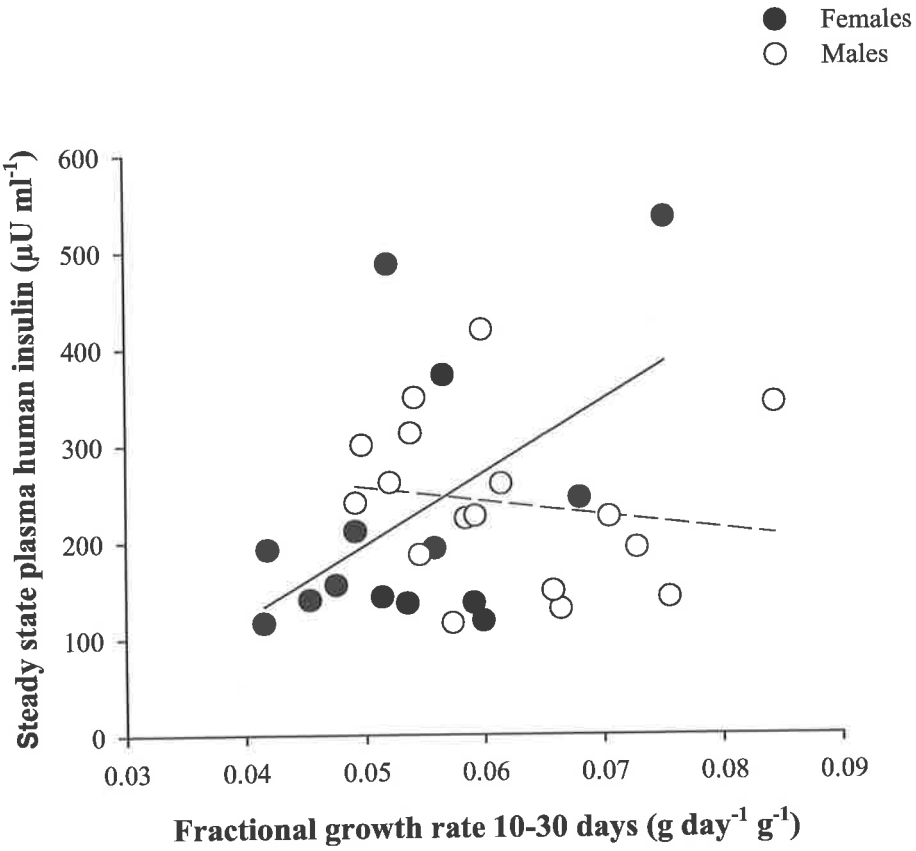

Figure 4.11 The relationship of steady state plasma human insulin concentrations to neonatal fractional growth rate in the aged guinea pig

Relationship of neonatal fractional growth rate to steady state plasma human insulin (females: r=0.52, n=14, p<0.03; males: r=-0.17, n=17, ns) during the HEC in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

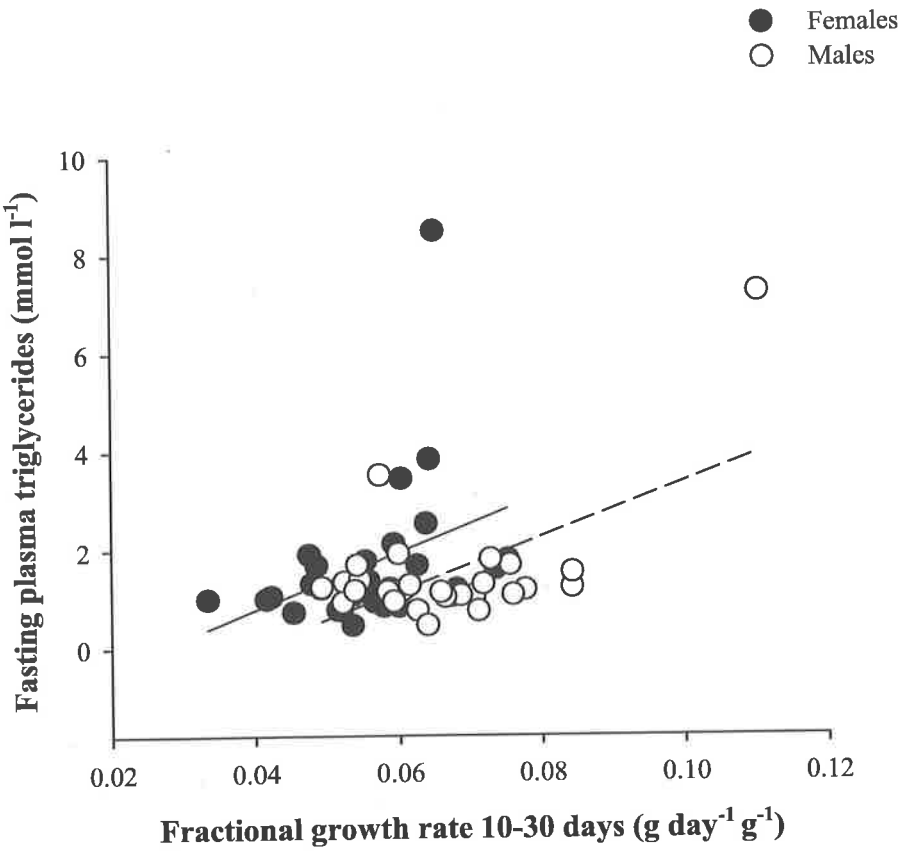

Figure 4.12 The relationship of fasting plasma triglyceride concentrations to neonatal fractional growth rate in the aged guinea pig

Relationship of neonatal fractional growth rate to fasting plasma triglyceride concentrations (females: $r=0.36$, $n=29$, $p<0.03$; males: $r=0.57$, $n=28$, $p<0.005$) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

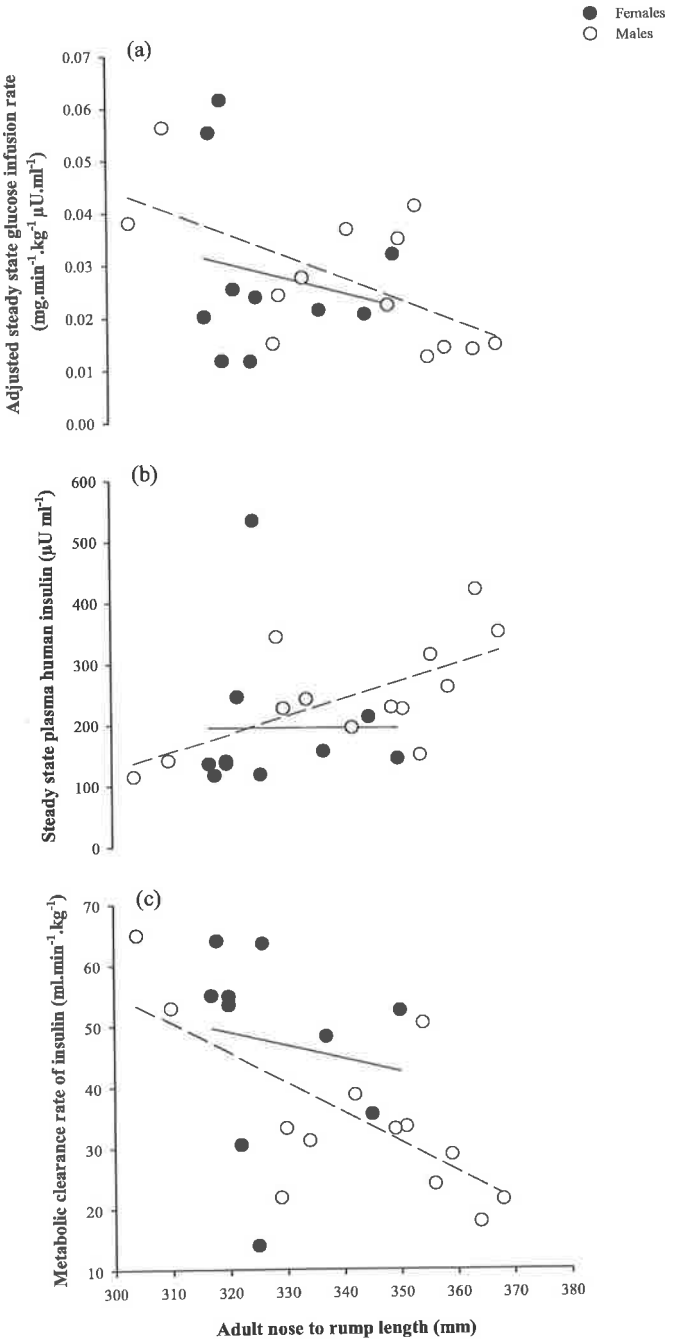

Figure 4.13 The relationship of whole body insulin sensitivity of glucose metabolism, steady state plasma human insulin and the metabolic clearance rate of insulin to adult nose to rump length in the aged guinea pig

Relationship of adult nose to rump length to the (a) adjusted steady state glucose infusion rate (females: r=-0.20, n=10, ns; males: r=-0.62, n=13, $p<0.02$) (b) steady state plasma human insulin (females: r=-0.004, n=10, ns; males: r=0.63, n=13, $p<0.02$) and (c) metabolic clearance rate of insulin (females: r=-0.16, n=10, ns; males: r=-0.70, n=13, $p<0.005$) during the HEC in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

In all male and female offspring combined, fasting plasma total cholesterol concentrations correlated negatively with adult body mass index (r=-0.48, p=0.001, n=39) (Table 4.4). In male offspring, fasting plasma total cholesterol concentrations correlated negatively with adult body mass index (r=-0.47, p=0.016, n=21) (Figure 4.14). In female offspring, fasting plasma total cholesterol concentrations correlated positively with adult nose to rump length (r=0.48, p=0.023, n=18) (Figure 4.15) and negatively with adult body mass index (r=-0.52, p=0.014, n=18) (Figure 4.14).

4.3.3.4 Partial correlations

The positive association between the adjusted steady state glucose infusion rate and birth nose to rump length in all offspring was independent of neonatal fractional growth rate (partial correlation (pc), r=0.34, p=0.043), but not adult weight. The negative association between the steady state plasma human insulin concentration and birth nose to rump length in all offspring was independent of neonatal fractional growth rate (pc: r=-0.38, p=0.024) and adult weight (pc: r=-0.46, p=0.015). The positive association between the metabolic clearance rate of insulin and birth nose to rump length in all offspring was independent of neonatal fractional growth rate (pc: r=0.35, p=0.038) and adult weight (pc: r=0.37, p=0.047). The negative association between the steady state plasma human insulin concentration and head length at birth in all offspring was not independent of neonatal fractional growth rate or adult weight. The negative associations between the adjusted steady state glucose infusion rate and metabolic clearance rate of insulin and ponderal index at birth in all offspring were independent of neonatal fractional growth rate (pc: r=-0.38, p=0.026 and r=-0.46, p=0.008), but not adult weight. The positive association between the steady state plasma human insulin concentration and ponderal index at birth in all offspring was independent of neonatal fractional growth rate (pc: r=0.39, p=0.021) and adult weight (r=0.36, p=0.049).

In male offspring, the negative association between the adjusted steady state glucose infusion rate and birth weight was independent of adult weight (pc: r=-0.70, p=0.003), but not neonatal fractional growth rate. The negative association between the adjusted steady state glucose infusion rate and birth weight to length ratio in male offspring was independent of neonatal fractional growth rate (pc: r=-0.62, p=0.009) and adult weight (pc: r=-0.56, p=0.029). The positive association between the steady

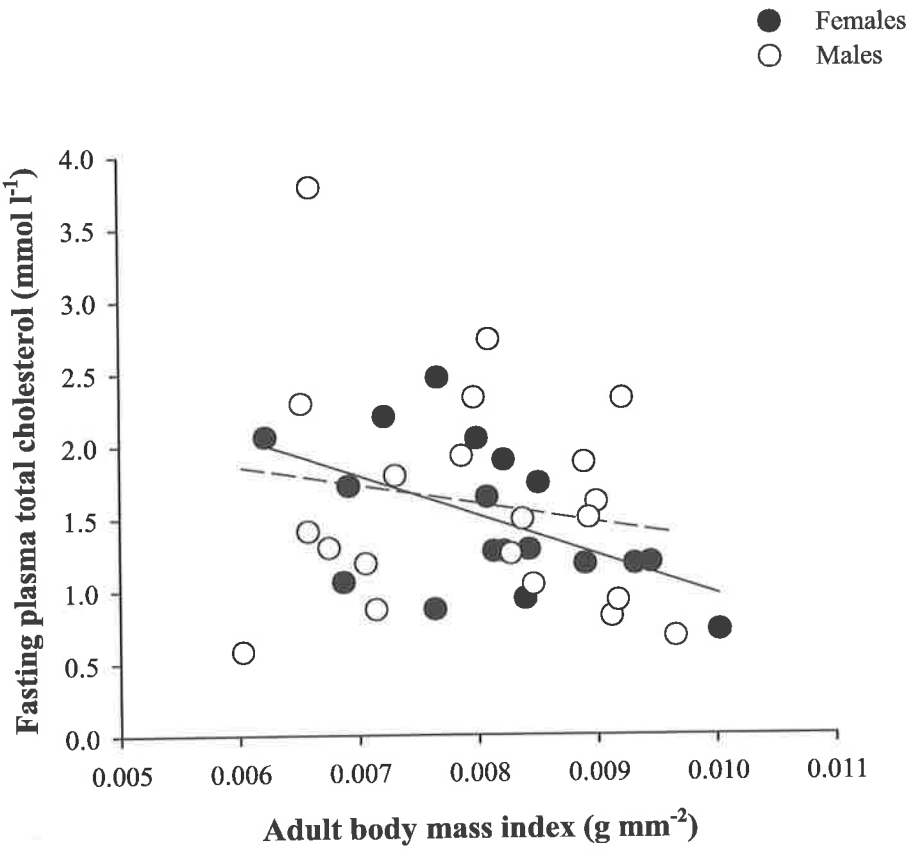

Figure 4.14 The relationship of fasting plasma total cholesterol concentrations to adult body mass index in the aged guinea pig

Relationship of adult body mass index to fasting plasma total cholesterol concentrations (females: r=-0.52, n=18, p<0.02; males: r=-0.47, n=21, p<0.02) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

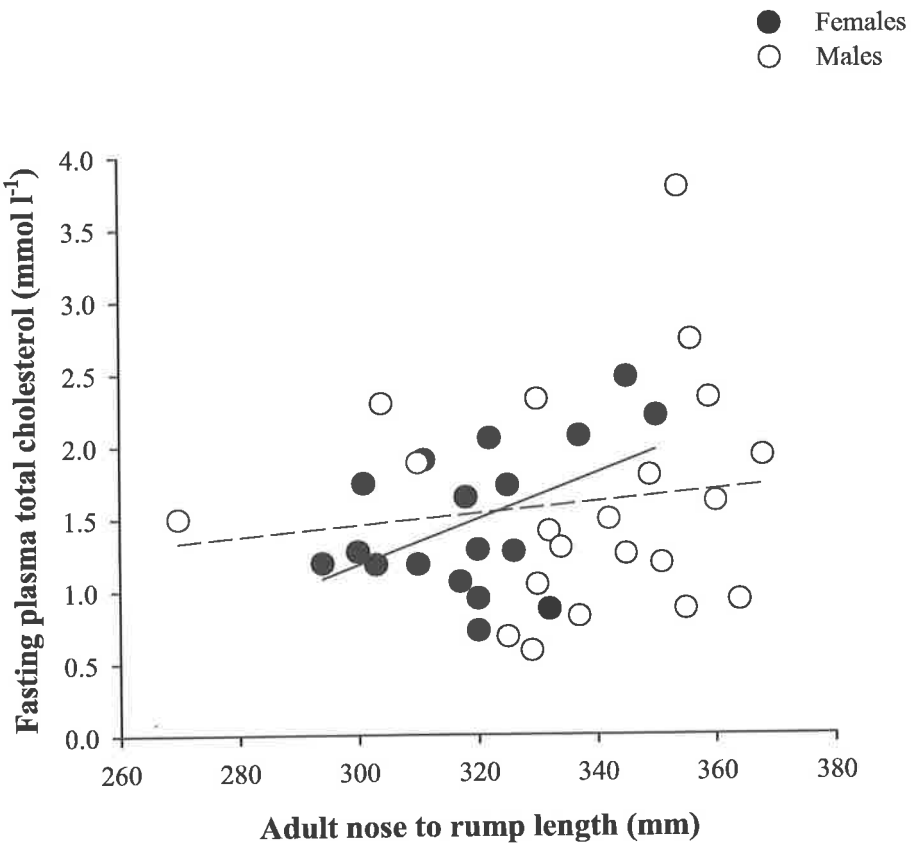

Figure 4.15 The relationship of fasting plasma total cholesterol concentrations to adult nose to rump length in the aged guinea pig

Relationship of adult nose to rump length to fasting plasma total cholesterol concentrations (females: r=0.48, n=18, p<0.03; males: r=-0.20, n=21, ns) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

state plasma human insulin concentration and birth weight to length ratio in male offspring was independent of neonatal fractional growth rate (pc: r=0.58, p=0.015), but not adult weight. The negative association between the metabolic clearance rate of insulin and birth weight to length ratio in male offspring was independent of neonatal fractional growth rate (pc: r=-0.58, p=0.016), but not adult weight.

In female offspring, the positive association between the adjusted steady state glucose infusion rate and birth weight was independent of neonatal fractional growth rate (pc: r=0.51, p=0.037), but not adult weight. The negative association between the steady state plasma human insulin concentration and birth weight in female offspring was not independent of neonatal fractional growth rate or adult weight. The positive associations between the adjusted steady state glucose infusion rate and metabolic clearance rate of insulin and birth nose to rump length in female offspring were independent of neonatal fractional growth rate (pc: r=0.59, p=0.021 and r=0.63, p=0.014) and adult weight (pc: r=0.65, p=0.028 and r=0.78, p=0.006). The negative association between the steady state plasma human insulin concentration and birth nose to rump length in female offspring was independent of neonatal fractional growth rate (pc: r=-0.57, p=0.028) and adult weight (pc: r=-0.80, p=0.005). The positive associations between the adjusted steady state glucose infusion rate and metabolic clearance rate of insulin and head length at birth in female offspring were independent of neonatal fractional growth rate (pc: r=0.70, p=0.011 and r=0.60, p=0.033) and adult weight (pc: r=0.70, p=0.017 and r=0.68, p=0.021). The negative association between the steady state plasma human insulin concentration and head length at birth in female offspring was independent of neonatal fractional growth rate (pc: r=-0.56, p=0.049) and adult weight (pc: r=-0.66, p=0.026). The negative association between the metabolic clearance rate of insulin and ponderal index at birth in female offspring was independent of neonatal fractional growth rate (pc: r=-0.69, p=0.007) and adult weight (pc: r=-0.75, p=0.01).

The positive association between the unadjusted steady state glucose infusion rate and neonatal fractional growth rate in all offspring was independent of birth weight (pc: r=0.36, p=0.027) and adult weight (pc: r=0.41, p=0.02). In female offspring, the positive association between the steady state plasma human insulin concentration and neonatal fractional growth rate was not independent of birth weight or adult weight.

The positive association between the fasting plasma free fatty acid concentration and birth weight in all offspring was independent of adult weight (pc: r=0.25, p=0.044), but not neonatal fractional growth rate. The positive associations between fasting plasma total cholesterol concentrations and nose to rump length and head width at birth in all offspring were independent of neonatal fractional growth rate (pc: r=0.36, p=0.005 and r=0.29, p=0.017) and adult weight (pc: r=0.40, p=0.004 and r=0.36, p=0.008). The negative association between the fasting plasma total cholesterol concentration and ponderal index at birth in all offspring was independent of neonatal fractional growth rate (pc: r=-0.32, p=0.01) and adult weight (pc: r=-0.32, p=0.018). The positive associations between fasting plasma free fatty acid and total cholesterol concentrations and the birth head width to abdominal circumference ratio in all offspring were independent of neonatal fractional growth rate (pc: r=0.28, p=0.022 and r=0.26, p=0.031) and adult weight (pc: r=0.27, p=0.036 and r=0.38, p=0.006).

In male offspring, the positive association between the fasting plasma total cholesterol concentration and birth nose to rump length was independent of neonatal fractional growth rate (pc: r=0.39, p=0.027) and adult weight (pc: r=0.56, p=0.004). The negative association between the fasting plasma total cholesterol concentration and ponderal index at birth in male offspring was independent of neonatal fractional growth rate (pc: r=-0.48, p=0.008) and adult weight (pc: r=-0.48, p=0.012).

The positive association between fasting plasma triglyceride concentrations and neonatal fractional growth rate in all offspring was independent of birth weight (pc: r=0.44, p<0.0001) and adult weight (pc: r=0.57, p<0.0001). In male offspring, the positive association between fasting plasma triglyceride concentrations and neonatal fractional growth rate was independent of birth weight (pc: r=0.62, p<0.0001) and adult weight (pc: r=0.64, p<0.0001). In female offspring, the positive association between fasting plasma triglyceride concentrations and neonatal fractional growth rate was independent of birth weight (pc: r=0.53, p=0.002) and adult weight (pc: r=0.42, p=0.025).

4.3.4 Relationship of whole body insulin sensitivity and circulating lipid profile to body composition in the aged guinea pig

4.3.4.1 Adiposity

In all male and female offspring combined, the adjusted steady state glucose infusion rate and metabolic clearance rate of insulin correlated negatively with subcutaneous adiposity (r=-0.34, p=0.05 and r=-0.42, p=0.018, respectively, n=25) and relative subcutaneous adiposity (r=-0.39, p=0.028 and r=-0.44, p=0.013, respectively, n=25) (Table 4.5).

In male offspring, the steady state plasma human insulin concentration correlated positively with combined and relative combined adiposity (r=0.62, p=0.012 and r=0.59, p=0.017, respectively, n=13) and subcutaneous and relative subcutaneous adiposity (r=0.65, p=0.006 and r=0.57, p=0.016, respectively, n=14) and the metabolic clearance rate of insulin correlated negatively with combined and relative combined adiposity (r=-0.55, p=0.025 and r=-0.58, p=0.019, respectively, n=13) and subcutaneous and relative subcutaneous adiposity (r=-0.62, p=0.009 and r=-0.62, p=0.009, respectively, n=14) (Table 4.6). In female offspring, parameters of adult whole body insulin sensitivity were not associated with any measures of adiposity (Table 4.7).

Parameters of the adult circulating lipid profile were not associated with any measures of adiposity in all male and female offspring combined (Table 4.5), or in male (Table 4.6) or female (Table 4.7) offspring only.

4.3.4.2 Skeletal muscle mass

In all male and female offspring combined, parameters of adult whole body insulin sensitivity were not associated with any measures of skeletal muscle mass (Table 4.5). In male offspring, the metabolic clearance rate of insulin correlated negatively with combined (r=-0.44, p=0.049, n=15) and mixed (r=-0.45, p=0.047, n=15) muscle mass (Table 4.6). In female offspring, parameters of adult whole body insulin sensitivity were not associated with any measures of skeletal muscle mass (Table 4.7).

In all male and female offspring combined, fasting plasma free fatty acid concentrations correlated positively with relative combined (r=0.33, p=0.013, n=47)

Table 4.5 Relationship of whole body insulin sensitivity and circulating lipid profile to adiposity, skeletal muscle mass and liver weight in the aged guinea pig

	Correlation coefficients (r)						
	SSGIR	AdjSSGIR	SSPHI	MCRi	FPFFA	FPTRIG	FPCHOL
Combined adiposity (g)	-0.13 (24)	-0.26 (24)	0.17 (24)	-0.27 (24)	-0.06 (42)	-0.23 (42)	-0.12 (42)
(% body weight)	-0.21 (24)	-0.30 (24)	0.13 (24)	-0.22 (24)	0.04 (42)	-0.23 (42)	-0.06 (42)
Visceral adiposity (g)	-0.10 (25)	-0.16 (25)	0.08 (25)	-0.15 (25)	-0.03 (45)	-0.20 (45)	-0.09 (45)
(% body weight)	-0.19 (25)	-0.19 (25)	0.03 (25)	-0.08 (25)	0.05 (45)	-0.20 (45)	-0.04 (45)
Subcutaneous adiposity (g)	-0.12 (25)	-0.34 (25)*	0.27 (25)	-0.42 (25)*	-0.05 (45)	-0.17 (45)	-0.09 (45)
(% body weight)	-0.15 (25)	-0.39 (25)*	0.28 (25)	-0.44 (25)*	0.02 (45)	-0.17 (45)	-0.02 (45)
Combined muscle (g)	0.11 (26)	-0.04 (26)	0.14 (26)	-0.26 (26)	0.17 (47)	-0.09 (47)	-0.05 (47)
(% body weight)	0.20 (26)	0.11 (26)	0.04 (26)	-0.07 (26)	0.33 (47)*	0.07 (47)	0.17 (47)
Mixed muscle (g)	0.12 (26)	-0.04 (26)	0.14 (26)	-0.26 (26)	0.17 (48)	-0.09 (48)	-0.04 (48)
(% body weight)	0.21 (26)	0.11 (26)	0.04 (26)	-0.08 (26)	0.33 (48)*	0.07 (48)	0.19 (48)
Type 2 muscle (g)	-0.07 (26)	-0.15 (26)	0.14 (26)	-0.20 (26)	0.07 (47)	-0.06 (47)	-0.21 (47)
(% body weight)	-0.06 (26)	0.00 (26)	0.02 (26)	0.07 (26)	0.09 (47)	0.10 (47)	-0.02 (47)
Liver (g)	-0.37 (26)*	-0.30 (26)	0.08 (26)	-0.09 (26)	-0.11 (48)	-0.17 (48)	-0.31 (48)*
(% body weight)	-0.43 (26)*	-0.17 (26)	-0.12 (26)	0.21 (26)	-0.13 (48)	-0.05 (48)	-0.15 (48)

r represents the correlation coefficient between each variable of adult whole body insulin sensitivity or circulating lipid profile and each variable of adult adiposity, skeletal muscle mass or liver weight. Numbers in parentheses represent the number of animals. Statistical significance of correlation coefficients: $^{*}P < 0.05$. SSGIR, steady state glucose infusion rate ($mg.min^{-1}.kg^{-1}$); AdjSSGIR, adjusted steady state glucose infusion rate ($mg.min^{-1}.kg^{-1}$ $\mu U.ml^{-1}$); SSPHI, steady state plasma human insulin, ($\mu U\ ml^{-1}$); MCRi, metabolic clearance rate of insulin; FPFFA, fasting plasma free fatty acids ($meq\ l^{-1}$); FPTRIG, fasting plasma triglycerides ($mmol\ l^{-1}$); FPCHOL, fasting plasma total cholesterol ($mmol\ l^{-1}$).

Table 4.6 Relationship of whole body insulin sensitivity and circulating lipid profile to adiposity, skeletal muscle mass and liver weight in aged male guinea pigs

	Correlation coefficients (*r*)						
	SSGIR	AdjSSGIR	SSPHI	MCRi	FPFFA	FPTRIG	FPCHOL
Combined adiposity (g)	-0.03 (13)	-0.33 (13)	0.62 (13)*	-0.55 (13)*	-0.12 (21)	-0.31 (21)	-0.13 (21)
(% body weight)	-0.04 (13)	-0.36 (13)	0.59 (13)*	-0.58 (13)*	-0.02 (21)	-0.28 (21)	-0.01 (21)
Visceral adiposity (g)	0.05 (14)	-0.16 (14)	0.41 (14)	-0.35 (14)	-0.02 (22)	-0.27 (22)	-0.05 (22)
(% body weight)	0.05 (14)	-0.17 (14)	0.40 (14)	-0.36 (14)	0.08 (22)	-0.24 (22)	0.08 (22)
Subcutaneous adiposity (g)	-0.15 (14)	-0.43 (14)	0.65 (14)**	-0.62 (14)**	-0.09 (24)	-0.27 (24)	-0.17 (24)
(% body weight)	-0.14 (14)	-0.42 (14)	0.57 (14)*	-0.62 (14)**	-0.03 (24)	-0.25 (24)	-0.09 (24)
Combined muscle (g)	-0.13 (15)	-0.31 (15)	0.41 (15)	-0.44 (15)*	0.19 (25)	-0.22 (25)	-0.23 (25)
(% body weight)	-0.22 (15)	-0.28 (15)	0.08 (15)	-0.23 (15)	0.29 (25)	0.00 (25)	0.01 (25)
Mixed muscle (g)	-0.13 (15)	-0.31 (15)	0.42 (15)	-0.45 (15)*	0.19 (25)	-0.23 (25)	-0.22 (25)
(% body weight)	-0.22 (15)	-0.31 (15)	0.13 (15)	-0.27 (15)	0.30 (25)	-0.01 (25)	0.02 (25)
Type 2 muscle (g)	-0.13 (15)	-0.23 (15)	0.22 (15)	-0.29 (15)	0.14 (25)	-0.06 (25)	-0.29 (25)
(% body weight)	-0.13 (15)	0.03 (15)	-0.32 (15)	0.21 (15)	0.07 (25)	0.22 (25)	-0.06 (25)
Liver (g)	-0.49 (15)*	-0.53 (15)*	0.51 (15)*	-0.36 (15)	-0.05 (25)	-0.12 (25)	-0.27 (25)
(% body weight)	-0.57 (15)*	-0.36 (15)	0.12 (15)	0.07 (15)	-0.14 (25)	0.11 (25)	-0.03 (25)

r represents the correlation coefficient between each variable of adult whole body insulin sensitivity or circulating lipid profile and each variable of adult adiposity, skeletal muscle mass or liver weight. Numbers in parentheses represent the number of animals. Statistical significance of correlation coefficients: $^{*}P < 0.05$, $^{**}P < 0.01$. SSGIR, steady state glucose infusion rate ($mg.min^{-1}.kg^{-1}$); AdjSSGIR, adjusted steady state glucose infusion rate ($mg.min^{-1}.kg^{-1}$ $\mu U.ml^{-1}$); SSPHI, steady state plasma human insulin, ($\mu U\ ml^{-1}$); MCRi, metabolic clearance rate of insulin; FPFFA, fasting plasma free fatty acids ($meq\ l^{-1}$); FPTRIG, fasting plasma triglycerides ($mmol\ l^{-1}$); FPCHOL, fasting plasma total cholesterol ($mmol\ l^{-1}$).

Table 4.7 Relationship of whole body insulin sensitivity and circulating lipid profile to adiposity, skeletal muscle mass and liver weight in aged female guinea pigs

	Correlation coefficients (r)						
	SSGIR	AdjSSGIR	SSPHI	MCRi	FPFFA	FPTRIG	FPCHOL
Combined adiposity (g)	-0.32 (11)	-0.18 (11)	-0.25 (11)	0.07 (11)	0.01 (21)	-0.12 (21)	-0.16 (21)
(% body weight)	-0.34 (11)	-0.26 (11)	-0.22 (11)	-0.01 (11)	0.14 (21)	-0.10 (21)	-0.10 (21)
Visceral adiposity (g)	-0.40 (11)	-0.20 (11)	-0.30 (11)	0.15 (11)	-0.05 (23)	-0.10 (23)	-0.24 (23)
(% body weight)	-0.48 (11)	-0.29 (11)	-0.33 (11)	0.12 (11)	0.01 (23)	-0.08 (23)	-0.19 (23)
Subcutaneous adiposity (g)	-0.23 (11)	-0.25 (11)	-0.12 (11)	-0.09 (11)	0.00 (21)	-0.02 (21)	-0.02 (21)
(% body weight)	-0.18 (11)	-0.35 (11)	0.00 (11)	-0.26 (11)	0.13 (21)	0.03 (21)	0.10 (21)
Combined muscle (g)	0.36 (11)	0.37 (11)	-0.15 (11)	0.07 (11)	0.18 (22)	0.15 (22)	0.26 (22)
(% body weight)	0.51 (11)	0.35 (11)	0.04 (11)	-0.07 (11)	0.38 (22)*	0.23 (22)	0.45 (22)*
Mixed muscle (g)	0.37 (11)	0.38 (11)	-0.16 (11)	0.08 (11)	0.19 (23)	0.16 (23)	0.24 (23)
(% body weight)	0.50 (11)	0.36 (11)	0.03 (11)	-0.06 (11)	0.37 (23)*	0.23 (23)	0.45 (23)*
Type 2 muscle (g)	0.04 (11)	0.00 (11)	0.07 (11)	-0.12 (11)	-0.07 (22)	-0.08 (22)	0.00 (22)
(% body weight)	0.28 (11)	-0.01 (11)	0.40 (11)	-0.37 (11)	0.12 (22)	-0.02 (22)	0.19 (22)
Liver (g)	-0.36 (11)	0.12 (11)	-0.50 (11)	0.49 (11)	-0.22 (23)	-0.38 (23)*	-0.51 (23)**
(% body weight)	-0.25 (11)	0.08 (11)	-0.32 (11)	0.34 (11)	-0.14 (23)	-0.35 (23)*	-0.34 (23)

r represents the correlation coefficient between each variable of adult whole body insulin sensitivity or circulating lipid profile and each variable of adult adiposity, skeletal muscle mass or liver weight. Numbers in parentheses represent the number of animals. Statistical significance of correlation coefficients: $^{*}P < 0.05$, $^{**}P < 0.01$. SSGIR, steady state glucose infusion rate ($mg.min^{-1}.kg^{-1}$); AdjSSGIR, adjusted steady state glucose infusion rate ($mg.min^{-1}.kg^{-1}$ $\mu U.ml^{-1}$); SSPHI, steady state plasma human insulin, ($\mu U\ ml^{-1}$); MCRi, metabolic clearance rate of insulin; FPFFA, fasting plasma free fatty acids ($meq\ l^{-1}$); FPTRIG, fasting plasma triglycerides ($mmol\ l^{-1}$); FPCHOL, fasting plasma total cholesterol ($mmol\ l^{-1}$).

and mixed (r=0.33, p=0.012, n=48) muscle mass (Table 4.5). In male offspring, parameters of the adult circulating lipid profile were not associated with any measures of skeletal muscle mass (Table 4.6). In female offspring, fasting plasma free fatty acid and total cholesterol concentrations correlated positively with relative combined (r=0.38, p=0.039 and r=0.45, p=0.018, respectively, n=22) and mixed muscle mass (r=0.37, p=0.041 and r=0.45, p=0.015, respectively, n=23) (Table 4.7).

4.3.4.3 Liver weight

In all male and female offspring combined, the steady state glucose infusion rate correlated negatively with liver (r=-0.37, p=0.032, n=26) and relative liver (r=-0.43, p=0.014, n=26) weight (Table 4.5). In male offspring, the steady state glucose infusion rate and adjusted steady state glucose infusion rate correlated negatively (r=-0.49, p=0.031 and r=-0.53, p=0.02, respectively, n=15) and the steady state plasma human insulin concentration correlated positively (r=0.51, p=0.027, n=15) with liver weight and the steady state glucose infusion rate correlated negatively (r=-0.57, p=0.013, n=15) with relative liver weight (Table 4.6). In female offspring, parameters of adult whole body insulin sensitivity were not associated with any measures of liver weight (Table 4.7).

In all male and female offspring combined, fasting plasma total cholesterol concentrations correlated negatively with liver weight (r=-0.31, p=0.016, n=48) (Table 4.5). In male offspring, parameters of the adult circulating lipid profile were not associated with any measures of liver weight (Table 4.6). In female offspring, fasting plasma triglyceride and total cholesterol concentrations correlated negatively (r=-0.38, p=0.036 and r=-0.51, p=0.006, respectively, n=23) with liver weight and fasting plasma triglyceride concentrations correlated negatively (r=-0.35, p=0.048, n=23) with relative liver weight (Table 4.7).

4.4 DISCUSSION

The current study has demonstrated that in the guinea pig, perturbed growth in early life alters both whole body insulin sensitivity of glucose metabolism, and the circulating lipid profile of aged offspring, but in a sex-specific manner. Specifically, small size at birth was associated with impaired whole body insulin sensitivity of glucose metabolism in aged female offspring, and adult whole body insulin sensitivity of glucose metabolism was reduced by 51% in low birth weight female offspring when compared to their high birth weight counterparts. In contrast in males, it was offspring that were large at birth that demonstrated reduced whole body insulin sensitivity of glucose metabolism as aged adults. However slow growing male offspring demonstrated reduced adult whole body insulin sensitivity of glucose metabolism and elevated fasting plasma free fatty acid concentrations when compared to their fast growing counterparts. Male offspring that were disproportionately growth restricted *in utero*, as indicated by a low ponderal index at birth, demonstrated increased fasting plasma total cholesterol concentrations as adults, however spontaneous fetal growth restriction did not alter fasting plasma lipid concentrations in aged females. An accelerated neonatal fractional growth rate was associated with elevated fasting plasma triglyceride concentrations in both aged male and female offspring however. Thus the outcomes for insulin sensitivity and lipid profiles in the aged guinea pig of variations in prenatal and early postnatal growth differ between males and females.

It is important to note that the definition of fetal growth restriction used in this study, namely a birth weight below the median, differs from clinical IUGR and SGA, and in fact variations in fetal growth and the programming phenomenon are typically on a continuum.

In aged female guinea pigs, impaired whole body insulin sensitivity of glucose metabolism was associated with poor fetal growth, as indicated by a reduced weight, length or head length at birth. These findings are consistent with the observations of epidemiological studies in a number of populations, which have demonstrated that a low weight or ponderal index at birth is associated with insulin resistance in adult life, albeit in both males and females (Jacquet *et al.*, 2000; Flanagan *et al.*, 2000; McKeigue *et al.*, 1998; Phillips *et al.*, 1994; Lithell *et al.*, 1996). In aged male

guinea pigs however, small size at birth was not associated with any change in whole body insulin sensitivity of glucose metabolism. Interestingly, a recent UK study of elderly men and women has shown that insulin resistance was inversely related to birth weight more strongly in women than in men (Phillips *et al.*, 2005).

A number of putative mechanisms have been proposed to explain the relationship between impaired fetal growth and insulin resistance in adult life. As skeletal muscle is a major target of insulin action, it has been suggested that structural or functional defects in this tissue, as a result of perturbed muscle development *in utero*, may explain in part the relationship between restricted fetal growth and insulin resistance postnatally. Decreased insulin stimulated peripheral glucose uptake in adult men and women of low birth weight has been associated with a reduced expression of the GLUT-4 glucose transporter in skeletal muscle (Jacquet *et al.*, 2001), while a reduced expression of proximal signalling molecules in the skeletal muscle of low birth weight adults has also been reported (Ozanne *et al.*, 2003), indicating that insulin resistance following perturbed fetal growth is characterised by post-receptor defects in skeletal muscle insulin signalling.

Maternal undernutrition in the guinea pig has been shown to impair the development of skeletal muscle in offspring (Dwyer *et al.*, 1995; Dwyer *et al.*, 1992), reducing the number of fibres within muscle (Dwyer *et al.*, 1995). A recent study in this cohort of aged guinea pigs has demonstrated that impaired fetal growth, as indicated by a reduced weight or length at birth, was associated with a reduction in the proportion of type I insulin-sensitive fibres, and an increase in the proportion of type IIa fibres which are less insulin sensitive, in the vastus lateralis of aged female offspring (JM Forbes *et al.*, unpublished observations). Furthermore, adult whole body insulin sensitivity of glucose metabolism in these animals was positively associated with the proportion of type I fibres, and negatively associated with the proportion of type IIa fibres. These findings suggest that the impaired whole body insulin sensitivity of glucose metabolism observed in the current study, in aged female offspring following spontaneous fetal growth restriction may be due in part to persistent alterations in skeletal muscle structure in response to an adverse intrauterine environment.

Increased activity of, and responsiveness to the HPAA has also been suggested as a possible link between restricted growth *in utero* and impaired insulin sensitivity in adult life. Low birth weight has been shown to be associated with increased 0900h plasma cortisol concentrations in men aged 64 years (Phillips *et al.*, 1998) and men and women aged 50 years in the UK (Phillips *et al.*, 2000), and in 20 year old men and women in Australia (Phillips *et al.*, 2000). In addition, fasting plasma cortisol concentrations have been shown to be inversely related to insulin sensitivity in elderly men (Phillips *et al.*, 1998). Elevated fasting cortisol levels may represent a stress response, due to the combination of fasting and the novel clinic setting in which the blood samples were taken (Phillips *et al.*, 2006). More recently, a study of boys and girls aged between 7 and 9 years has shown that birth weight was inversely related to salivary cortisol responses to psychological stress in boys, while in girls birth weight was inversely related to morning peak cortisol levels (Jones *et al.*, 2006), suggesting that there are sex differences in the relationship between birth weight and the HPAA response to acute psychological stress. Fetal growth restriction may cause intrinsic changes in skeletal muscle increasing the effects of cortisol in this target tissue. For example, an increase in glucocorticoid receptors or a reduction in cortisol binding globulins or 11 beta hydroxy steroid dehydrogenase-2 (11BHSD-2), the enzyme responsible for cortisol inactivation, may increase skeletal muscle sensitivity to cortisol.

The present study has shown that spontaneous fetal growth restriction, as indicated by a low birth weight, is associated with increased adrenal size in the aged guinea pig (see Chapter 3), which may reflect an increased sensitivity or exposure of this gland to the trophic actions of ACTH. In addition, a recent study in this cohort of aged guinea pigs has demonstrated that impaired fetal growth, as indicated by thinness, or a reduced weight, length or abdominal circumference at birth, was associated with increased salivary cortisol concentrations in aged female offspring (S Grover *et al.*, unpublished observations). Previous studies in the same species have also shown that maternal dexamethasone treatment (Dean *et al.*, 1999; Liu *et al.*, 2001) or nutrient restriction (Lingas *et al.*, 2001) during late gestation programmes HPAA function in adolescent offspring in a sex-specific manner, with females demonstrating a greater susceptibility to programming of the HPAA than males. These findings suggest that the impaired insulin action observed in aged female guinea pigs following perturbed intrauterine growth, could be due to in part to an

increased activity of the HPAA. In aged male guinea pigs however, small size at birth did not alter salivary cortisol concentrations or adrenal size. These findings could explain in part, why spontaneous fetal growth restriction is associated with impaired insulin sensitivity in aged female, but not male, guinea pigs.

Increased activity of the SNS established *in utero* as a result of restricted fetal growth, is another mechanism by which small size at birth and insulin resistance in adult life may be linked. Catecholamines are able to induce insulin resistance, while low birth weight babies have been shown to have increased heart rate, an index of SNS activity, when compared with controls during sleep (Spassov *et al.*, 1994). In addition, a direct relationship between adult pulse rate, which is an index of SNS activity, and birth weight has been shown (Phillips *et al.*, 1997). Recently, low birth weight has been shown to be associated with an enhanced heart rate response to psychological stressors in women, but not men (Ward *et al.*, 2004), while a study of boys and girls aged between 7 and 9 years has shown that cardiac SNS activation both at rest and during stress, was greater in girls who were small in size at birth, however this relationship was not as strong in males (Jones *et al.*, 2005). These findings suggest that there are sex differences in the relationship between birth weight and the sympathoadrenal response to acute psychological stress. Additionally, experimental studies have demonstrated that low birth weight is associated with increased plasma noradrenalin concentrations in juvenile pigs (Poore *et al.*, 2003) and elevated heart rate in young adult guinea pigs (Persson *et al.*, 1992). In the present study, heart rate was inversely related to size at birth (see Chapter 6) and positively associated with fasting plasma insulin levels, in aged female offspring (data not shown). These findings suggest that the impaired insulin sensitivity observed in these animals following spontaneous fetal growth restriction may be due, at least partly to increased activity of the SNS. In aged male guinea pigs however, small size at birth did not alter resting heart rate or fasting plasma insulin levels. These findings could explain in part, why spontaneous fetal growth restriction is associated with impaired insulin sensitivity in aged female, but not male, guinea pigs.

In male guinea pigs however, it was excessive fetal growth, as indicated by an increased weight or weight to length ratio at birth, which was associated with impaired whole body insulin sensitivity of glucose metabolism in aged offspring. This relationship between large size at birth and impaired adult insulin action has

been observed in several human populations, most notably the Pima Native Americans (McCance *et al.*, 1994; Dabelea *et al.*, 1999). The majority of individuals who are born large for gestational age are thought to be overgrown as a result of gestational diabetes. In gestational diabetes, the fetus is exposed to elevated levels of glucose, amino acids and insulin which accelerates the growth of lean body mass and fat in the short term (Vohr *et al.*, 1997), and predisposes the individual to insulin resistance, impaired glucose tolerance and NIDDM in adult life (McCance *et al.*, 1994; Dabelea *et al.*, 1999). Interstingly, in this study, the majority of male guinea pigs who were large at birth came from medium or large, rather than small litters. It is possible however that these larger guinea pigs may have been exposed to a different metabolic environment than their smaller littermates before birth, perhaps because of their position in the uterus, which may explain, at least in part, their altered metabolic function as adults.

In the current study we have shown that disproportionate fetal growth, as indicated by a reduced ponderal index at birth, was associated with elevated fasting plasma total cholesterol concentrations in aged male offspring. These results are consistent with the findings in human populations of increased plasma total cholesterol concentrations in children and young adults who were thin at birth (Moore 1997). Disproportionate or asymmetrical fetal growth restriction is thought to be indicative of nutrient deprivation in late gestation, when the growth of the brain is sustained at the expense of the trunk (Robinson *et al.*, 1996). The liver, which grows rapidly during late gestation, may be particularly compromised by a reduction in the supply of nutrients at this time.

Studies in the rat have shown that the offspring of dams fed a reduced-protein diet during pregnancy and lactation have large structural and functional changes in their liver, including a reduction in the number of liver lobules (Burns *et al.*, 1997). Studies have also shown that enzymes involved in cholesterol metabolism are sectioned or zoned in specific areas within the liver (Jungermann *et al.*, 1989). In the rat, manipulation of maternal or perinatal nutrition has been shown to exert long-term effects on the hepatic activity of HMG CoA reductase and cholesterol 7α-hydroxylase, key enzymes of cholesterol metabolism (Innis 1985). Therefore, the increased plasma total cholesterol concentrations observed here in aged male offspring following disproportionate growth *in utero* may be due at least in part to

changes in the expression of key enzymes involved in cholesterol metabolism, as a result of structural changes in the liver.

In addition to excessive fetal growth, a low fractional growth rate during the neonatal period also impaired whole body insulin sensitivity of glucose metabolism in aged male guinea pigs. These observations are consistent with the findings of a recent Finnish study, which has shown that babies of above-average birth weight are at an increased risk of developing NIDDM in adult life if their growth in the first few months after birth falters (Eriksson *et al.*, 2003). The risk of developing NIDDM in adult life was greatest in children whose birth weights were greater than 3.5 kg and who also demonstrated low rates of linear growth during the first 3 months of life. It was suggested that growth faltering during early infancy is associated with a long-term impairment of insulin metabolism which in turn impairs their ability to meet the challenge of the rapid childhood increase in BMI seen in many of these children (Eriksson *et al.*, 2003).

In the general population, insulin resistance is strongly associated with a central pattern of obesity, in which a high proportion of body fat is deposited in intra-abdominal depots (Stern *et al.*, 1986). This relationship is thought to be mediated by the elevated free fatty acid concentrations that accompany increased central adiposity, and which have been shown to impair the insulin sensitivity of liver (Rebrin *et al.*, 1996) skeletal muscle (Magnan *et al.*, 1996) and adipose tissue (Van Epps Fung *et al.*, 1997). Plasma free fatty acid concentrations are largely determined by the action of insulin to suppress adipocyte lipolysis, as in the physiological state insulin suppresses hormone sensitive lipase and promotes re-esterification of non-esterified free fatty acids (Yki-Jarvinen *et al.*, 1988; Weiland *et al.*, 1980). Exposure of skeletal muscle to elevated plasma free fatty acid concentrations has been shown to impair insulin-stimulated glucose uptake in both rats (Kim *et al.*, 1996; Park *et al.*, 1998; Jucker *et al.*, 1997; Griffin *et al.*, 1999) and humans (Kelley *et al.*, 1993; Boden *et al.*, 1994; Ferrannini *et al.*, 1983).

Interestingly, in this study a low fractional growth rate during the neonatal period, both reduced whole body insulin sensitivity of glucose metabolism, and also increased fasting plasma free fatty acid concentrations in the aged male guinea pig. While no significant association between whole body insulin sensitivity of glucose

metabolism and fasting plasma concentrations of free fatty acids was observed in the current study, it remains possible that the relationship between poor neonatal growth and impaired insulin action may be mediated by chronic exposure to elevated plasma free fatty acid concentrations.

Plasma non-esterified free fatty acids are an important determinant of plasma triglyceride concentrations, since the rate of supply of non-esterified free fatty acids to the liver is a major factor controlling hepatic triglyceride secretion (Byrne *et al.*, 1992; Kissebah *et al.*, 1974). Hepatic triglyceride synthesis is controlled by the availability of non-esterified free fatty acid substrates and by the enzymes phosphatidate phosphohydrolase and diacylglycerol acyltransferase. Both of these enzymes are stimulated by non-esterified free fatty acids (Cascales *et al.*, 1984; Haagsman *et al.*, 1981). Consequently failure to adequately suppress non-esterified free fatty acids leads to an increase in VLDL triglyceride levels.

In the current study, male and female guinea pigs that experienced accelerated growth as neonates had higher fasting plasma triglyceride concentrations as aged adults. Considering that poor neonatal growth increased fasting plasma free fatty acid concentrations in aged male guinea pigs, and hepatic triglyceride synthesis is controlled in part by the availability of non-esterified free fatty acids, this positive association is somewhat surprising. However, while it is well recognised that plasma free fatty acids stimulate VLDL production (Lewis *et al.*, 1995) and are an important source of VLDL triglyceride fatty acids (Havel *et al.*, 1970; Barter *et al.*, 1973; Diraison *et al.*, 1998), an important contribution to the hepatocyte fatty acid pool also comes from three sources other than plasma free fatty acids, including *de novo* lipogenesis, cytoplasmic triglyceride stores, and intracellular lipolysis of lipoproteins taken up directly by the liver (Diraison *et al.*, 1998; Parks *et al.*, 1999). In addition, while hypertriglyceridaemia is due primarily to VLDL overproduction, reduced VLDL clearance does play a role in some instances (Lewis *et al.*, 1996). Therefore, the elevated fasting plasma triglyceride concentrations observed in aged male guinea pigs following accelerated growth in the neonatal period may be due to factors other than an increased supply of plasma free fatty acids, including a reduced VLDL clearance rate.

In aged female guinea pigs, in addition to the positive association with neonatal growth, fasting plasma triglyceride levels were negatively associated with adult liver weight. Interestingly, aged female guinea pigs that displayed high rates of growth during the neonatal period demonstrated reduced liver weights when compared to their slow growing counterparts (see Chapter 3). These findings suggest that the relationship between accelerated neonatal growth and elevated fasting plasma triglyceride concentrations may be mediated by alterations in hepatic structure and function.

In summary, spontaneous fetal growth restriction in the guinea pig reduced whole body insulin sensitivity of glucose metabolism in aged female offspring, while in males excessive fetal growth was associated with impaired insulin action in aged adults. Fasting plasma total cholesterol concentrations were increased in aged male offspring that were disproportionately growth restricted *in utero*. Altered postnatal growth also influenced the circulating lipid profile of aged offspring, with elevated fasting plasma triglyceride concentrations observed in male and female offspring who experienced accelerated neonatal growth. The aged male guinea pig that grew slowly as a neonate also demonstrated reduced adult whole body insulin sensitivity of glucose metabolism and elevated fasting free fatty acid concentrations when compared to its fast growing counterparts. Therefore it appears that in the guinea pig, perturbed growth in early life programmes whole body insulin sensitivity and lipid metabolism in aged offspring, but differently with sex. As insulin resistance is thought to be the primary underlying defect in the IRS, it seems plausible that the perinatal programming of other components of this syndrome in the aged guinea may also occur in a sex-specific manner.

CHAPTER 5

EARLY LIFE INFLUENCES ON GLUCOSE TOLERANCE AND INSULIN SECRETION IN THE AGED GUINEA PIG

5.1 INTRODUCTION

Epidemiological studies have established that small size at birth is associated with an increased risk of impaired glucose tolerance and NIDDM in adult life (Hales *et al.*, 1991; Phipps *et al.*, 1993; Lithell *et al.*, 1996; Ravelli *et al.*, 1998). In addition to these studies in older adults, studies in children (Yajnik *et al.*, 1995; Law *et al.*, 1995) and young adults (Robinson *et al.*, 1992) have shown that plasma glucose levels in the basal state and after an oral glucose load are inversely related to birth weight. In addition, postnatal catch-up growth in terms of weight and height has also been associated with an increased risk of impaired glucose tolerance and NIDDM in childhood (Crowther *et al.*, 1998) and adult life (Forsen *et al.*, 2000).

Several experimental models of IUGR have demonstrated that perturbed growth in early life is associated with a loss of glucose tolerance (Garofano *et al.*, 1999; Hales *et al.*, 1996; Petry *et al.*, 2001; Simmons *et al.*, 2001; Poore *et al.*, 2002) and both increased (Poore *et al.*, 2002) and reduced (Jansson *et al.*, 1999; Simmons *et al.*, 2001) insulin secretion in response to a glucose challenge, in adult offspring. However, only a small number of studies in non-human species have investigated the effect of suboptimal growth *in utero* on postnatal glucose tolerance and insulin secretion in aged offspring. Further to this, few experimental studies have examined the influence of early postnatal growth on adult glucose homeostasis.

We have previously shown that moderate maternal feed restriction in the guinea pig (85% *ad libitum* intake) causes fasting hyperinsulinaemia and an increased insulin response to an IVGTT, in young adult male offspring (Kind *et al.*, 2003). In addition, male offspring that experienced accelerated neonatal growth, demonstrated elevated fasting plasma insulin concentrations as young adults (Kind *et al.*, 2003).

The aims of the studies described in Chapter 5 were therefore to determine the effects of (1) spontaneous fetal growth restriction, due to natural variations in litter

size, and (2) accelerated neonatal growth, on glucose tolerance and insulin secretion, in the aged guinea pig. The influence of sex on the impact of altered perinatal growth on glucose tolerance and insulin secretion in the aged adult was also assessed, as were the relationships between adult size and body composition and glucose tolerance and insulin secretion. We hypothesised that spontaneous fetal growth restriction and accelerated neonatal fractional growth rate for weight in the guinea pig would increase fasting plasma glucose and insulin concentrations, and decrease glucose tolerance and insulin secretion in response to an IVGTT, in aged male and female offspring.

5.2 MATERIALS AND METHODS

5.2.1 Animals

Nulliparous, 3 to 4 month old female guinea pigs (IMVS Tri-coloured) were obtained from the Gilles Plains Animal Resource Centre (Gilles Plains, SA, Australia), and following a two-week acclimatisation period, females in oestrous were pair mated with male guinea pigs (IMVS Tri-coloured) overnight (as described in Section 2.2.1). At 60 days gestation, pregnant animals were transferred to plastic tubs containing paper bedding, where they gave birth. Offspring were weighed at birth, and the nose to rump length, abdominal circumference, and head length and head width of each pup was measured. A total of 27 pups born to 17 mothers were randomly assigned to this study of aged guinea pigs (12 male and 15 female), with the remaining offspring allocated to other studies (as described in Section 2.2.1). A description of the total number of litters used and their size is summarised in Appendix A. Of the 27 pups assigned to this study, 19 had at least one other litter mate in the study while 8 did not. Fasting plasma glucose concentrations obtained from an additional 27 animals prior to the HEC (described in Chapter 4) were included in the analyses. The size at birth and neonatal growth rate characteristics of these additional animals were largely similar to those of animals assigned to this study. All pups were weighed daily from birth to 30 days of age, and the neonatal absolute ($AGR_{10\text{-}30}$) (g day^{-1}) and fractional ($FGR_{10\text{-}30}$) (g day^{-1} g^{-1}) growth rates for weight were calculated from 10 days of age to weaning at 30 days of age (as described in Section 3.2.1), following which pups were transferred to individual wire-bottomed cages and weaned onto normal guinea pig chow *ad libitum*. Postnatal food intakes were not able to be measured for logistic reasons.

At 412 ± 4 days of age catheters were inserted into the right carotid artery and jugular vein under general anaesthesia (as described in Section 2.2.1). Catheter patency was maintained by daily flushing with 800 µl of heparinized saline (250 IU ml^{-1}, Multiparin, Fisons Pharmaceuticals, NSW, Australia). Animals were allowed at least 5 days postoperative recovery before the commencement of *in vivo* studies. It was not possible to obtain all data from all animals due to human resource limitations, and the number of observations for each experimental data set is indicated in each table or figure. All procedures in this study were reviewed and approved by the University of Adelaide Animal Ethics Committee.

5.2.2 Intravenous glucose tolerance test

At 418 ± 4 days of age, and following a 16-hour overnight fast, an IVGTT was performed to determine glucose tolerance and insulin secretion (as described in Section 2.2.3).

Glucose tolerance was measured as the area under the glucose concentration curve (AUGC). Absolute insulin secretion was measured as the area under the insulin concentration curve (AUIC) and relative insulin secretion was calculated as AUIC divided by AUGC. Plasma insulin and glucose concentrations throughout the IVGTT were plotted using SigmaPlot (Jandel Scientific Software, CA, USA). Areas under the glucose (AUGC) or insulin (AUIC) curves were calculated as follows. The mean plasma glucose or insulin concentrations prior to dextrose administration were used as the baseline and the area under the plasma glucose or insulin profile determined using SigmaScan Pro image analysis software (Jandel Scientific Software, CA, USA). The glucose tolerance index (K_G), which provides an estimate of the rate of glucose elimination after glucose dose was also calculated. The slope of the regression line obtained from the plot of the natural logarithm-transformed plasma glucose concentrations between 2 and 60 minutes was expressed as a percentage per minute (Kind *et al.*, 2003).

5.2.3 Plasma metabolite and hormone analyses

Guinea pig insulin concentrations in plasma were analysed in duplicate by radioimmunoassay using guinea pig insulin standards, as described previously (Gorray *et al.*, 1980; Kind *et al.*, 2003) with an intra-assay coefficient of variation of 8.4% and an interassay coefficient of variation of 10.9%. Plasma glucose concentrations were measured in duplicate by enzymatic colorimetric analysis on a COBAS Mira automated centrifugal analyser using a commercially available kit and control sera, with intra and interassay coefficients of variation below 5% (as described in Section 2.2.5)

5.2.4 Body composition

At 428 ± 5 days of age, and following a 20 hour-overnight fast, animals were sacrificed by an intravenous overdose of sodium pentobarbitone (Virbac, NSW, Australia), and a post mortem was performed between 1400h and 1600h. Body weight and nose to rump length were measured and the body mass index was calculated (weight/nose to rump length^2). Selected skeletal muscles and adipose depots were dissected out, weighed immediately, summed, and expressed as a percentage of body weight at post mortem, to give an index of the percentage of body weight composed of mixed and type 2 muscle and visceral and subcutaneous adipose tissue (as described in Section 2.2.6). In addition, all individual skeletal muscle and adipose depot weights were summed and expressed as a percentage of body weight at post mortem, to give an index of the percentage of body weight composed of skeletal muscle (combined muscle) and adipose tissue (combined adiposity) respectively. The pancreas and liver were also dissected out and weighed. All adipose depot, skeletal muscle, and pancreas and liver weights were expressed in absolute terms as well as in relative terms as a percentage of body weight at post mortem.

5.2.5 Statistical analysis

All statistical analyses were carried out using SPSS for Windows (Version 13.0, SPSS Inc., Chicago, IL, USA). To examine the effect of size at birth on adult glucose tolerance and insulin secretion, offspring were classed into two groups, those with birth weights greater than (high birth weight) or less than (low birth weight) the median birth weight for the cohort of guinea pigs described in this thesis, which was 95.55 grams. Males and females were also analysed separately, classing each as high or low birth weight, using the median birth weight for each sex (94.8 grams for males and 99.58 grams for females). The definition of fetal growth restriction in experimental studies in non-human species varies, and this approach of below versus above the median size at birth has been commonly used in these studies, as well as in some human studies. The effect of birth weight class on adult glucose tolerance and insulin secretion was assessed by a single between factor ANOVA in all animals combined, and in males and females separately. The effects of birth weight class, sex and their interaction on adult glucose tolerance and insulin secretion were examined

by a two between factor ANOVA. Specific comparisons were carried out by Bonferroni post hoc tests.

To examine the effect of neonatal fractional growth rate on adult glucose tolerance and insulin secretion, offspring were classed into two groups as either 'high growers' or 'low growers' using the following approach. In order to classify animals according to growth, males and females were separated, and the neonatal fractional growth rate was plotted against birth weight for each sex separately. Animals above and below the regression line were classified as 'high growers' and 'low growers' respectively. The effect of growth rate class on adult glucose tolerance and insulin secretion was assessed by a single between factor ANOVA in all animals combined, and in males and females separately. A two between factor ANOVA was used to determine the effect of growth rate class, sex and their interaction, on adult glucose tolerance and insulin secretion. Specific comparisons were carried out by Bonferroni post hoc tests.

Relationships between adult glucose tolerance and insulin secretion and size at birth, neonatal growth rates and adult size and body composition were examined using simple correlation and multiple linear regression analyses, in all animals combined and in male and female offspring separately. One sided p-values were used to test *a priori* hypotheses regarding the relationships between adult glucose tolerance and insulin secretion and size at birth and neonatal growth rates, based on similar relationships reported in humans.

For all statistical tests, significance was accepted at $P<0.05$. All data are presented as mean ± S.E.M.

5.3 RESULTS

5.3.1 Effect of birth weight class on adult glucose tolerance and insulin secretion in the aged guinea pig

In all male and female offspring combined, low birth weight increased fasting plasma glucose concentrations (+9%) ($p<0.04$) and decreased relative insulin secretion (AUIC:AUGC) (-45%) ($p<0.05$) (Table 5.1). Low birth weight altered a number of parameters of adult glucose tolerance and insulin secretion differently with sex, such that fasting plasma glucose and insulin concentrations and the ratio of fasting plasma insulin to glucose tended to increase with low birth weight in females, but to decrease in males ($p<0.05$ for all) (Table 5.1). In male offspring only, fasting plasma insulin concentrations (-58%) ($p<0.02$) (Figure 5.1b), the ratio of fasting plasma insulin to glucose (-51%) ($p<0.03$) (Figure 5.1c) and insulin secretion in absolute (AUIC) (-60%) ($p<0.01$) (Figure 5.2a) and relative terms (AUIC:AUGC) (-68%) ($p<0.005$) (Figure 5.2b) were reduced in low compared to high birth weight offspring. In female offspring only, fasting plasma glucose concentrations were increased in low compared to high birth weight offspring (+26%) ($p<0.001$) (Figure 5.1a).

5.3.2 Effect of neonatal fractional growth rate class on glucose tolerance and insulin secretion in the aged guinea pig

Neonatal fractional growth rate class did not alter any parameter of adult glucose tolerance or insulin secretion in all male and female offspring combined (Table 5.2), or in male or female offspring only (data not shown). Neonatal fractional growth rate class did alter the fasting plasma insulin to glucose ratio differently with sex however, such that it tended to decrease with high neonatal fractional growth rate in females, but increase in males ($p<0.05$) (Table 5.2). Inclusion of birth weight as a co-variate did not alter these outcomes.

Table 5.1 Effect of birth weight class and sex on glucose tolerance and insulin secretion in the aged guinea pig

	Birth weight class				ANOVA *P*-value		
	Low birth weight		High birth weight		BW	S	BW x S
	Males (n=7)	Females (n=6)	Males (n=5)	Females (n=9)			
Fasting plasma glucose (mmol l^{-1})*	8.35 ± 0.36	10.34 ± 0.60	8.91 ± 0.42	8.25 ± 0.29	0.035	NS	0.001
Fasting plasma insulin (ng ml^{-1})	8.34 ± 1.73	18.54 ± 3.68	19.83 ± 4.84	11.43 ± 2.49	NS	NS	0.004
Fasting plasma insulin to glucose ratio	0.95 ± 0.20	1.59 ± 0.28	1.92 ± 0.42	1.39 ± 0.32	NS	NS	0.039
Area under the glucose curve (mmol l^{-1} min^{-1})	724 ± 94	695 ± 161	570 ± 116	583 ± 61	NS	NS	NS
Area under the insulin curve (ng ml^{-1} min^{-1})	1736 ± 329	3131 ± 905	4300 ± 910	3095 ± 821	NS	NS	NS
AUIC:AUGC	2.49 ± 0.50	4.47 ± 0.75	7.82 ± 1.56	5.22 ± 1.13	0.045	NS	NS
Glucose tolerance index, K_G (% min^{-1})	0.97 ± 0.14	0.95 ± 0.15	1.05 ± 0.11	1.08 ± 00.13	NS	NS	NS

Data are presented as means ± S.E.M. n refers to the number of animals. Statistical significance was assumed at $P < 0.05$. NS, not significant. *Contains data from a larger cohort of low birth weight (males, n=13; females, n=12) and high birth weight animals (males, n=15; females, n=17). AUIC:AUGC, ratio of area under the insulin curve to area under the glucose curve. ANOVA: effect of birth weight (BW), sex (S) and their interaction (BW x S).

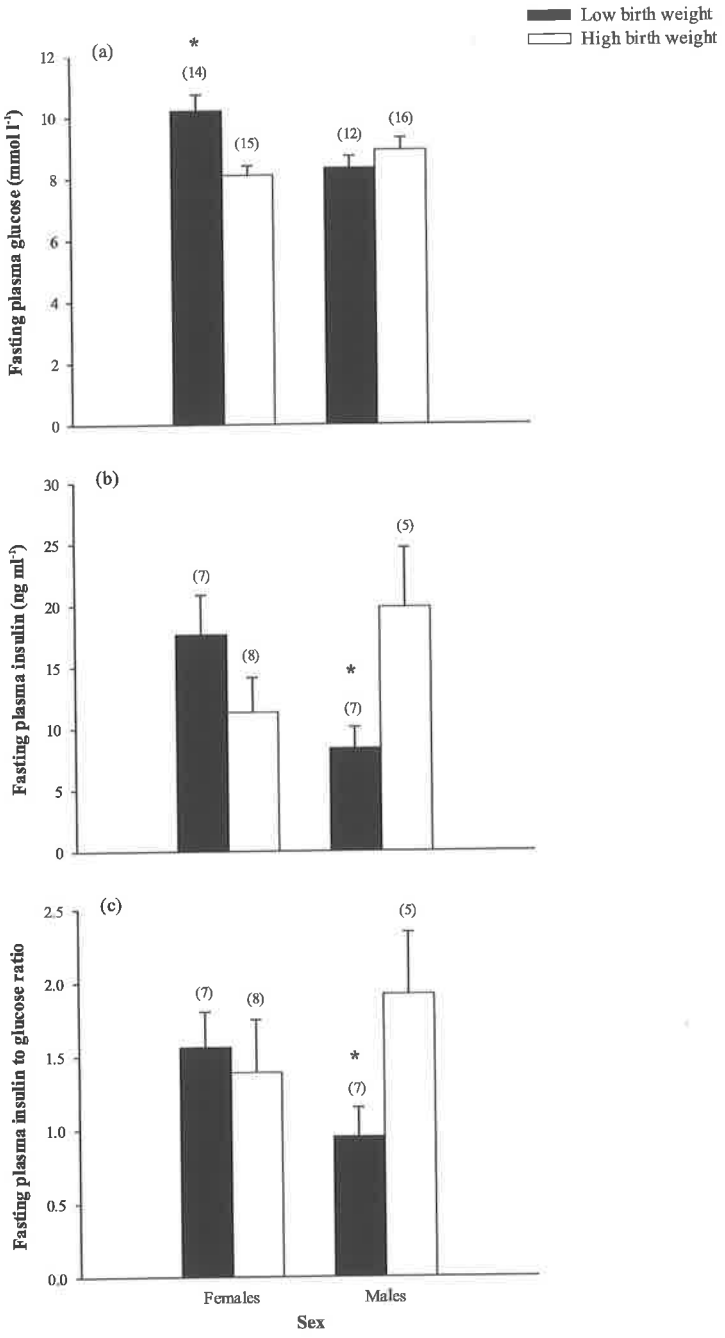

Figure 5.1 The effect of birth weight class and sex on fasting plasma glucose and insulin concentrations and the ratio of fasting plasma insulin to glucose in the aged guinea pig

Fasting plasma glucose (a) and insulin (b) concentrations and the ratio of fasting plasma insulin to glucose (c) in aged adult guinea pigs of low and high birth weight. Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. *$P < 0.05$ compared with high birth weight offspring of the same sex.

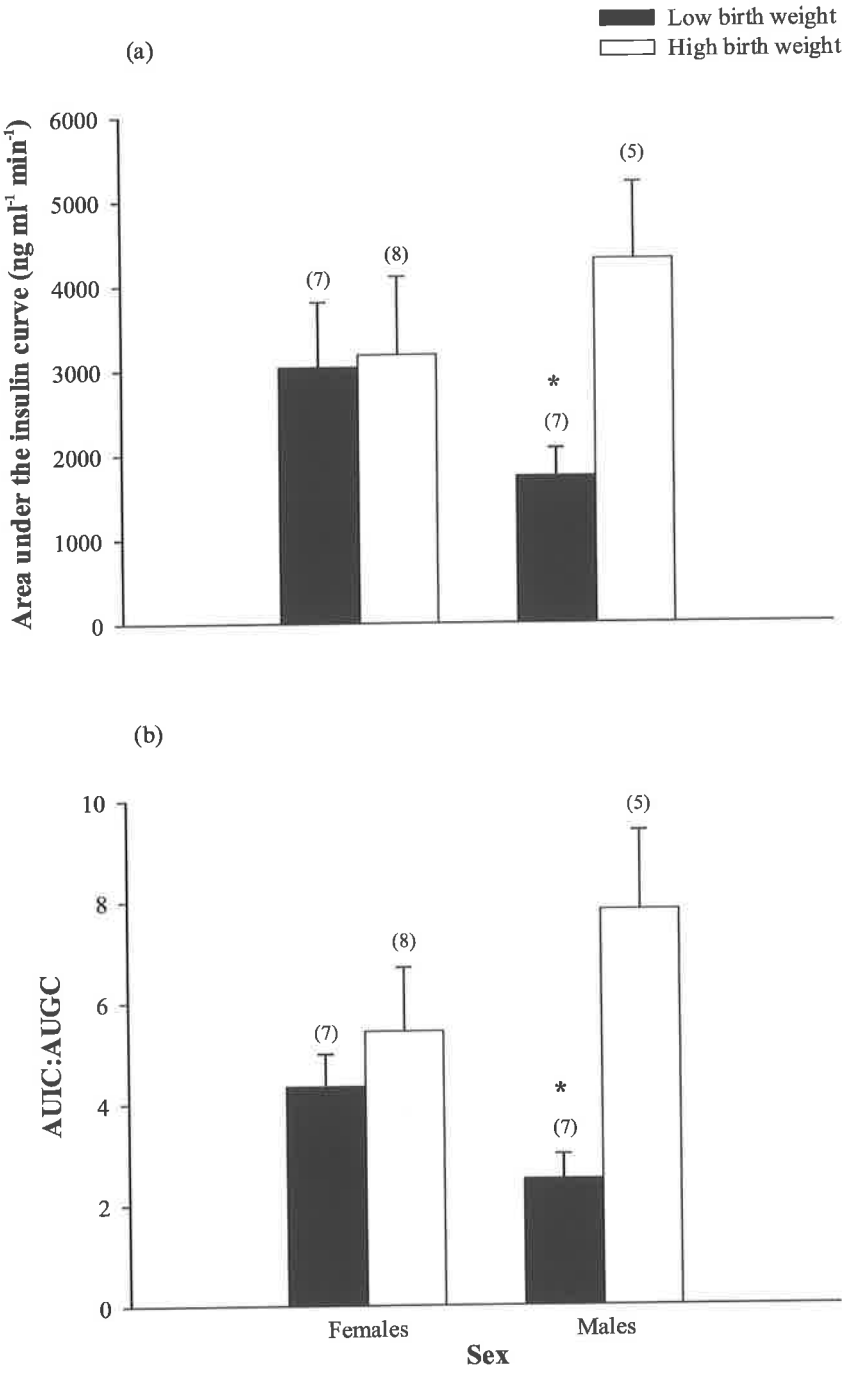

Figure 5.2 The effect of birth weight class and sex on insulin secretion in the aged guinea pig

The area under the insulin curve (a) and the ratio of the area under the insulin curve to the area under the glucose curve (AUIC:AUGC) (b) during the IVGTT in aged adult guinea pigs of low and high birth weight. Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. *$P < 0.05$ compared with high birth weight offspring of the same sex.

Table 5.2 Effect of neonatal fractional growth rate class and sex on glucose tolerance and insulin secretion in the aged guinea pig

	Neonatal fractional growth rate class				ANOVA *P*-value		
	Low growth rate		High growth rate		GR	S	GR x S
	Males (*n*=3)	Females (*n*=11)	Males (*n*=9)	Females (*n*=4)			
Fasting plasma glucose (mmol l^{-1})*	8.43 ± 0.32	9.16 ± 0.51	8.85 ± 0.45	9.04 ± 0.44	NS	NS	NS
Fasting plasma insulin (ng ml^{-1})	9.06 ± 3.21	16.32 ± 2.75	14.49 ± 3.44	8.64 ± 1.54	NS	NS	NS
Fasting plasma insulin to glucose ratio	1.01 ± 0.33	1.68 ± 0.26	1.47 ± 0.31	0.88 ± 0.14	NS	NS	0.048
Area under the glucose curve (mmol l^{-1} min^{-1})	592 ± 109	642 ± 91	683 ± 93	590 ± 119	NS	NS	NS
Area under the insulin curve (ng ml^{-1} min^{-1})	2625 ± 820	3484 ± 750	2864 ± 708	2081 ± 673	NS	NS	NS
AUIC:AUGC	4.18 ± 0.84	5.42 ± 0.92	4.89 ± 1.38	3.56 ± 0.87	NS	NS	NS
Glucose tolerance index, K_G (% min^{-1})	1.15 ± 0.28	1.02 ± 0.10	0.96 ± 0.08	1.06 ± 0.25	NS	NS	NS

Data are presented as means ± S.E.M. *n* refers to the number of animals. Statistical significance was assumed at $P < 0.05$. NS, not significant.
*Contains data from a larger cohort of low growth rate (males, *n*=13; females, *n*=18) and high growth rate animals (males, *n*=15; females, *n*=11).
AUIC:AUGC, ratio of area under the insulin curve to area under the glucose curve. ANOVA: effect of neonatal fractional growth rate class (GR), sex (S) and their interaction (GR x S).

5.3.3 Relationship of glucose tolerance and insulin secretion to size at birth, neonatal growth rate and adult size in the aged guinea pig

5.3.3.1 Size at birth

In all male and female offspring combined, the fasting plasma glucose concentration correlated negatively with birth nose to rump length, head width at birth and the head width to abdominal circumference ratio at birth (r=-0.27, p=0.025; r=-0.34, p=0.006 and r=-0.23, p=0.05, respectively, n=54), the fasting plasma insulin concentration correlated negatively with head width at birth (r=-0.43, p=0.013, n=27), glucose tolerance (AUGC) correlated negatively with birth weight and birth weight to length ratio (r=-0.33, p=0.048 and r=-0.36, p=0.034, respectively, n=27), absolute insulin secretion (AUIC) correlated negatively with head width and the head width to abdominal circumference ratio at birth (r=-0.39, p=0.022 and r=-0.34, p=0.044, respectively, n=27) and relative insulin secretion (AUIC:AUGC) correlated positively with birth weight to length ratio (r=0.36, p=0.033, n=27) and negatively with head width to abdominal circumference ratio at birth (r=-0.35, p=0.036, n=27) (Table 5.3).

In male offspring, absolute insulin secretion correlated positively with birth weight to length ratio (r=0.50, p=0.048, n=12) (Figure 5.5c) and head length at birth (r=0.55, p=0.032, n=12) (Figure 5.6c).

In female offspring, fasting plasma glucose and insulin concentrations and glucose tolerance (AUGC) negatively with birth weight (r=-0.54, p=0.001, n=29; r=-0.45, p=0.045, n=15 and r=-0.45, p=0.046, n=15) (Figures 5.3a, b and c), fasting plasma glucose and insulin concentrations correlated negatively with birth nose to rump length (r=-0.44, p=0.009, n=28 and r=-0.47, p=0.041, n=15) (Figures 5.4a and b), fasting plasma glucose concentrations and glucose tolerance (AUGC) correlated negatively with the birth weight to length ratio (r=-0.38, p=0.022, n=28 and r=-0.44, p=0.049, n=15) (Figures 5.5a and b), fasting plasma glucose and insulin concentrations correlated negatively with head length at birth (r=-0.36, p=0.031, n=28 and r=-0.45, p=0.046, n=15) (Figures 5.6a and b), and fasting plasma glucose and insulin concentrations, the fasting plasma insulin to glucose ratio and absolute insulin secretion (AUIC) correlated negatively with head width at birth (r=-0.36, p=0.031, n=28; r=-0.56, p=0.015, n=15; r=-0.55, p=0.016, n=15 and r=-0.54, p=0.019, n=15) (Figures 5.7a, b, c and d).

Table 5.3 Relationship of glucose tolerance and insulin secretion to size at birth in the aged guinea pig

	Correlation coefficients (r)						
	FPG	FPI	I:G	AUGC	AUIC	AUIC:AUGC	K_G
BWT	-0.21 (57)	-0.04 (27)	0.09 (27)		0.12 (27)	0.31 (27)	0.21 (27)
BNRL	-0.27 (54)*	-0.09 (27)	-0.08 (27)	-0.20 (27)	-0.03 (27)	0.14 (27)	0.02 (27)
BWT:BNRL	-0.10 (54)	0.00 (27)	0.19 (17)	-0.36 (27)*	0.20 (27)	0.36 (27)*	0.31 (27)
BPI	0.18 (54)	0.13 (27)	0.27 (27)	-0.08 (27)	0.23 (27)	0.18 (27)	0.21 (27)
BAC	0.04 (54)	-0.17 (27)	-0.01 (27)	-0.24 (27)	0.14 (27)	0.23 (27)	0.08 (27)
BHL	-0.13 (54)	-0.15 (27)	-0.07 (27)	-0.16 (27)	0.12 (27)	0.16 (27)	-0.08 (27)
BHW	-0.34 (54)**	-0.43 (27)*	-0.32 (27)	-0.31 (27)	-0.39 (27)*	-0.23 (27)	0.30 (27)
BHW:BAC	-0.23 (54)*	-0.09 (27)	-0.15 (27)	0.06 (27)	-0.34 (27)*	-0.35 (27)*	0.06 (27)

r represents the correlation coefficient between each variable of adult glucose tolerance or insulin secretion and each variable of size at birth. Numbers in parentheses represent the number of animals. Partial correlations: all significant correlations highlighted in light grey were independent of $FGR_{10\text{-}30}$ and those highlighted in dark grey were independent of adult weight, all remaining significant correlations were independent of $FGR_{10\text{-}30}$ and adult weight. Statistical significance of correlation coefficients: $^{*}P < 0.05$ $^{**}P < 0.01$. FPG, fasting plasma glucose (mmol l^{-1}); FPI, fasting plasma insulin (ng ml^{-1}); I:G, fasting plasma insulin to glucose ratio; AUGC, area under the glucose curve (mmol l^{-1} min^{-1}); AUIC, area under the insulin curve (ng ml^{-1} min^{-1}); AUIC:AUGC, the ratio of the area under the insulin curve to the area under the glucose curve; K_G, the glucose tolerance index (% min^{-1}), provides an estimate of the rate of glucose elimination between 2 and 60 minutes of the intravenous glucose tolerance test. BW, birth weight (g); BNRL, birth nose to rump length (mm); BW:BNRL, birth weight to nose to rump length ratio (g mm^{-1}); BPI, birth ponderal index (g mm^{-3}); BAC, birth abdominal circumference (mm); BHL, birth head length (mm); BHW, birth head width (mm); BHW:BAC, birth head width to abdominal circumference ratio.

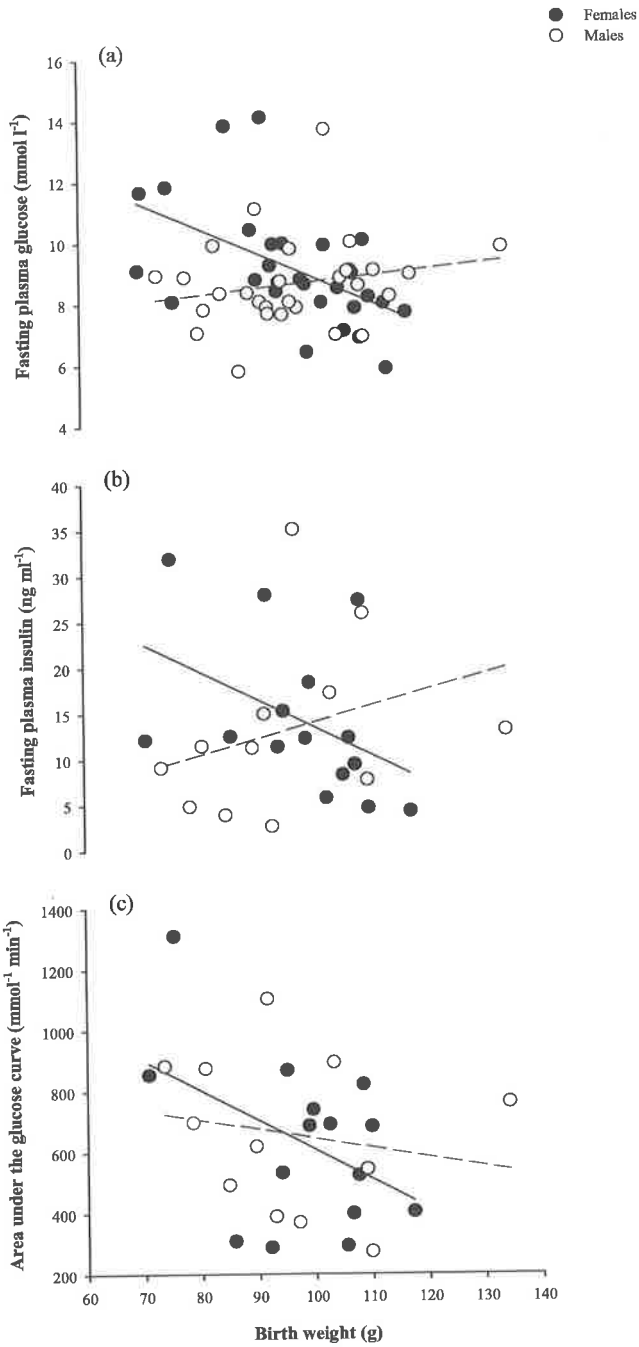

Figure 5.3 The relationship of fasting plasma glucose and insulin concentrations and glucose tolerance to birth weight in the aged guinea pig

Relationship of birth weight to fasting plasma (a) glucose (females: r=-0.54, n=29, p<0.005; males: r=0.19, n=28, ns) and (b) insulin concentrations (females: r=-0.45, n=15, p<0.05; males: r=0.31, n=12, ns) and (c) the area under the glucose curve (females: r=-0.45, n=15, p<0.05; males: r=-0.20, n=12, ns) during the IVGTT in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

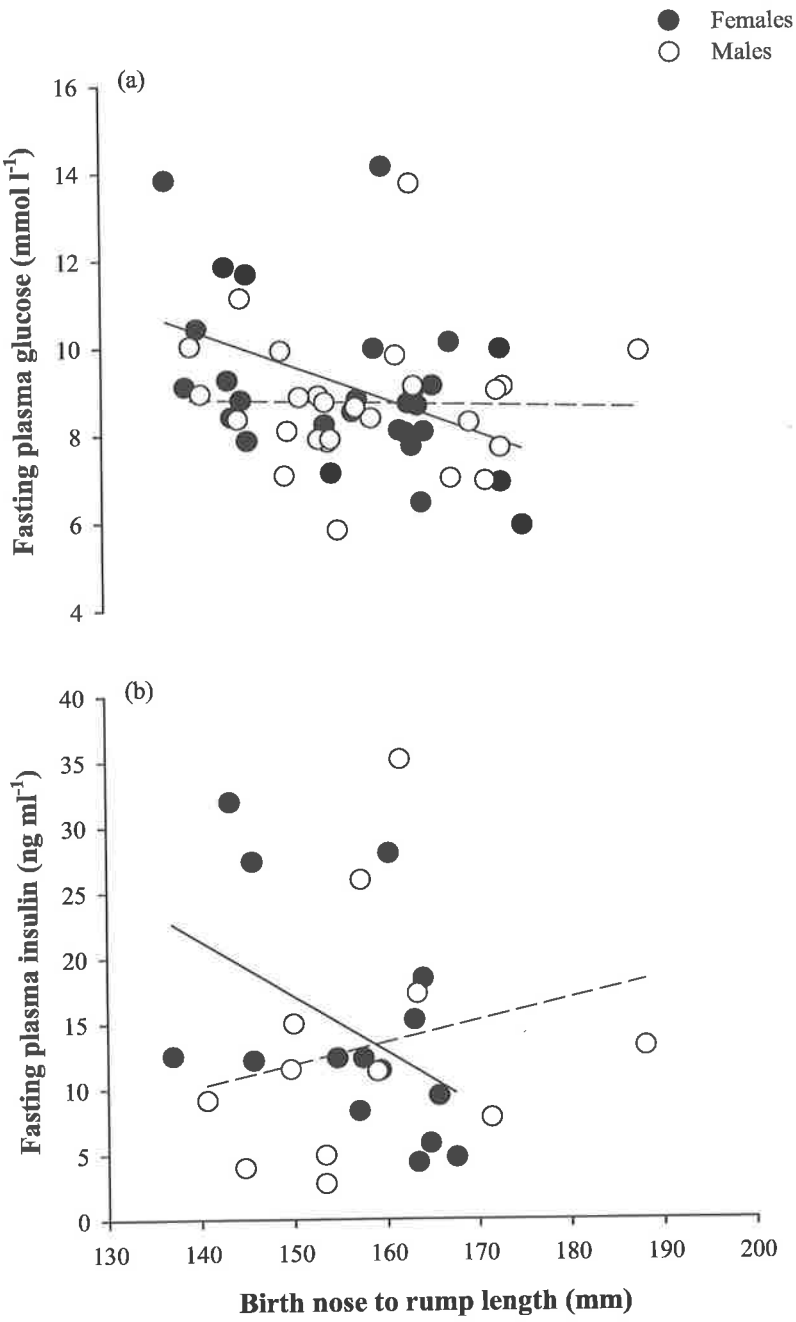

Figure 5.4 The relationship of fasting plasma glucose and insulin concentrations to nose to rump length at birth in the aged guinea pig

Relationship of nose to rump length at birth to fasting plasma (a) glucose (females: r=-0.44, n=28, p<0.01; males: r=-0.04, n=26, ns) and (b) insulin (females: r=-0.47, n=15, p<0.05; males: r=0.23, n=12, ns) concentrations in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

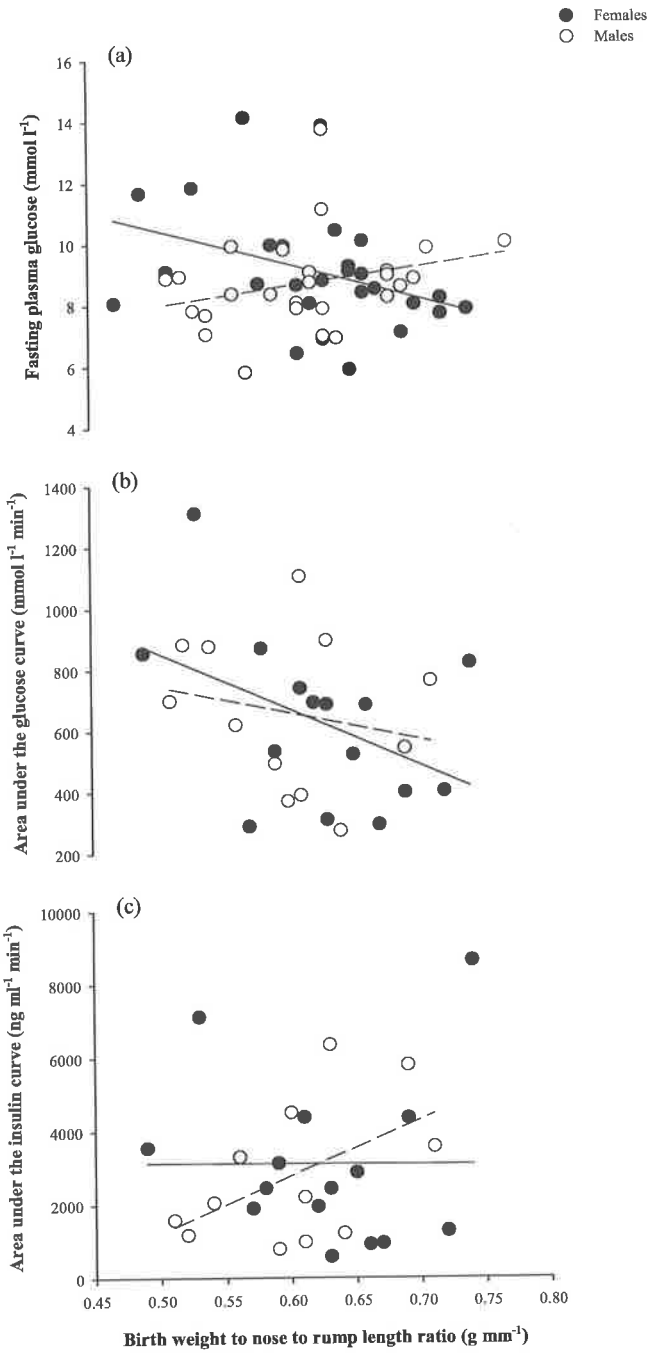

Figure 5.5 The relationship of fasting plasma glucose concentration, glucose tolerance and insulin secretion to weight to length ratio at birth in the aged guinea pig

Relationship of birth weight to length ratio to (a) fasting plasma glucose concentration (females: r=-0.38, n=28, p<0.03; males: r=0.27, n=26, ns) and area under the (b) glucose curve (females: r=-0.44, n=15, p<0.05; males: r=-0.21, n=12, ns) and (c) insulin curve (females: r=-0.01, n=15, ns; males: r=0.50, n=12, p<0.04) during the IVGTT in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

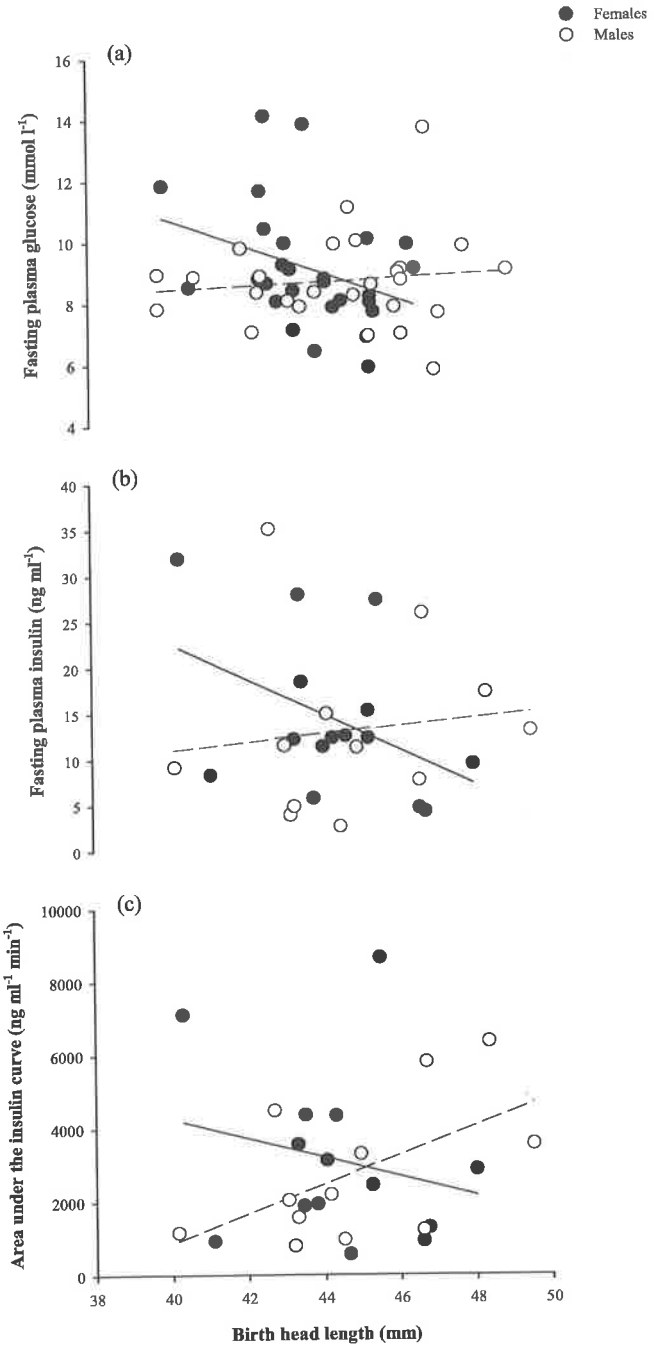

Figure 5.6 The relationship of fasting plasma glucose and insulin concentrations and insulin secretion to head length at birth in the aged guinea pig

Relationship of head length at birth to fasting plasma (a) glucose (females: r=-0.36, n=28, p<0.04; males: r=0.10, n=26, ns) and (b) insulin concentrations (females: r=-0.45, n=15, p<0.05; males: r=0.12, n=12, ns) and (c) area under insulin curve (females: r=-0.23, n=15, ns; males: r=0.55, n=12, p<0.04) during the IVGTT in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

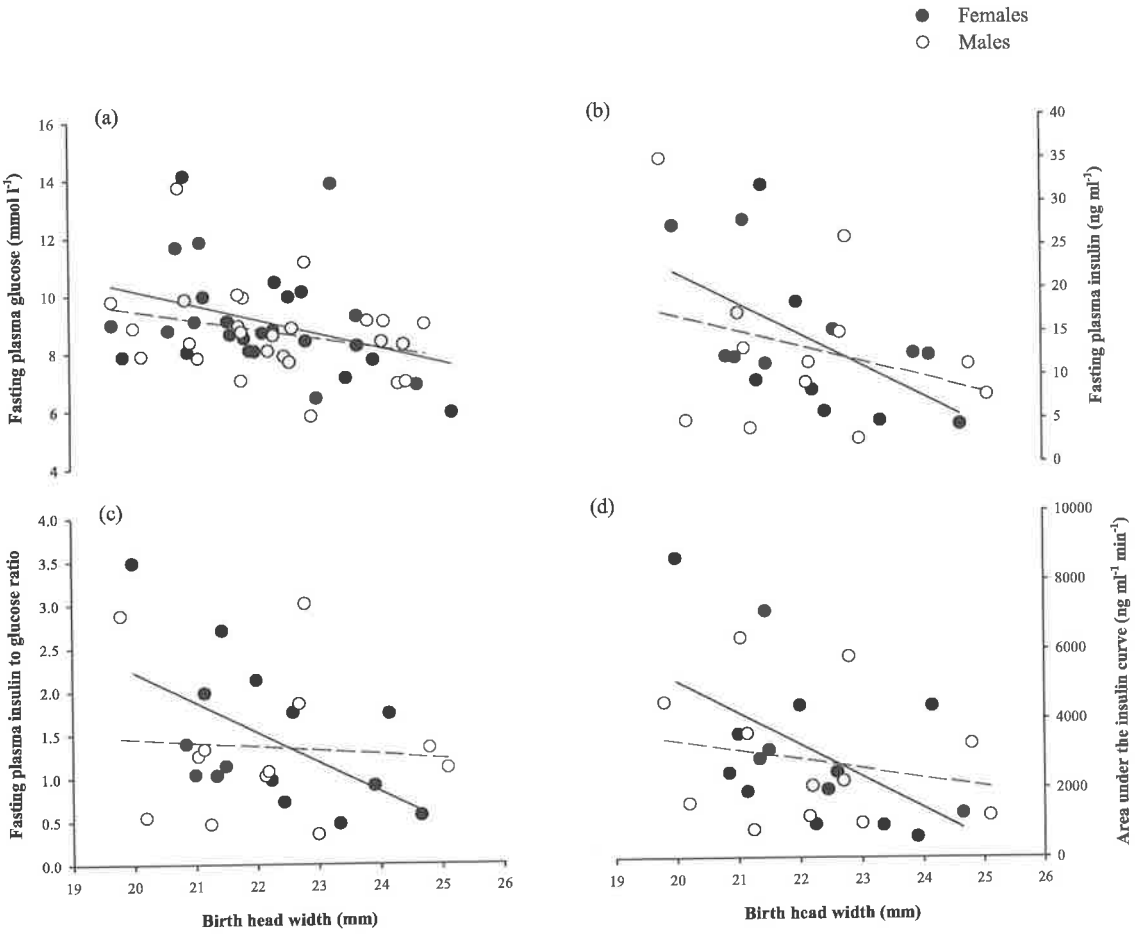

Figure 5.7 The relationship of fasting plasma glucose and insulin concentrations, the ratio of fasting plasma insulin to glucose and insulin secretion to head width at birth in the aged guinea pig

Relationship of head width at birth to fasting plasma (a) glucose (females: r=-0.36, n=28, p<0.04; males: r=-0.31, n=26, ns) and (b) insulin concentrations (females: r=-0.56, n=15, p<0.02; males: r=-0.31, n=12, ns) (c) ratio of fasting plasma insulin to glucose (females: r=-0.55, n=15, p<0.02; males: r=-0.09, n=12, ns) and (d) area under insulin curve (females: r=-0.54, n=15, p<0.02; males: r=-0.23, n=12, ns) during the IVGTT in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

5.3.3.2 *Neonatal growth rate*

In all male and female offspring combined, parameters of adult glucose tolerance and insulin secretion were not associated with any measures of neonatal growth (Table 5.4). In male offspring, fasting plasma glucose and insulin concentrations and the fasting plasma insulin to glucose ratio correlated positively with neonatal absolute growth rate (r=0.41, p=0.016, n=28; r=0.56, p=0.028 n=12 and r=0.51, p=0.047, n=12) (Figures 5.8a, b and c) and absolute (AUIC) and relative (AUIC:AUGC) insulin secretion correlated positively with neonatal absolute growth rate (r=0.50, p=0.05 and r=0.60, p=0.02, respectively, n=12) (Figures 5.9a and b). In female offspring, the fasting plasma glucose concentration correlated positively with neonatal fractional growth rate (r=0.44, p=0.008, n=29) (Figure 5.10).

5.3.3.3 *Adult size*

In all male and female offspring combined, the glucose tolerance index (K_G) correlated negatively with adult weight (r=-0.43, p=0.03, n=20) and fasting plasma glucose concentrations correlated positively with adult body mass index (r=0.33, p=0.019, n=39) (Table 5.4). In male offspring, the fasting plasma insulin to glucose ratio correlated positively (r=0.64, p=0.023, n=10) (Figure 5.11a) and the glucose tolerance index (K_G) correlated negatively (r=-0.60, p=0.032, n=10) (Figure 5.11b) with adult weight. In female offspring, the fasting plasma glucose concentration correlated negatively (r=-0.45, p=0.029, n=18) (Figure 5.12a) and the fasting plasma insulin to glucose ratio and relative insulin secretion (AUIC:AUGC) correlated positively (r=0.75, p=0.026 and r=0.92, p=0.002, respectively, n=7) (Figures 5.12b and c) with adult nose to rump length.

5.3.3.4 *Partial correlations*

The negative association between the fasting plasma glucose concentration and birth nose to rump length in all offspring was independent of neonatal fractional growth rate (partial correlation (pc), r=-0.24, p=0.039) and adult weight (pc: r=-0.28, p=0.032). The negative associations between fasting plasma glucose and insulin concentrations and head width at birth in all offspring were independent of neonatal fractional growth rate (pc: r=-0.31, p=0.012 and r=-0.43, p=0.015) and adult weight (pc: r=-0.42, p=0.002 and r=-0.48, p=0.019). The negative association between fasting plasma glucose concentrations and the birth head width to abdominal

Table 5.4 Relationship of glucose tolerance and insulin secretion to neonatal growth rate and adult size in the aged guinea pig

	Correlation coefficients (r)						
	FPG	FPI	I:G	AUGC	AUIC	AUIC:AUGC	K_G
AGR_{10-30}	0.03 (57)	-0.01 (27)	-0.04 (27)	-0.15 (27)	-0.09 (27)	0.09 (27)	0.01 (27)
FGR_{10-30}	0.19 (57)	0.00 (27)	-0.09 (27)	0.08 (27)	-0.11 (27)	-0.11 (27)	-0.12 (27)
AW	0.09 (48)	0.21 (20)	0.32 (20)	0.30 (20)	0.15 (20)	0.13 (20)	-0.43 (20)*
ANRL	-0.13 (39)	0.22 (15)	0.38 (15)	-0.03 (15)	0.22 (15)	0.35 (15)	-0.28 (15)
ABMI	0.33 (39)*	0.10 (15)	-0.05 (15)	0.38 (15)	0.04 (15)	-0.08 (15)	-0.27 (15)

r represents the correlation coefficient between each variable of adult glucose tolerance or insulin secretion and each variable of neonatal growth or adult size. Numbers in parentheses represent the number of animals. Statistical significance of correlation coefficients: $^*P < 0.05$. FPG, fasting plasma glucose (mmol l^{-1}); FPI, fasting plasma insulin (ng ml^{-1}); I:G, fasting plasma insulin to glucose ratio; AUGC, area under the glucose curve (mmol l^{-1} min^{-1}); AUIC, area under the insulin curve (ng ml^{-1} min^{-1}); AUIC:AUGC, the ratio of the area under the insulin curve to the area under the glucose curve; K_G, the glucose tolerance index (% min^{-1}), provides an estimate of the rate of glucose elimination between 2 and 60 minutes of the intravenous glucose tolerance test. AGR_{10-30}, absolute growth rate (10-30 days) (g day^{-1}); FGR_{10-30}, fractional growth rate (10-30 days) (g day^{-1} g^{-1}); AW, adult weight (g); ANRL, adult nose to rump length (mm); ABMI, adult body mass index (g mm^{-2}).

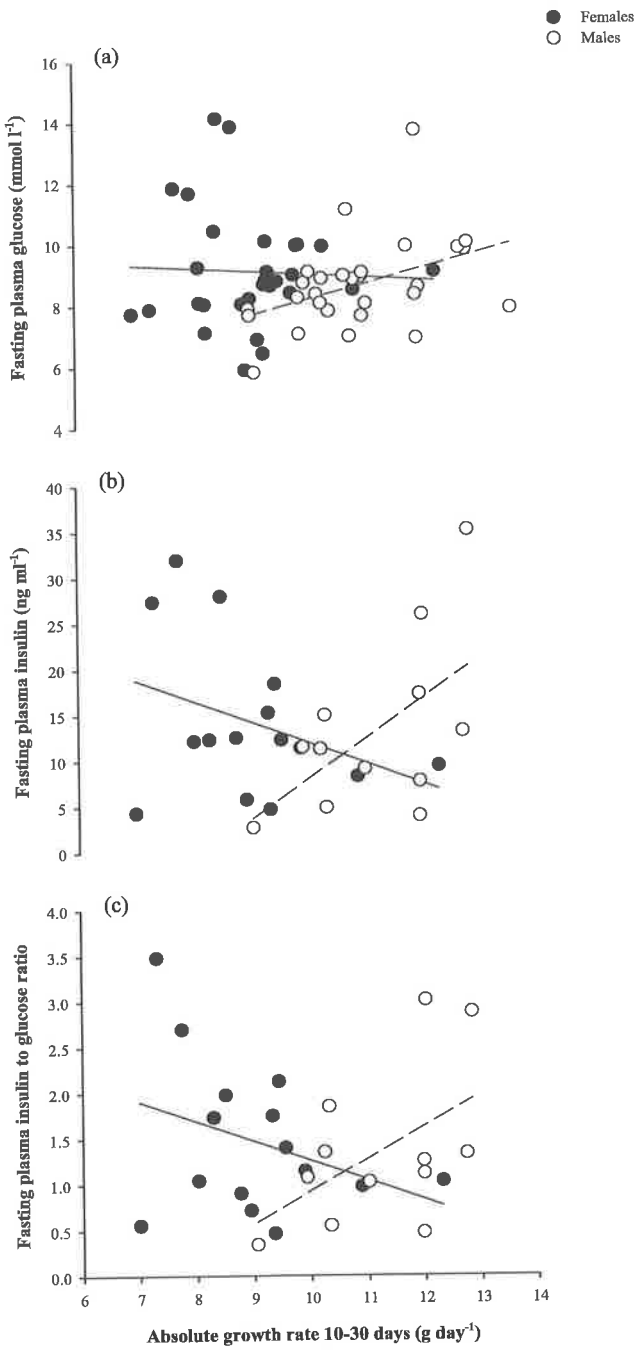

Figure 5.8 The relationship of fasting plasma glucose and insulin concentrations and the ratio of fasting plasma insulin to glucose to neonatal absolute growth rate in the aged guinea pig

Relationship of neonatal absolute growth rate to fasting plasma (a) glucose (females: r=-0.05, n=29, ns; males: r=0.41, n=28, $p<0.02$) and (b) insulin concentrations (females: r=-0.36, n=15, ns; males: r=0.56, n=12, $p<0.05$) and (c) ratio of fasting plasma insulin to glucose (females: r=-0.35, n=15, ns; males: r=0.51, n=12, $p<0.05$) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

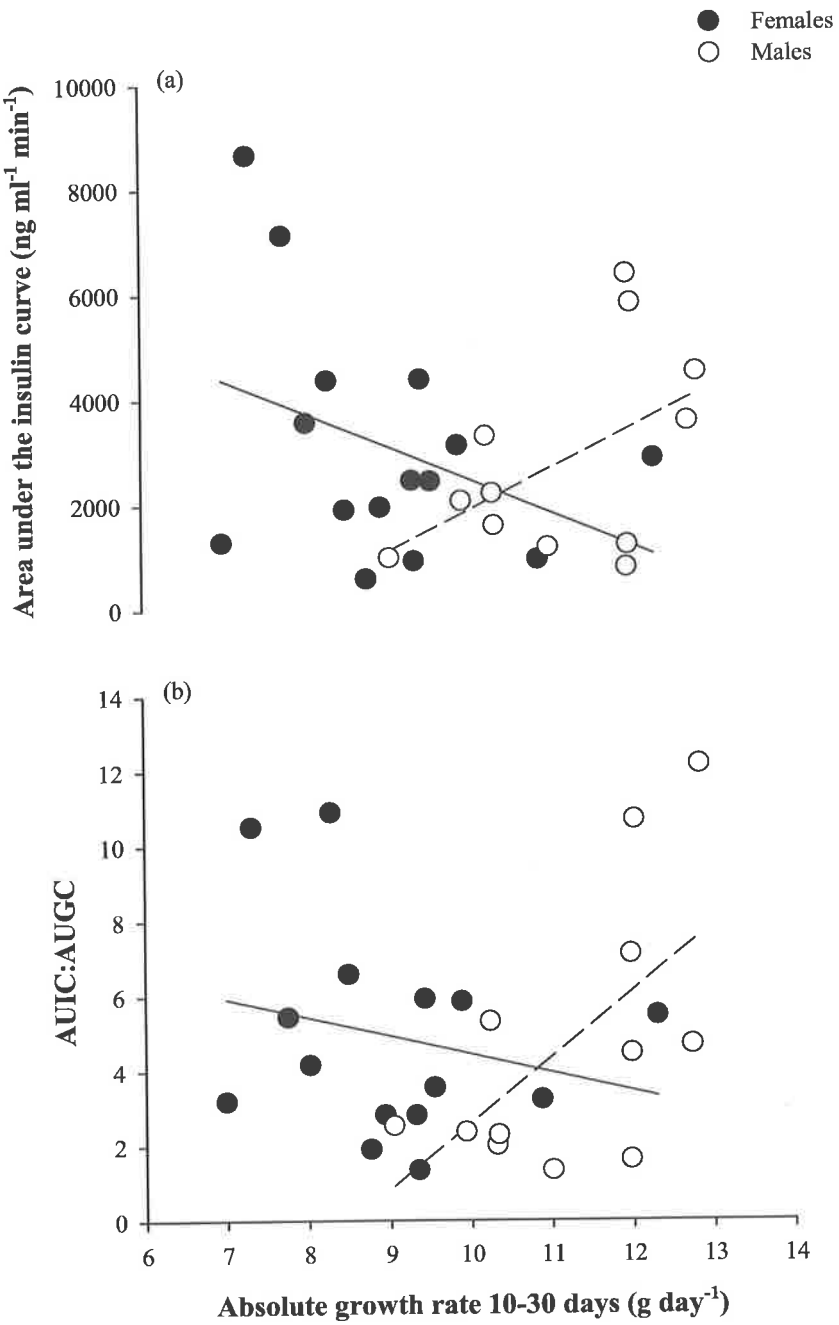

Figure 5.9 The relationship of insulin secretion to neonatal absolute growth rate in the aged guinea pig

Relationship of neonatal absolute growth rate to (a) area under the insulin curve (females: r=-0.37, n=15, ns; males: r=0.50, n=12, $p<0.05$) and (b) ratio of area under the insulin curve to area under the glucose curve (females: r=-0.24, n=15, ns; males: r=0.60, n=12, $p<0.03$) during the IVGTT in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

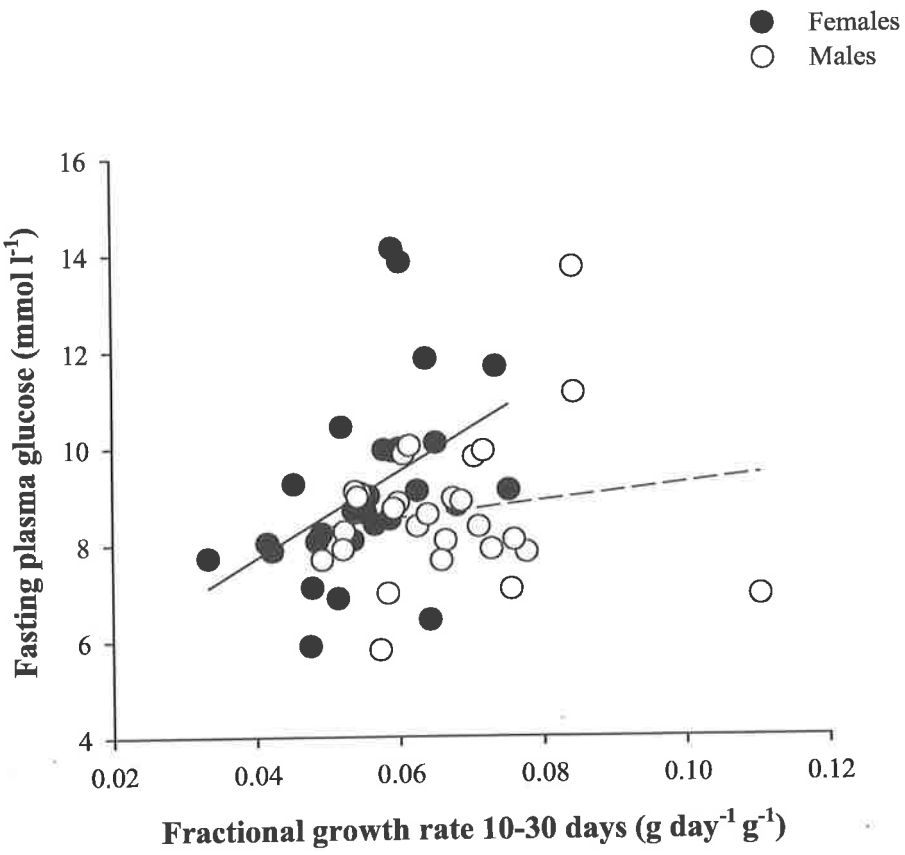

Figure 5.10 The relationship of fasting plasma glucose concentration to neonatal fractional growth rate in the aged guinea pig

Relationship of neonatal fractional growth rate to fasting plasma glucose concentration (females: r=0.44, n=29, p<0.01; males: r=0.15, n=28, ns) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

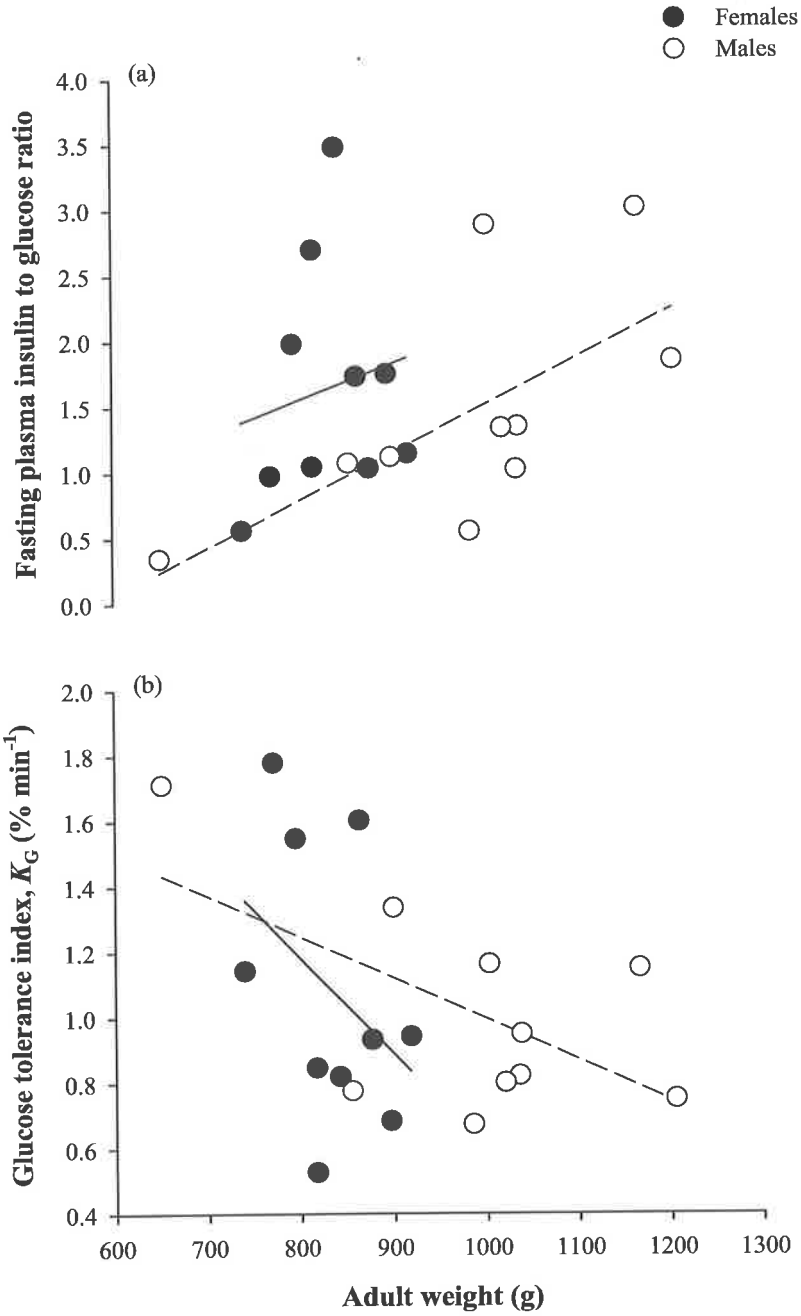

Figure 5.11 The relationship of the ratio of fasting plasma insulin to glucose and the glucose tolerance index to adult weight in the aged guinea pig

Relationship of adult weight to (a) ratio of fasting plasma insulin to glucose (females: r=0.17, n=10, ns; males: r=0.64, n=10, p<0.03) and (b) glucose tolerance index (K_G) during the IVGTT (females: r=-0.39, n=10, ns; males: r=-0.60, n=10, p<0.04) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

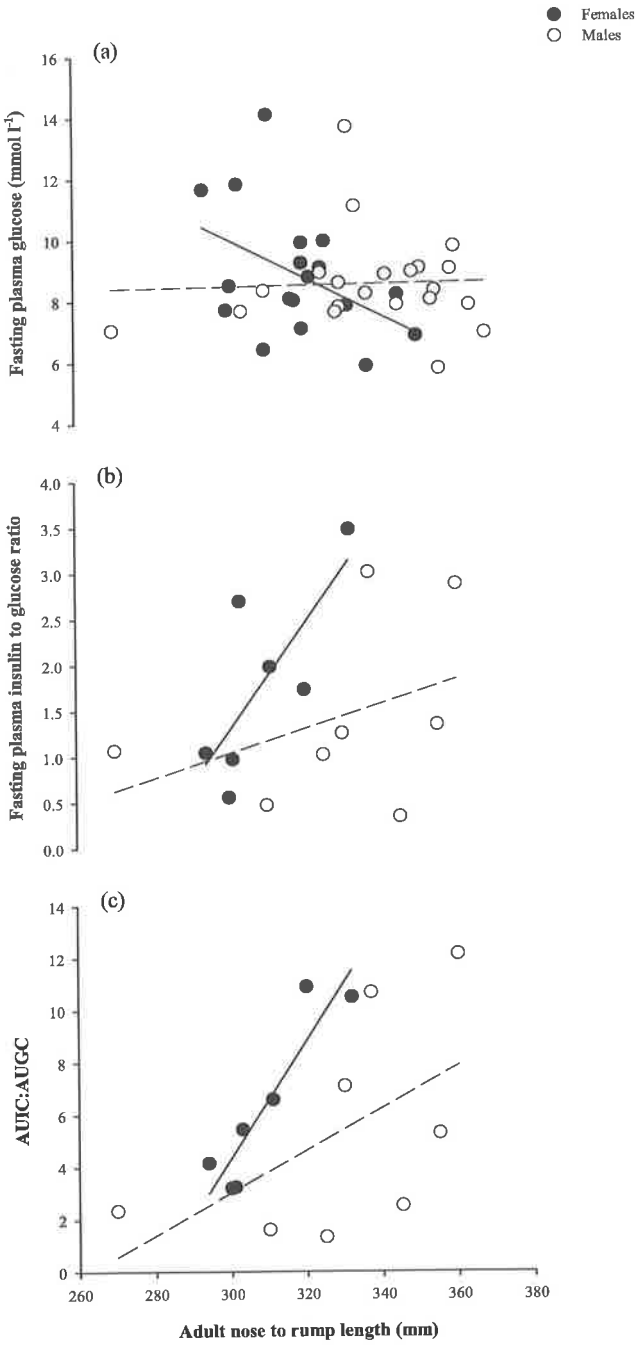

Figure 5.12 The relationship of fasting plasma glucose concentration, ratio of fasting plasma insulin to glucose and insulin secretion to adult nose to rump length in the aged guinea pig

Relationship of adult nose to rump length to (a) fasting plasma glucose concentration (females: r=-0.45, n=18, p<0.03; males: r=0.22, n=21, ns) (b) ratio of fasting plasma insulin to glucose (females: r=0.75, n=7, p<0.03; males: r=0.48, n=8, ns) and (c) ratio of the area under the insulin curve to area under the glucose curve during the IVGTT (females: r=0.92, n=7, p<0.005; males: r=0.35, n=8, ns) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

circumference ratio in all offspring was not independent of adult weight or neonatal fractional growth rate. The negative association between glucose tolerance (AUGC) and birth weight in all offspring was independent of adult weight (pc: r=-0.49, p=0.016), but not of neonatal fractional growth rate. The negative association between glucose tolerance (AUGC) and the birth weight to length ratio in all offspring was independent of neonatal fractional growth rate (pc: r=-0.35, p=0.038) and adult weight (pc: r=-0.40, p=0.043). The negative association between absolute insulin secretion (AUIC) and head width at birth in all offspring was independent of neonatal fractional growth rate (pc: r=-0.39, p=0.024), but not of adult weight. The negative association between absolute insulin secretion (AUIC) and the head width to abdominal circumference ratio at birth in all offspring was not independent of adult weight or neonatal fractional growth rate. The positive association between relative insulin secretion (AUIC:AUGC) and the birth weight to length ratio in all offspring was independent of neonatal fractional growth rate (pc: r=0.34, p=0.043) and adult weight (pc: r=-0.45, p=0.027). The negative association between relative insulin secretion (AUIC:AUGC) and the birth head width to abdominal circumference ratio in all offspring was independent of neonatal fractional growth rate (pc: r=-0.34, p=0.047) and adult weight (pc: r=-0.43, p=0.034).

In male offspring, the positive association between absolute insulin secretion (AUIC) and the birth weight to length ratio was not independent of neonatal fractional growth rate or adult weight. The positive association between absolute insulin secretion (AUIC) and head length at birth weight in male offspring was independent of neonatal fractional growth rate (pc: r=0.57, p=0.035), but not adult weight.

In female offspring, the negative associations between fasting plasma glucose concentrations and birth weight and nose to rump length were independent of neonatal fractional growth rate (pc: r=-0.37, p=0.027 and r=-0.38, p=0.026) and adult weight (pc: r=-0.58, p=0.002 and r=-0.39, p=0.041). The negative associations between fasting plasma glucose concentrations and head length and width at birth and the birth weight to length ratio in female offspring were independent of adult weight (pc: r=-0.46, p=0.017, r=-0.54, p=0.005 and r=-0.50, p=0.01), but not of neonatal fractional growth rate. The negative association between the fasting plasma insulin concentration and birth weight in female offspring was independent of neonatal fractional growth rate (pc: r=-0.53, p=0.025), but not adult weight. The

negative associations between fasting plasma insulin concentrations and the birth weight to length ratio and head length at birth in female offspring were not independent of neonatal fractional growth rate or adult weight. The negative association between the fasting plasma insulin concentration and head width at birth in female offspring was independent of neonatal fractional growth rate (pc: r=-0.56, p=0.019), and adult weight (pc: r=-0.60, p=0.045). The negative association between the fasting plasma insulin to glucose ratio and head width at birth in female offspring was independent of neonatal fractional growth rate (pc: r=-0.65, p=0.006), but not adult weight. The negative association between glucose tolerance (AUGC) and birth weight in female offspring was independent of adult weight (pc: r=-0.62, p=0.037), but not neonatal fractional growth rate. The negative association between glucose tolerance (AUGC) and the birth weight to length ratio in female offspring was not independent of adult weight or neonatal fractional growth rate. The negative association between absolute insulin secretion (AUIC) and head width at birth in female offspring was independent of neonatal fractional growth rate (pc: r=-0.62, p=0.009), but not adult weight.

The positive association between fasting plasma glucose concentrations and neonatal fractional growth rate in female offspring was independent of adult weight (pc: r=0.44, p=0.02), but not of birth weight.

5.3.4 Relationship of glucose tolerance and insulin secretion to body composition in the aged guinea pig

5.3.4.1 Adiposity

In all male and female offspring combined, the fasting insulin to glucose ratio was correlated positively with absolute combined adiposity (r=0.43, p=0.049, n=16), absolute insulin secretion (AUIC) was correlated positively with relative combined and visceral adiposity (r=0.56, p=0.013, n=16 and r=0.54, p=0.013, n=17), relative insulin secretion (AUIC:AUGC) was correlated positively with absolute and relative combined adiposity (r=0.51, p=0.022 and r=0.63, p=0.004, respectively, n=16), and relative visceral (r=0.44, p=0.038, n=17) and subcutaneous adiposity (r=0.44, p=0.031, n=19) and the glucose tolerance index (K_G) was correlated negatively with absolute and relative visceral adiposity (r=-0.45, p=0.035 and r=-0.44, p=0.04, respectively, n=17) (Table 5.5).

Table 5.5 Relationship of glucose tolerance and insulin secretion to adiposity, skeletal muscle mass and pancreas and liver weight in the aged guinea pig

	Correlation coefficients (r)						
	FPG	FPI	I:G	AUGC	AUIC	AUIC:AUGC	K_G
Combined adiposity (g)	0.16 (42)	0.32 (16)	0.43 (16)*	0.15 (16)	0.42 (16)	0.51 (16)*	-0.33 (16)
(% body weight)	0.14 (42)	0.35 (16)	0.42 (16)	0.08 (16)	0.56 (16)*	0.63 (16)†	-0.27 (16)
Visceral adiposity (g)	0.09 (45)	0.32 (17)	0.40 (17)	0.31 (17)	0.40 (17)	0.36 (17)	-0.45 (17)*
(% body weight)	0.08 (45)	0.35 (17)	0.39 (17)	0.30 (17)	0.54 (17)*	0.44 (17)*	-0.44 (17)*
Subcutaneous adiposity (g)	0.17 (45)	0.28 (19)	0.34 (19)	0.06 (19)	0.27 (19)	0.38 (19)	-0.38 (19)
(% body weight)	0.16 (45)	0.29 (19)	0.32 (19)	-0.02 (19)	0.31 (19)	0.44 (19)*	-0.34 (19)
Combined muscle (g)	0.02 (47)	0.33 (20)	0.53 (20)**	0.22 (20)	0.44 (20)*	0.38 (20)*	-0.33 (20)
(% body weight)	-0.14 (47)	0.02 (20)	0.08 (20)	-0.23 (20)	0.26 (20)	0.21 (20)	0.33 (20)
Mixed muscle (g)	0.01 (48)	0.33 (20)	0.53 (20)**	0.22 (20)	0.44 (20)*	0.38 (20)*	-0.33 (20)
(% body weight)	-0.15 (48)	0.03 (20)	0.10 (20)	-0.23 (20)	0.28 (20)	0.22 (20)	0.32 (20)
Type 2 muscle (g)	0.16 (47)	0.22 (20)	0.32 (20)	0.23 (20)	0.31 (20)	0.17 (20)	-0.19 (20)
(% body weight)	0.05 (47)	-0.14 (20)	-0.20 (20)	-0.19 (20)	-0.02 (20)	-0.11 (20)	0.39 (20)*
Pancreas (g)	0.10 (48)	0.22 (20)	0.34 (20)	0.02 (20)	0.40 (20)*	0.53 (20)**	0.07 (20)
(% body weight)	0.08 (48)	0.13 (20)	0.19 (20)	-0.10 (20)	0.35 (20)	0.48 (20)*	0.27 (20)
Liver (g)	0.06 (48)	0.13 (20)	0.23 (20)	0.13 (20)	0.23 (20)	0.27 (20)	-0.31 (20)
(% body weight)	-0.09 (48)	-0.08 (20)	-0.05 (20)	-0.17 (20)	0.16 (20)	0.22 (20)	0.08 (20)

r represents the correlation coefficient between each variable of adult glucose tolerance or insulin secretion and each variable of adult adiposity, skeletal muscle mass or pancreas or liver weight. Numbers in parentheses represent the number of animals. Statistical significance of correlation coefficients: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{\dagger}P < 0.005$. FPG, fasting plasma glucose (mmol l^{-1}); FPI, fasting plasma insulin (ng ml^{-1}); I:G, fasting plasma insulin to glucose ratio; AUGC, area under the glucose curve (mmol l^{-1} min^{-1}); AUIC, area under the insulin curve (ng ml^{-1} min^{-1}); AUIC:AUGC, the ratio of the area under the insulin curve to the area under the glucose curve; K_G, the glucose tolerance index (% min^{-1}), provides an estimate of the rate of glucose elimination between 2 and 60 minutes of the intravenous glucose tolerance test.

In male offspring, the fasting plasma glucose concentration and relative insulin secretion (AUIC:AUGC) were correlated positively with combined adiposity in absolute (r=0.61, p=0.002, n=21 and r=0.68, p=0.046, n=7) and relative terms (r=0.55, p=0.005, n=21 and r=0.70, p=0.039, respectively, n=7), the fasting plasma insulin concentration and absolute insulin secretion (AUIC) were correlated positively with combined adiposity (r=0.70, p=0.041 and r=0.74, p=0.027, respectively, n=7) and visceral adiposity (r=0.72, p=0.035, and r=0.68, p=0.048, respectively, n=7), the fasting plasma glucose concentration, absolute insulin secretion (AUIC) and relative insulin secretion (AUIC:AUGC) were correlated positively with subcutaneous adiposity in absolute terms (r=0.62, p=0.001, n=24; r=0.60, p=0.032, n=10 and r=0.55, p=0.049, n=10), fasting plasma glucose concentrations were correlated positively with subcutaneous adiposity in relative terms (r=0.55, p=0.003, n=24) and the fasting plasma insulin to glucose ratio was correlated positively with combined adiposity in absolute terms (r=0.68, p=0.046, n=7) (Table 5.6).

In female offspring, relative insulin secretion (AUIC:AUGC) was correlated positively with absolute and relative combined adiposity (r=0.62, p=0.037 and r=0.65, p=0.028, respectively, n=9) and absolute and relative subcutaneous adiposity (r=0.66, p=0.026 and r=0.71, p=0.017, respectively, n=9) and absolute insulin secretion (AIUC) was correlated positively with relative visceral adiposity (r=0.57, p=0.042, n=10) (Table 5.7).

5.3.4.2 Skeletal muscle mass

In all male and female offspring combined, the fasting plasma insulin to glucose ratio, absolute insulin secretion (AUIC) and relative insulin secretion (AUIC:AUGC) were correlated positively with combined muscle (r=0.53, p=0.008, r=0.44; p=0.027, and r=0.38, p=0.049, respectively, n=20) and mixed muscle mass (r=0.53, p=0.009, r=0.44, p=0.027; r=0.38, p=0.048, respectively, n=20) and the glucose tolerance index (K_G) was correlated positively with relative type 2 muscle mass (r=0.40, p=0.042, n=20) (Table 5.5).

Table 5.6 Relationship of glucose tolerance and insulin secretion to adiposity, skeletal muscle mass and pancreas and liver weight in aged male guinea pigs

	Correlation coefficients (r)						
	FPG	FPI	I:G	AUGC	AUIC	AUIC:AUGC	K_G
Combined adiposity (g)	0.61 (21)†	0.70 (7)*	0.68 (7)*	-0.14 (7)	0.74 (7)*	0.68 (7)*	0.06 (7)
(% body weight)	0.55 (21)**	0.62 (7)	0.49 (7)	-0.45 (7)	0.57 (7)	0.70 (7)*	0.28 (7)
Visceral adiposity (g)	0.30 (22)	0.72 (7)*	0.66 (7)	0.13 (10)	0.68 (7)*	0.55 (7)	-0.21 (7)
(% body weight)	0.26 (22)	0.64 (7)	0.44 (7)	-0.25 (10)	0.49 (7)	0.59 (7)	0.05 (7)
Subcutaneous adiposity (g)	0.62 (24)†	0.51 (10)	0.52 (10)	-0.06 (10)	0.60 (10)*	0.55 (10)*	-0.40 (10)
(% body weight)	0.55 (24)†	0.40 (10)	0.34 (10)	-0.22 (10)	0.46 (10)	0.50 (10)	-0.31 (10)
Combined muscle (g)	0.28 (25)	0.53 (10)	0.73 (10)**	0.29 (10)	0.73 (10)**	0.44 (10)	-0.37 (10)
(% body weight)	-0.14 (25)	-0.30 (10)	-0.31 (10)	-0.50 (10)	-0.14 (10)	-0.03 (10)	0.74 (10)**
Mixed muscle (g)	0.28 (25)	0.54 (10)	0.74 (10)**	0.30 (10)	0.74 (10)**	0.45 (10)	-0.38 (10)
(% body weight)	-0.13 (25)	-0.28 (10)	-0.28 (10)	-0.49 (10)	-0.11 (10)	-0.01 (10)	0.74 (10)**
Type 2 muscle (g)	0.24 (25)	0.16 (10)	0.36 (10)	0.05 (10)	0.38 (10)	0.22 (10)	-0.12 (10)
(% body weight)	-0.18 (25)	-0.54 (10)	-0.53 (10)	-0.52 (10)	-0.44 (10)	-0.25 (10)	0.72 (10)*
Pancreas (g)	0.23 (25)	0.50 (10)	0.66 (10)*	-0.07 (10)	0.55 (10)*	0.51 (10)	-0.15 (10)
(% body weight)	0.12 (25)	0.20 (10)	0.29 (10)	-0.63 (10)*	0.19 (10)	0.43 (10)	0.42 (10)
Liver (g)	0.37 (25)*	0.52 (10)	0.54 (10)	0.29 (10)	0.48 (10)	0.36 (10)	-0.48 (10)
(% body weight)	0.07 (25)	0.17 (10)	0.01 (10)	-0.16 (10)	0.05 (10)	0.18 (10)	0.08 (10)

r represents the correlation coefficient between each variable of adult glucose tolerance or insulin secretion and each variable of adult adiposity, skeletal muscle mass or pancreas or liver weight. Numbers in parentheses represent the number of animals. Statistical significance of correlation coefficients: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{\dagger}P < 0.005$. FPG, fasting plasma glucose (mmol l^{-1}); FPI, fasting plasma insulin (ng ml^{-1}); I:G, fasting plasma insulin to glucose ratio; AUGC, area under the glucose curve (mmol l^{-1} min^{-1}); AUIC, area under the insulin curve (ng ml^{-1} min^{-1}); AUIC:AUGC, the ratio of the area under the insulin curve to the area under the glucose curve; K_G, the glucose tolerance index (% min^{-1}), provides an estimate of the rate of glucose elimination between 2 and 60 minutes of the intravenous glucose tolerance test.

Table 5.7 Relationship of glucose tolerance and insulin secretion to adiposity, skeletal muscle mass and pancreas and liver weight in aged female guinea pigs

	Correlation coefficients (r)						
	FPG	FPI	I:G	AUGC	AUIC	AUIC:AUGC	K_G
Combined adiposity (g)	-0.08 (21)	0.15 (9)	0.31 (9)	0.27 (9)	0.52 (9)	0.62 (9)*	-0.39 (9)
(% body weight)	-0.14 (21)	0.20 (9)	0.37 (9)	0.33 (9)	0.58 (9)	0.65 (9)*	-0.42 (9)
Visceral adiposity (g)	-0.04 (23)	0.18 (10)	0.32 (10)	0.43 (10)	0.50 (10)	0.39 (10)	-0.51 (10)
(% body weight)	-0.08 (23)	0.24 (10)	0.37 (10)	0.50 (10)	0.57 (10)*	0.42 (10)	-0.53 (10)
Subcutaneous adiposity (g)	-0.06 (21)	0.26 (9)	0.41 (9)	0.14 (9)	0.50 (9)	0.66 (9)*	-0.35 (9)
(% body weight)	-0.11 (21)	0.35 (9)	0.50 (9)	0.16 (9)	0.55 (9)	0.71 (9)*	-0.35 (9)
Combined muscle (g)	-0.13 (22)	0.31 (10)	0.57 (10)*	0.17 (10)	0.66 (10)*	0.68 (10)*	-0.30 (10)
(% body weight)	-0.20 (22)	0.25 (10)	0.39 (10)	-0.02 (10)	0.42 (10)	0.41 (10)	0.00 (10)
Mixed muscle (g)	-0.14 (23)	0.29 (10)	0.56 (10)*	0.15 (10)	0.64 (10)*	0.67 (10)*	-0.29 (10)
(% body weight)	-0.21 (23)	0.24 (10)	0.40 (10)	-0.03 (10)	0.42 (10)	0.43 (10)	0.00 (10)
Type 2 muscle (g)	0.13 (22)	0.53 (10)	0.44 (10)	0.64 (10)*	0.57 (10)*	0.24 (10)	-0.32 (10)
(% body weight)	0.11 (22)	0.20 (10)	0.03 (10)	0.14 (10)	0.08 (10)	-0.14 (10)	0.10 (10)
Pancreas (g)	0.04 (23)	0.08 (10)	0.17 (10)	0.06 (10)	0.37 (10)	0.65 (10)*	0.18 (10)
(% body weight)	0.03 (23)	0.08 (10)	0.15 (10)	0.05 (10)	0.35 (10)	0.60 (10)*	0.23 (10)
Liver (g)	-0.16 (23)	-0.30 (10)	-0.06 (10)	-0.04 (10)	0.20 (10)	0.28 (10)	-0.13 (10)
(% body weight)	-0.24 (23)	-0.41 (10)	-0.17 (10)	-0.16 (10)	0.12 (10)	0.19 (10)	0.05 (10)

r represents the correlation coefficient between each variable of adult glucose tolerance or insulin secretion and each variable of adult adiposity, skeletal muscle mass or pancreas or liver weight. Numbers in parentheses represent the number of animals. Statistical significance of correlation coefficients: $^*P < 0.05$. FPG, fasting plasma glucose (mmol l^{-1}); FPI, fasting plasma insulin (ng ml^{-1}); I:G, fasting plasma insulin to glucose ratio; AUGC, area under the glucose curve (mmol l^{-1} min^{-1}); AUIC, area under the insulin curve (ng ml^{-1} min^{-1}); AUIC:AUGC, the ratio of the area under the insulin curve to the area under the glucose curve; K_G, the glucose tolerance index (% min^{-1}), provides an estimate of the rate of glucose elimination between 2 and 60 minutes of the intravenous glucose tolerance test.

In male offspring, the fasting plasma insulin to glucose ratio and absolute insulin secretion (AUIC) were correlated positively with combined muscle (r=0.73, p=0.009 and r=0.73, p=0.008, respectively, n=10) and mixed muscle mass (r=0.74, p=0.008 and r=0.74, p=0.007, respectively, n=10) and the glucose tolerance index (K_G) was correlated positively with relative combined, mixed and type 2 muscle mass (r=0.74, p=0.007; r=0.74, p=0.008 and r=0.72, p=0.01, respectively, n=10) (Table 5.6).

In female offspring, the fasting plasma insulin to glucose ratio, absolute insulin secretion (AUIC) and relative insulin secretion (AUIC:AUGC) were correlated positively with combined muscle (r=0.57, p=0.041; r=0.66, p=0.019, and r=0.68, p=0.015, respectively, n=10) and mixed muscle mass (r=0.56, p=0.047; r=0.64, p=0.024 and r=0.67, p=0.017, respectively, n=10) and glucose tolerance (AUGC) and absolute insulin secretion (AUIC) were correlated positively with type 2 muscle mass (r=0.64, p=0.023 and r=0.57, p=0.043, n=10) (Table 5.7).

5.3.4.3 Pancreas and liver weights

In all male and female offspring combined, absolute insulin secretion (IAUC) was correlated positively with pancreas weight (r=0.40, p=0.042, n=20) and relative insulin secretion (AUIC:AUGC) was correlated positively with pancreas and relative pancreas weight (r=0.53, p=00.008 and r=0.48, p=0.017, respectively, n=20) (Table 5.5). In male offspring, the fasting plasma insulin to glucose ratio and absolute insulin secretion (AUIC) were correlated positively with pancreas weight (r=0.66, p=0.019 and r=0.55, p=0.05, respectively, n=10), glucose tolerance (AUGC) was correlated negatively with relative pancreas weight (r=-0.63, p=0.025, n=10) and the fasting plasma glucose concentration was correlated positively with liver weight (r=0.37, p=0.034, n=25) (Table 5.6). In female offspring, relative insulin secretion (AUIC:AUGC) was correlated positively with pancreas and relative pancreas weight (r=0.65, p=0.021 and r=0.60, p=0.034, respectively, n=10) (Table 5.7).

5.4 DISCUSSION

We have shown that small size at birth was associated with increased fasting plasma concentrations of insulin and glucose, an increased insulin response to an IVGTT and impaired glucose tolerance, in the aged female guinea pig. By contrast, male guinea pigs that were large at birth demonstrated an increased insulin response to an IVGTT as aged adults, with normal fasting plasma glucose concentrations and glucose tolerance. When animals were divided into groups with birth weights above or below the median birth weight, adult fasting plasma glucose concentrations were increased in low birth weight female offspring when compared to their high birth weight counterparts. Conversely in males, adult fasting plasma concentrations of insulin and the insulin response to an IVGTT were increased in high birth weight compared to low birth weight offspring. In addition, an accelerated neonatal fractional growth rate was predictive of elevated fasting plasma glucose concentrations in aged female offspring.

It is important to note that the definition of fetal growth restriction used in this study, namely a birth weight below the median, differs from clinical IUGR and SGA, and in fact variations in fetal growth and the programming phenomenon are typically on a continuum.

In the aged female guinea pig, fasting hyperinsulinaemia and hyperglycaemia, impaired glucose tolerance and an increased insulin response to an IVGTT were associated with poor fetal growth, as indicated by a reduced weight, length, weight to length ratio, head length or head width at birth. These results are consistent with the observations of studies in human populations, which have demonstrated that a low weight or ponderal index at birth was associated with impaired glucose tolerance and NIDDM (Hales *et al.*, 1991; Phipps *et al.*, 1993; Lithell *et al.*, 1996). In aged male guinea pigs however, small size at birth was not associated with any change in fasting insulin or glucose levels, or glucose tolerance or insulin secretion in response to an IVGTT. Interestingly, a recent UK study of elderly men and women has shown that in men, the measurements of insulin and glucose during an OGTT were correlated more strongly with weight at 1 year than birth weight, while in women, the associations between birth weight and glucose and insulin measurements were more robust than the associations with weight at 1 year (Phillips *et al.*, 2005).

The inverse relationship between size at birth and postnatal glucose intolerance may be U-shaped or reversed in Native Americans, Mexican-Americans, urban Indians and other populations at a high risk for diabetes. The weakening or reversal of the inverse relationship between size at birth and glucose intolerance is largely due to the effects of gestational diabetes. In gestational diabetes, maternal hyperglycaemia causes fetal hyperinsulinaemia, which in turn increases fetal growth, particularly in subcutaneous fat depots (Freinkel 1980). In Pima Native Americans, the risk of diabetes was much higher in the offspring of mothers who were diabetic during pregnancy than in the offspring of mothers who developed diabetes subsequently (Pettitt *et al.* 1998). In this population, a U-shaped relationship between birth weight and the prevalence of diabetes in adult life has been observed, with an increased prevalence of diabetes in those individuals with birth weights less than 2.5 kg or more than 4.5 kg (McCance *et al.*, 1994). Studies in non-human species have also shown that maternal hyperglycaemia exerts long-term effects on glucose homeostasis in offspring. The induction of maternal hyperglycaemia in female rats has been shown to cause insulin resistance, glucose intolerance and impaired β cell function in adult offspring (Aerts *et al.*, 1988; Grill *et al.*, 1991)

In the current study, in male guinea pigs, excessive fetal growth, as indicated by an increased weight, weight to length ratio or head length at birth, was associated with fasting hyperinsulinaemia and an increased insulin response to an IVGTT in the aged adult, which is consistent with the findings in human populations (McCance *et al.*, 1994; Dabelea *et al.*, 1999). However, fasting glucose levels and glucose tolerance in adult life were not influenced by size at birth in male offspring, suggesting that a deterioration of glucose homeostasis in these animals following excessive fetal growth is yet to develop, but may with increasing age. Interstingly, in this study, the majority of male guinea pigs who were large at birth came from medium or large, rather than small litters. It is possible however that these larger guinea pigs may have been exposed to a different metabolic environment than their smaller littermates before birth, perhaps because of their position in the uterus, which may explain, at least in part, their altered metabolic function as adults.

A number of mechanisms have been proposed to explain the relationship between perturbed growth in early life and impaired glucose tolerance and NIDDM in adult life. The nutritional perturbations that reduce fetal growth also impair the

development of tissues involved in the regulation of glucose metabolism, including the endocrine pancreas. IUGR infants display a marked reduction in the size of their endocrine pancreas (Van Assche *et al.*, 1979). Studies in animals have reported that fetal malnutrition is associated with a persistent impairment of pancreatic β cell function and development (Dahri *et al.*, 1991; Garofano *et al.*, 1997). In the rat, maternal protein restriction throughout pregnancy reduced the proliferation rate, size, and insulin content of pancreatic islets in fetuses at the end of pregnancy (Dahri *et al.*, 1991; Snoeck *et al.*, 1990).

Endocrine pancreas islet hypertrophy and hyperplasia of β cells have long been recognised as typical characteristics in fetuses and newborn babies of diabetic mothers (Cardell 1953; D'Agostino *et al.*, 1963; Jackson *et al.*, 1958; Naeye 1965). Experimental studies in animals have revealed that when the mother has diabetes during pregnancy, increased amounts of glucose reach the fetus by facilitated transfer through the placenta (Aerts *et al.*, 1990; Kervran *et al.*, 1978). In order to cope with this oversupply of glucose, adaptations occur in fetal insulin production and action. Consequently, the development of the fetal islets of Langerhans is enhanced, resulting in hypertrophy of the endocrine pancreas and hyperplasia of β cells. In addition to this increase in the number of fetal β cells, the biosynthetic activity of these insulin producing cells is also improved (Aerts *et al.*, 1990).

In the current study, adult pancreas weight was positively associated with abdominal circumference at birth (see Chapter 3) and the insulin response to an IVGTT in both male and female guinea pigs, and was negatively associated with glucose intolerance in male offspring. These findings suggest that impaired pancreatic development as a result of a perturbed intrauterine environment may have long-term adverse consequences for insulin-regulated glucose homeostasis in the guinea pig.

Increased activity of, and responsiveness to the HPAA has been suggested as a link between restricted growth *in utero* and impaired glucose tolerance and NIDDM in adult life. Studies in 64 year old men (Phillips *et al.*, 1998) and 50 year old men and women (Phillips *et al.*, 2000) in the UK, and 20 year old men and women in Australia (Phillips *et al.*, 2000) have shown that low birth weight is associated with increased 0900h plasma cortisol concentrations. In addition, fasting plasma cortisol concentrations have been shown to be positively related to fasting plasma glucose

concentrations (Phillips *et al.*, 1998). The relationship between impaired fetal growth and elevated fasting cortisol levels is thought to represent prenatal programming of the cortisol stress response, due to the combination of fasting and the novel clinic setting in which the blood samples were taken (Phillips *et al.*, 2006). Recently, a study of boys and girls aged between 7 and 9 years has shown that birth weight was inversely related to salivary cortisol responses to psychological stress in boys, while in girls birth weight was inversely related to morning peak cortisol levels (Jones *et al.*, 2006), suggesting that in humans, there are sex differences in the relationship between birth weight and the HPAA response to acute psychological stress. Programming of the liver is particularly sensitive to increased circulating levels of cortisol, with a number of key hepatic processes, including many enzymes controlling the production and fate of metabolic fuels, regulated by glucocorticoids. Rats exposed to dexamethasone, a synthetic glucocorticoid which is a poor substrate for 11BHSD-2, during the last week of pregnancy were smaller at birth and demonstrated glucose intolerance and increased expression of phosphoenolpyruvate carboxykinase (PEPCK), the rate limiting enzyme of gluconeogenesis, as adults (Nyirenda *et al.*, 1998).

The present study has shown that spontaneous fetal growth restriction, as indicated by a low birth weight, is associated with increased adult adrenal size (see Chapter 3), which may reflect an increased sensitivity of this gland to the trophic actions of ACTH. In addition, a recent study in this cohort of aged guinea pigs has demonstrated that impaired fetal growth, as indicated by thinness, or a reduced weight, length or abdominal circumference at birth, was associated with increased salivary cortisol concentrations in female offspring (S Grover *et al.*, unpublished observations). In the guinea pig, previous studies have demonstrated that females are more susceptible to programming of HPAA function than males (Dean *et al.*, 1999; Liu *et al.*, 2001; Lingas *et al.*, 2001). These findings suggest that the impaired glucose tolerance and fasting hyperglycaemia observed in aged female guinea pigs following perturbed intrauterine growth, may be due to in part to an increased activity of the HPAA. In aged male guinea pigs however, small size at birth did not alter salivary cortisol concentrations or adrenal size. These findings could explain in part, why spontaneous fetal growth restriction is associated with impaired glucose tolerance and fasting hyperglycaemia in aged female, but not male, guinea pigs.

We have shown that combined, subcutaneous and visceral adiposity were positively associated with fasting glucose and insulin concentrations in aged male offspring, and were positively associated with the insulin response to IVGTT in both male and female aged offspring. Increased adiposity may stimulate insulin secretion by increasing the levels of circulating free fatty acids. Exposure of the β cells to increased free fatty acid levels results initially in an oversecretion of insulin, however with chronic exposure, β cell stores are depleted and as a result insulin secretion is impaired (Mason *et al.*, 1999). Interestingly, fasting free fatty acid concentrations in this study were not associated with any measure of glucose tolerance or insulin secretion in both male and female aged offspring, however several other adipocyte-derived factors have also been shown to play a role in β cell dysfunction, including leptin, adiponectin, TNF-α, and IL-6 (Zhao *et al.*, 2005), and these may warrant further investigation.

In humans, increased rates of impaired glucose tolerance and NIDDM were observed in elderly men and women who were light, short or thin at birth but had caught up in terms of weight and height at 7 years of age, and continued to grow at an accelerated rate to 15 years of age (Forsen *et al.*, 2000). However, as catch-up growth occurs primarily during the first 2 to 3 months of life in humans, some have argued that body size measurements at 7 years of age may reflect the development of obesity in childhood, rather than catch-up growth after fetal growth restriction (Kind *et al.*, 2003). In the current study, female offspring that experienced accelerated growth as neonates had higher fasting plasma glucose concentrations as adults.

Fasting glycaemia reflects hepatic glucose production once glycogen stores have been exhausted and the response of this process to fasting plasma insulin levels. Therefore, increased fasting plasma glucose concentrations may reflect increased endogenous glucose production and reduced hepatic insulin sensitivity to prevailing circulating insulin. Notably, whole body insulin sensitivity was reduced in low birth weight or small at birth females (see Chapter 4), but whether this reflects impaired hepatic or skeletal muscle insulin sensitivity or both, is not known. Many metabolic processes in the liver are sectioned or zoned within specific areas. For example glucokinase, the insulin sensitive enzyme in the rate limiting step in the glycolytic pathway, is located primarily in the perivenous zone of liver parenchyma (Lawrence et *al.*, 1986), while other enzymes like PEPCK, which is involved in

gluconeogenesis, are located in the periportal regions of the liver (Wimmer *et al.*, 1990). Studies in animals have shown that the offspring of dams fed a reduced-protein diet during pregnancy and lactation have large structural and functional changes in their liver, including a reduction in liver lobule size (Burns *et al.*, 1997). The offspring of dams that were protein restricted during pregnancy have demonstrated a decreased expression of glucokinase in the perivenous regions in the parenchyma of rat livers, whereas PEPCK activity was increased in the periportal regions (Desai *et al.*, 1997a). Therefore there is impairment in the ability of the liver to utilise glucose as a fuel source through glycolysis, and an increase in the activity of enzymes such as PEPCK which are required for glucose production. In addition, postnatal weight gain in the rat has also been shown to influence the increase in hepatic gluconeogenic enzyme activities induced by prenatal protein restriction (Desai *et al.*, 1997b).

Interestingly, female guinea pigs that displayed high rates of growth during the neonatal period also demonstrated reduced adult liver weights when compared to their slow growing littermates (see Chapter 3). Therefore, the increased fasting glucose concentrations observed in aged female offspring that experienced accelerated neonatal growth, may be due at least in part to changes in the expression of key gluconeogenic enzymes, as a result of structural changes in the liver.

In summary, spontaneous fetal growth restriction in the guinea pig increased fasting insulin and glucose concentrations and the insulin response to an IVGTT, and impaired glucose tolerance in aged female offspring. In males, excessive fetal growth was associated with an increased insulin response to an IVGTT in aged adults. Altered postnatal growth also influenced adult glucose homeostasis, with elevated fasting glucose concentrations observed in female offspring that experienced accelerated growth in the neonatal period. Therefore it appears that in the guinea pig, perturbed growth in early life results in the development of impaired glucose tolerance and NIDDM in aged female offspring, due primarily to an impairment of whole body insulin sensitivity (see Chapter 4) and the inability of the pancreas to adequately compensate for this defect. In males however, the defect in adult insulin action following excessive growth in early life (see Chapter 4) is still adequately compensated for by the pancreas at this age and normal glucose tolerance is able to be maintained.

CHAPTER 6

EARLY LIFE INFLUENCES ON BLOOD PRESSURE IN THE AGED GUINEA PIG

6.1 INTRODUCTION

Population studies have demonstrated that low birth weight as a result of poor fetal growth, as well as disproportionate growth *in utero*, as indicated by a low ponderal index or reduced head circumference at birth (Barker *et al.*, 1993), is associated with an increased risk of hypertension in adult life (Barker *et al.*, 1989). A systematic review of the published literature has reported that in both males and females, and in all ages and races, blood pressure fell by approximately 2 mmHg with each kilogram increase in birth weight, and this negative relationship was amplified with increasing age in adult life (Huxley *et al.*, 2000). In addition to impaired growth *in utero*, accelerated postnatal growth of skeletal and soft tissues has also been associated with raised blood pressure in adult life (Huxley *et al.*, 2000; Law *et al.*, 2002; Eriksson *et al.*, 2000).

Maternal feed (Vickers *et al.*, 2000; Woodall *et al.*, 1996) or protein restriction (Langley *et al.*, 1994; Langley-Evans *et al.*, 1996) in the rat has been shown to increase systolic blood pressure in male and female adult offspring. A number of these studies used tail-cuff plethysmography to measure blood pressure, a technique that is known to place animals under stress (Tonkiss *et al.*, 1998), suggesting that it maybe the blood pressure response to stressors that is increased by prenatal perturbation. More recently, a study in the chronically catheterised juvenile pig has demonstrated that low birth weight and thinness at birth, as a result of natural variations in litter size, were associated with increased mean arterial blood pressure (Poore *et al.*, 2002). Similarly, severe maternal undernutrition during early gestation has been shown to increase pulse pressure in the young adult sheep (Gardner *et al.*, 2004). By contrast, only a few studies in non-human species have investigated the influence of early postnatal growth on adult blood pressure.

Previously we have demonstrated that moderate maternal feed restriction (85% *ad libitum* intake) in the guinea pig increases systolic blood pressure in chronically catheterised young adult male offspring (Kind *et al.*, 2002). A reduced head width at birth was also associated with raised systolic and mean arterial blood pressure in these young adult offspring of *ad libitum* fed and feed-restricted mothers (Kind *et al.*, 2002). Other parameters of basal cardiovascular function in the young adult guinea pig, including heart rate and pulse pressure, were unaltered by maternal feed restriction (Kind *et al.*, 2002). Additionally, male offspring of *ad libitum* fed mothers that experienced accelerated neonatal growth, demonstrated elevated systolic blood pressure as young adults (Kind *et al.*, 2002).

The aims of the studies described in Chapter 6 were therefore to determine the effects of (1) spontaneous fetal growth restriction and (2) accelerated neonatal growth, on resting systolic, diastolic and mean arterial blood pressure, pulse pressure and heart rate, in the aged guinea pig. The influence of sex on the impact of altered perinatal growth on resting blood pressure and heart rate in the aged adult was also assessed, as were the relationships between adult size and body composition and resting blood pressure and heart rate. We hypothesised that spontaneous fetal growth restriction and accelerated neonatal fractional growth rate for weight in the guinea pig will increase resting systolic, diastolic and mean arterial blood pressure, pulse pressure and heart rate, in aged male and female offspring.

6.2 MATERIALS AND METHODS

6.2.1 Animals

Nulliparous, 3 to 4 month old female guinea pigs (IMVS Tri-coloured) were obtained from the Gilles Plains Animal Resource Centre (Gilles Plains, SA, Australia), and following a two-week acclimatisation period, females in oestrous were pair mated with male guinea pigs (IMVS Tri-coloured) overnight (as described in Section 2.2.1). At 60 days gestation, pregnant animals were transferred to plastic tubs containing paper bedding, where they gave birth. Offspring were weighed at birth, and the nose to rump length, abdominal circumference, and head length and head width of each pup was measured. A total of 24 pups born to 15 mothers were randomly assigned to this study of aged guinea pigs (12 male and 12 female), with the remaining offspring allocated to other studies (as described in Section 2.2.1). A description of the total number of litters used and their size is summarised in Appendix A. Of the 24 pups assigned to this study, 15 had at least one other litter mate in this study while 9 did not. All pups were weighed daily from birth to 30 days of age, and the neonatal absolute (AGR_{10-30}) (g day^{-1}) and fractional (FGR_{10-30}) (g day^{-1} g^{-1}) growth rates for weight were calculated from 10 days of age to weaning at 30 days of age (as described in Section 3.2.1), following which pups were transferred to individual wire-bottomed cages and weaned onto normal guinea pig chow *ad libitum*. Postnatal food intakes were not able to be measured for logistic reasons.

At 410 ± 3 days of age catheters were inserted into the right carotid artery and jugular vein under general anaesthesia (As described in Section 2.2.1). Catheter patency was maintained by daily flushing with 800 µl of heparinized saline (250 IU ml^{-1}, Multiparin, Fisons Pharmaceuticals, NSW, Australia). Animals were allowed at least 5 days postoperative recovery before the commencement of *in vivo* studies. It was not possible to obtain all data from all animals due to human resource limitations, and the number of observations for each experimental data set is indicated in each table or figure. All procedures in this study were reviewed and approved by the University of Adelaide Animal Ethics Committee.

6.2.2 Blood pressure

At 415 ± 3 days of age resting systolic, diastolic and mean arterial blood pressure and heart rate were measured directly from the carotid artery catheter, for a period of 2 hours between 1000h and 1200h (as described in Section 2.2.4). Arterial blood pressure and heart rate were measured using MacLab chart software on a Power Macintosh computer. Pulse pressure was calculated as the difference between the systolic and diastolic blood pressures.

6.2.3 Body composition

At 426 ± 5 days of age, and following a 20 hour-overnight fast, animals were sacrificed by an intravenous overdose of sodium pentobarbitone (Virbac, NSW, Australia), and a post mortem was performed between 1400h and 1600h. Body weight and nose to rump length were measured and the body mass index was calculated (weight/nose to rump length^2). Selected skeletal muscles and adipose depots were dissected out, weighed immediately, summed, and expressed as a percentage of body weight at post mortem, to give an index of the percentage of body weight composed of mixed and type 2 muscle and visceral and subcutaneous adipose tissue (as described in Section 2.2.6). In addition, all individual skeletal muscle and adipose depot weights were summed and expressed as a percentage of body weight at post mortem, to give an index of the percentage of body weight composed of skeletal muscle (combined muscle) and adipose tissue (combined adiposity) respectively. The following organs and glands were also dissected out and weighed: adrenals, kidneys and brain. The heart was dissected out, weighed and then separated into the right and left ventricles, which were weighed individually. All adipose depot, skeletal muscle, organ and gland weights were expressed in absolute terms as well as in relative terms as a percentage of body weight at post mortem.

6.2.4 Statistical analysis

All statistical analyses were carried out using SPSS for Windows (Version 13.0, SPSS Inc., Chicago, IL, USA). To examine the effect of size at birth on adult blood pressure, offspring were classed into two groups, those with birth weights greater than (high birth weight) or less than (low birth weight) the median birth weight for

the cohort of guinea pigs described in this thesis, which was 95.55 grams. Males and females were also analysed separately, classing each as high or low birth weight, using the median birth weight for each sex (94.8 grams for males and 99.58 grams for females). The definition of fetal growth restriction in experimental studies in non-human species varies, and this approach of below versus above the median size at birth has been commonly used in these studies, as well as in some human studies. The effect of birth weight class on adult blood pressure was assessed by a single between factor ANOVA in all animals combined, and in males and females separately. The effects of birth weight class, sex and their interaction, on adult blood pressure were examined by a two between factor ANOVA. Specific comparisons were carried out by Bonferroni post hoc tests.

To examine the effect of neonatal fractional growth rate on adult blood pressure, offspring were classed into two groups as either 'high growers' or 'low growers' using the following approach. In order to classify animals according to growth, males and females were separated, and the neonatal fractional growth rate was plotted against birth weight for each sex separately. Animals above and below the regression line were classified as 'high growers' and 'low growers' respectively. The effect of growth rate class on adult blood pressure was assessed by a single between factor ANOVA in all animals combined, and in males and females separately. A two between factor ANOVA was used to determine the effect of growth rate class, sex and their interaction, on adult blood pressure. Specific comparisons were carried out by Bonferroni post hoc tests.

Relationships between adult blood pressure, size at birth, neonatal growth rates and adult size and body composition were examined using simple correlation and multiple linear regression analyses, in all animals combined and in male and female offspring separately. One sided p-values were used to test *a priori* hypotheses regarding the relationships between adult blood pressure and size at birth and neonatal growth rates, based on similar relationships reported in humans.

For all statistical tests, significance was accepted at $P<0.05$. All data are presented as mean ± S.E.M.

6.3 RESULTS

6.3.1 Effect of birth weight class on blood pressure in the aged guinea pig

Birth weight class did not alter systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, pulse pressure or heart rate in all male and female offspring combined (Table 6.1). Low birth weight did alter mean arterial blood pressure differently with sex, such that mean arterial blood pressure tended to be lower in males, but to increase in females ($p<0.03$) (Table 6.1).

In male offspring only, birth weight class did not influence any parameter of adult blood pressure (data shown for systolic blood pressure and mean arterial blood pressure only, Figures 6.1a and b).

In female offspring only, birth weight class did not alter diastolic blood pressure, pulse pressure or heart rate (data not shown), but did alter systolic blood pressure and mean arterial blood pressure. Specifically, systolic blood pressure (+11%) ($p<0.02$) and mean arterial blood pressure (+7%) ($p<0.05$) were increased in low birth weight compared to high birth weight female offspring (Figures 6.1a and b).

6.3.2 Effect of neonatal fractional growth rate class on blood pressure in the aged guinea pig

Neonatal fractional growth rate class did not alter systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, pulse pressure or heart rate in all male and female offspring combined. High neonatal fractional growth rate did alter pulse pressure differently with sex however, such that pulse pressure tended to decrease with a high neonatal fractional growth rate in females, but to increase in males ($p<0.03$) (Table 6.2).

In male offspring only, neonatal fractional growth rate class did not alter systolic, diastolic or mean arterial blood pressure (data not shown), but did alter pulse pressure and heart rate (Figures 6.2b and c). Fast growing males demonstrated a higher pulse pressure (+34%) ($p<0.04$) and heart rate (+13%) ($p<0.02$) than their slow growing counterparts (Figures 6.2b and c).

Table 6.1 Effect of birth weight class and sex on blood pressure in the aged guinea pig

	Birth weight class				ANOVA *P*-value		
	Low birth weight		High birth weight		BW	S	BW x S
	Males (*n*=8)	Females (*n*=4)	Males (*n*=4)	Females (*n*=8)			
Systolic Blood Pressure (mmHg)	84.6 ± 3.6	90.0 ± 4.2	89.9 ± 4.3	83.8 ± 2.5	NS	NS	NS
Diastolic Blood Pressure (mmHg)	58.0 ± 1.8	60.3 ± 1.4	62.5 ± 3.1	61.3 ± 2.4	NS	NS	NS
Mean Arterial Blood Pressure (mmHg)	71.2 ± 2.3	75.2 ± 1.7	79.6 ± 3.4	72.9 ± 1.9	NS	NS	0.025
Pulse Pressure (mmHg)	26.6 ± 2.8	29.8 ± 4.8	27.4 ± 2.5	22.5 ± 2.8	NS	NS	NS
Heart Rate (beats min^{-1})	263 ± 10	256 ± 11	279 ± 8	247 ± 13	NS	NS	NS

Data are presented as means ± S.E.M. *n* refers to the number of animals. ANOVA: effect of birth weight class (BW), sex (S) and their interaction (BW x S). Statistical significance was assumed at $P < 0.05$. NS, not significant.

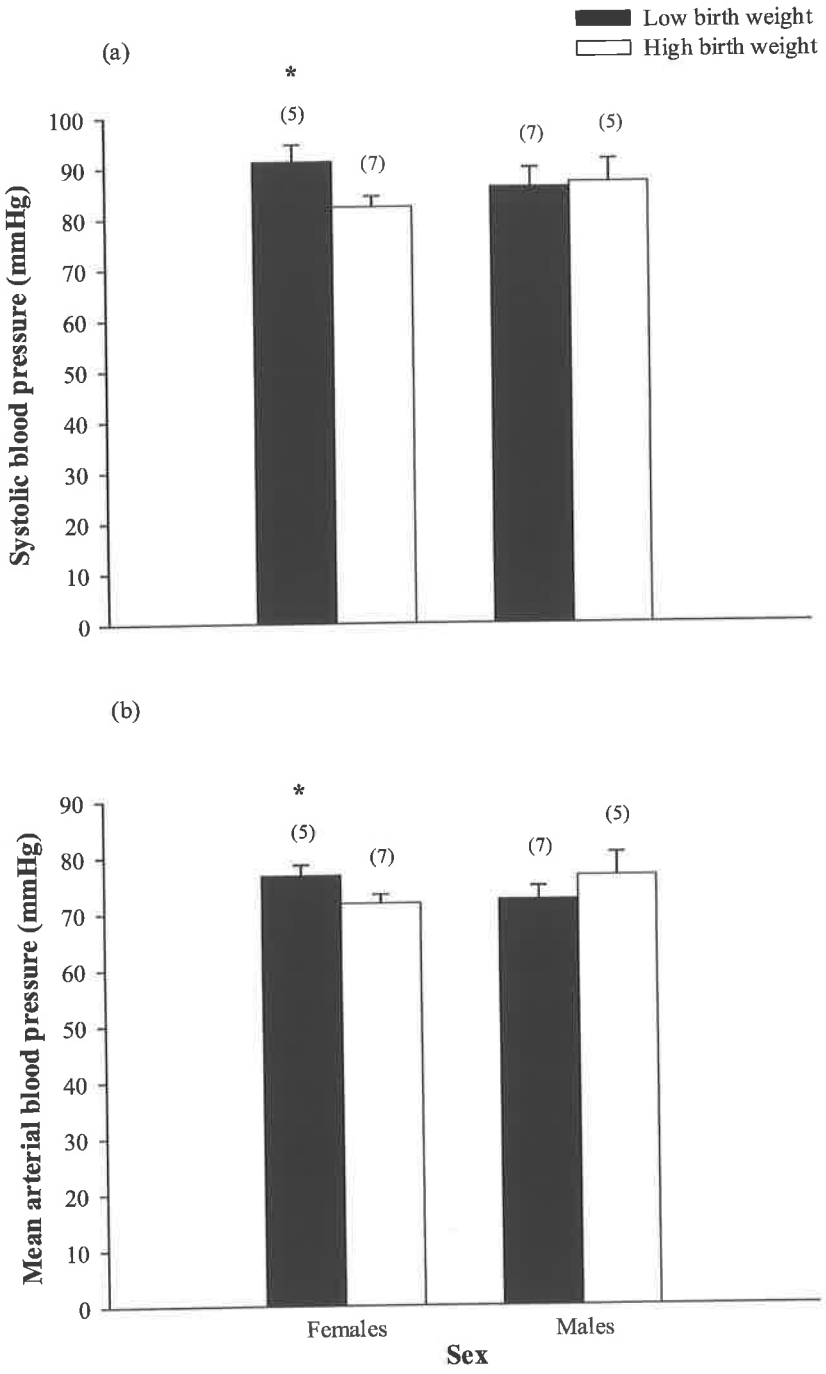

Figure 6.1 The effect of birth weight class and sex on systolic and mean arterial blood pressure in the aged guinea pig

Systolic (a) and mean arterial (b) blood pressure in aged adult guinea pigs of low and high birth weight. Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. *$P < 0.05$ compared with high birth weight offspring of the same sex.

Table 6.2 Effect of neonatal fractional growth rate class and sex on blood pressure in the aged guinea pig

	Neonatal fractional growth rate class				ANOVA *P*-value		
	Low growth rate		High growth rate		GR	S	GR x S
	Males (*n*=4)	Females (*n*=6)	Males (*n*=8)	Females (*n*=6)			
Systolic Blood Pressure (mmHg)	83.3 ± 7.1	89.7 ± 2.9	87.9 ± 2.5	82.0 ± 2.8	NS	NS	NS
Diastolic Blood Pressure (mmHg)	61.4 ± 4.1	61.6 ± 2.9	58.6 ± 1.5	60.3 ± 1.7	NS	NS	NS
Mean Arterial Blood Pressure (mmHg)	73.0 ± 5.2	75.7 ± 1.9	74.5 ± 2.3	71.7 ± 1.8	NS	NS	NS
Pulse Pressure (mmHg)	21.9 ± 4.7	28.1 ± 4.3	29.3 ± 1.8	21.8 ± 2.6	NS	NS	0.021
Heart Rate (beats min^{-1})	247 ± 16	249 ± 16	279 ± 5	252 ± 12	NS	NS	NS

Data are presented as means ± S.E.M. *n* refers to the number of animals. ANOVA: effect of neonatal fractional growth rate class (GR), sex (S) and their interaction (GR x S). Statistical significance was assumed at $P < 0.05$. NS, not significant.

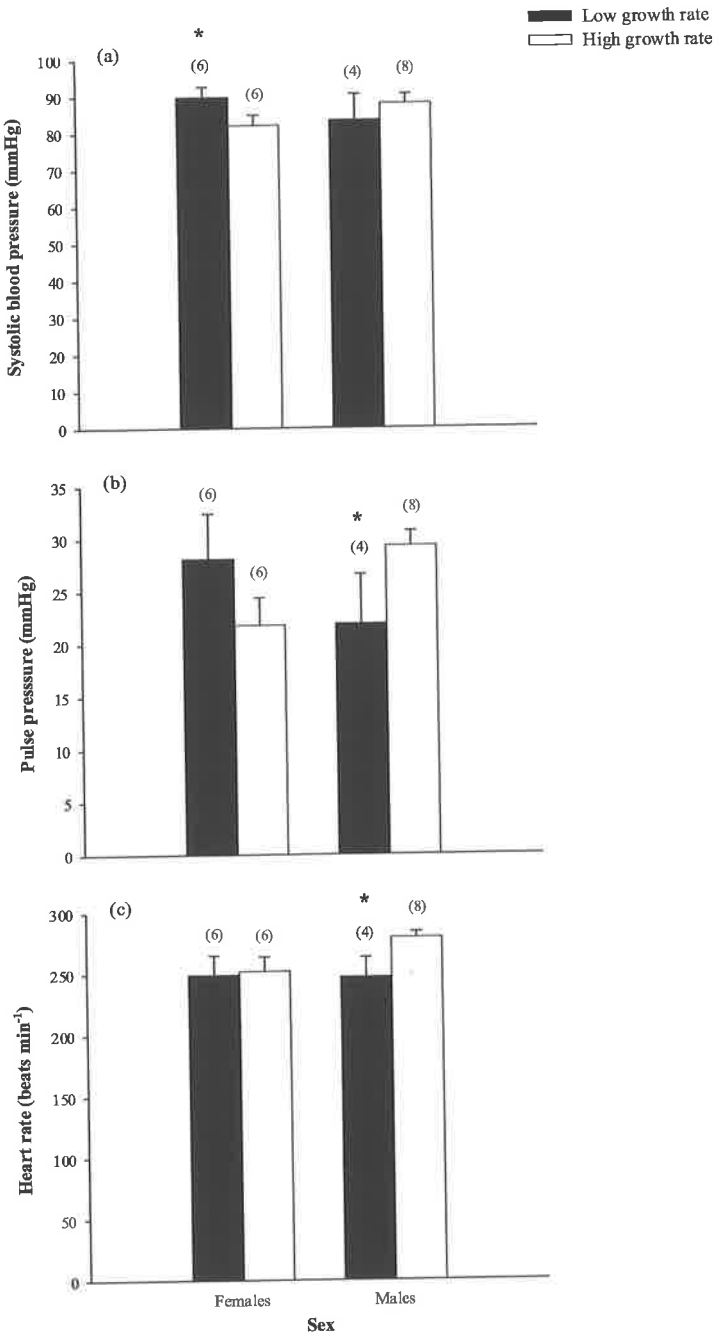

Figure 6.2 The effect of neonatal fractional growth rate class and sex on systolic blood pressure, pulse pressure and heart rate in the aged guinea pig

Systolic blood pressure (a) pulse pressure (b) and heart rate (c) in aged adult guinea pigs of low and high neonatal fractional growth rate. Data are presented as means ± SEM. Numbers in parentheses represent the number of animals. *$P < 0.05$ compared with high growth rate offspring of the same sex.

In female offspring only, neonatal fractional growth rate class did not alter diastolic blood pressure, mean arterial blood pressure, pulse pressure or heart rate (data not shown), but did alter systolic blood pressure, with slow growing females demonstrating a higher systolic blood pressure (+9%) ($p<0.05$) than their fast growing counterparts (Figure 6.2a). Inclusion of birth weight as a co-variate did not alter these outcomes.

6.3.3 Relationship of blood pressure to size at birth, neonatal growth rate and adult size in the aged guinea pig

6.3.3.1 Size at birth

In all male and female offspring combined, diastolic blood pressure correlated positively with head width at birth ($r=0.36$, $p=0.043$, $n=24$), pulse pressure and heart rate correlated negatively with head width at birth ($r=-0.37$, $p=0.037$ and $r=-0.43$, $p=0.017$, respectively, $n=24$) and pulse pressure correlated negatively with the birth weight to length ratio ($r=-0.35$, $p=0.048$, $n=24$) (Table 6.3).

In male offspring, adult blood pressure parameters were not associated with any measures of size at birth (data shown for selected associations only, Figures 6.3a, b and c and Figures 6.4a and b).

In female offspring, systolic blood pressure, pulse pressure and heart rate correlated negatively with abdominal circumference at birth ($r=-0.67$, $p=0.009$; $r=-0.59$, $p=0.021$ and $r=-0.56$, $p=0.030$, respectively, $n=12$) (Figures 6.3a, b and c) and pulse pressure and heart rate correlated negatively with head width at birth ($r=-0.62$, $p=0.016$, and $r=-0.57$, $p=0.027$, respectively, $n=12$) (Figures 6.4a and b).

6.3.3.2 Neonatal growth rate

In all male and female offspring combined, heart rate correlated positively with neonatal absolute growth rate ($r=0.63$, $p<0.001$, $n=24$) and diastolic blood pressure correlated negatively and heart rate correlated positively with neonatal fractional growth rate ($r=-0.36$, $p=0.042$ and $r=0.54$, $p=0.003$, respectively, $n=24$) (Table 6.4).

In male offspring, mean arterial blood pressure correlated positively with neonatal absolute growth rate ($r=0.51$, $p=0.044$, $n=12$) (Figure 6.5b) and pulse pressure and

Table 6.3 Relationship of blood pressure to size at birth in the aged guinea pig

	Correlation coefficients (*r*)				
	SBP	DBP	MAP	PP	HR
BWT	-0.08 (24)	0.29 (24)	0.10 (24)	-0.29 (24)	-0.24 (24)
BNRL	0.01 (24)	0.26 (24)	0.18 (24)	-0.18 (24)	-0.11 (24)
BWT:BNRL	-0.15 (24)	0.26 (24)	0.03 (24)	[redacted]	-0.30 (24)
BPI	-0.17 (24)	-0.04 (24)	-0.20 (24)	-0.16 (24)	-0.18 (24)
BAC	-0.16 (24)	0.20 (24)	-0.13 (24)	-0.32 (24)	-0.33 (24)
BHL	0.11 (24)	0.34 (24)	0.12 (24)	-0.13 (24)	-0.22 (24)
BHW	-0.11 (24)	[redacted]	-0.06 (24)	-0.37 (24)*	-0.43 (24)*
BHW:BAC	0.09 (24)	0.05 (24)	0.07 (24)	0.07 (24)	0.00 (24)

r represents the correlation coefficient between each variable of adult blood pressure and each variable of size at birth. Numbers in parentheses represent the number of animals. Partial correlations: all significant correlations highlighted in dark grey were independent of adult weight. Statistical significance of correlation coefficients: $^{*}P < 0.05$. SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); MAP, mean arterial blood pressure (mmHg); PP, pulse pressure (mmHg); HR, heart rate (beats min^{-1}). BWT, birth weight (g); BNRL, birth nose to rump length (mm); BW:BNRL, birth weight to nose to rump length ratio (g mm^{-1}); BPI, birth ponderal index (g mm^{-3}); BAC, birth abdominal circumference (mm); BHL, birth head length (mm); BHW, birth head width (mm); BHW:BAC, birth head width to abdominal circumference ratio.

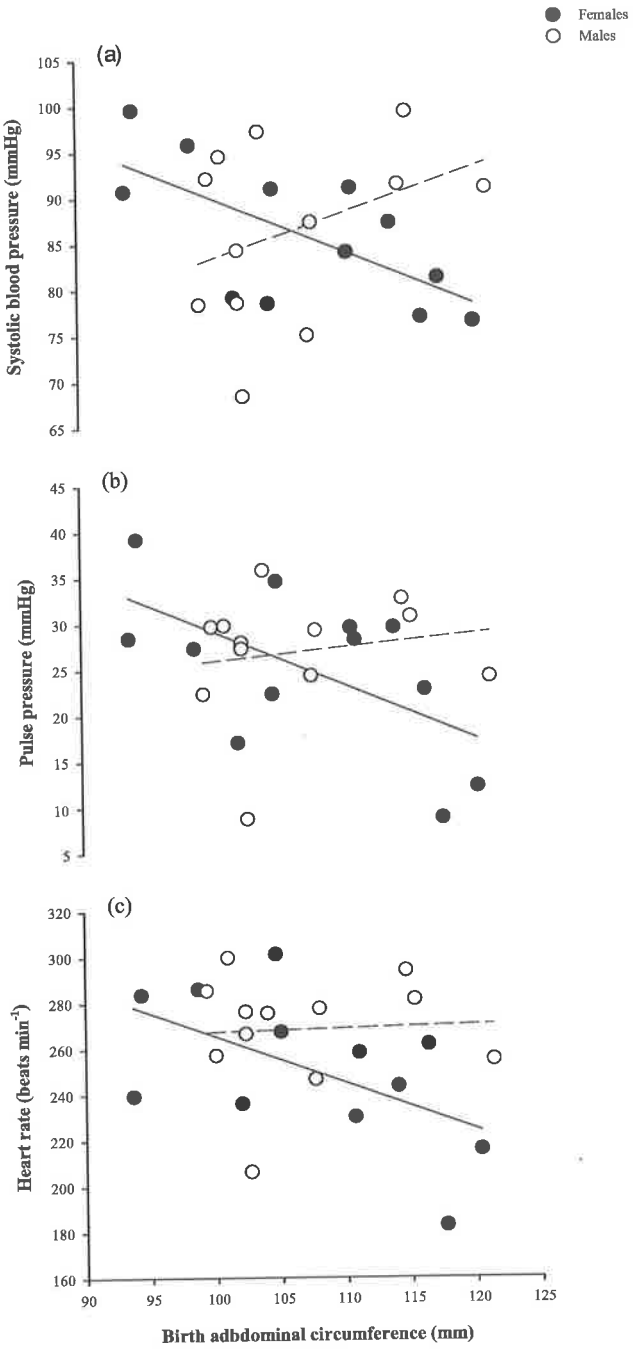

Figure 6.3 Relationship of systolic blood pressure, pulse pressure and heart rate to abdominal circumference at birth in the aged guinea pig

Relationship of abdominal circumference at birth to (a) systolic blood pressure (females: r=-0.67, n=12, p<0.01; males: r=0.37, n=12, ns) (b) pulse pressure (females: r=-0.59, n=12, p<0.05; males: r=0.15, n=12, ns) and (c) heart rate (females: r=-0.56, n=12, p<0.05; males: r=0.04, n=12, ns) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

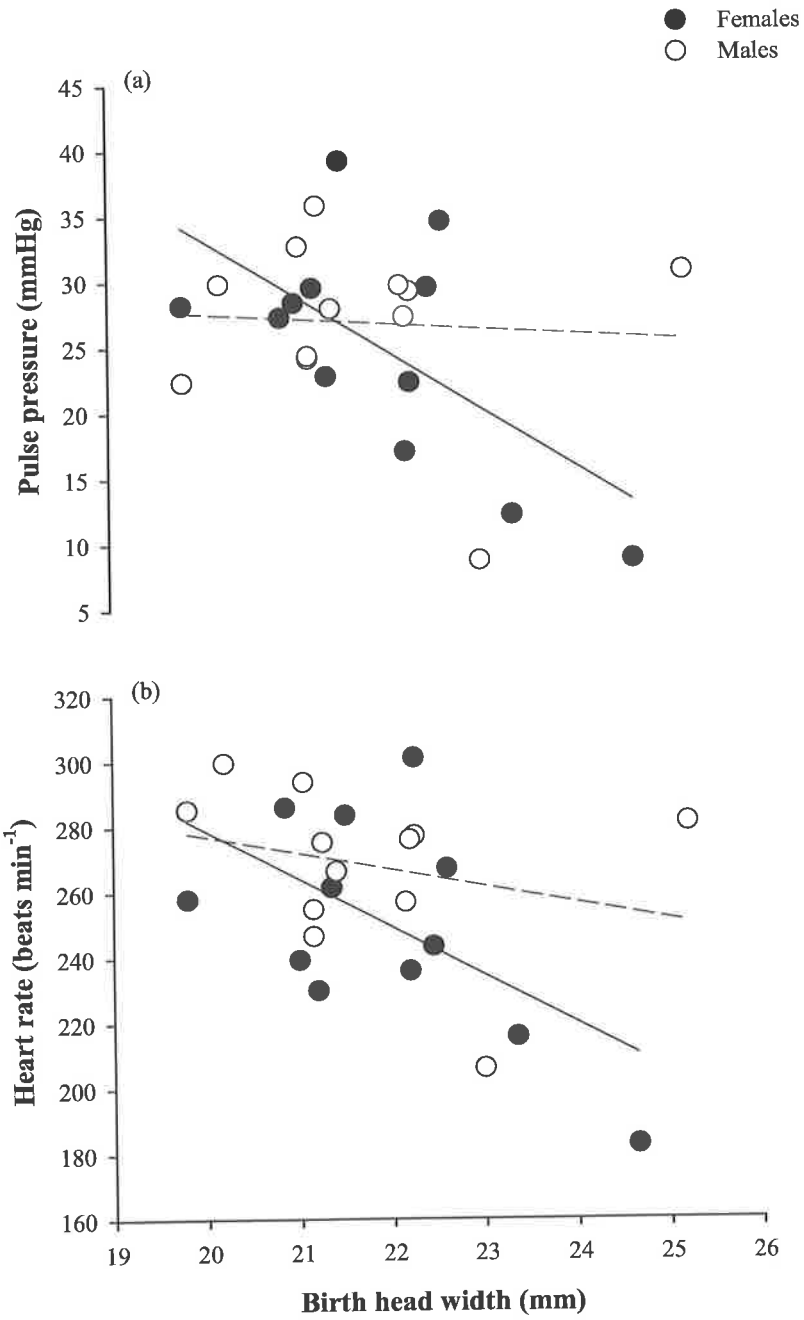

Figure 6.4 The relationship of pulse pressure and heart rate to head width at birth in the aged guinea pig

Relationship of head width at birth to (a) pulse pressure (females: r=-0.62, n=12, $p<0.05$; males: r=-0.08, n=12, ns) and (b) heart rate (females: r=-0.57, n=12, $p<0.05$; males: r=-0.29, n=12, ns) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

Table 6.4 Relationship of blood pressure to neonatal growth rate and adult size in the aged guinea pig

	Correlation coefficients (r)				
	SBP	DBP	MAP	PP	HR
$AGR_{10\text{-}30}$	0.10 (24)	-0.25 (24)	0.11 (24)	0.29 (24)	0.63 (24)††
$FGR_{10\text{-}30}$	0.06 (24)	-0.36 (24)*	-0.12 (24)	0.32 (24)	0.54 (24)†
AW	0.57 (24)†	0.23 (24)	0.57 (24)†	0.46 (24)*	0.52 (24)**
ANRL	0.57 (13)*	-0.12 (13)	0.38 (13)	0.70 (13)†	0.59 (13)*
ABMI	0.15 (13)	0.25 (13)	0.36 (13)	-0.06 (13)	-0.04 (13)

r represents the correlation coefficient between each variable of adult blood pressure and each variable of neonatal growth or adult size. Numbers in parentheses represent the number of animals. Statistical significance of correlation coefficients: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{\dagger}P < 0.005$, $^{\dagger\dagger}P < 0.001$. SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); MAP, mean arterial blood pressure (mmHg); PP, pulse pressure (mmHg); HR, heart rate (beats min^{-1}). $AGR_{10\text{-}30}$, absolute neonatal growth rate (10-30 days) (g day^{-1}); $FGR_{10\text{-}30}$, fractional neonatal growth rate (10-30 days) (g day^{-1} g^{-1}); AW, adult weight (g); ANRL, adult nose to rump length (mm); ABMI, adult body mass index (g mm^{-2}).

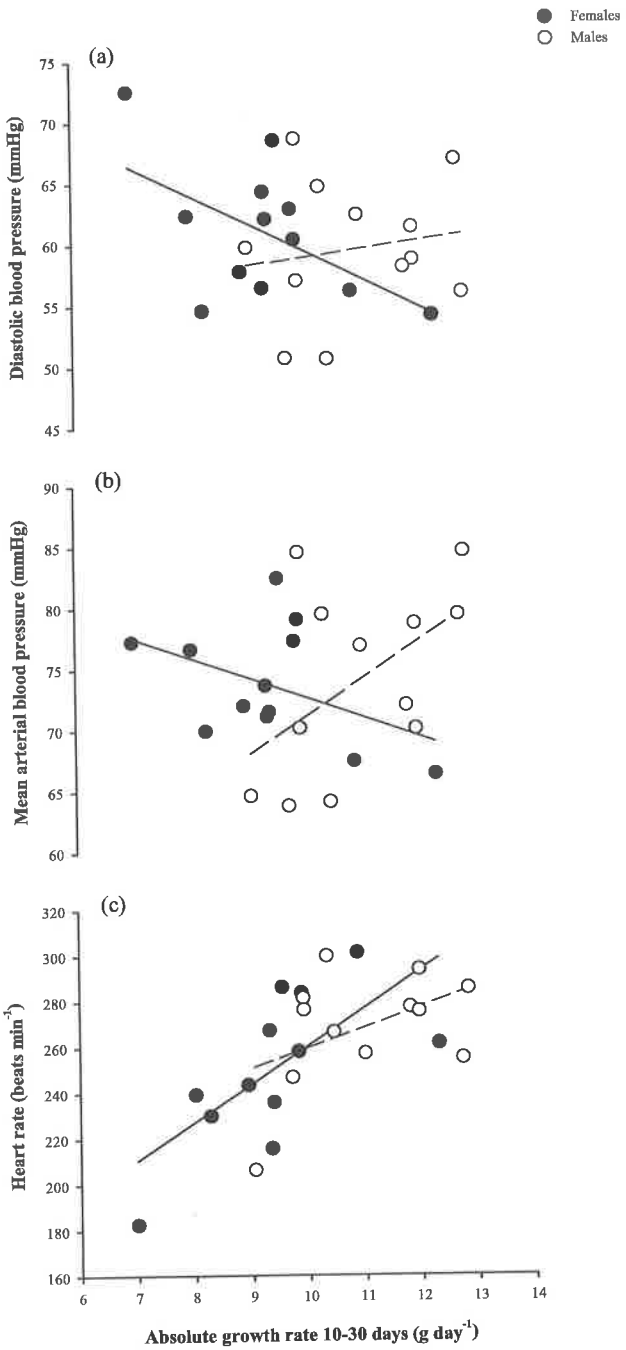

Figure 6.5 The relationship of diastolic and mean arterial blood pressure and heart rate to neonatal absolute growth rate in the aged guinea pig

Relationship of neonatal absolute growth rate to (a) diastolic blood pressure (females: $r=-0.55$, $n=12$, $p<0.05$; males: $r=0.15$, $n=12$, ns) (b) mean arterial blood pressure (females: $r=-0.45$, $n=12$, ns; males: $r=0.51$, $n=12$, $p<0.05$) and (c) heart rate (females: $r=0.68$, $n=12$, $p<0.01$; males: $r=0.44$, $n=12$, ns) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

heart rate correlated positively with neonatal fractional growth rate (r=0.53, p=0.037 and r=0.59, p=0.021, respectively, n=12) (Figures 6.6a and b).

In female offspring, diastolic blood pressure correlated negatively and heart rate correlated positively associated neonatal absolute growth rate (r=-0.55, p=0.033 and r=0.68, p=0.007, respectively, n=12) (Figures 6.5a and c).

6.3.3.3 Adult size

In all male and female offspring combined, systolic blood pressure, mean arterial blood pressure, pulse pressure and heart rate correlated positively with adult weight (r=0.57, p=0.002; r=0.57, p=0.020; r=0.46, p=0.012, and r=0.52, p=0.005, respectively, n=24), and systolic blood pressure, pulse pressure and heart rate correlated positively with adult nose to rump length (r=0.57, p=0.020; r=0.70, p=0.004, and r=0.59, p=0.017, respectively, n=13) (Table 6.4).

In male offspring, systolic blood pressure, mean arterial blood pressure, pulse pressure and heart rate correlated positively with adult weight (r=0.73, p=0.003; r=0.72, p=0.004; r=0.63, p=0.015 and r=0.59, p=0.021, respectively, n=12) (Figures 6.7a, b, c and d) and systolic blood pressure, pulse pressure and heart rate correlated positively with adult nose to rump length (r=0.74, p=0.011; r=0.90, p=0.001 and r=0.82, p=0.003, respectively, n=9) (Figures 6.8a, b and c).

In female offspring, adult blood pressure parameters were not associated with any measures of adult size (data shown for selected associations only, Figures 6.7a, b, c and d and Figures 6.8a, b and c).

6.3.3.4 Partial correlations

The positive association between diastolic blood pressure and head width at birth in all offspring was independent of adult weight (partial correlation (pc), r=0.44, p=0.019), but not of neonatal fractional growth rate. The negative association between pulse pressure and the birth weight to length ratio in all offspring was independent of adult weight (pc: r=-0.35, p=0.049), but not of neonatal fractional growth rate. The negative association between pulse pressure and head width at birth in all offspring was not independent of adult weight or neonatal fractional growth rate. The negative association between heart rate and head width at birth in all

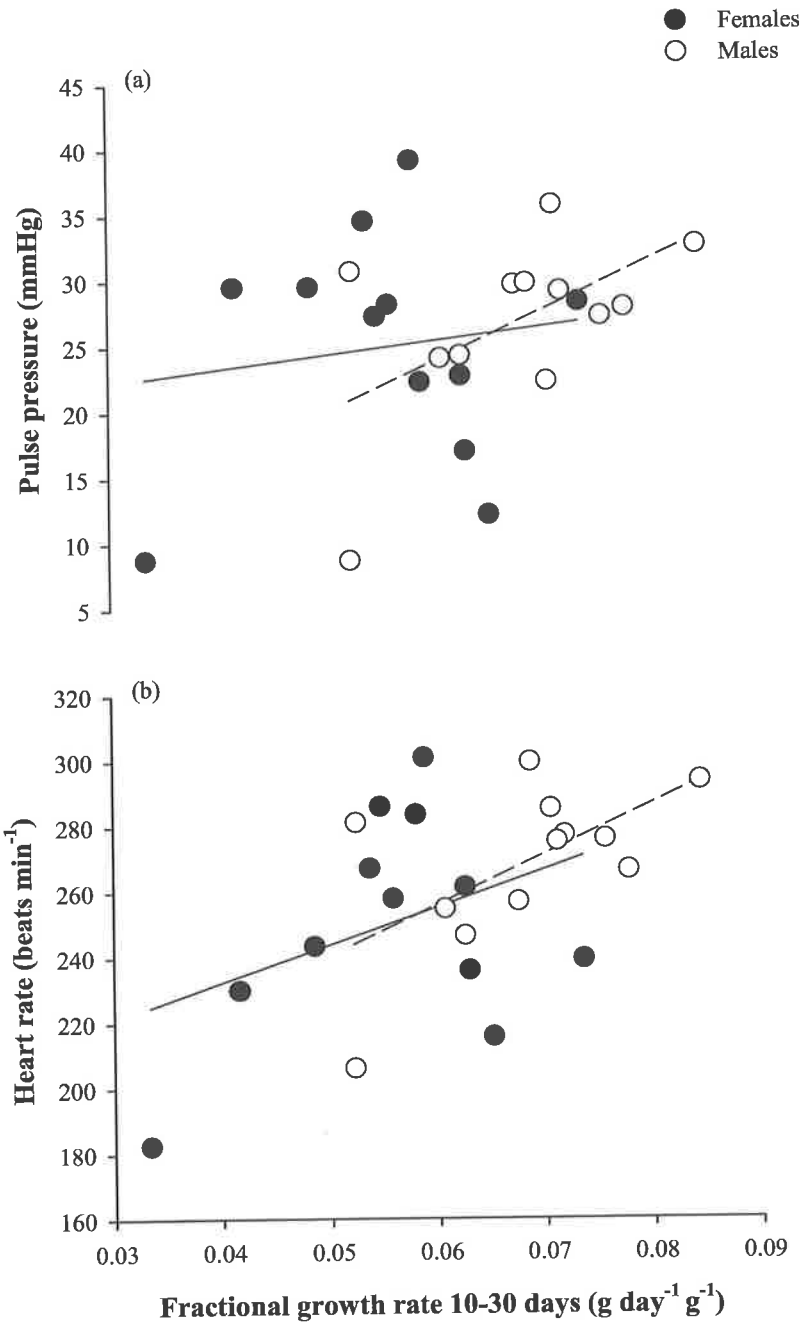

Figure 6.6 The relationship of pulse pressure and heart rate to neonatal fractional growth rate in the aged guinea pig

Relationship of neonatal fractional growth rate to (a) pulse pressure (females: r=0.14, n=12, ns; males: r=0.53 n=12, $p<0.05$) and (b) heart rate (females: r=0.39, n=12, ns; males: r=0.59, n=12, $p<0.05$) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

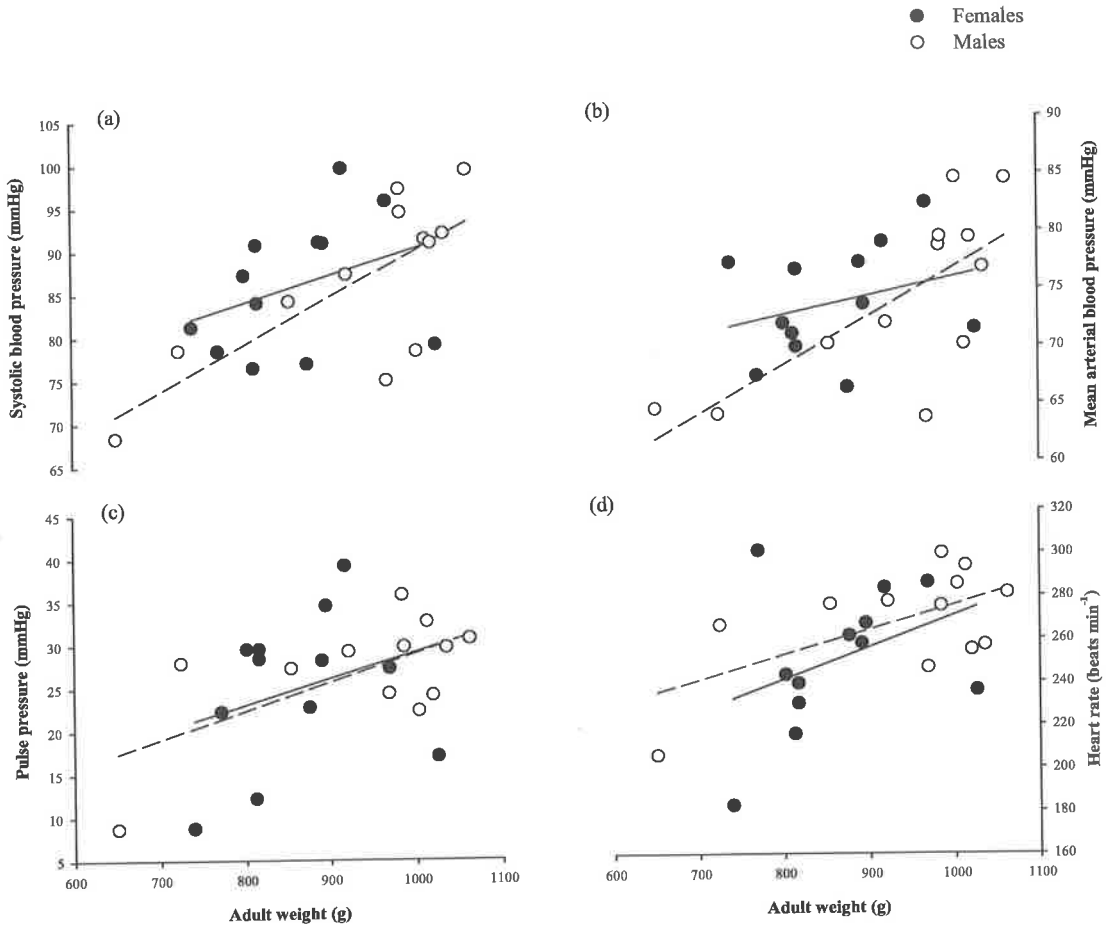

Figure 6.7 The relationship of systolic and mean arterial blood pressure, pulse pressure and heart rate to adult weight in the aged guinea pig

Relationship of adult weight to (a) systolic blood pressure (females: r=0.34, n=12, ns; males: r=0.73, n=12, p<0.005) (b) mean arterial blood pressure (females: r=0.28, n=12, ns; males: r=0.72, n=12, p<0.005) (c) pulse pressure (females: r=0.29, n=12, ns; males: r=0.63, n=12, p<0.05) and (d) heart rate (females: r=0.38, n=12, ns; males: r=0.59, n=12, p<0.05) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

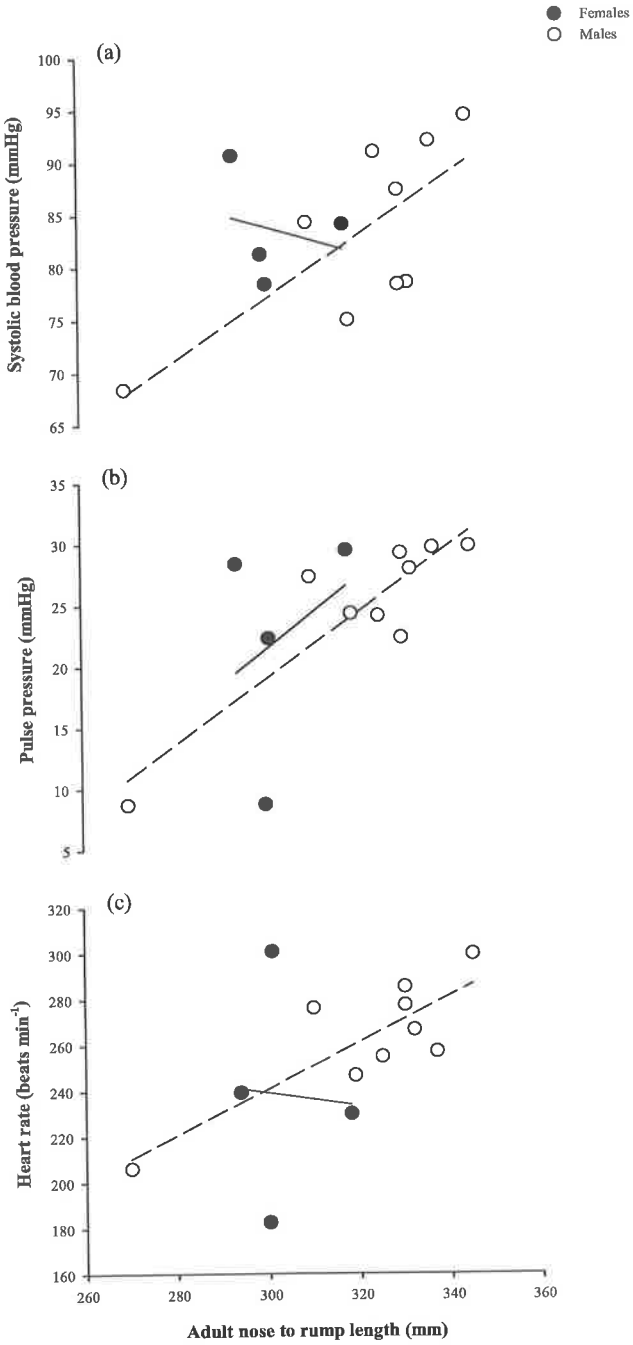

Figure 6.8 The relationship of systolic blood pressure, pulse pressure and heart rate to adult nose to rump length in the aged guinea pig

Relationship of adult nose to rump length to (a) systolic blood pressure (females: r=-0.25, n=4, ns; males: r=0.74, n=9, $p<0.01$) (b) pulse pressure (females: r=0.32, n=4, ns; males: r=0.90, n=9, $p<0.005$) and (c) heart rate (females: r=-0.06, n=4, ns; males: r=0.82, n=9, $p<0.005$) in aged adult guinea pigs. Females are represented by the solid regression line and solid circles and males are represented by the dashed regression line and open circles.

offspring was independent of adult weight (pc: r=-0.37, p=0.040), but not of neonatal fractional growth rate.

In female offspring, systolic blood pressure and pulse pressure were negatively associated with abdominal circumference at birth independent of neonatal fractional growth rate (pc: r=-0.74, p=0.005 and r=-0.59, p=0.028) and adult weight (pc: r=-0.62, p=0.022 and r=-0.54, p=0.044). In contrast, the negative association between heart rate and abdominal circumference at birth in females was not independent of neonatal fractional growth rate or adult weight. The negative association of pulse pressure with head width at birth in females was independent of neonatal fractional growth rate (pc: r=-0.62, p=0.021) and adult weight (pc: r=-0.57, p=0.033). In contrast, the negative association of heart rate and head width at birth in females was not independent of neonatal fractional growth rate or adult weight.

The negative association between diastolic blood pressure and neonatal fractional growth rate in all offspring was independent of adult weight (pc: r=-0.47, p=0.013), but not of birth weight. The positive association between heart rate and neonatal fractional growth rate in all offspring was independent of birth weight (pc: r=0.51, p=0.007) and adult weight (pc: r=0.47, p=0.012).

In male offspring, the positive association between pulse pressure and neonatal fractional growth rate was independent of adult weight (pc: r=0.65, p=0.015) but not birth weight, however the positive association between heart rate and neonatal fractional growth rate in male offspring was independent of birth weight (pc: r=0.61, p=0.023) and adult weight (pc: r=0.71, p=0.007).

6.3.4 Relationship of blood pressure to body composition in the aged guinea pig

6.3.4.1 Adiposity

In all male and female offspring combined, pulse pressure correlated positively with both absolute and relative combined adiposity (r=0.51, p=0.047 and r=0.51, p=0.046, respectively, n=12), absolute and relative visceral adiposity (r=0.55, p=0.02 and r=0.57, p=0.016, respectively, n=14) and absolute and relative subcutaneous adiposity (r=0.50, p=0.028 and r=0.48, p=0.036, respectively, n=15) (Table 6.5).

Table 6.5 Relationship of blood pressure to adiposity and skeletal muscle mass in the aged guinea pig

	Correlation coefficients (r)				
	SBP	DBP	MAP	PP	HR
Combined adiposity (g)	0.31 (12)	-0.19 (12)	0.40 (12)	0.51 (12)*	0.41 (12)
(% body weight)	0.23 (12)	-0.29 (12)	0.21 (12)	0.51 (12)*	0.35 (12)
Visceral adiposity (g)	0.36 (14)	-0.19 (14)	0.38 (14)	0.55 (14)*	0.42 (14)
(% body weight)	0.29 (14)	-0.30 (14)	0.19 (14)	0.57 (14)*	0.38 (14)
Subcutaneous adiposity (g)	0.41 (15)	-0.08 (15)	0.49 (15)*	0.50 (15)*	0.60 (15)*
(% body weight)	0.32 (15)	-0.18 (15)	0.35 (15)	0.48 (15)*	0.57 (15)*
Combined muscle (g)	0.30 (16)	-0.02 (16)	0.30 (16)	0.33 (16)	0.13 (16)
(% body weight)	-0.56 (16)*	-0.11 (16)	-0.59 (16)**	-0.51 (16)*	-0.66 (16)†
Mixed muscle (g)	0.30 (17)	-0.04 (17)	0.29 (17)	0.35 (17)	0.14 (17)
(% body weight)	-0.56 (17)*	-0.15 (17)	-0.59 (17)**	-0.48 (17)*	-0.64 (17)†
Type 2 muscle (g)	0.35 (16)	0.37 (16)	0.33 (16)	0.09 (16)	-0.14 (16)
(% body weight)	-0.30 (16)	0.32 (16)	-0.31 (16)	-0.56 (16)*	-0.71 (16)†

r represents the correlation coefficient between each variable of adult blood pressure and each variable of adult adiposity or skeletal muscle mass. Numbers in parentheses represent the number of animals. Statistical significance of correlation coefficients: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{\dagger}P < 0.005$. SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); MAP, mean arterial blood pressure (mmHg); PP, pulse pressure (mmHg); HR, heart rate (beats min^{-1}).

Heart rate correlated positively with absolute and relative subcutaneous adiposity (r=0.60, p=0.01 and r=0.57, p=0.013 respectively, n=15) and mean arterial blood pressure correlated positively with subcutaneous adiposity in absolute terms only (r=0.49, p=0.031, n=15) in all male and female offspring combined (Table 6.5).

In male offspring, pulse pressure and heart rate correlated positively with subcutaneous adiposity in absolute (r=0.63, p=0.035 and r=0.81, p=0.004, respectively, n=9) and relative terms (r=0.60, p=0.044 and r=0.79, p=0.006, respectively n=9) and mean arterial blood pressure correlated positively with subcutaneous adiposity in absolute terms only (r=0.67, p=0.025, n=9) (Table 6.6).

In female offspring, systolic blood pressure correlated positively with visceral and combined adiposity in absolute (r=0.85, p=0.004, n=8 and r=0.80, p=0.028, n=6 respectively) and relative terms (r=0.85, p=0.004, n=8 and r=0.82, p=0.023, n=6 respectively) and pulse pressure correlated positively with visceral adiposity in absolute and relative terms (r=0.82, p=0.007 and r=0.78, p=0.011, respectively, n=8) and combined adiposity in absolute terms only (r=0.74, p=0.048, n=6) (Table 6.7).

6.3.4.2 Skeletal muscle mass

In all male and female offspring combined, systolic blood pressure, mean arterial pressure, pulse pressure and heart rate correlated negatively with the relative weight of combined muscle (r=-0.56, p=0.011; r=-0.59, p=0.008; r=-0.51, p=0.022 and r=-0.66, p=0.003, respectively, n=16) and mixed muscle (r=-0.56, p=0.010; r=-0.59, p=0.007; r=-0.48, p=0.025 and r=-0.64, p=0.003, respectively, n=17). Pulse pressure and heart rate correlated negatively associated with type 2 muscle in relative terms (r=-0.56, p=0.012 and r=-0.71, p=0.001, respectively, n=16) in all offspring combined (Table 6.5).

In male offspring, systolic blood pressure and mean arterial pressure correlated negatively with the relative weight of combined muscle (r=-0.70, p=0.018 and r=-0.83, p=0.003, respectively, n=9) and of mixed muscle (r=-0.70, p=0.019 and r=-0.82, p=0.003, respectively, n=9), pulse pressure and heart rate correlated negatively with type 2 muscle in relative terms (r=-0.58, p=0.05 and r=-0.77, p=0.008, respectively, n=9) and heart rate correlated negatively with combined muscle in relative terms (r=-0.62, p=0.039, n=9) (Table 6.6).

Table 6.6 Relationship of blood pressure to adiposity and skeletal muscle mass in aged male guinea pigs

	Correlation coefficients (r)				
	SBP	DBP	MAP	PP	HR
Combined adiposity (g)	-0.30 (6)	-0.09 (6)	0.56 (6)	-0.51 (6)	0.33 (6)
(% body weight)	-0.61 (6)	-0.62 (6)	-0.11 (6)	-0.16 (6)	0.37 (6)
Visceral adiposity (g)	-0.18 (6)	0.04 (6)	0.59 (6)	-0.52 (6)	0.19 (6)
(% body weight)	-0.54 (6)	-0.56 (6)	-0.16 (6)	-0.12 (6)	0.26 (6)
Subcutaneous adiposity (g)	0.53 (9)	0.08 (9)	0.67 (9)*	0.63 (9)*	0.81 (9)†
(% body weight)	0.34 (9)	-0.17 (9)	0.41 (9)	0.60 (9)*	0.79 (9)**
Combined muscle (g)	0.47 (9)	0.11 (9)	0.38 (9)	0.52 (9)	0.31 (9)
(% body weight)	-0.70 (9)*	-0.50 (9)	-0.83 (9)†	-0.50 (9)	-0.62 (9)*
Mixed muscle (g)	0.45 (9)	0.07 (9)	0.36 (9)	0.54 (9)	0.33 (9)
(% body weight)	-0.70 (9)*	-0.52 (9)	-0.82 (9)†	-0.47 (9)	-0.58 (9)
Type 2 muscle (g)	0.48 (9)	0.53 (9)	0.39 (9)	0.18 (9)	-0.02 (9)
(% body weight)	-0.33 (9)	0.17 (9)	-0.47 (9)	-0.58 (9)*	-0.77 (9)**

r represents the correlation coefficient between each variable of adult blood pressure and each variable of adult adiposity or skeletal muscle mass. Numbers in parentheses represent the number of animals. Statistical significance of correlation coefficients: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{\dagger}P < 0.005$. SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); MAP, mean arterial blood pressure (mmHg); PP, pulse pressure (mmHg); HR, heart rate (beats min^{-1}).

Table 6.7 Relationship of blood pressure to adiposity and skeletal muscle mass in aged female guinea pigs

	Correlation coefficients (r)				
	SBP	DBP	MAP	PP	HR
Combined adiposity (g)	0.80 (6)*	-0.09 (6)	0.49 (6)	0.74 (6)*	0.34 (6)
(% body weight)	0.82 (6)*	0.01 (6)	0.56 (6)	0.69 (6)	0.27 (6)
Visceral adiposity (g)	0.85 (8)†	-0.15 (8)	0.50 (8)	0.82 (8)**	0.41 (8)
(% body weight)	0.85 (8)†	-0.10 (8)	0.54 (8)	0.78 (8)*	0.36 (8)
Subcutaneous adiposity (g)	0.67 (6)	-0.15 (6)	0.36 (6)	0.67 (6)	0.38 (6)
(% body weight)	0.67 (6)	-0.06 (6)	0.41 (6)	0.62 (6)	0.33 (6)
Combined muscle (g)	0.32 (7)	-0.16 (7)	0.17 (7)	0.37 (7)	-0.32 (7)
(% body weight)	-0.37 (7)	0.48 (7)	0.08 (7)	-0.62 (7)	-0.81 (7)*
Mixed muscle (g)	0.39 (8)	-0.07 (8)	0.28 (8)	0.38 (8)	-0.24 (8)
(% body weight)	-0.35 (8)	0.46 (8)	0.07 (8)	-0.60 (8)	-0.82 (8)**
Type 2 muscle (g)	-0.06 (7)	0.06 (7)	0.04 (7)	-0.08 (7)	-0.61 (7)
(% body weight)	-0.51 (7)	0.50 (7)	-0.03 (7)	-0.75 (7)*	-0.69 (7)*

r represents the correlation coefficient between each variable of adult blood pressure and each variable of adult adiposity or skeletal muscle mass. Numbers in parentheses represent the number of animals. Statistical significance of correlation coefficients: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{\dagger}P < 0.005$. SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); MAP, mean arterial blood pressure (mmHg); PP, pulse pressure (mmHg); HR, heart rate (beats min^{-1}).

In female offspring, heart rate correlated negatively with the relative weight of combined, mixed and type 2 muscle (r=-0.81, p=0.013, n=7; r=-0.82, p=0.007, n=8 and r=-0.69, p=0.044, n=7, respectively) and pulse pressure correlated negatively with type 2 muscle in relative terms (r=-0.75, p=0.027, n=7) (Table 6.7).

6.3.4.3 Organ and gland weights

In all male and female offspring combined, systolic blood pressure, pulse pressure and heart rate correlated positively with adrenal weight (r=0.49, p=0.023; r=0.43, p=0.044 and r=0.47, p=0.029, respectively, n=17) (Table 6.8). Systolic blood pressure, mean arterial blood pressure, pulse pressure and heart rate correlated negatively with relative brain weight (r=-0.49, p=0.028; r=-0.45, p=0.04; r=-0.57, p=0.011 and r=-0.58, p=0.009, respectively, n=16) and systolic blood pressure and pulse pressure correlated positively with left ventricle weight (r=0.46, p=0.033 and r=0.44, p=0.040, respectively, n=17) in all offspring combined (Table 6.8). Systolic blood pressure and mean arterial blood pressure correlated negatively with relative kidney weight (r=-0.46, p=0.032 and r=-0.51, p=0.018, respectively, n=17) and heart rate correlated positively with kidney weight (r=0.59, p=0.006, n=17) in all offspring combined (Table 6.8).

In male offspring, systolic blood pressure, mean arterial blood pressure, pulse pressure and heart rate correlated positively with adrenal weight (r=0.89, p=0.001; r=0.60, p=0.045; r=0.78, p=0.006 and r=0.64, p=0.032, respectively, n=9) and kidney weight (r=0.81, p=0.004; r=0.64, p=0.032; r=0.62, p=0.037 and r=0.64, p=0.032, respectively, n=9) (Table 6.9). Systolic blood pressure and pulse pressure correlated positively with heart weight (r=0.61, p=0.042 and r=0.58, p=0.049, respectively, n=9), left ventricle weight (r=0.64, p=0.032 and r=0.58, p=0.049, respectively, n=9) and relative adrenal weight (r=0.59, p=0.048 and r=0.60, p=0.043, respectively, n=9) and systolic blood pressure correlated positively with brain weight (r=0.59, p=0.047, n=9) in male offspring (Table 6.9).

In female offspring, systolic blood pressure and mean arterial pressure correlated negatively with relative kidney weight (r=-0.70, p=0.026 and r=-0.73, p=0.019, respectively, n=8) and relative right ventricle weight (r=-0.75, p=0.015 and r=-0.75, p=0.015, respectively, n=8), pulse pressure and heart rate correlated negatively with relative brain weight (r=-0.83, p=0.011 and r=-0.91, p=0.002, respectively, n=7), and

Table 6.8 Relationship of blood pressure to organ and gland weights in the aged guinea pig

	Correlation coefficients (r)				
	SBP	DBP	MAP	PP	HR
Adrenals (g)	0.49 (17)*	0.13 (17)	0.39 (17)	0.43 (17)*	0.47 (17)*
(% body weight)	0.17 (17)	0.03 (17)	-0.03 (17)	0.16 (17)	0.30 (17)
Kidneys (g)	-0.03 (17)	-0.17 (17)	-0.08 (17)	0.09 (17)	0.59 (17)**
(% body weight)	-0.46 (17)*	-0.21 (17)	-0.51 (17)*	-0.33 (17)	0.15 (17)
Heart (g)	0.41 (17)	0.03 (17)	0.17 (17)	0.41 (17)	0.24 (17)
(% body weight)	0.11 (17)	-0.07 (17)	-0.16 (17)	0.17 (17)	0.01 (17)
Left Ventricle (g)	0.46 (17)*	0.07 (17)	0.27 (17)	0.44 (17)*	0.27 (17)
(% body weight)	0.20 (17)	-0.03 (17)	-0.02 (17)	0.24 (17)	0.08 (17)
Right Ventricle (g)	0.08 (17)	-0.22 (17)	-0.13 (17)	0.26 (17)	0.16 (17)
(% body weight)	-0.15 (17)	-0.30 (17)	-0.37 (17)	0.07 (17)	-0.02 (17)
Brain (g)	0.17 (16)	0.24 (16)	0.25 (16)	0.01 (16)	0.24 (16)
(% body weight)	-0.49 (16)*	0.08 (16)	-0.45 (16)*	-0.57 (16)*	-0.58 (16)**

r represents the correlation coefficient between each variable of adult blood pressure and each adult organ or gland weight. Numbers in parentheses represent the number of animals. Statistical significance of correlation coefficients: $^{*}P < 0.05$, $^{**}P < 0.01$. SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); MAP, mean arterial blood pressure (mmHg); PP, pulse pressure (mmHg); HR, heart rate (beats min^{-1}).

Table 6.9 Relationship of blood pressure to organ and gland weights in aged male guinea pigs

	Correlation coefficients (r)				
	SBP	DBP	MAP	PP	HR
Adrenals (g)	0.89 (9)†	0.46 (9)	0.60 (9)*	0.78 (9)**	0.64 (9)*
(% body weight)	0.59 (9)*	0.21 (9)	0.09 (9)	0.60 (9)*	0.44 (9)
Kidneys (g)	0.81 (9)†	0.52 (9)	0.64 (9)*	0.62 (9)*	0.64 (9)*
(% body weight)	-0.29 (9)	-0.08 (9)	-0.46 (9)	-0.31 (9)	-0.25 (9)
Heart (g)	0.61 (9)*	0.26 (9)	0.27 (9)	0.58 (9)*	0.24 (9)
(% body weight)	0.25 (9)	-0.03 (9)	-0.13 (9)	0.36 (9)	0.01 (9)
Left Ventricle (g)	0.64 (9)*	0.31 (9)	0.40 (9)	0.58 (9)*	0.29 (9)
(% body weight)	0.34 (9)	0.03 (9)	0.04 (9)	0.43 (9)	0.13 (9)
Right Ventricle (g)	0.35 (9)	-0.09 (9)	0.00 (9)	0.54 (9)	0.18 (9)
(% body weight)	0.05 (9)	-0.31 (9)	-0.29 (9)	0.33 (9)	0.01 (9)
Brain (g)	0.59 (9)*	0.52 (9)	0.51 (9)	0.34 (9)	0.56 (9)
(% body weight)	-0.50 (9)	-0.17 (9)	-0.57 (9)	-0.51 (9)	-0.41 (9)

r represents the correlation coefficient between each variable of adult blood pressure and each adult organ or gland weight. Numbers in parentheses represent the number of animals. Statistical significance of correlation coefficients: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{\dagger}P < 0.005$. SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); MAP, mean arterial blood pressure (mmHg); PP, pulse pressure (mmHg); HR, heart rate (beats min^{-1}).

mean arterial pressure correlated negatively with kidney weight (r=-0.73, p=0.02, n=8) and right ventricle weight (r=-0.75, p=0.015, n=8) (Table 6.10).

Table 6.10 Relationship of blood pressure to organ and gland weights in aged female guinea pigs

	Correlation coefficients (r)				
	SBP	DBP	MAP	PP	HR
Adrenals (g)	0.30 (8)	-0.40 (8)	-0.02 (8)	0.52 (8)	0.31 (8)
(% body weight)	-0.46 (8)	-0.13 (8)	-0.39 (8)	-0.30 (8)	-0.00 (8)
Kidneys (g)	-0.53 (8)	-0.52 (8)	-0.73 (8)*	-0.10 (8)	0.58 (8)
(% body weight)	-0.70 (8)*	-0.33 (8)	-0.73 (8)*	-0.37 (8)	0.38 (8)
Heart (g)	0.11 (8)	-0.45 (8)	-0.20 (8)	0.40 (8)	0.28 (8)
(% body weight)	-0.27 (8)	-0.19 (8)	-0.31 (8)	-0.11 (8)	-0.02 (8)
Left Ventricle (g)	0.12 (8)	-0.36 (8)	-0.13 (8)	0.35 (8)	0.30 (8)
(% body weight)	-0.18 (8)	-0.21 (8)	-0.24 (8)	-0.01 (8)	0.11 (8)
Right Ventricle (g)	-0.54 (8)	-0.61 (8)	-0.75 (8)*	-0.04 (8)	0.05 (8)
(% body weight)	-0.75 (8)*	-0.36 (8)	-0.75 (8)*	-0.40 (8)	-0.21 (8)
Brain (g)	-0.55 (7)	-0.11 (7)	-0.45 (7)	-0.36 (7)	-0.30 (7)
(% body weight)	-0.67 (7)	0.47 (7)	-0.15 (7)	-0.83 (7)*	-0.91 (7)†

r represents the correlation coefficient between each variable of adult blood pressure and each adult organ or gland weight. Numbers in parentheses represent the number of animals. Statistical significance of correlation coefficients: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{\dagger}P < 0.005$. SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); MAP, mean arterial blood pressure (mmHg); PP, pulse pressure (mmHg); HR, heart rate (beats min^{-1}).

6.4 DISCUSSION

This study has demonstrated that in the guinea pig, small size at birth is associated with elevated systolic blood pressure, pulse pressure and heart rate in aged female offspring, but not males. Specifically, adult systolic blood pressure and mean arterial blood pressure were increased by 11% and 7% respectively, in low birth weight female guinea pigs when compared to their high birth weight counterparts. In addition, an accelerated neonatal fractional growth rate was associated with elevated pulse pressure and heart rate in aged male guinea pigs, but not in females. Slow growing female guinea pigs demonstrated increased adult systolic blood pressure when compared to their fast growing counterparts, while fast growing male guinea pigs demonstrated increased adult pulse pressure and heart rate when compared to their slow growing counterparts. Therefore in aged female guinea pigs, poor growth before and after birth increases resting blood pressure and heart rate, while in aged male guinea pigs, resting blood pressure and heart rate were increased by rapid growth after birth. In males, the relationships between neonatal growth and adult blood pressure and heart rate were independent of the effect of adult weight.

It is important to note that the definition of fetal growth restriction used in this study, namely a birth weight below the median, differs from clinical IUGR and SGA, and in fact variations in fetal growth and the programming phenomenon are typically on a continuum.

In the current study, spontaneous fetal growth restriction in the guinea pig, as indicated by a reduced head width or abdominal circumference at birth, was associated with elevated systolic blood pressure, pulse pressure and heart rate in aged female offspring, with those classed as low birth weight demonstrating higher systolic and mean arterial blood pressure, compared to their high birth weight counterparts. These findings are consistent with the observations of epidemiological studies in a number of populations, which have demonstrated that adult systolic blood pressure is inversely related to both weight and head circumference at birth, however these associations were present in both males and females (Huxley *et al.*, 2000), in contrast to the findings in the guinea pig in the current study.

Interestingly, guinea pigs subjected to moderate maternal feed restriction and probably greater challenges *in utero*, demonstrated increased systolic blood pressure

as young adults, however in contrast to the present study, this was seen in male but not female offspring (Kind *et al.*, 2002). In offspring of *ad libitum* fed and feed-restricted mothers, combined data showed that systolic blood pressure and mean arterial blood pressure were negatively associated with head width at birth (Kind *et al.*, 2002). In addition, similar to the findings in the current study, an increased neonatal growth rate was associated with higher systolic blood pressure in young adult male, but not female, offspring of *ad libitum* fed mothers (Kind *et al.*, 2002).

Increased activity of, and responsiveness to the HPAA has been suggested as a link between restricted growth *in utero* and hypertension in adult life. Low birth weight has been shown to be associated with increased 0900h plasma cortisol concentrations in men aged 64 years (Phillips *et al.*, 1998) and men and women aged 50 years in the UK (Phillips *et al.*, 2000), and in 20 year old men and women in Australia (Phillips *et al.*, 2000). In addition, fasting plasma cortisol concentrations have been shown to be directly related to blood pressure in elderly men (Phillips *et al.*, 1998). Elevated fasting cortisol levels may represent a stress response, due to the combination of fasting and the novel clinic setting in which the blood samples were taken (Phillips *et al.*, 2006). More recently, a study of boys and girls aged between 7 and 9 years has shown that birth weight was inversely related to salivary cortisol responses to psychological stress in boys, while in girls birth weight was inversely related to morning peak cortisol levels (Jones *et al.*, 2006), suggesting that there are sex differences in the relationship between birth weight and the HPAA response to acute psychological stress. In the rat, maternal exposure to a low protein diet (Langley-Evans 1997) or dexamethasone administration (Levitt *et al.*, 1996) during pregnancy increased blood pressure in adult offspring, due perhaps to an increased binding capacity of glucocorticoid receptors in vascular tissues (Langley-Evans 1997).

The present study has shown that spontaneous fetal growth restriction, as indicated by a low birth weight, is associated with increased adult adrenal size (see Chapter 3), which may reflect an increased sensitivity of this gland to the trophic actions of ACTH. Previous studies in the same species have also shown that maternal dexamethasone treatment (Dean *et al.*, 1999; Liu *et al.*, 2001) or nutrient restriction (Lingas *et al.*, 2001) during late gestation programmes HPAA function in a sex-specific manner, with females demonstrating a greater susceptibility to programming of the HPAA than males. These findings suggest that the elevated blood pressure

observed in aged female guinea pigs following perturbed intrauterine growth, could be due to in part to an increased activity of the HPAA. In aged male guinea pigs however, small size at birth did not alter adrenal size. These findings could explain in part, why spontaneous fetal growth restriction is associated with elevated blood pressure in aged female, but not male, guinea pigs.

Elevated SNS activity established *in utero* may be another mechanism linking small size at birth with increased blood pressure in adult life. Low birth weight babies have been shown to have increased heart rate, an index of SNS activity, when compared with controls during sleep (Spassov *et al.*, 1994). Additionally, a direct relationship between adult pulse rate, which is an index of SNS activity, and birth weight has been shown (Phillips *et al.*, 1997). More recently, low birth weight has been shown to be associated with an enhanced heart rate response to psychological stressors in women, but not men (Ward *et al.*, 2004), while a study of boys and girls aged between 7 and 9 years has shown that cardiac SNS activation both at rest and during stress, was greater in girls who were small in size at birth, however this relationship was not as strong in males (Jones *et al.*, 2005). These findings suggest that there are sex differences in the relationship between birth weight and the sympathoadrenal response to acute psychological stress. In addition, experimental studies have demonstrated that low birth weight is associated with increased plasma noradrenalin concentrations in juvenile male and female pigs (Poore *et al.*, 2003) and elevated heart rate in young adult male and female guinea pigs (Persson *et al.*, 1992).

We have demonstrated that small size at birth was associated with an elevated adult heart rate in female offspring, suggesting that the negative association between size at birth and postnatal blood pressure in aged female guinea pigs may be due to increases in SNS activity or innervation initiated *in utero*. Interestingly, elevated fasting plasma insulin levels, also a marker of increased SNS activity, were observed in aged female offspring following spontaneous fetal growth restriction (see Chapter 5). In addition, resting systolic blood pressure, pulse pressure and heart rate were positively associated with fasting plasma insulin levels in aged female guinea pigs ($r=0.66$, $p=0.03$; $r=0.77$, $p=0.008$ and $r=0.63$, $p=0.04$, respectively, $n=9$) (data not shown), but not in males. These findings suggest that the elevated blood pressure observed in these animals following spontaneous fetal growth restriction may be due, at least partly to increased activity of the SNS. In aged male guinea pigs however,

small size at birth did not alter resting heart rate or fasting plasma insulin levels. These findings could explain in part, why spontaneous fetal growth restriction is associated with elevated blood pressure in aged female, but not male, guinea pigs.

Alterations in the development of key organs involved in the control of cardiovascular function, including the kidney, have been suggested as potential mechanisms through which poor growth *in utero* may programme blood pressure in postnatal life. Restricted fetal growth is thought to reduce the number of nephrons, which in turn increases glomerular capillary pressure, leading to the development of glomerular sclerosis (Mackenzie *et al.*, 1995; Brenner *et al.*, 1993). As a result of this sclerosis, further nephrons are lost, resulting in hypertension. Restricted intrauterine growth has been shown to reduce nephron numbers in both humans and animals (Merlet-Benichou *et al.*, 1993). In the rat, BUAL during late gestation has been shown to significantly reduce glomeruli number in full-term fetal kidneys (Pham *et al.*, 2003). In the same species, maternal protein restriction throughout pregnancy has been shown to reduce nephron number in the offspring in early postnatal and adult life (Langley-Evans, Welham *et al.*, 1999; Vehaskari *et al.*, 2001; Woods *et al.*, 2001), and decrease renal function (Nwagwu *et al.*, 2000; Woods *et al.*, 2001). More recently, it has been shown that in humans the number of nephrons ranged from 227,327 to 1,825,380 and that there was a strong correlation with birth weight, so that it was estimated that the number of nephrons increased by 257,426 for each kilogram increase in birth weight (Hughson *et al.*, 2003). It has been suggested that during childhood the growth of the kidney lags behind increases in body weight, leading to a rise in blood pressure as the body attempts to maintain renal homeostasis (Weder *et al.*, 1994). Studies in adult men and women have shown that plasma renin concentrations were related to size at birth (Martyn *et al.*, 1996), while a low weight or ponderal index at birth was associated with microalbuminuria (Yudkin *et al.*, 1997), suggesting that fetal growth may be linked to renal function adult life.

In the current study, although growth restriction *in utero* did not influence adult kidney weight in either male or female guinea pigs, both systolic and mean arterial blood pressure were negatively associated with kidney weight in aged female offspring, suggesting that there may be a renal component to the hypertension observed in these animals.

We have shown that spontaneous fetal growth restriction in the guinea pig is associated with elevated pulse pressure in aged female offspring. Pulse pressure is the difference between the systolic and diastolic blood pressures, and is thought to reflect arterial compliance. One possible explanation for the association between low birth weight and raised pulse pressure in adult life is that in fetuses whose growth is retarded, there is an impairment in the synthesis of elastin during a critical period of blood vessel development. The elasticity of the larger arteries depends on the scleroprotein elastin, which is laid down *in utero* and during infancy and thereafter turns over slowly (Rucker *et al.*, 1977). In growth-retarded fetuses there are changes in blood flow in several vascular beds, including the descending aorta and cerebral vasculature (Al-Ghazali *et al.*, 1989). These are 'brain sparing' adaptations which lead to preferential perfusion of the brain at the expense of the trunk. If sustained they may lead to reduced growth of the abdominal viscera and stunting at birth. Reduced blood flow in the large arteries of the trunk and legs may be associated with reduced elastin deposition. As a result of the relative deficiency in elastin, the compliance of the aorta and large arteries is reduced, which in turn leads to higher pulse pressures. Over time, the gradual loss of elastin that accompanies ageing and its replacement with collagen will tend to amplify the increase in blood pressure and may also predispose to left ventricular hypertrophy and cardiovascular disease. A study in the UK has shown that small size at birth was associated with reduced compliance in the large arteries of the trunk and legs, as indicated by a higher aPWV (Martyn *et al.*, 1995). This relationship persisted after adjustment for current blood pressure, suggesting that reduced aortic compliance was a primary event, rather than a consequence of raised blood pressure. More recently, fetal undernutrition in the rat, induced via a maternal low-protein diet, has been shown to cause a decrease in aortic wall thickness and total elastin content of the aorta, in young adult male offspring (Skilton *et al.*, 2006).

A number of metabolic abnormalities including insulin resistance, hyperglycaemia and hyperinsulinaemia are also thought to promote arterial stiffening (Toto-Moukouo *et al.*, 1986; Taquet *et al.*, 1993; Amar *et al.*, 1995; Salomaa *et al.*, 1995; Kupari *et al.*, 1994). Elevated insulin levels have been shown to cause hypertrophy of the vascular wall, resulting in the proliferation of smooth muscle cells, an increase in the number and size of monocytes, as well as increases in collagen (DeFronzo *et al.*, 1991; King *et al.*, 1999). Hyperglycaemia has been shown to stimulate collagen

synthesis and cause glycation of proteins in the arterial wall, which in turn leads to cross linking between protein fibres, causing vascular damage (Feener *et al.*, 1997). In the present study, small size at birth was associated with insulin resistance (see Chapter 4) and fasting hyperglycaemia and hyperinsulinaemia (see Chapter 5) in aged female guinea pigs, suggesting that the elevated pulse pressure observed in these animals may be due in part to their impaired metabolic function. In aged male guinea pigs however, small size at birth did not alter whole body insulin sensitivity of glucose metabolism or fasting plasma glucose or insulin levels. These findings could explain in part, why spontaneous fetal growth restriction is associated with elevated pulse pressure in aged female, but not male, guinea pigs.

We have demonstrated that accelerated neonatal growth was associated with elevated pulse pressure and heart rate in aged male offspring, an observation that is consistent with the findings of previous epidemiological studies which have reported that neonatal or childhood catch-up growth is associated with an increased risk of high blood pressure in men and women (Huxley *et al.*, 2000; Eriksson *et al.*, 2000; Law *et al.*, 2002).

The mechanisms responsible for the association between accelerated neonatal or childhood growth and hypertension in adult life remain unclear. One target for perinatal programming of cardiovascular function is the final number of cardiomyocytes within the heart. Cardiac myocytes become terminally differentiated before birth and their rate of maturation is influenced by the load on the heart. During growth restriction *in utero*, the diversion of oxygenated blood away from the trunk to sustain the growth of the brain also increases peripheral resistance and the load on the heart (Al-Ghazali *et al.*, 1989). This early pressure loading in turn leads to fewer, but larger myocytes, and echocardiography has demonstrated that growth-restricted fetuses have hypertrophy of both ventricles. Subsequent catch-up growth in postnatal life is thought to be achieved by overgrowth of, and excessive metabolic demand on this limited cell mass. Interestingly, in the current study, fast growing male guinea pigs demonstrated increased adult left ventricle weights when compared to their slow growing counterparts. Therefore, the increased blood pressure observed in aged male guinea pigs following accelerated growth in the neonatal period, may be due at least in part to changes in vascular development, in particular an enlargement of the left ventricle.

We have also shown that slow growing female guinea pigs demonstrated increased systolic blood pressure and left ventricular weights as aged adults, when compared to their fast growing counterparts. Failure of infant growth has been shown to be highly predictive of coronary heart disease in men (Osmond *et al.*, 1993), with those who weighed least at 1 year of age three times more likely to develop or die from the disease than those who were large (Osmond *et al.*, 1993; Fall *et al.*, 1995). In addition, a study of children and adults in France has demonstrated that low weight at 1 year is associated with later concentric left ventricular hypertrophy (Zureik *et al.*, 1996). There is evidence that infants who fail to put on weight have become resistant to growth hormone, which takes over control of growth from insulin in late fetal life (Barker *et al.*, 1993). Growth hormone resistance is associated with high circulating concentrations of the hormone, which in turn may cause cardiac enlargement and atheroma in blood vessels, as is the case in patients with tumours that produce excessive growth hormone. Growth hormone does not play the same role in postnatal growth and development in the guinea pig as it does in the human and other mammalian species (Keightley *et al.*, 1996). In the guinea pig, the regulation of postnatal growth is primarily controlled by IGF-I and II (Keightley *et al.*, 1996). Therefore, it is possible that similar to failure of infant growth in the human, poor neonatal growth in the guinea pig may reflect resistance to the actions of IGF-I and II, and the subsequent rise in circulating concentrations of these growth factors could contribute to the left ventricular hypertrophy observed in these animals.

In summary, spontaneous fetal growth restriction in the guinea pig increased systolic blood pressure, pulse pressure and heart rate in aged female offspring, but failed to alter any parameter of basal cardiovascular function in aged males. Altered postnatal growth also influenced the blood pressure of aged guinea pigs of both sexes, with an elevated pulse pressure and heart rate observed in males following accelerated growth in the neonatal period. In addition, slow growing female offspring demonstrated increased adult systolic blood pressure when compared to their fast growing counterparts. These changes in basal cardiovascular function may be due, at least in part to alterations in vascular structure and metabolic function.

CHAPTER 7

GENERAL DISCUSSION

In human populations, both perturbed perinatal growth (Barker *et al.*, 1993; Phillips *et al.*, 1994; Mi *et al.*, 2000; Valdez *et al.*, 1994; Yarbrough *et al.*, 1998) and ageing (Ford *et al.*, 2002) have been identified as risk factors for the development of the metabolic and haemodynamic abnormalities that characterise the IRS. The consequences of restricted prenatal growth for postnatal function have been investigated in a range of experimental models of IUGR, mainly in the rat. The results of these studies have shown that some, but not all of the aspects of postnatal metabolic and cardiovascular function that are programmed in humans, are also programmed in the rat. Further to this, relatively few studies in non-human species have investigated the effects of perturbed early postnatal growth on adult function, or whether ageing amplifies the effects of events in early life. We have previously shown that in the guinea pig, spontaneous fetal growth restriction and neonatal catch-up growth are associated with some, but not all of the elements of the IRS in young adult offspring (DM Horton *et al.*, unpublished observations; Kind *et al.*, 1999; Kind *et al.*, 2002; Kind *et al.*, 2003). Therefore this study was designed to determine firstly, whether the IRS develops and is exacerbated with increasing age in the guinea pig as it is in the human, and secondly whether the development of this syndrome is more pronounced in aged adults that have undergone spontaneous fetal growth restriction and accelerated growth in the neonatal period. In addition, the effects of ageing and perturbed growth in early life on body composition of the aged guinea pig were investigated.

We have demonstrated for the first time that elements of the IRS develop with increasing age in the guinea pig, accompanied by an increase in visceral adiposity in females and a reduction in subcutaneous adiposity and skeletal muscle mass in both males and females. Whole body insulin sensitivity of glucose metabolism was reduced in aged female guinea pigs when compared to their young adult counterparts, however this age-related impairment of insulin action was not observed in male guinea pigs. The increase in visceral adiposity in aged female guinea pigs may have contributed to their impaired insulin action, by altering the expression of

adipocytokines such as TNF-α and adiponectin, which are known to influence insulin sensitivity (Feingold *et al.*, 1992; Hotamisligil *et al.*, 1994; Altomonte *et al.*, 2003). In the future, it may be informative to measure the expression of TNF-α and adiponectin in adipose tissue and circulating levels of these in guinea pigs to determine if these adipocyte-derived factors are altered with increasing age and are directly related to insulin sensitivity. In addition, the absence of an age-related increase in visceral adiposity in male guinea pigs could explain, at least in part, their unaltered insulin sensitivity as aged adults. Both male and female guinea pigs demonstrated a reduction in skeletal muscle mass with ageing, which could also have contributed to the age-related decline in insulin sensitivity observed in females. More specifically, concentrations of GLUT-4, the protein responsible for glucose uptake in skeletal muscle, have been shown to fall with increasing age in both men and women (Houmard *et al.*, 1995), suggesting that a defect in glucose transport may contribute to the insulin resistance of ageing. We may be able to measure GLUT-4 expression in the skeletal muscle of these guinea pigs in the future, in order to determine whether a defect in this protein contributes to the impaired insulin action observed in female guinea pigs with increasing age. Fasting plasma glucose, triglyceride and cholesterol concentrations were increased in aged male and female guinea pigs when compared to their young adult counterparts, however fasting plasma insulin concentrations were increased in female, but not male, guinea pigs with increasing age. An age-related rise in resting systolic, diastolic and mean arterial blood pressure was also observed in male and female guinea pigs; however pulse pressure was elevated in aged male, but not female offspring. A number of the age-related metabolic, hormonal and anthropometric changes reported in the present study could also have contributed to the observed increase in blood pressure with ageing. For example, the rise in fasting plasma triglyceride and total cholesterol concentrations with increasing age could have contributed to the formation of atherosclerotic plaques, reducing the distensibility of the peripheral arterial system, in turn leading to an increase in blood pressure (Lakatta *et al.*, 1999; Lakatta *et al.*, 1993; Robert 1999). Similarly, the impaired insulin action, fasting hyperinsulinaemia and increased visceral adiposity reported in females, and the fasting hyperglycaemia reported in males and females, with increasing age could also have contributed to the age-related rise in resting blood pressure, by promoting

arterial stiffening (Toto-Moukouo *et al.*, 1986; Taquet *et al.*, 1993; Amar *et al.*, 1995; Salomaa *et al.*, 1995).

An accumulation of molecular damage as a result of defective repair systems has been identified as one of the possible causes of ageing (Kirkwood *et al.*, 1995). It has been suggested that many of the degenerative changes that occur with increasing age, including the deterioration in vascular function and insulin sensitivity, are the consequence of cell deaths caused by a failure to replace defective DNA, RNA and proteins (Kirkwood 1977; Kirkwood *et al.*, 1975). This hypothesis has been supported by developments in our understanding of Werner's syndrome, a condition characterised by premature ageing and which is caused by a mutation in the gene coding for helicase, an enzyme required for DNA repair (Yu *et al.*, 1996). The shortening of telomeres, specialised nucleoprotein complexes which stabilise the chromosome during mitosis and protect it from end-degrading enzymes, has been shown to occur with increasing age in humans (Hastie *et al.*, 1990). The significance of telomeric attrition for the development of cardiovascular and metabolic disease in humans is still unclear, however an inverse relationship between pulse pressure, which can be an indicator of the biological age of the large arteries, and telomere length has been demonstrated (Jeanclos *et al.*, 2000). Similarly, a reduction in telomere length was observed in the white blood cells of individuals with insulin dependent diabetes mellitus when compared to controls, suggesting a link between telomeric attrition and impaired glucose homeostasis (Jeanclos *et al.*, 1998). Recently, perturbed growth in prenatal and early postnatal life has been shown to impair molecular repair systems in the rat, increasing the rate of age-related telomere shortening in certain organs (Jennings *et al.*, 1999).

Growth restriction during fetal life as a result of maternal protein restriction, followed by postnatal catch-up growth, was associated with a shorter life span and an age-related shortening of kidney telomeres in male rats (Jennings *et al.*, 1999). The findings from this study suggest that the rate of ageing, at least in some systems, may be programmed by events in early life, providing a mechanistic basis for the association between altered prenatal and early postnatal growth and later disease. The present study has demonstrated that neither spontaneous fetal growth restriction nor accelerated neonatal growth had any significant effect on the longevity of male or female guinea pigs. In the future however, it maybe useful to determine whether

the guinea pig exhibits age-related telomeric attrition in key organs involved in metabolic and cardiovascular function, and whether this is more pronounced following perturbed perinatal growth.

In the current study, spontaneous fetal growth restriction in the guinea pig reduced the weight, nose to rump length, weight to length ratio, abdominal circumference and head length and width of offspring at birth. In this polytocous species, spontaneous fetal growth restriction occurs as a result of large litter size, presumably because of the increased competition for uterine blood flow and delivery of essential maternal substrates such as oxygen, glucose, and amino acids to each developing placenta and fetus (Saintonge *et al.*, 1981). Therefore, we hypothesise that the fetal guinea pig from a large litter will be hypoxic, hypoglycaemic and will have reduced circulating levels of amino acids *in utero*, compared to offspring from small litters. We further hypothesise that similar to fetal growth restriction in other species, spontaneous fetal growth restriction in the guinea pig will be associated with increased levels of catabolic hormones such as cortisol, catecholamines and glucagon and reduced levels of anabolic hormones such as insulin, insulin-like growth factors and thyroid hormone (Robinson *et al.*, 1994). These metabolic and endocrine changes in the growth restricted fetal guinea pig may in turn permanently programme postnatal metabolic and cardiovascular function via a number of mechanisms. In order to properly test these hypotheses in the future, it would be important to firstly characterise the effect of variable litter size on the metabolic and hormonal environment of the fetal and maternal guinea pig in late gestation, before embarking on specific studies to determine the effects of either maternal oxygen deprivation, or carbohydrate or protein deficiency, on fetal and postnatal growth and adult function.

We also observed that low birth weight guinea pigs demonstrated higher fractional growth rates for weight during the neonatal period than their high birth weight counterparts, however their absolute growth rates were lower during this period and they remained smaller in terms of weight and nose to rump length as aged adults. A recent study in our laboratory has characterised the physiological basis of this neonatal catch-up growth in the guinea pig following spontaneous fetal growth restriction. It appears that increased insulin and IGF-1 sensitivity of glucose metabolism, as well as increased plasma free T_3:T_4, indicative of increased

circulating levels of active thyroid hormone, are major factors driving postnatal catch-up growth in the guinea pig (Campbell 2006).

Interestingly, this study has demonstrated that in the aged guinea pig, the perinatal programming of the IRS occurs in a sex-specific manner.

In aged female guinea pigs, spontaneous fetal growth restriction, as indicated by a reduced size at birth, was associated with decreased whole body insulin sensitivity of glucose metabolism, increased fasting concentrations of glucose and insulin, an increased insulin response to IVGTT and impaired glucose tolerance. Similarly, spontaneous fetal growth restriction impaired basal cardiovascular function, increasing resting systolic and mean arterial blood pressure, pulse pressure and heart rate in aged female guinea pigs. Accelerated neonatal growth was associated with increased fasting levels of glucose and triglycerides, while poor neonatal growth was associated with raised systolic blood pressure, in aged females.

In aged male guinea pigs in contrast, excessive fetal growth, as indicated by large size at birth, was associated with decreased whole body insulin sensitivity of glucose metabolism, increased fasting levels of insulin and an increased insulin response to IVGTT, while disproportionate fetal growth was associated with increased fasting levels of total cholesterol. Poor neonatal growth was associated with decreased whole body insulin sensitivity of glucose metabolism and increased fasting levels of free fatty acids, while accelerated neonatal growth was associated with increased fasting levels of triglycerides, and an increased resting pulse pressure and heart rate, in aged males.

In the current study therefore, spontaneous fetal growth restriction was associated with many of the metabolic and cardiovascular abnormalities that characterise the IRS, in aged female, but not male, guinea pigs. As described earlier, metabolic abnormalities including insulin resistance, hyperglycaemia and hyperinsulinaemia may contribute to a rise in blood pressure, by promoting arterial stiffening (Toto-Moukouo *et al.*, 1986; Taquet *et al.*, 1993; Amar *et al.*, 1995; Salomaa *et al.*, 1995; Kupari *et al.*, 1994). We have shown in the present study that female guinea pigs that are small in size at birth, as a result of restricted growth *in utero*, are insulin resistant, glucose intolerant and display fasting hyperinsulinaemia and hyperglycaemia as aged adults. These metabolic abnormalities could have

contributed to the raised blood pressure and heart rate observed in growth restricted female guinea pigs as aged adults, and in fact fasting plasma insulin levels were positively associated with systolic blood pressure, pulse pressure and heart rate in these animals. In male guinea pigs however, small size at birth was not associated with any change in insulin action, glucose tolerance or fasting plasma glucose or insulin levels in aged adults. These findings could explain, at least in part, why spontaneous fetal growth restriction is associated with elevated blood pressure and heart rate in aged female, but not male, guinea pigs.

Previous epidemiological studies in humans and experimental studies in non-human species have employed correlation analysis to identify potential links between early life exposures and outcomes in later life. The associations examined in this thesis were selected to provide additional tests of the hypotheses based on the findnings of previous studies. While the use of correleation analysis to explore the relationship of physiological outcomes to size at birth does increase the possibility of type 1 errors, it does enable comparison with a range of studies in humans and other species and provides a broad basis for future, more targeted studies.

The observation in this study, that different patterns of early growth were responsible for the programming of adult metabolic and cardiovascular dysfunction, parallels that seen in 50 year old men and women who were exposed *in utero* to the Dutch Winter famine of 1944-1945. In this group of people, prenatal exposure to famine in mid and late-gestation reduced adult glucose tolerance (Ravelli *et al.*, 1998), but had a negligible effect on fetal growth and adult blood pressure (Roseboom *et al.*, 1999), suggesting that glucose metabolism and blood pressure may be programmed *in utero* via different maternal influences.

Permanent changes in organ size and structure and alterations in cell type are mechanisms by which some adult-onset degenerative diseases are thought to be programmed. In the current study, the rise in blood pressure observed in aged male and female guinea pigs following altered prenatal and early postnatal growth may be explained, at least in part, by an enlargement of the left ventricle. Similarly, the reduction in pancreas and liver weights seen in aged female guinea pigs following perturbed perinatal growth, could possibly have contributed to their impaired glucose tolerance and fasting hyperglycaemia. In aged male guinea pigs, the impaired whole

body insulin sensitivity of glucose metabolism observed following excessive fetal growth could not be clearly explained by changes in adult body composition. Interestingly in the male guinea pig, an increase in adult adiposity, which has previously been associated with impaired insulin action (Lemieux 2001), was observed here following suboptimal, rather than excessive growth *in utero*, thus they are clearly dissociated. The findings from recent subsidiary studies in this cohort of aged guinea pigs have helped elucidate some of the mechanisms by which perturbed perinatal growth may program the alterations in adult metabolic and cardiovascular function reported in the present study.

Skeletal muscle is a key target of insulin action and structural or functional defects in this tissue, as a result of its impaired development during growth restriction *in utero*, have been suggested as possible causes of postnatal insulin resistance. In recent times, impaired fat oxidation and the subsequent accumulation of intracellular lipid have been identified as putative causes of skeletal muscle insulin resistance. Carnitine palmitoyl transferase I (CPTI) is the rate limiting enzyme for transport of long chain fatty acyl-CoAs (LCFA-CoAs) into the mitochondria for oxidation (Ruderman *et al.*, 1999; Rasmussen *et al.*, 1999). Malonyl-CoA is an allosteric inhibitor of CPTI, and factors which increase malonyl-CoA concentrations are able to reduce fat oxidation and increase the intracellular concentration of lipids, including LCFA-CoAs (Ruderman *et al.*, 1999; Rasmussen *et al.*, 1999). This increase in intracellular lipid is able to impair insulin action in skeletal muscle via a number of mechanisms, including regulatory effects on key enzymes involved in glucose metabolism (Kelley *et al.*, 2000; Schmitz-Peiffer 2000; Ruderman *et al.*, 1999; Thompson *et al.*, 2000). Interestingly, increased malonyl CoA levels and a reduced expression of CPTI have been observed in the livers of juvenile and young adult male rats following fetal growth restriction induced by uterine artery ligation (Lane *et al.*, 2001).

Recently, a study in this cohort of aged guinea pigs has demonstrated that the concentration of intramyocellular lipid in the *vastus lateralis*, determined histologically using Oil-Red O staining, was negatively associated with both birth weight and whole body insulin sensitivity of glucose metabolism in female offspring (JA Owens, unpublished observations). As discussed above, in the guinea pig neonatal catch-up growth following spontaneous fetal growth restriction is

characterised by an increased sensitivity to insulin and other anabolic hormones (N Campbell *et al.*, unpublished observations). In addition, spontaneous fetal growth restriction in this species is associated with early onset hyperphagia (DM Horton *et al.*, unpublished observations). It is possible that both the neonatal hypersensitivity to insulin and hyperphagia which occur following spontaneous fetal growth restriction increase glucose availability and its insulin-stimulated entry into skeletal muscle cells, which in turn increases malonyl-CoA concentrations. This rise in malonyl-CoA levels will reduce fat oxidation, leading to an accumulation of intracellular LCFA-CoAs which may then impair glucose metabolism and insulin signalling in skeletal muscle. In contrast to females, aged male guinea pigs that were small in size at birth did not demonstrate an increase in the concentration of intramyocellular lipid in the *vastus lateralis*. These findings could explain, at least in part, why spontaneous fetal growth restriction is associated with impaired insulin sensitivity of glucose metabolism in aged female, but not male, guinea pigs. In the future, it may be possible to utilise pharmacological interventions to establish whether the increased levels of intramyocellular lipid observed in aged female guinea pigs following suboptimal growth *in utero*, do in fact play a role in their impaired insulin action. For example, we could treat these animals with ureido-fibrate-5, a peroxisome proliferator-activated receptor-α (PPARα) agonist which is able to stimulate mitochondrial fat oxidation in skeletal muscle, and then measure its effect on insulin sensitivity of glucose metabolism and intracellular concentrations of malonyl-CoA, LCFA-CoAs and other lipid products.

Lasting changes in the pattern of hormone release and tissue sensitivity to specific hormones are mechanisms by which some adult metabolic and cardiovascular disorders may be programmed. Studies in non-human species have shown that exposure to environmental perturbations in prenatal life, such as deficiencies in essential maternal substrates, can cause excessive increases in fetal cortisol levels during late gestation (Robinson *et al.*, 1994), due to either maternal or fetal stress. In addition, prenatal stress can cause permanent alterations in the biological response to stress in adult offspring (Reul *et al.*, 1994; Barbazanges *et al.*, 1996), which in turn has adverse consequences for blood pressure and carbohydrate metabolism (Levitt *et al.*, 1996; Lindsay *et al.*, 1996). It is thought that environmental perturbations and excessive exposure to cortisol *in utero* cause adult dysfunction in part, by programming increased activity of, and responsiveness to the HPAA in adult

offspring. A UK study of men aged 64 years reported that low birth weight was associated with increased 0900h plasma cortisol concentrations (Phillips *et al.*, 1998), and this finding has since been reported in 20 year old men and women in Australia, as well as in 50 year old men and women who were born in Preston in the UK (Phillips *et al.*, 2000). This relationship between small size at birth and elevated fasting cortisol levels may represent a stress response, due to the combination of fasting and the novel clinic setting in which the blood samples were taken (Phillips *et al.*, 2006). A recent study of boys and girls aged between 7 and 9 years has shown that birth weight was inversely related to salivary cortisol responses to psychological stress in boys, while in girls birth weight was inversely related to morning peak cortisol levels (Jones *et al.*, 2006), suggesting sex differences in the relationship between birth weight and the HPAA response to acute psychological stress. In contrast, in the guinea pig, maternal dexamethasone treatment (Dean *et al.*, 1999; Liu *et al.*, 2001) or nutrient restriction (Lingas *et al.*, 2001) during late gestation have been shown to programme HPAA function females, but not males.

A recent subsidiary study in this cohort of aged guinea pigs has demonstrated that impaired fetal growth, as indicated by thinness, or a reduced weight, length or abdominal circumference at birth, was associated with increased salivary cortisol concentrations in female offspring (S Grover *et al.*, unpublished observations). In addition, the present study has demonstrated that in aged females, spontaneous fetal growth restriction is associated with increased adrenal size, which may reflect an increased sensitivity of this gland to the trophic actions of ACTH. In aged male guinea pigs however, small size at birth did not alter salivary cortisol concentrations or adrenal size. These findings could explain in part, why spontaneous fetal growth restriction is associated with impaired metabolic and cardiovascular function in aged female, but not male, guinea pigs. It may be possible to determine experimentally whether the increased HPAA activity observed in aged female guinea pigs following perturbed intrauterine growth, does in fact play a role in their impaired metabolic and cardiovascular function. Metyrapone is a drug that reduces cortisol synthesis, by inhibiting 11 β-hydroxylase, an enzyme which is necessary for the conversion of 11-deoxycortisol to cortisol (Gower 1974). In the guinea pig, metyrapone administration has been shown to decrease plasma cortisol concentrations *in vivo* (Werner 1988), as well as cortisol production by isolated adrenal cells (De Coster *et al.*, 1985). We could administer metyrapone to female guinea pigs from large litters

as juveniles (40 days of age), before insulin resistance and its related disorders are known to develop, and then examine whether the subsequent reduction in circulating cortisol levels prevents the onset of metabolic and cardiovascular dysfunction in adulthood.

Increased activity of the SNS established *in utero* as a result of restricted fetal growth, is another mechanism by which small size at birth and impaired metabolic and cardiovascular function in adult life may be linked. Low birth weight babies have been shown to have increased heart rate, an index of SNS activity, when compared with controls during sleep (Spassov *et al.*, 1994). In addition, a direct relationship between adult pulse rate, which is an index of SNS activity, and birth weight has been demonstrated (Phillips *et al.*, 1997). More recently, low birth weight has been shown to be associated with an enhanced heart rate response to psychological stressors in women, but not men (Ward *et al.*, 2004), while a study of boys and girls aged between 7 and 9 years has shown that cardiac SNS activation both at rest and during stress, was greater in girls who were small in size at birth, however this relationship was not as strong in males (Jones *et al.*, 2005). These findings suggest that there are sex differences in the relationship between birth weight and the sympathoadrenal response to acute psychological stress. Additionally, experimental studies have demonstrated that low birth weight is associated with increased plasma noradrenalin concentrations in juvenile pigs (Poore *et al.*, Fowden 2003) and elevated heart rate in young adult guinea pigs (Persson *et al.*, 1992). In the present study, heart rate was inversely related to size at birth (see Chapter 6) and positively associated with fasting plasma insulin levels, which is also an indicator of increased SNS activity, in aged female guinea pigs (data not shown). These findings suggest that the impaired insulin sensitivity and elevated blood pressure observed in these animals following spontaneous fetal growth restriction may be due, at least partly to increased activity of the SNS. In aged male guinea pigs however, small size at birth did not alter resting heart rate or fasting plasma insulin levels. These findings could explain in part, why spontaneous fetal growth restriction is associated with impaired insulin sensitivity and elevated blood pressure in aged female, but not male, guinea pigs. In the future, it may be useful to measure catecholamine levels in these guinea pigs, in order to determine whether a defect in SNS activity contributes to the impaired insulin action and cardiovascular function observed in aged female guinea pigs following spontaneous fetal growth restriction.

In conclusion, the guinea pig appears to be a suitable animal model of ageing, displaying many of the metabolic, cardiovascular and anthropometric changes seen in humans. Furthermore, perturbed prenatal and early postnatal growth also influence and promote the development of the IRS in the aged guinea pig, but in a sexually dimorphic pattern. The mechanisms responsible for the emergence of this syndrome in a sex-specific manner remain to be determined. However the findings of recent subsidiary studies in this cohort of aged guinea pigs suggest that it may be due in part to alterations in the set point of the HPAA, as well as defects in skeletal muscle structure and metabolic function. This study has been the first to examine the effects of altered perinatal growth on the development of the IRS in an aged, chronically catheterised experimental model employing a precocial species such as the guinea pig. The knowledge gained from these investigations may aid in the design of interventions to more effectively prevent or ameliorate the onset and progression of the IRS in humans, either following perturbed growth in early life or more generally.

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APPENDIX A

Summary of the total number of litters used in this thesis and their size		
Litter size	**Pregnant guinea pigs**	**No. of offspring used in study**
1	4	4
2	22	35
3	32	63
4	22	62
5	4	8
Total	84	172