

**LACK OF DEMONSTRABLE HARMFUL EFFECT OF
HIGHER PROTEIN WEIGHT LOSS DIETS
ON BONE HEALTH AND NEPHROPATHY
IN TYPE 2 DIABETES**

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THESIS ABSTRACT

The optimal macronutrient composition of diets to maximize weight loss has been a subject of great interest in obesity research. High protein weight loss diets have gained widespread popularity. However their safety regarding osteoporosis risk and progression of diabetic nephropathy has been questioned.

With regard to bone, higher dietary protein could cause a low grade metabolic acidosis, leading to hypercalciuria with loss of calcium from bone, causing osteoporosis. Recent studies suggest the situation is more complicated. The interaction with other dietary components such as calcium, fruit and vegetables is important. Historically, nephrologists have advocated lower protein diets in subjects with renal impairment to slow the decline in glomerular filtration rate. Whether this could be extrapolated to subjects with nephropathy from type 2 diabetes remains unclear. These subjects are often obese and potential candidates for high protein diets. If weight loss was achieved, this should slow the deterioration in renal function both directly as well as by improving lipid, blood pressure and glycaemic control which are risk factors for diabetic nephropathy. This thesis aims to test the hypothesis that higher protein diets are harmful to bone and renal health.

We designed two clinical trials to test the effect of higher protein weight loss diets compared to lower protein weight loss diets on bone health, in overweight but otherwise healthy post-menopausal women over 2 years and on decline in glomerular filtration rate

over 1 year in subjects with early nephropathy from type 2 diabetes. These two trials were conducted at the Commonwealth Scientific and Industrial Research Organisation in collaboration with the Centre of Clinical Research Excellence in Human Nutrition at the University of Adelaide. As a separate sub-study, I analysed the accuracies of the three Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations using creatinine, cystatin C and both, compared to radioisotope methods in our obese subjects from the renal study. These results and accompanying discussion are presented in a combined narrative and publication format as per University of Adelaide guidelines.

The results of my studies did not demonstrate any signal of increased bone loss from higher protein diets; our results are consistent with a modest beneficial effect based on lower bone turnover with higher dietary protein. With the renal study, there was no evidence that the higher protein diet accelerated decline in GFR in type 2 diabetes. The main benefit came from weight loss; subjects who hyperfiltered (estimated GFR greater than 120 ml/min/1.73m²) had a decrease in eGFR. Subjects with a baseline eGFR between 40-120 ml/min/1.73m² had an increase in GFR with weight loss. I also demonstrated that after weight loss, the CKD-EPI equations using cystatin C or both cystatin C and creatinine had higher precision compared to the equation using creatinine alone. This reflected the loss of lean mass with weight loss, which has a bigger influence on serum creatinine than cystatin C.

Therefore, in post-menopausal women and subjects with early type 2 diabetic nephropathy, higher protein weight loss diets are not harmful to bone and renal health.

THESIS DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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ACKNOWLEDGEMENTS

There are many people who have been of great assistance to me over the course of my PhD. Firstly I am very grateful for the mentorship and guidance of my principal supervisor, Professor Peter Clifton throughout my PhD. Peter conceived the idea of studying the areas of bone and renal health and the link with dietary protein. He was instrumental in conducting the two major clinical trials at the CSIRO from design to completion. I have learnt a lot from him about forward planning and attention to detail which is required for such an enterprise. I am also appreciative of his insightful comments and analysis as we have translated the results from the trials into manuscripts and this PhD. I also would like to acknowledge the support of my co-supervisors, Professors Michael Horowitz and Professor Gary Wittert firstly in pointing out the benefits of undertaking a PhD and for their support for my career in Endocrinology over the last 15 years. With Professor Clifton, they were able to facilitate the financial and intellectual collaboration between the CSIRO and the Centre of Clinical Research Excellence in Human Nutrition at the University of Adelaide which has supported this research.

I also would like to acknowledge the expertise of my other co-authors on the manuscripts presented here. Our renal trial would not have been possible without the expertise of Dr Eva Pedersen our lead dietician who worked tirelessly with subjects to encourage dietary compliance and weight loss of our subjects as well as intellectual input into the manuscripts as acknowledged on the “statements of authorship”. A/Prof Jennifer Keogh was instrumental in designing and monitoring trial diets for our bone study and also

critically reviewed and advised on the manuscript. We were also able to draw on the vast experience and insight of Professor B E Christopher Nordin as we designed our bone trial.

We would not have been able to undertake the trials without the generosity and goodwill of our many subjects who volunteered their time and effort for the sake of science. We are very much indebted to them. Many clinic staff at the CSIRO played important roles in the conduct of this trial such as organising appointments for subjects, venepuncture and specimen processing, assisting with adherence to diets and storing of data. Special thanks to Julia Weaver, Lindy Lawson, Kathryn Bastiaans, Rosemary McArthur, Anne McGuffin and Pennie Taylor. I have also benefitted from the statistical advice provided by Ms Kylie Lange from Adelaide University and Mr. Jim Wang from the Queen Elizabeth Hospital.

Finally it is always a challenge to strike the right balance between time spent on my clinical work as an Endocrinologist, the time required to undertake a PhD and home life. I have always been grateful for the love and support of my parents Joyce and Cyril. However it has been the presence of my two children Daniel (who was 3 years old when I started this PhD) and Sonali who was born during my PhD who have kept me grounded and provided me with much joy and delight during this time. However most important of all, I must acknowledge the love, support and constant encouragement of my wife Shilpa as I attempted to balance my PhD with all the competing priorities in my life. She has been the bedrock of our family and I dedicate this thesis to her.

RESEARCH PRESENTATIONS

2010: CSIRO Research meeting (oral)

2011: Endocrine Grand Round, Royal Adelaide Hospital – Oral Presentation

2012: International Congress of Endocrinology/European College of Endocrinology
Combined

Meeting, Florence, Italy – Oral Presentation

2012: European Congress of Obesity, Lyon France – Poster Presentation

2012: Endocrine Grand Round, Royal Adelaide Hospital – Oral Presentation

2013: School of Medicine, Seminar Program, Adelaide University

2013: International Diabetes Federation, Melbourne, Australia –Poster Presentation

CHAPTER 1: INTRODUCTION

1.1 General Introduction

The incidence of obesity and morbid obesity has steadily increased over the last 50 years in developed countries¹. This has led to a strong interest in strategies to induce weight loss including surgical and non-surgical methods. High protein diets, involving the partial substitution of dietary carbohydrate with protein, have become a very popular method employed by overweight and obese patients to lose weight over the last 15 years². Several randomized clinical trials have shown that higher protein diets are comparable to or superior than lower protein weight loss diets in achieving weight loss, improving cardiovascular risk profile and preserving lean body mass³.

However, there have been concerns about the safety of higher protein diets if used long term on bone health in subjects at risk of osteoporosis and on progression of renal disease in subjects with impaired renal function. These are outlined below.

1.2 Dietary Protein and Bone Health

In 1968, Wachman and Bernstein first suggested the possibility that higher protein diets, such as found in the Western world, could be a risk factor for osteoporosis and may explain the higher risk of hip fracture in developed countries compared to developing countries⁴. They hypothesized that Western diets containing meat, a source of “acid ash”, would cause a low grade metabolic acidosis which after buffering by bone would lead to hypercalciuria. They estimated that the expected loss of 2 mEq of calcium daily would result in the loss of about 15% bone per decade and cause osteoporosis. However in practice the picture has proven more complicated. Subsequent cross-sectional and

longitudinal studies have yielded mixed results. For example, the Framingham Osteoporosis Study suggested that elderly men and women with lower protein intake had greater bone loss at the femur and spine⁵. The Rancho Bernardo study showed a beneficial effect on bone density of women consuming animal but not vegetable protein⁶. Conversely, the Nurses Health Study there was a relative risk of 1.22 of forearm fracture in women consuming more than 95 g day protein compared to less than 68 g daily⁷

These discrepant results may be due to the interplay of other factors which influence the effect of dietary protein on bone. Important confounders that might explain discrepancies between studies include the interaction of dietary calcium with dietary protein and varying fruit and green vegetable intake in different studies^{8,9}. The latter are a source of dietary potassium which can have an important alkalinizing effect and neutralize the acidifying effect of dietary protein. These issues are well summarised in a review by Heaney¹⁰. Also increased salt intake, by antagonising renal tubular calcium resorption, promotes bone loss¹¹. These issues are discussed in detail in Chapter 2.

1.3 Dietary Protein and Renal Disease

The link between dietary protein and renal function was first suggested in 1951 by Thomas Addis¹². He suggested that loss of kidney mass stimulated hypertrophy of the remaining renal tissue, the extent of which depended on the amount of dietary protein. Since then much research has been conducted looking at the role of increased dietary protein and renal disease generally and type 1 diabetes specifically. It has been suggested that excess dietary protein could accelerate nephropathy¹³. These recommendations have been extrapolated to type 2 diabetes, even though the evidence is mixed. In addition the role of co-existing

obesity and the potential improvement in renal function with weight loss have not always been considered.

It is also important to consider that the effect of dietary protein in renal failure may vary depending on how advanced the condition is. Renal dysfunction is a spectrum that ranges from microalbuminuria, in the presence of normal or even elevated glomerular filtration rate (GFR) called hyperfiltration, to end stage renal failure with significantly reduced GFR requiring dialysis. The most recent classification of chronic kidney disease which considers both GFR and degree of proteinuria is shown below. It is referred to in this thesis^{14,15}.

Table 1: Revised chronic kidney disease classification based upon glomerular filtration rate and albuminuria^{14,15}

GFR stages	GFR (mL/min/1.73 m ²)	Terms
G1	>90	Normal or high
G2	60 to 89	Mildly decreased
G3a	45 to 59	Mildly to moderately decreased
G3b	30 to 44	Moderately to severely decreased
G4	15 to 29	Severely decreased
G5	<15	Kidney failure (add D if treated by dialysis)

Albuminuria	AER (mg/day)	Terms
A1	<30	Normal to mildly increased
A2	30 to 300	Moderately increased
A3	>300	Severely increased

Any study of renal function in obese populations should consider the accuracy of the different methods of measuring renal function in this group and how well the different methods have been validated. The “gold standard” method to determine GFR is traditionally considered to be by measuring the clearance from blood or urine of exogenous markers such as iothalamate, ⁵¹Cr-ethylenediaminetetraacetic acid (EDTA) and ^{99m}Tc-diethylenetriamine.^{16,17} Unfortunately, these are not convenient to use in day to day clinical practice. However there is increasing awareness that many of the surrogate measures of GFR that we use in clinical practice are either not validated or sometimes unsatisfactory in the overweight and obese population who may have relatively normal or even elevated GFR.¹⁸ These include measured creatinine clearance from 24 hour urine collections, serum creatinine and creatinine based equations such as the MDRD and also the equations designed for obese populations such as the Cockcroft-Gault formula and the Salazar Corcoran equation.¹⁹⁻²³ This is because creatinine production is related to fat free (lean) mass such as muscle which is often increased in obesity and leads to an underestimation of GFR with formulae such as the MDRD formula and overestimation with weight-based formulae which is enhanced by sarcopenic obesity.

There have been attempts to circumvent the issue and improve the accuracy of GFR measurement. One approach includes using lean body weight rather than total weight in formulae such as the Cockcroft-Gault.²⁴ Another approach has been to adjust for body surface area (BSA). GFR from radioisotope methods can be expressed in absolute terms or expressed as ml/min/1.73 m² which was the average adult BSA in 1916²⁵. Some creatinine based equations such as the MDRD formula always express GFR as ml/min/1.73 m² whilst some studies have shown adjusting GFR from the Cockcroft Gault formula for BSA in this way has sometimes lead to estimations of GFR which correlate more closely to the gold standard inulin clearance^{26,27}

Another surrogate marker that has been used to detect early kidney dysfunction in diabetes is cystatin C.²⁸ The concentration of cystatin C, a non-glycosylated protein produced by nucleated cells can be used to estimate GFR either alone or incorporated into equations with creatinine. However cystatin C levels are higher in the obese even after adjusting for renal function^{18,29}. This may be due to increased cystatin C production by adipose cells raising concerns about its reliability in the obese.

Recently, Inker et al. have added two new formulae based on the CKD-EPI formula to incorporate both cystatin C and also both cystatin C and creatinine³⁰. These formulae are less affected with age, sex and race although there were limited measurements of muscle mass in the populations from which these equations were derived³¹

1.4 Rationale for Thesis and Hypotheses

The purpose of this thesis was firstly to review the evidence for a deleterious effect of high protein diets compared to a normal protein diet on bone and renal health respectively.

Based on the historical opinion from the literature discussed above, the hypotheses that I investigated were that:

1. Higher protein diets would cause greater bone loss than normal protein diets
2. Higher protein diets would cause greater decline in glomerular filtration rate (GFR) than normal protein diets.

I could then design two randomized clinical trials to compare the safety of a higher protein diet on post-menopausal women at risk of osteoporosis and obese subjects with diabetes with early renal dysfunction respectively. Because I was comparing two weight loss diets, in each case of varying protein content, I had to confine myself to obese or overweight subjects. In the case of the renal study, I chose subjects who had early nephropathy from type 2 diabetes as our cohort to study. This was because type 2 diabetes is the commonest cause of renal dysfunction in Australia which would aid recruitment and because such subjects are commonly overweight or obese compared to subjects with renal dysfunction from other causes such as glomerulonephritis. Moreover as mentioned above, the evidence for a harmful effect of higher dietary protein was not as convincing for type 2 diabetic nephropathy compared to renal disease generally. These clinical trials were conducted at the Commonwealth Scientific and Industrial Research Organization (CSIRO), an Australian Government Research Centre, which has conducted much work testing the efficacy of high protein weight loss diets under the leadership of Professor Peter Clifton, my primary supervisor. This research was also supported by the Centre of Clinical

Research Excellence in Human Nutrition from the University of Adelaide with the assistance of my two co-supervisors, Professor Michael Horowitz and Professor Gary Wittert.

In both trials, in conjunction with Professor Clifton, I designed the study, obtained ethical approval and assisted in subject recruitment and monitoring throughout the study. With the Renal Study, there was an ethical requirement for subjects to have stable glycaemic, blood pressure and lipid control prior to starting dietary treatment and for medications to be adjusted as weight loss occurred during the study. Subjects were each reviewed by me on multiple occasions for this purpose. Finally Professor Clifton and I were responsible for data analysis and manuscript preparation, submission and revision.

As part of the trial studying the effect of higher protein diets on renal disease, I carried out measurement and estimation of GFR/creatinine clearance by all standard methods. This included radioisotope methods, measured creatinine clearance from 24 hour urine collections, cystatin C and measured serum creatinine. The latter allowed us to estimate GFR from accepted formulae such as the Modification of Diet in Renal Disease (MDRD) equation. As mentioned above, there is some controversy in the literature about the best method to estimate GFR in obese cohorts. I published a review of this topic which forms this basis of Chapter 5 of this thesis. However during the period of this thesis, three new equations to estimate GFR incorporating creatinine, cystatin C and creatinine and cystatin C were devised- the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations^{30,32}.

The CKD-EPI equation incorporating creatinine is now used in many countries to estimate GFR and was introduced to Australia in 2013. I decided to compare the utility of these equations to measure GFR in obese populations as there was minimal data on this topic. This manuscript has now been published and forms part of the body of my thesis.

1.5 Format of Thesis

This thesis is by publication, supplemented by narrative, as per University of Adelaide guidelines. I have presented 6 manuscripts encompassing my work during the PhD. Two of these are reviews and four of these are results from the two clinical trials. None of these articles were solicited by the journals. All of these papers were submitted to appropriate bone, nutritional or renal journals, underwent peer review by 2-4 reviewers and further revisions until the reviewers and editors were satisfied. The format of this thesis is:

Chapter 1: General Introduction

Chapter 2: Review of The Interaction between Dietary protein and Bone Health.

(Manuscript 1: Jesudason D, Clifton P. J Bone Miner Metab. Jan 2011; 29(1):1-14).

This paper was published in The Journal of Bone and Mineral Metabolism and reviews recent cross-sectional and longitudinal studies looking at the relationship between dietary protein and bones. It discusses the reasons for the conflicting results between different studies due to the confounding effect of other factors. These include the degree of calcium sufficiency in the diet, the role of fruit and vegetables in the diet to neutralize dietary acid and subject characteristics such as age and sex.

Chapter 3: Comparison of 2 weight-loss diets of different protein content on bone health: a randomized trial. (**Manuscript 2:** Jesudason D, Nordin BC, Keogh J, Clifton P. *Am J Clin Nutr.* Nov 2013; 98(5):1343-1352).

This manuscript was published in the American Journal of Clinical Nutrition and summarizes the results of our 24 month clinical trial studying the effect of dietary protein on bone health. I studied obese post-menopausal women at risk of future osteoporosis and was careful to ensure calcium sufficiency and adequate fruit and vegetables in both diets to ensure a balanced pH. Prior to our study, I am not aware of any randomized controlled trial comparing 2 diets of different protein content on bone health, exceeding 12 months duration.

Chapter 4: Introduction to Dietary Protein and Renal Health.

This chapter summaries the existing literature linking dietary protein and renal disease generally and then type 2 diabetes more specifically. This provides an introduction to the trial which is presented in Chapter 6 as a published paper. I note that the general concerns that have been raised between higher protein diets and advanced renal disease, including from type 1 diabetes, cannot be completely extrapolated to type 2 diabetes. This is because any weight loss induced by the higher protein diets would be beneficial to renal health either directly or from an improvement in risk factors such as glycaemic, lipid or blood pressure control. This may particularly be the case in early nephropathy.

Chapter 5: Interpreting different measures of glomerular filtration rate in obesity and weight loss: pitfalls for the clinician. (**Manuscript 3:** Jesudason DR, Clifton P. *Int J Obes (Lond).* Nov 2012; 36 (11):1421-1427). As discussed above, there is controversy in the literature on the best methods to measure renal function in the obese compared to

radioisotope methods. This chapter reviews the difficulties in using our current methods to accurately determine renal function in overweight and obese cohorts and was published in the International Journal of Obesity.

Chapter 6: Weight loss diets in people with type 2 diabetes and renal disease: a randomized controlled trial of the effect of different dietary protein amounts. (**Manuscript 4:** Jesudason D, Nordin BC, Keogh J, Clifton P. Am J Clin Nutr. Nov 2013; 98(5):1343-1352).

This chapter summarizes the results of my 12 month study comparing two diets of different dietary protein content on main renal endpoints and was published in the American Journal of Clinical Nutrition. I chose obese subjects with early nephropathy from type 2 diabetes for the reasons mentioned above. Subjects only underwent the dietary intervention after a period of stabilization of their blood pressure, lipids and glycaemic control by an endocrinologist (me).

Chapter 7: High protein weight loss diets in obese subjects with type 2 diabetes mellitus. (**Manuscript 5:** Pedersen E, Jesudason DR, Clifton PM. Nutr Metab Cardiovasc Dis. May 2014;24(5):554-562).

This chapter summarized the effect of the two different diets on secondary outcomes such as glycaemic control and lipids from the renal study and was published in Nutrition, Metabolism and Cardiovascular Diseases. This manuscript is included with my thesis as I was responsible for the study design and metabolic management of all subjects prior to starting the study and during the 12 months of the trial. However the continuous glucose monitoring and data analysis for this was primarily conducted by Ms Eva Pedersen, the study dietician who is acknowledged as first author on this paper.

Chapter 8: Utility of CKD-EPI formulae in Obese Diabetic Subjects Before and After Weight Loss. (**Manuscript 6:** Jesudason D, Pedersen E, Clifton P. Am J Kidney Dis 2014 Jul; 64(1):159-6).

Subsequent to the completion of the renal trial, the CKD-EPI creatinine equation was adopted for widespread use by Australian laboratories to estimate GFR as had occurred overseas. Because of the reliance of this equation on serum creatinine which is affected by muscle mass, I wondered whether the 2 other CKD-EPI equations using cystatin C and both cystatin C and creatinine would be more accurate in obese populations. This chapter evaluates the utility of the three different CKD-EPI equations to accurately estimate GFR in our overweight and obese cohorts at baseline and after weight loss. We had available radioisotope measurements of GFR, serum creatinine and cystatin C both before and after weight loss on our subjects.

Chapter 9: Conclusions and future directions

This chapter summaries my main conclusions from this thesis and whether I have demonstrated any harm from higher dietary protein in the context of weight loss in the cohorts of obese subjects at risk of osteoporosis and with early nephropathy from type 2 diabetes.

Chapter 10: References

Whilst the references for the 6 publications are included in each respective manuscript, this chapter lists references for the other chapters in this thesis.

**CHAPTER 2: MANUSCRIPT 1- THE
INTERACTION BETWEEN DIETARY PROTEIN
AND BONE HEALTH.**

Publication: J Bone Miner Metab. Jan 2011; 29(1):1-14

Statement of Authorship

Title of Paper	The interaction between dietary protein and bone health
Publication Status	<input checked="" type="radio"/> Published, <input type="radio"/> Accepted for Publication, <input type="radio"/> Submitted for Publication, <input type="radio"/> Publication style
Publication Details	Jesudason D, Clifton P. The interaction between dietary protein and bone health. J Bone Miner Metab 2011;29(1):1-14. doi: 10.1007/s00774-010-0225-9.

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	David Jesudason		
Contribution to the Paper	Reviewed existing literature and clinical trials regarding the link between dietary protein and bone health and prepared manuscript reviewing this topic and submitted to peer review journal. Made all necessary changes until manuscript was accepted for publication.		
Signature		Date	15/7/14

Name of Co-Author	Peter Clifton		
Contribution to the Paper	Critically reviewed manuscript after every version change with suggestions on how to further improve the quality of the paper.		
Signature		Date	17/7/14

Name of Co-Author			
Contribution to the Paper			
Signature		Date	

Name of Co-Author			
Contribution to the Paper			
Signature		Date	

Jesudason, D. & Clifton, P. (2011) The interaction between dietary protein and bone health.
Journal of Bone and Mineral Metabolism, v. 29(1), pp. 1-14

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of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

<http://doi.org/10.1007/s00774-010-0225-9>

**CHAPTER 3: MANUSCRIPT 2 - COMPARISON
OF 2 WEIGHT-LOSS DIETS OF DIFFERENT
PROTEIN CONTENT ON BONE HEALTH: A
RANDOMIZED TRIAL.**

Publication: Am J Clin Nutr Nov 2013; 98(5):1343-1352.

Statement of Authorship

Title of Paper	Comparison of 2 weight-loss diets of different protein content on bone health: a randomized trial
Publication Status	<input checked="" type="radio"/> Published, <input type="radio"/> Accepted for Publication, <input type="radio"/> Submitted for Publication, <input type="radio"/> Publication style
Publication Details	Jesudason D, Nordin BC, Keogh J, Clifton P. Comparison of 2 weight-loss diets of different protein content on bone health: a randomized trial. Am J Clin Nutr 2013;98(5):1343-52. doi: 10.3945/ajcn.113.058586.

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	David Jesudason		
Contribution to the Paper	Co-designed trial protocol, responsible for submission to Research Ethics Committee, determined eligibility of screened subjects to participate, responsible for trial conduct, monitored results and adverse events of all subjects during trial, analyzed data and wrote manuscript and all revisions after peer review.		
Signature		Date	23.4.14

Name of Co-Author	B E Christopher Nordin		
Contribution to the Paper	Co-designed trial protocol. Critically reviewed the original and revised manuscript.		
Signature		Date	23/4/14

Name of Co-Author	Jennifer Keogh		
Contribution to the Paper	Designed and monitored all trial diets. Critically reviewed the original and revised manuscript.		
Signature		Date	26/4/14

Name of Co-Author	Peter Clifton		
Contribution to the Paper	Developed initial concept of conducting this trial, co-designed trial protocol. Responsible with DJ for trial conduct. Assisted with data analysis. Critically reviewed all versions of the manuscript and made suggestions to improve the quality of the paper		
Signature		Date	24/4/14

Jesudason, D., Nordin, B.E.C., Keogh, J. & Clifton, P. (2013) Comparison of 2 weight-loss diets of different protein content on bone health: a randomized trial.
The American Journal of Clinical Nutrition, v. 98(5), pp. 1343-1352

NOTE:

This publication is included after page 24 in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

<http://doi.org/10.3945/ajcn.113.058586>

**CHAPTER 4: DIETARY PROTEIN, RENAL
DISEASE AND DIABETIC NEPHROPATHY**

Dietary Protein, Renal Disease and Diabetic Nephropathy

This chapter reviews the evidence for a link between increased dietary protein and renal disease generally and then looks more specifically at type 1 and then type 2 diabetes as a prelude to my subsequent study which is presented in chapter 6 as a published manuscript.

4.1 The Effect of Dietary Protein on Renal Dysfunction

Over 30 years ago, Brenner hypothesised in the *New England Journal of Medicine* that the high dietary protein enjoyed by modern humans would increase renal blood flow. This would cause hyperfiltration and increase intrarenal hypertension and contribute over time to glomerular sclerosis and ultimately deteriorating renal function³³. Because healthy humans have nephrons which are surplus to requirements, the effect of the higher protein diets would be manifest where there is already nephron loss. These include disease states such as diabetes or where there is surgical ablation of nephrons. This is consistent with studies in rats where a higher dietary protein had no effect histologically on the kidneys of rats with pre-existing normal nephron number but accelerated the histologic changes associated with renal ablation compared to a low protein diet³⁴⁻³⁶.

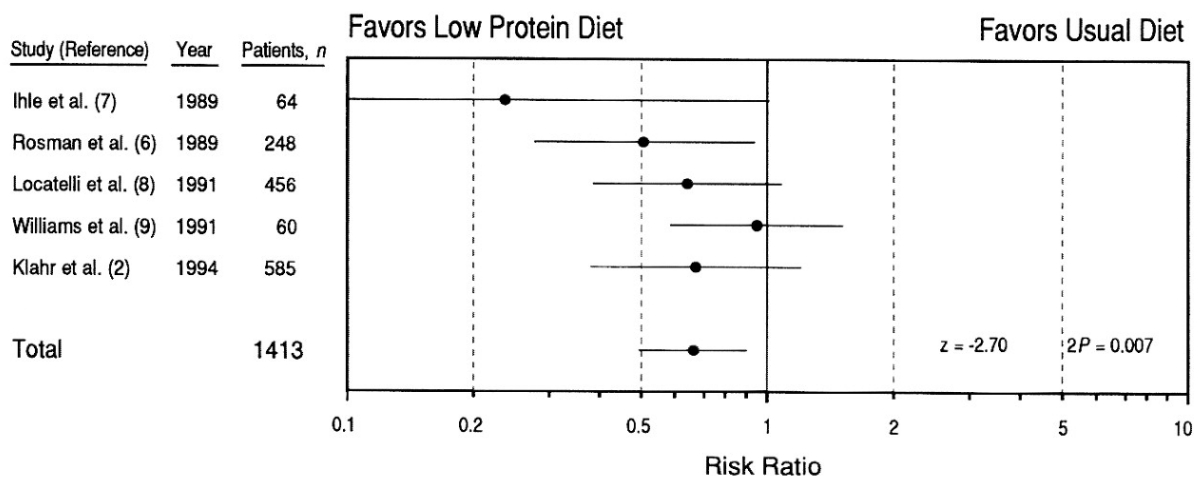
In humans the results are conflicting. Viberti et al. compared the effect in normal subjects of a low protein diet of 43g/day compared with a more usual 75g/day for 3 weeks. The former lowered renal plasma flow by 10% and GFR by about 20% whilst also halving urinary albumin excretion³⁷. After an acute 80 g of meat protein challenge, GFR increased by 7-13% as renal plasma flow increased due to a fall in renal vascular resistance. Filtration fraction did not change. On both diets, oral protein loading produced a 200-300%

increase in the urinary excretion and fractional clearance of albumin and IgG. Conversely Chan et al. compared the effect of a protein rich meal (1.5g/kg protein) on renal haemodynamics in 12 healthy volunteers and 12 subjects with chronic glomerular disease. Whilst the high protein increased renal plasma flow by 10% in normal subjects compared to 33% in patients with renal disease, there was no increase in albumin or IgG excretion³⁸.

Pedrini performed a meta-analysis looking at five randomized controlled trials between 1966 and 1994 using low protein diets in non-diabetic renal disease. There were 1413 patients followed for a mean length of 18 to 36 months³⁹ (Figure 1). A low-protein diet significantly reduced the risk for renal failure or death (relative risk, 0.67 [95% CI, 0.50 to 0.89]) as shown below. Notably the low protein diet was not always complied with as well as the normal protein diet. The need for dialysis was reduced in the low protein groups even though rates of decline in creatinine clearance were often not different.

Figure 1: Meta-analysis of dietary protein and non-diabetic renal disease.

Pedrini MT et al. *Ann Intern Med.* Apr 1 1996; 124(7):627-632.

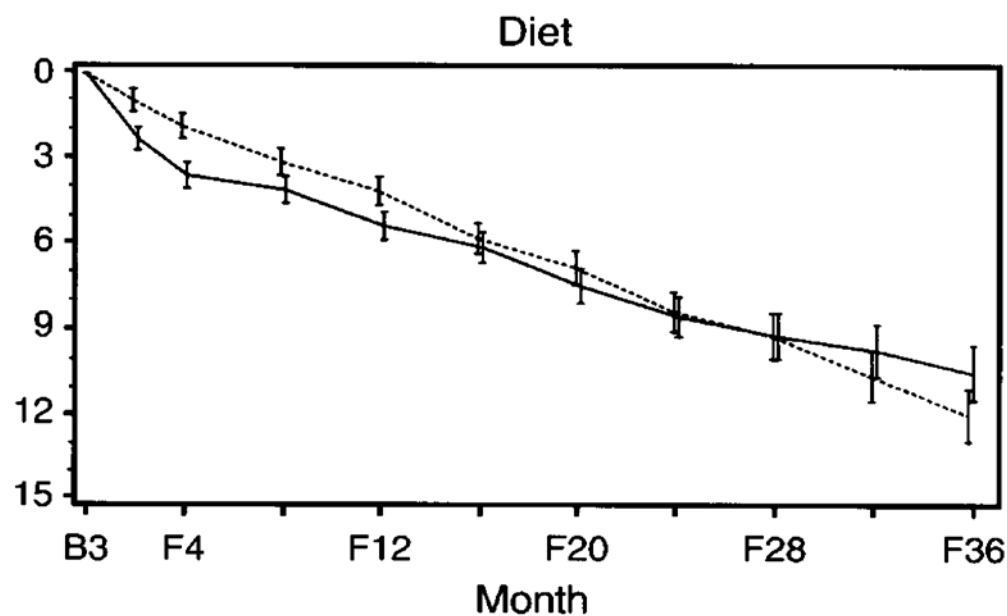


This issue was examined through a multi-centre trial by Klahr's group in 1994⁴⁰ (Figure 2). This study examined two cohorts. MDRD Study 1 looked at 585 subjects with moderate renal disease (GFR 25- 55 mL/min/1.73m²) assigned to either a usual protein or low protein diet (1.3 vs. 0.58 g protein/kg body weight). There was a non-linear decline in GFR. There was a greater fall in GFR in the low protein group initially. This was thought to reflect a haemodynamic response to the drop in protein intake rather than a true decline in renal function. Over 3 years there was no significant change in mean GFR decline between groups and no significant correlation between GFR decline and achieved protein intake.

Figure 2: Estimated Mean (\pm SE) Decline in GFR (ml/min) from baseline in Study 1.

Usual protein diet – dashed line and low protein diet – solid line.

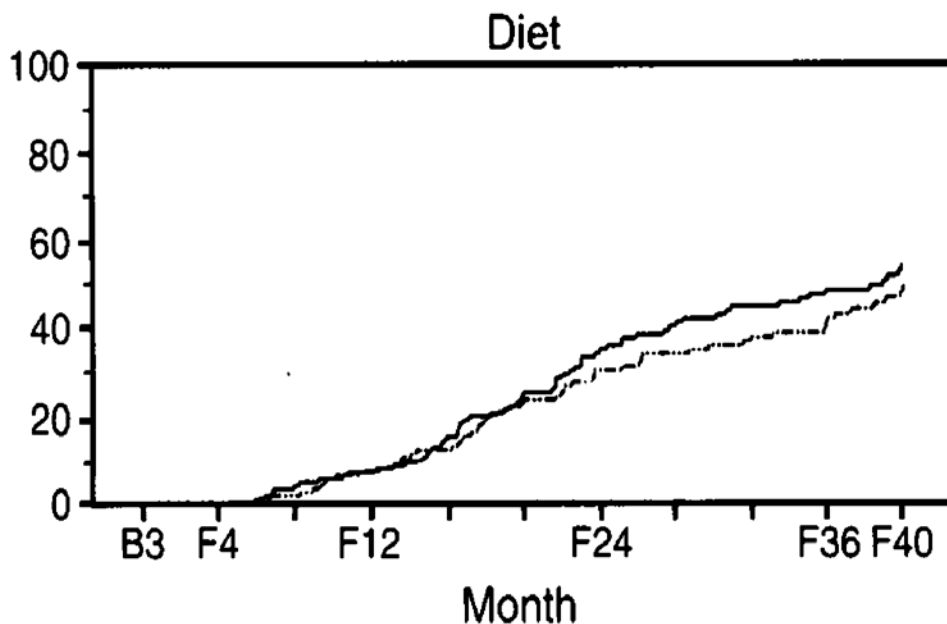
Klahr S. et al. N Engl J Med 1994; 330(13): 877-84.



The second cohort, MDRD study 2 compared the effect of a low protein and a very low protein diet (0.58 vs. 0.28 g/kg body weight) in 255 patients with a baseline GFR of 13-24 mL/min/1.73m² (Figure 3). Although the relative risk of death or renal failure was 0.93 (0.65-1.33) in patients assigned to the very low-protein diet group compared with the low - protein diet groups, similar to the Pedrini meta-analysis, this was not significant. Subsequent secondary analyses found a correlation between achieved protein intake and rate of decline in GFR⁴¹. It suggested that for each 0.2g/kg/day reduction in dietary protein, 1.15ml/min/year of GFR was preserved (p=0.01). This suggests that although the effects of low protein diets in nephropathy generally are not always consistent, there is evidence that as renal dysfunction advances, there may be a benefit of low protein diets.

Figure 3: The occurrence (%) of patients with end Stage Renal Disease or Death in Study 2. Low protein diet- solid line and very low protein diet- dashed line.

(Klahr S. et al. N Engl J Med 1994; 330(13): 877-84.



4.2 Type 1 Diabetes

Jones et al. found that baseline GFR appeared to be a key determinant of the renal haemodynamic response to a protein challenge in subjects with type 1 diabetes. In those who were hyperfiltering, the expected GFR rise after a protein challenge was attenuated although this could be restored after 3 weeks on a low protein diet⁴². Rudberg et al. showed in type 1 diabetes patients of 5 – 20 years duration, GFR declined after 10 days of a low protein diet compared to a usual protein isocaloric diet (10% vs 20% protein as calories). There was a greater decrease in those who were hyperfiltering⁴³. This was accompanied by a decline in albumin excretion rate and systolic blood pressure and was independent of glycaemic control. In this study the renal plasma flow was relatively unchanged but filtration fraction was decreased with a low protein diet. This was quite different to other studies where diabetes was of less than 5 years duration, where the decrease in GFR was accompanied by a drop in renal plasma blood flow.

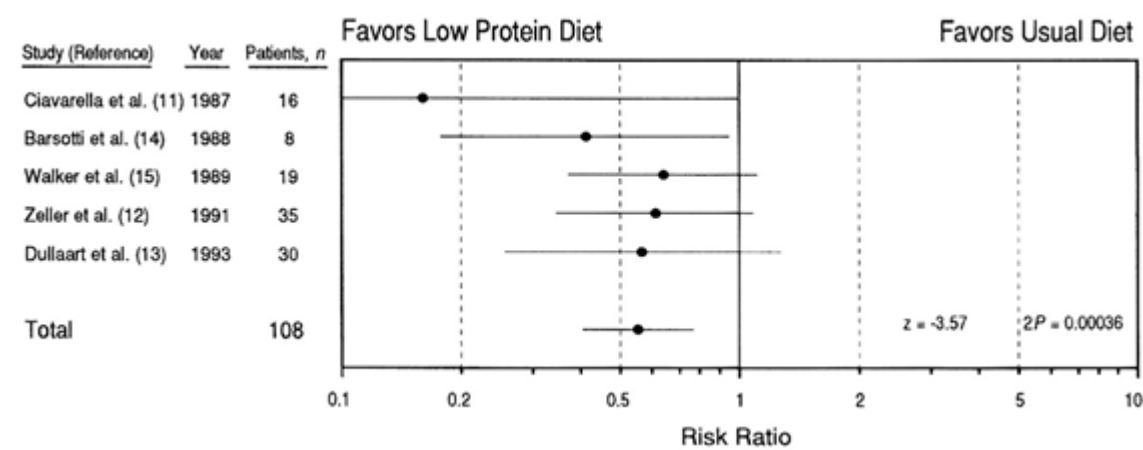
Other longer term studies suggested that lower protein diets would retard the GFR decline in type 1 diabetes⁴⁴. For example a study of 35 subjects with type 1 diabetes and nephropathy, a low protein diet of 0.6 g/kg ideal body weight halved the GFR decline over 37 months compared to a diet of 1.0g/kg ideal body weight⁴⁵. In a group of 19 type 1 diabetes patients with proteinuria, who consumed a normal-protein diet (1.13 g/kg per day) for a mean 29 months, then switched to a low-protein diet (0.67 g/kg per day) for 33 months, the mean rate of decline in glomerular filtration rate fell from 0.61 ml/min per month with the normal-protein diet to 0.14 ml/min per month with the low-protein diet. This was still significant after adjustment for blood pressure, energy intake, and

glycosylated haemoglobin⁴⁶. There was a significant fall in albumin excretion from 467 mg/24 h on the normal-protein diet to 340mg/24h on the low-protein diet.

In a separate meta-analysis by Pedrini, five studies of 108 patients with type 1 diabetes studied for 9 -35 months were analysed³⁹. This is shown in Figure 4. A low protein diet significantly slowed the increase in urinary albumin level or the decline in glomerular filtration rate or creatinine clearance (relative risk, 0.56 [CI, 0.40 to 0.77]). A Cochrane review in 2000 of protein intake in patients with type 1 diabetes also concluded that a protein restricted diet (0.3-0.8g/kg) slowed the progression of diabetic nephropathy towards renal failure⁴⁷. However it remained unclear what level of protein restriction was desirable or could be acceptable to patients and not cause malnutrition. Furthermore a lot of the trials used proxy indicators such as creatinine clearance or reduction in albumin excretion rather than outcomes such as time to dialysis or prevention of end stage renal failure (ESRF). These trials were confined to type 1 diabetes patients and the benefit in the type 2 population was not examined. Even where benefit was shown, it was often modest⁴⁸. However, in a 4 year study of 82 type 1 diabetes patients consuming 1.02 g/kg/day protein as compared with 0.89 (0.83 to 0.95) in the low-protein diet group, the low protein diet did not alter the rate of decline of creatinine clearance but did reduce the need for dialysis or death from 27% to 10%⁴⁹. Therefore, analogous to the situation with nephropathy generally, there is some supporting evidence albeit not definitive for the role of protein restriction in type 1 diabetes, particularly at the later stages of renal failure.

Figure 4: Meta-analysis of dietary protein and type 1 diabetes with renal disease.

Pedrini MT et al. *Ann Intern Med.* Apr 1 1996; 124(7):627-632.



4.3 Type 2 diabetes mellitus and nephropathy

The increasing incidence of Type 2 diabetes has been described as a global health crisis which will affect over 350 million people by 2030^{50,51}. Accordingly, the prevalence of diabetic macrovascular and microvascular complications will increase. Diabetes has now become the main cause of end stage renal disease (ESRD) worldwide. By 2015, approximately 70 % of all cases of ESRD will be due to diabetes⁵².

Strategies to reduce the progression of diabetic nephropathy that have been well validated include good glycaemic, blood pressure and lipid control as well as angiotensin receptor blockade⁵³. As obesity, driven by excessive caloric intake has been posited as driving the increased incidence of diabetes, it is logical to examine firstly if dietary weight loss per se will reduce the progression of diabetic nephropathy and secondly to consider the role of

macronutrients⁵⁰. For the purposes of this review the role of dietary protein will be considered both in its own right and in the context of weight loss.

4.4 Obesity, weight loss and renal dysfunction?

It is now accepted that both current and past obesity is a risk factor for nephropathy both in persons with diabetes and without diabetes^{54,55}. In logistic regression analysis, this has been shown to be independent of sex, age, disease duration, hypertension, dyslipidemia, HbA1c, and diabetic retinopathy. Wang's meta-analysis of studies linking weight and chronic kidney disease has shown that compared with normal-weight individuals, overweight individuals ($25 \leq \text{BMI} < 30 \text{ kg/m}^2$) had a relative risk of kidney disease of 1.40 (95% CI 1.30-1.50). For obese individuals the relative risk is 1.83 (1.57-2.13)⁵⁵. Similarly, 320,252 adults who participated in a health check-up between 1964 and 1985 were then followed up for 8,347,955 person-years. There was a seven fold increase in relative risk of end stage renal disease for those with a BMI $\geq 40 \text{ kg/m}^2$ compared with those with a BMI $\leq 25 \text{ kg/m}^2$ ⁵⁶.

Does weight loss in the obese reverse or slow the progression of renal disease? Weight loss induced by reduced calorie diets as well as by bariatric surgery can ameliorate proteinuria within weeks, even when weight loss is modest. The aetiology is multifactorial and may relate to improved blood pressure, lipid profile, glycaemic control and insulin sensitivity, decreased activation of the renin-angiotensin system and reduction in glomerular hyperfiltration where this is present^{57,58}.

A recent meta-analysis including thirteen studies examined the issue of whether weight loss would be beneficial for subjects with chronic kidney disease and obesity⁵⁹. In those subjects who were morbidly obese with a BMI ≥ 40 kg/m² and hyperfiltration defined as GFR > 125 ml/min, bariatric surgery decreased BMI and decreased GFR (weighted mean difference -25.56 ml/min; 95% CI -36.23 to -14.89) as well as albuminuria, and systolic blood pressure. In those subjects without hyperfiltration who had non-surgical weight loss, the BMI decreased significantly (weighted mean difference -3.67 kg/m²; 95% CI -6.56 to -0.78). This was associated with a significant decrease in proteinuria (weighted mean difference -1.31 g/24 h; 95% CI -2.11 to -0.51) and systolic BP with no further decrease in GFR during a mean follow-up of 7.4 months.

4.5 Dietary Protein and Type 2 Diabetes

The effect of protein restriction on renal function is less well studied in subjects with type 2 diabetes. A Japanese study of 14 type 2 patients with nephropathy followed for a month compared the effects of a low protein (0.8 g/kg/day) diet compared to a standard protein (1.2 g/kg/day) diet⁶⁰. This resulted in a significant difference in protein intake of 41 g/day versus 68 g/day. Urinary albumin excretion decreased in the lower protein group from 3.2 g/day to 1.9 g/day. No other significant changes were seen. As body weight was measured at baseline but not subsequently, the fall in albumin excretion may have been related to a fall in body weight.

A 12 month study of 121 type 2 patients with diabetes with microalbuminuria, randomized subjects to either 0.8 g/kg/day or to continue on 1.2 g/kg/day⁶¹. After 12 months,

albuminuria in the lower dietary protein group decreased by 12 % compared to control in the intention to treat analysis. However a significant confounder was that the subjects in the higher protein group gained weight at 6 months whereas the lower dietary protein group remained weight stable which may have been responsible for the differences seen. Conversely a study of 67 type 2 diabetes patients followed for 1-4 years found no beneficial effect of a 0.6 g/kg/day protein restriction nor did a study of 106 subjects with type 2 diabetes conducted by Okada et al.^{62,63}. A prospective study by Dussol et al. of a mixed cohort of 63 type 1 and type 2 diabetes patients with excellent blood pressure and angiotensin blockade showed no benefit of protein restriction⁶⁴. Two studies of dietary restriction by Meloni et al. on subjects with nephropathy incorporating subjects with type 1 and type 2 diabetes as well as without diabetes showed no benefit of a lower protein diet of 0.6-0.8g/kg/day in those with type 2 diabetes^{65,66}.

In fact a trial of 160 type 2 diabetes patients showed no advantage of protein restriction and a possible harmful effect on albuminuria after 28 months of intervention⁶⁷. However in this as well as some of the other studies above, adherence to the prescribed diet was limited with a convergence of protein intake between the 2 groups after 12 months.

Therefore unlike the situation in subjects without diabetes and type 1 diabetes, the evidence for a beneficial effect of protein restriction in type 2 diabetes with impaired renal function is limited. What could be the reason for this difference? One factor much more common with type 2 diabetic nephropathy compared with other forms of nephropathy is the co-existence of obesity. There is evidence that weight loss per se could slow down the

progression of renal dysfunction both directly as well as by acting through control of risk factors like glycaemic and hypertensive control as discussed below.

4.6 Why consider High Protein Weight Loss Diets?

Give the benefits of weight reduction in the chronic kidney disease cohort, what diet should be used? We know that high protein weight loss diets are popular and are frequently used in the general population³. A cross-sectional analysis conducted from 1999-2006 examined the lifestyle factors of 10,971 overweight and obese adult participants in the National Health and Nutrition Examination Surveys⁶⁸. They found that the percentage of energy derived from protein was similar between the subjects with and without chronic kidney disease. Amongst participants pursuing weight loss, dietary interventions utilized by CKD and non-CKD participants were similar; specifically a significant proportion of the CKD population used diets that had high-protein content. The authors argued that the safety of a high protein diet in this cohort was unknown. If high protein diets are used by obese patients with type 2 diabetes, what do we know about their effect in kidney dysfunction?

4.7 High protein weight loss diets and risk factors for type 2 diabetic nephropathy

The dramatic weight loss of 20-30% induced by bariatric surgery in people with type 2 diabetes can normalise sugars and reduce the dosage of insulin or oral medication in most people. However even modest non-surgical weight loss of 5-10% can have a profound effect on glycaemic control of diabetes especially in the short term. A CSIRO study demonstrated a fall in HbA1c of 1% or more⁶⁹. A recent meta-analysis of high protein

weight loss diets of 4-24 weeks duration analysed 9 trials with a total of 418 subjects with diabetes⁷⁰. The protein intake ranged from 25-32% of total daily energy in the high protein group and 15-20% of total daily energy in the control group. The high-protein diets resulted in greater weight loss (pooled mean difference: -2.08, (95% CI -3.25, -0.90 kg) compared to control diets. High-protein diets significantly decreased HbA1c by 0.52%, but did not affect the fasting glucose. There were no differences in lipids. In addition, weight loss reduces blood pressure which is a major risk factor for renal disease as above. The pooled net changes in systolic and diastolic blood pressure were -3.13 (95% CI -6.58, 0.32) mmHg and -1.86 (95% CI -4.26, 0.56) mmHg, respectively. Unfortunately, it is hard to find high protein weight loss trials of 12 or more months in diabetes patients where the effects on glycaemic control and other metabolic parameters are studied.

However there is evidence that even in the absence of weight loss, replacing carbohydrate with protein from 15 to 30% total daily calories can reduce Hba1c modestly by 0.5% over 5 weeks. A combination of increases in protein and fat and larger decreases in carbohydrate can decrease HbA1c by 2.1%⁷¹. The longevity of this improvement in metabolic control if the dietary changes are sustained is uncertain. In subjects without diabetes who consumed an Atkins diet high in fat and protein, but low in carbohydrate, the weight loss at 12 months is greater than on a conventional high carbohydrate diet (by about 2.5kg) without any adverse effects. In this case, the difference in fasting glucose was not significant⁷².

Dietary protein when substituted for carbohydrates and combined with fibre has been shown both in randomized trials and in population studies (particularly vegetable protein)

to be associated with lower blood pressure.⁷³⁻⁷⁵ In type 2 diabetes where the metabolic syndrome including hypertension can co-exist, improved blood pressure control could be reasonably expected to be beneficial⁵³.

4.8 Epidemiological Studies

In a study of 1,150 patients with type 1 diabetes for greater than 5 years, 75 cases of microalbuminuria were identified. When diet was compared to matched subjects without microalbuminuria, total protein intake from any source was not associated with microalbuminuria⁷⁶. Higher consumers of fish protein (greater than the 75th percentile) who had a mean intake 9.35 g fish protein/day, i.e., approximately 53 g fish/day had lower odds ratios for microalbuminuria than individuals consuming less fish protein (mean 2.72 g/day) (crude odds ratio 0.49 and 95% CI 0.25-0.97). Another large multi-ethnic study of 4381 subjects analyzing the link between multiple dietary elements and microalbuminuria found no link with dietary protein⁷⁷.

In a cohort of 680 Caucasians, with a spectrum of glycaemic control ranging from normality to overt type 2 diabetes, a link between homocysteine levels and the presence of microalbuminuria was sought⁷⁸. As a separate finding, it was noted that every 0.1 g/kg/day increment of protein intake was also associated with an increased risk for microalbuminuria after adjustment for age, sex, classical risk factors and serum total homocysteine [odds ratio 1.20 (1.08 to 1.32)].

The Nurses Health study investigators examined prospectively the changes in estimated GFR over 11 years amongst 1624 women aged 42 to 68⁷⁹. No association with dietary

protein amongst women with baseline estimated GFR greater than 80 ml/min/1.73m² was found. In those women with mild renal dysfunction i.e.an estimated GFR of 55-80 ml/min/1.73m², a reduction in GFR of 1.69ml/min per 10g of dietary protein was seen. Non-dairy animal protein was more strongly associated with reduction than other proteins.

4.9 Revised American Diabetes Association Recommendations

Protein restriction was previously recommended for patients with diabetes with impaired renal function. In 2008 the American Diabetes Association (ADA) stated that for type 2 diabetes patients, protein consumption of 15-20% of total energy (TE) was not associated with increased risk of developing diabetes related nephropathy. It cautioned that it was unclear how protein intake >20% TE would affect renal function in type 2 patients⁸⁰.

This recommendation was somewhat tempered by 2011 with the advent of several trials with low carbohydrate diets as well as a Cochrane review and meta-analysis which found no significant benefit on GFR with a low protein diet either with type 1 or type 2 diabetes^{81,82}. The ADA noted that the “optimal macronutrient distribution of weight loss diets has not been established”⁸³. However it was still suggested that protein intake should be reduced to 0.8-1.0g/kg body weight/day in diabetes patients with early nephropathy and to 0.8 g/kg body weight daily in those with latter stages of CKD. However no attempt was made to differentiate between Type 1 and Type 2 diabetes and to consider the issue of macronutrient composition in the context of weight loss per se rather than in general.

However in 2014, the American Diabetes Association further modified their nutritional recommendations regarding protein restriction in both Type 1 and Type 2 diabetes^{84,85}. Their Level A recommendation for people with either Type 1 or 2 diabetes and kidney disease (i.e. with micro or macroalbuminuria) was that “reducing the amount of dietary protein below the usual intake is not recommended because it does not alter glycaemic measures, cardiovascular risk measures, or the course of glomerular filtration rate (GFR) decline.” The ADA noted though that in some of the studies, the low protein intervention group consumed a higher protein diet than the control group with protein intakes being 0.7-1.1 g/kg/ body weight daily. This suggested the need for further studies. The evidence cited for the benefit of weight loss with diabetes in improving cardiovascular risk factors like lipids, blood pressure and glycaemic control was much stronger. However no specific recommendation was made by the ADA regarding weight loss in those with diabetic nephropathy.

4.10 Summary

Available evidence favouring the use of a protein restricted diet is stronger in non-diabetic renal disease than in diabetic nephropathy. In non-diabetic renal disease, a higher than usual protein diet might have an adverse effect. This is particularly true where renal dysfunction is more advanced. In type 1 diabetes with renal disease, the evidence for protein restriction is less secure and is no longer dogmatically advocated for all patients.

In patients with type 2 diabetes with renal disease the data is even less clear. In the absence of any other changes, a higher than usual protein intake might increase albumin excretion. However, if the high protein intake was associated with weight loss, then albuminuria

might fall due to the direct effect of weight reduction as well as the associated improvements in blood pressure, glycaemic control and lipids.

Thus a weight loss study comparing 2 diets with higher and lower protein content in obese subjects with type 2 diabetes would help answer this important question. Because the harmful effects of higher protein diets in other forms of renal disease seem to be more manifest with more advanced renal failure, ideally type 2 subjects with early nephropathy (eg microalbuminuria with hyperfiltration or modestly reduced GFR) should be studied in the first instance. This was the rationale for the design of our renal trial as presented in Chapter 6.

**CHAPTER 5: MANUSCRIPT 3 - INTERPRETING
DIFFERENT MEASURES OF GLOMERULAR
FILTRATION RATE IN OBESITY AND WEIGHT
LOSS: PITFALLS FOR THE CLINICIAN.**

Publication: Int J Obes (Lond). Nov 2012; 36(11):1421-1427.

Statement of Authorship

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Publication Status	<input checked="" type="radio"/> Published, <input type="radio"/> Accepted for Publication, <input type="radio"/> Submitted for Publication, <input type="radio"/> Publication style
Publication Details	Jesudason DR, Clifton P. Interpreting different measures of glomerular filtration rate in obesity and weight loss: pitfalls for the clinician. Int J Obes (Lond) 2012;36(11):1421-7. doi: 10.1038/ijo.2011.242.

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	David Jesudason		
Contribution to the Paper	Reviewed existing literature and clinical trials regarding the utility of the different ways to measure glomerular filtration rate in the obese, both before and after weight loss. Prepared manuscript reviewing this topic and submitted to peer review journal. Made all necessary changes until manuscript was accepted for publication.		
Signature		Date	10/7/14

Name of Co-Author	Peter Clifton		
Contribution to the Paper	Critically reviewed manuscript after every version change with suggestions on how to further improve the quality of the paper.		
Signature		Date	10/7/14

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Signature		Date	

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Signature		Date	

Jesudason, D. & Clifton, P. (2012) Interpreting different measures of glomerular filtration rate in obesity and weight loss: pitfalls for the clinician.
International Journal of Obesity, v. 36, pp. 1421-1427

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**CHAPTER 6: MANUSCRIPT 4 - WEIGHT LOSS
DIETS IN PEOPLE WITH TYPE 2 DIABETES
AND RENAL DISEASE: A RANDOMIZED
CONTROLLED TRIAL OF THE EFFECT OF
DIFFERENT DIETARY PROTEIN AMOUNTS.**

Publication: Am J Clin Nutr. Nov 2013; 98(5):1343-1352.

Statement of Authorship

Title of Paper	Weight-loss diets in people with type 2 diabetes and renal disease: a randomized controlled trial of the effect of different dietary protein
Publication Status	<input checked="" type="radio"/> Published, <input type="radio"/> Accepted for Publication, <input type="radio"/> Submitted for Publication, <input type="radio"/> Publication style
Publication Details	Jesudason DR, Pedersen E, Clifton PM. Weight-loss diets in people with type 2 diabetes and renal disease: a randomized controlled trial of the effect of different dietary protein amounts. Am J Clin Nutr 2013;98(2):494-501. doi: 10.3945/ajcn.113.060889.

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Name of Principal Author (Candidate)	David Jesudason		
Contribution to the Paper	Assisted with study design, responsible for ethics submission. Determined eligibility of screened subjects. As study endocrinologist, stabilized cardiovascular risk factors for all subjects prior to trial and reviewed all results during trial and provided medical management frequently during trial. Contributed to data analysis and manuscript preparation.		
Signature		Date	24/4/14

Name of Co-Author	Eva Pedersen		
Contribution to the Paper	As lead dietitian provided all aspects of dietary care including designing both weight loss diets, regularly reviewing all subjects prior to and during study for dietary instruction. Contributed to data analysis and manuscript preparation.		
Signature		Date	26/4/2014

Name of Co-Author	Peter Clifton		
Contribution to the Paper	Conceived and designed study and supervised the other co-authors as they provided medical and dietetic care respectively. Contributed to data analysis and to manuscript preparation. Corresponding author on manuscript.		
Signature		Date	28/4/14

Name of Co-Author			
Contribution to the Paper			
Signature		Date	

Jesudason, D., Pedersen, E. & Clifton, P. (2013) Weight-loss diets in people with type 2 diabetes and renal disease: a randomized controlled trial of the effect of different dietary protein amounts. *The American Journal of Clinical Nutrition*, v. 98(2), pp. 494-501

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**CHAPTER 7: MANUSCRIPT 5 - HIGH
PROTEIN WEIGHT LOSS DIETS IN OBESE
SUBJECTS WITH TYPE 2 DIABETES MELLITUS.**

Publication: Nutr Metab Cardiovasc Dis. May 2014;
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Title of Paper	High protein weight loss diets in obese subjects with type 2 diabetes mellitus
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Publication Details	Pedersen E, Jesudason DR, Clifton PM. High protein weight loss diets in obese subjects with type 2 diabetes mellitus. Nutrition, metabolism, and cardiovascular diseases : NMCD 2013. doi: 10.1016/j.numecd.2013.11.003. Epub ahead of print.

Author Contributions

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Name of Principal Author (Candidate)	David Jesudason		
Contribution to the Paper	Assisted with study design, ethics submission and subject suitability for main study. As study endocrinologist, stabilized diabetic risk factors for all subjects prior to trial and reviewed all results during trial and provided medical management frequently during trial. Contributed to data analysis and manuscript preparation and revision.		
Signature		Date	24.4.14

Name of Co-Author	Eva Pedersen		
Contribution to the Paper	As lead dietitian responsible for managing diet of all subjects in the original trial. Exclusively responsible for the continuous glucose monitoring sub-study reported here. Contributed to data analysis. Primary role in manuscript preparation and revision.		
Signature		Date	26/04/2014

Name of Co-Author	Peter Clifton		
Contribution to the Paper	Conceived and designed original study. Contributed to data analysis and to manuscript preparation.		
Signature		Date	24/4/14

Name of Co-Author			
Contribution to the Paper			
Signature		Date	

Pedersen, E., Jesudason, D.R. & Clifton, P.M. (2013) High protein weight loss diets in obese subjects with type 2 diabetes.

Nutrition, Metabolism and Cardiovascular Diseases, v. 24(5), pp. 554-562

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**CHAPTER 8: MANUSCRIPT 6 - UTILITY OF
CHRONIC KIDNEY DISEASE EPIDEMIOLOGY
COLLABORATION (CKD-EPI) EQUATIONS
IN OBESE DIABETIC INDIVIDUALS BEFORE
AND AFTER WEIGHT LOSS.**

Publication: Am J Kidney Dis. 2014 Jul; 64(1):159-61.

Statement of Authorship

Title of Paper	Utility of Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Equations in Obese Diabetic Individuals Before and After Weight Loss
Publication Status	<input type="radio"/> Published, <input checked="" type="radio"/> Accepted for Publication, <input type="radio"/> Submitted for Publication, <input type="radio"/> Publication style
Publication Details	Jesudason D, Pedersen E, Clifton PM. Utility of Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Equations in Obese Diabetic Individuals Before and After Weight Loss . American Journal Kidney Diseases - in press (corrected proof available on-line).

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	David Jesudason		
Contribution to the Paper	Assisted with study design, eligibility of screened subjects and endocrine management of subjects prior and during original study. Conceived idea for this paper comparing the accuracy of the 3 CKD-EPI equations in this subset. Responsible for all data analysis and primary contributor to manuscript before and after revision.		
Signature		Date	24.4.14

Name of Co-Author	Eva Pedersen		
Contribution to the Paper	As lead dietician, provided comprehensive dietary management for all subjects before and after weight loss in this trial. Critically reviewed and contributed to manuscript.		
Signature		Date	26/4/2014

Name of Co-Author	Peter Clifton		
Contribution to the Paper	Conceived and co-designed original study upon which this secondary analysis was made. Critically reviewed and contributed to manuscript.		
Signature		Date	26/4/14

Name of Co-Author			
Contribution to the Paper			
Signature		Date	

Jesudason, D., Pedersen, E. & Clifton, P. (2014) Utility of chronic kidney disease epidemiology collaboration (CKD-EPI) equations in obese diabetic individuals before and after weight loss. *American Journal of Kidney Diseases*, v. 64(1), pp. 159-161

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CHAPTER 9: CONCLUSIONS AND FUTURE

DIRECTIONS

9.1 Dietary Protein and Bone

The purpose of my thesis was to investigate whether higher protein weight loss diets were deleterious to bone health and kidney health as hypothesized by Wachman and Bernstein in 1968⁴ and Addis in 1951¹² respectively. I chose as the cohort for study overweight or obese women at risk of osteoporosis and overweight or obese subjects with early diabetic nephropathy from type 2 diabetes. The results of my clinical trials, interpreted in the context of recent research, suggest that there is no evidence that higher dietary protein is harmful to bones. On the contrary, higher dietary protein may have a mildly net beneficial effect. In the case of early renal disease, the harmful effects of higher dietary protein may have been overstated. However, there are some caveats to this statement as discussed below.

9.2 Dietary Acid, Calcium and Protein Interaction

My review of recent literature showed that the initial hypothesis that the acid load from dietary protein caused hypercalciuria and led to osteoporosis over time was already being seen as too simplistic. Firstly dietary protein could increase calcium absorption which would attenuate the loss of calcium from hypercalciuria. Secondly if diets with higher protein were balanced with sufficient calcium then the protein might also be protective through an effect on bone matrix. Thirdly the high potassium content of dietary fruit and green vegetables might have an alkalizing effect which would balance the acid load from dietary protein. Indeed those studies which controlled for these factors often did not show a harmful effect of dietary protein. In fact the meta-

analysis by Darling et al. showed a modest beneficial effect of dietary protein on bone health⁸⁶.

The randomized controlled trial described in this thesis showed that confounding factors were successfully overcome. There was calcium adequacy in both diets of over 1000mg daily. In fact the dietary calcium intake became higher over the two years in the higher protein group. The absence of a time by diet effect on urine pH showed the acid load of the higher protein diet was ameliorated by other dietary macronutrients. Because weight loss and altered body fat can cause both artefactual as well as real changes in DXA bone density measurement, it was also important that matched weight loss and matched changes in body composition were achieved. As shown in figure 2, in Manuscript 2, there is a relationship between loss of bone at each of the sites and loss of weight and loss of fat mass. However the relationship was not strong and only accounts for a small part of the change in bone density. Because of the inconsistent results of trials assessing potential differences of protein source such as meat, dairy or vegetable protein on bone health, I did not attempt to differentiate between these different sources of protein in this trial.

9.3 Lack of harm of Dietary Protein on Bone

There was no evidence from the study that higher dietary protein is harmful to bone health so this hypothesis is refuted. Moreover, consistent with the meta-analysis, there was a signal that there might even be a modest beneficial effect as shown by the significantly higher bone turnover in the lower protein group. Although there was no

diet or diet by time effect on bone density, percentage bone loss was non-significantly higher at most sites with the lower protein diet.

An important consideration here is whether we were adequately powered to make firm conclusions based on the subject attrition in this trial. Our power calculations were based on the knowledge that total body bone density declines at about 0.4% per year whilst data from the Danish Osteoporosis Prevention Study showed that hip/femoral neck and spine decline at about 0.9-1.3% per year^{87,88}. Based on the assumption that 200 participants would complete the study, we would have 80% power to detect a 1.6% difference ($p < 0.05$) in density between the two groups based on a 4% SD. Based on our 186 subjects left after 1 year and 136 completers after 2 years, we were powered at 78% and 65% to detect such a difference at end of 1 and 2 years respectively. On the basis of the greater bone loss at all sites at 2 years in the lower protein group and on the results of the bone markers, it is exceedingly unlikely that the hypothesis that protein is harmful to bone would be supported. However we concede that there is the possibility that we did not detect a beneficial effect on bone from higher protein diets. Hence we speculated in our manuscript that a much larger trial or a trial conducted over a much longer period or a trial that achieved greater protein separation between groups, may yield a beneficial effect of dietary protein on bone density. However this would suggest that any anabolic effect of dietary protein on bone, if it existed is likely to be very modest. At best, its clinical utility would only be targeted at high risk populations.

9.4 Subject Attrition and Compliance to Diet in Bone Study

This study also confirmed the well documented difficulties in preventing subject attrition over a 2 year weight loss study, although weight loss was maintained in those who remained (although this would have been a factor in maintaining participation in the study). We were not able to do an intention to treat analysis as we could not perform bone densities on the large number of non-completers. However we were able to demonstrate (Table 7 of Manuscript 2) that the non-completers were equally matched in both dietary groups which provided some reassurance that our analysis of completers is still valid.

I was not as successful at achieving greater dietary separation between the groups as I had wanted. The urinary nitrogen results suggested that a 16g separation in dietary protein after 1 year and a 13.4 g separation after 2 years was achieved. This occurred more by increasing dietary protein in the high protein group than by reducing dietary protein in the lower protein group. The diet records were consistent with the dietary separation as above but suggested that this was achieved by maintaining dietary protein intake in the higher protein group simultaneously with reducing overall caloric intake by approximately 1400kJ daily to increase percentage of dietary protein intake. Conversely dietary protein was reduced in the lower protein intake group to achieve the difference in dietary protein between the 2 groups. Either way, the fact that greater dietary protein separation between groups could not be achieved in a highly regulated clinical trial with strong dietetic support, highlights the potential difficulties if changes in dietary protein were ever advocated for bone health in free living individuals.

9.5 A Threshold Effect for Dietary Protein on Bone?

There was another issue raised but not completely answered by this research. Assuming dietary protein is beneficial to bone health, is there a threshold below which insufficient dietary protein is harmful and above which dietary protein is beneficial to bone health? The reason our two dietary groups were labelled High Protein (HP) and High Normal Protein (HNP) rather than Normal Protein was because the editors of AJCN pointed out that this latter group's protein intake exceeded the daily recommended protein intake of 0.8g/kg/day. However the editors conceded that this protein intake was consistent with the reported daily protein intake of Australian women and in that sense was "normal"⁸¹. It is possible that increasing dietary protein above current average daily intake might afford no benefit in the general population compared to increasing dietary protein in groups with very low dietary protein such as some elderly subjects who may be malnourished. This would require separate clinical trials to investigate this hypothesis.

9.6 Dietary Protein and Fractures

Finally there were too few fractures to ascribe any difference in fracture rate to dietary protein. As pointed out in the manuscript, the aetiology of fractures extend beyond bone density and even bone strength to other issues such as muscle strength and falls risk which may or may not be affected by dietary protein. This would require quite a different clinical trial and analysis to tease out the effects of dietary protein on other outcomes.

9.7 Dietary Protein, Renal Function and Obesity

My review of the relationship between dietary protein and renal health suggested that the relationship between the two was not straightforward. Factors to be considered included whether a short or longer time frame was used, the degree of protein restriction discussed, the degree of renal dysfunction of the subject population and whether one was studying renal disease generally or type 1 or type 2 diabetes.

Acute increase in dietary protein increases GFR within days. However epidemiological as well as some clinical studies suggest increased dietary protein accelerates the annual decline in GFR and increase in albumin excretion in non-diabetic renal disease and possibly type 1 diabetes. However the results of studies of protein restriction in type 2 diabetic nephropathy do not show similarly convincing evidence of benefit from lower protein diets. This may reflect the fact that many type 2 diabetes patients are overweight or obese and the higher protein diets may be better for weight loss. This weight loss, by itself, and due to a concomitant improvement in lipid, blood pressure and glycaemic control may actually slow the progression of nephropathy. My review also suggested that any beneficial role for protein restriction may be more likely in very advanced renal disease rather than early nephropathy.

For this reason I targeted obese subjects with type 2 diabetes with early nephropathy for the renal study. These subjects had microalbuminuria and either were hyperfiltering or had only mildly reduced GFR (i.e. grade 1 – 3a).

9.8 Outcomes of Renal Trial

I was successful in stabilizing glycaemic, blood pressure and lipid control to eliminate these confounding factors. As weight loss was matched in both groups, any change in GFR could be ascribed to the difference in dietary protein between groups. A protein difference between groups of 19g over the 12 months was achieved although we had aspired for a bigger difference. This was similar to the bone trial. Our groups were labelled moderate protein and standard protein rather than high and low protein because our actual protein intakes were 1.22g/kg day and 0.93 /kg day; the latter being at the higher end of the range for previous low protein trials.

There was no change with time or diet for our main endpoint of isotope measured GFR. Subjects who hyperfiltered (estimated GFR greater than 120 ml/min/1.73m²) had a decrease in eGFR of 15ml/min/1.73m² with weight loss. This might be postulated to be beneficial. The remainder, who had an eGFR between 40-120 ml/min/1.73m², had an increase in GFR of 4 ml/min/1.73m² with weight loss. There was no change in albuminuria. This study demonstrates no harmful effect of higher dietary protein on renal function in early diabetic nephropathy. In fact, if adopted in the context of weight loss, there may even be a beneficial effect.

9.9 Limitations of the Renal Trial

Nevertheless, there are a few caveats when interpreting our renal endpoints. As described in the manuscript we had difficulties recruiting eligible subjects. We had hoped to enrol at least 150 subjects but obtained only 76. As described in the

manuscript, this gave us an 80% power ($p < 0.05$) to see a difference of eGFR of 8.9 ml/min/1.73m². As only 45 subjects completed study, our power to detect a statistical significant change of this size dropped to 60%. This leaves the possibility that there was an indeed deleterious effect of the higher protein diet which we were underpowered to pick up. Also an eGFR difference of 8.9 ml/min/1.73m² is quite a blunt measure and true differences of lower magnitude would still be clinically important. The gold standard iGFR did suggest a borderline significant reduction in the higher protein group ($P=0.086$). However, a reduction in GFR may be beneficial in those who hyperfilter (as did occur), whilst those with GFR less than 120 ml/min/1.73m² would benefit from an increase in GFR (as also occurred). In retrospect I would have been better off devising two substudies to analyse separately those who were hyperfiltering as well as those who had impaired GFR from the outset.

It is acknowledged that albuminuria was our main criteria for enrolment to facilitate screening whilst our main endpoint was alteration in GFR which might seem counterintuitive as they describe overlapping but partially different cohorts. However by setting a lower limit of 40 ml/min/1.73m² for GFR as an additional criteria, I was aiming to partially overcome this limitation as well as capture subjects who may have had a “normal GFR” and yet had early kidney dysfunction by way of albuminuria. However because albuminuria was the main study enrolment point and because of our limited study numbers the eGFR appears unbalanced at enrolment (moderate protein group 98 ± 28 vs standard protein group 91 ± 30 ml/min/1.73m²).

9.10 Metabolic Effects of the Renal Trial

In our subsequent publication the metabolic effects of the two diets beyond weight loss were teased out. It should be stressed that the blood pressure, glycaemic and lipid control for our renal trial were secondary endpoints and that the power assumptions we made in terms of recruitment for the trial were based on the renal endpoints. Because subjects had excellent glycaemic, hypertensive and lipid control by design prior to starting the diet, it was harder to demonstrate improvement. Moreover subjects who improved further with weight loss were in a position to have their medications reduced. Nevertheless we demonstrated significant improvement in fasting glucose with weight loss but no treatment effect of dietary protein and a significant reduction in HbA1c in favour of the higher dietary protein group at 6 months which was not sustained at 12 months. Diastolic blood pressure was also significantly better in the higher protein group at 12 months although the significantly improved HDL was not different between groups. One could reasonably conclude on the basis of this study that in the situation of early diabetic nephropathy, weight loss itself rather than the level of dietary protein is a more important factor in determining metabolic improvement although the effects of the higher protein diet are reassuring.

It should be noted that the higher protein cohort was labelled “High Protein” in this manuscript rather than “Moderate Protein” as in the renal manuscript in Chapter 5. These labels reflected the views of the reviewers/editors of the respective journals.

Traditionally, the “normal” dietary protein content has been considered to be 15% of caloric intake and diets aspiring to constitute 30% of caloric intake such as used here

are labelled “High Protein Diets”⁸⁹. However high protein weight loss diets often achieve their target by only modestly increasing their dietary protein but reducing overall caloric intake and hence increasing the percentage of calories as protein. This occurred during our renal study where protein intake in the higher protein group was 1.22g/kg/day. On balance, this group could have been labelled “High Protein.”

9.11 Impact of Renal Study

This study attracted much interest and was the subject of an accompanying editorial⁹⁰. As pointed out, although a bigger protein difference between the two groups was not achieved and although the standard protein group did not consume a traditional low protein diet, this study could successfully claim that a high protein diet was not harmful. One would have to await further trials before extrapolating these conclusions to subjects with more advanced renal disease.

9.12 Difficulties in Determining GFR in the Obese

One important issue that I became aware of during this renal study was the limitations of our current methods to evaluate GFR in obese populations. Whilst radioisotope methods are considered the gold standard, many of the other methods commonly used in clinical practice either have significant limitations or are not validated in overweight or obese populations. This particularly applies to creatinine based methods and estimating equations because creatinine generation is increased in the obese (due to higher muscle mass) and altered during weight loss as muscle mass is lost. Moreover the tradition of adjusting eGFR to a body surface area of 1.73m² seems counterintuitive

in the obese. This topic became an important area of research for this thesis. My review of this topic suggested that of all available methods at the time of publication, in obese populations and after weight loss, the Cockcroft Gault equation adjusted for lean body mass compared most favourably with radioisotope methods.

In our renal study, although renal function was measured by radioisotope methods, 24 hour urine creatinine collections, cystatin C and the full gamut of creatinine based equations, the manuscripts reported renal function using radioisotope methods (iGFR) as the gold standard. In addition, eGFR using the MDRD equation was performed at frequent intervals throughout the study. The MDRD equation was the method used to estimate GFR by the Institute of Medical and Veterinary Science, the laboratory who performed our biochemical analyses (similar to all accredited Australian laboratories at the time). As our review suggested, this equation whilst well validated generally, would have benefited from being tested in an obese cohort. Our original intention was to perform an analysis comparing eGFR by the MDRD equation before and after weight loss in our obese cohort compared to the gold standard.

9.13 CKD-EPI Equations and Obesity

However, during the period of this study the CKD-EPI equation using creatinine measurements to estimate GFR was published. Around the time of the completion of the study, CKD-EPI superseded the MDRD equation in pathology reports from Australian and North American laboratories. With the subsequent publication of the CKD-EPI equations using cystatin C and using both creatinine and cystatin C, I had the

opportunity to estimate GFR before and after weight loss amongst the completers. I was able to compare the three CKD-EPI equations with radioisotope measurement. Despite modest subject numbers, I was able to show that despite no differences between the three equations at baseline in this cohort, after weight loss, the equations using cystatin C or both cystatin C and creatinine had higher precision compared to the equation using creatinine alone. This would reflect the change of body composition and loss of lean mass with weight loss which has a bigger influence on serum creatinine than cystatin C. Because of the modest subject numbers, my results are hypothesis generating and ideally would need confirmation in a larger study. My ability to detect small differences in renal function between the two diet groups was quite low.

9.14 No Evidence of Harm from High Protein Diets

In conclusion, in this thesis I have tested the hypothesis that higher protein diets are harmful to bone health and in early diabetic nephropathy. Based on the findings, the results of my research are compatible with recent work which suggests that there is no evidence of harm and even the possibility of a slight beneficial effect on bone health as long as the diet is calcium replete and not acidic.

In type 2 diabetic nephropathy, if the high protein diet is accompanied by weight loss, there are likely to be significant metabolic benefits. This may be helpful in terms of slowing the deterioration in GFR in those with normal or reduced baseline GFR or alternatively decrease some of the increased GFR in subjects who are hyperfiltering. My renal results are only hypothesis generating, due to the limitations discussed in this

thesis. These results can only be applied to those in the earlier stages of nephropathy and cannot be extrapolated to subjects with more advanced renal disease.

9.15 Future Directions

This body of work opens up future avenues for research. Given I have demonstrated no harm and suspect a beneficial effect of a higher protein diet in post-menopausal women, further studies might aim to achieve a greater protein separation between groups to ascertain if a more clinically significant improvement can be demonstrated. Ideally this should be demonstrated not just with bone markers but with bone density and ideally even fracture risk. This would be a resource intensive trial and require follow up of the cohort for much longer eg 5 years. This would improve the statistical power of our study to pick up smaller changes in bone density. I chose a cohort of post-menopausal women at risk of future osteoporosis; I might be better able to demonstrate a beneficial effect if I had chosen a cohort with established osteoporosis or who were older and hence at higher risk. However I would have to control for any osteoporotic medications that they were taking. There have also been very few longer term high protein trials in the frail elderly and this might also be a suitable cohort to study.

In terms of high protein diets and renal disease, any research would have to proceed more cautiously and incrementally. This is due to the longstanding concern about higher protein diets and renal disease generally and type 1 diabetes as opposed to type 2 diabetes. It would be important to confirm my findings with larger studies. This would overcome the statistical power limitations present here and allow us to make much

more secure conclusions and to detect smaller changes in GFR. Consideration could be given to measuring GFR using the CKD-EPI equation using cystatin C if this equation was further validated in obese populations. Initially it would be useful to focus on early nephropathy in type 2 diabetes with albuminuria and test our findings in two cohorts separately i.e. those subjects who have hyperfiltration with a $GFR > 120 \text{ ml/min/1.73m}^2$ and those with a $GFR > 40 \text{ ml/min/1.73m}^2$. This would allow us to use both albuminuria and GFR as screening tests and ensure that these parameters were better balanced at baseline. I would seek to confirm both that there is no signal of harm with higher protein diets and also to verify my findings that in the context of weight loss, hyperfiltration is reduced but GFR is increased in those with reduced GFR at baseline.

The next step would be to compare two diets with different dietary protein but with a much lower protein content for the lower protein group eg 0.6-0.8 g/kg daily. If the lower protein diet was superior in terms of reducing the decline in renal function in the context of weight loss, this would imply that it is not so much that there is a spectrum of increasing harm as dietary protein is increased. Rather, whilst a high protein diet was not harmful compared to a normal protein diet, a very low protein diet may have some intrinsic benefit.

Finally, my thesis also demonstrated the difficulties in clinical practice in using surrogate markers of renal function during situations of obesity and weight loss. The CKD-EPI eGFR equation using creatinine is now routinely used on pathology reports in Australia and many other countries. This research has generated the hypothesis that

the other two CKD-EPI equations using cystatin C may be superior and should be tested in different obese populations (eg at varying ages and with and without diabetes) to confirm or refute our findings.

CHAPTER 10: REFERENCES

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