High protein diets, weight loss, glycaemic control and renal function in type 2 diabetes mellitus

A thesis submitted for the degree of doctor of philosophy by

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November 2011

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Declaration of originality

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Acknowledgements

I would like to sincerely thank my supervisor Professor Peter Clifton for giving me the chance to pursue this challenging but rewarding project. Thank you for always being there and for your encouragement, motivation and patience.
Thank you to my supervisors Professors Manny Noakes and Gary Wittert for your support throughout my candidature.
I would also like to thank Ass Prof Jennifer Keogh for your friendship and fruitful conversations.
This thesis would not have been possible without the help of the volunteers, clinic staff: Julia Weaver, Lindy Lawson, Rosemary McArthur, Pennie Taylor, Xenia Cleanthous and David Jesudason for their assistance with project management, taking blood samples and assisting with dietary counselling, and for your friendship, I am deeply grateful. Thank you to Kylie Lange for patiently guiding me through the statistical challenge. I also thank Vanessa Russell for analysis of samples and with helping me understand the analysis methods.
Thank you to Kathryn Bastiaans for teaching me how to manage the huge amount of data in a rational way. A special thanks to Karma Pearce for sharing your knowledge of CGMS with me and for your support.
I am indebted to Dr Erin Symonds for guiding me through and helping me make sense of the gastric emptying study, your help was patiently and selflessly given. Thank you to the Centre for Paediatric and Adolescent Gastroenterology, Women and Children's hospital for analyzing the GE breath-tests.
Thank you to my fellow students Carly Moores, Razinah Sharif, Penelope Main, Kacie Dickinson and to Dr Sasja Beestra-Hill for your support and for listening to me when I needed it.
Thank you to friends and family who patiently supported me and most of all thank you to Kim, Mette and Marc for being interested and for your ongoing support.
Most importantly, thank you Steen for believing in me and pushing me forward even when it meant putting "life" on hold. Thank you for showing such pride in me, that means a lot.
I acknowledge the scholarship from The Centre of Clinical Research Excellence for funding my tuition and the acute study.

Abbreviations

ACCORD	The Action to Control Cardiovascular Risk in Diabetes
ACE	Angiotensin Converting Enzyme
ACEi	Angiotensin Converting Enzyme inhibitor
AER	Albumin Excretion Rate
AFM	Abdominal Fat Mass
Alb/cr	Albumin to creatinine ratio
ANCOVA	ANalysis Of COVAriance between groups
ANOVA	ANalysis Of VAriance between groups
ARB	ATII Receptor Blocker
ATII	Angiotensin II
ATP	Adenosine Triphosphate
AUC	Area Under the Curve
BF	Body Fat
BG	Blood Glucose
BMI	Body Mass Index
BMR	Basal Metabolic Rate
BP	Blood Pressure
BW	Body Weight
CCK	CholeCystoKinin
CHO	Carbohydrate
CKD	Chronic Kidney Disease
Cr	Creatinine
CrCl	Creatinine Clearance
CRF	Chronic Renal Failure
Da Qing	The Da Qing IGT and Diabetes Study

DBP	Diastolic Blood Pressure
DCCT	The Diabetes Control and Complication Trial
DECODE	Diabetes Epidemiology: COllaborative analysis of Diagnostic criteria in Europe
DXA	Dual-Energy X-ray Absorptiometry
DITE	Diet Induced Thermic Effect
DOB	Date of birth
eGFR	estimated GFR
ESRD	End Stage Renal Disease
FBG	Fasting Blood Glucose
FFM	Fat Free Mass
FFQ	Food Frequency Questionnaire
GE	Gastric Emptying
GFR	Glomerular Filtration Rate
GI	Glycaemic index
GLP_1	Glucagon Like Peptide 1
Gmax	Peak BG
HbA1c	Glycated haemoglobin A1c
HCLF	High Carbohydrate, Low fat diets
HDL	High Density Lipoprotein
HPD	High Protein Diet
HR	Hazard Ratio
IBW	Ideal Body Weight
IDF	International Diabetes Federation
IGF	Impaired Fasting blood Glucose
iGFR	isotope tracer GFR

IGT	Impaired Glucose Tolerance
IHD	Ischemic Heart Disease
KD	Kidney Disease
KDOQI	Kidney Disease Outcomes Quality Initiative
kJ	kilo Joule
LBM	Lean Body Mass
LDL	Low Density Lipoproteins
Look AHEAD	The Action for HEAlth and Diabetes
LPD	Low Protein Diet
LPh	Low Phosphorus
MAP	Mean Arterial Pressure
MDRD	Modification of Diet in renal disease
MI	Myocardial Infarction
Mo	Month
MUFA	Monounsaturated Fatty Acids
NHANES	The National Health and Nutrition Examination Survey
NOCHO	No Carbohydrate
OGTT	Oral Glucose Tolerance Test
OR	Odds Ratio
PA	Physical Activity
PPG	PostPrandial blood Glucose
PREVEND	The Prevention iof Renal and Vascular End Stage Disease
PUFA	Polyunsaturated Fatty Acids
RAAS	Renin-Angiotensin Aldosterone System
RCT	Randomized Controlled Trials
REE	Resting Energy Expenditure

RENAAL	Reduction of Endpoints in NIDDM with the AII Antagonist Losartan
RMR	Resting Metabolic Rate
RR	Relative Risk
SAFA	Saturated Fatty Acids
SBP	Systolic Blood Pressure
sLPD	supplemented (with keto acids) LPD
SNGFR	Single Nephron Glomerular Filtration Rate
SPD	Standard Protein Diet
sVLPD	supplemented Very Low Protein Diet
T>10	Time spent with a BG above 10 mmol/L
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
T-Chol	Total Cholesterol
TE	Total Energy
TEE	Total Energy Expenditure
TG	TriGlycerides
T_x	Time
UKPDS	United Kingdom Prospective Diabetes Study
UPD	Usual Protein Diet
USRDS	The United States Renal Data System Coordinating Centre
UUE	Urinary Urea Excretion
VAS	Visual Analogue Scale
VLPD	Very Low Protein Diets
WHO	World Health Organization

Abstract

The evidence for the efficacy of weight loss diets with a higher protein to carbohydrate (CHO) ratio has increased. However, the long-term effect of higher protein diets (HPD) on renal function in individuals with type 2 diabetes is lacking.

The studies in this thesis focus on the effect of altering the macronutrient composition towards a higher protein to carbohydrate ratio on renal function, HbA1c and lipids in individuals with type 2 diabetes mellitus (T2DM) and microalbuminuria.

The main study was a 12 month randomized weight loss study in 56 volunteers. A 6 MJ high protein diet (HPD: protein 30% total energy (TE) equal to 90-120g/d, carbohydrate [CHO] 40%TE, fat 30%TE) was contrasted with a 6 MJ standard protein diet (SPD: protein 20%TE equal to 55-70g/d, CHO 50%TE, fat 30%TE).

This study showed a significant decrease in weight (-10.5kg HPD and -7.5kg SPD), fat mass (-9% HPD and -8% SPD) and increased fat free mass (+6% in both groups) with no significant difference between diets.

Renal function, measured as isotope GFR, calculated GFR and serum cystatin C, was unaffected by either diet. Microalbuminuria was reduced in HPD (AER: -12.0±9.1 µg/min and + 1.0±17.0 µg/min in SPD) with a borderline significant treatment effect after adjustment for baseline values (p=0.059). Glycaemic control (HbA1c -0.9 HPD and -0.5 SPD), high density lipoprotein cholesterol (+ 0.1 mmol/L in both groups), and triglycerides (HPD -0.8 and SPD -0.5mmol/L), improved similarly in both groups. There was a decreased diastolic BP in the HPD group (-2.5 mmHg) and an increase in SPD (+5.2 mmHg; p=0.03).

The major contributor to diabetes nephropathy is hyperglycaemia. In study 2 (a sub-study to the main study) and study 3, a short term meal intervention study, we investigated the effect of changing macronutrient composition and CHO timing on glycaemic control using a continuous glucose monitoring system.

These studies showed a significant decrease in time spent with blood glucose (BG) above 10 mmol/L, maximal BG level and area under the BG curve indicating an overall beneficial effect of altering the CHO to protein ratio on

glycaemic control. Changing the CHO content of breakfast had no effect on lunch glucose levels.

In conclusion:

This study is the first to examine the long-term efficacy and safety of higher protein diets in individuals with T2DM and microalbuminuria. Both diets had positive effects on cardiovascular risk factors with no changes in renal function.

Chapter 1: Literature review

1.1 **Introduction**

1.1.1 Diabetes types

There are two main types of diabetes, Type 1 diabetes (T1DM) which is characterized by a lack of insulin production resulting in an absolute requirement for treatment with exogenous administered insulin. Of the total diabetes population T1DM accounts for 10-15% [1].

Type 2 diabetes (T2DM) which is characterized by an insulin resistance and insulin insufficiency is related to obesity and sedentary lifestyle with a strong genetic factor. T2DM accounts for 85-90 % of all cases of diabetes [1].

The focus of this thesis will be type 2 diabetes mellitus.

1.2 **Diagnosing diabetes**

The current criteria for diagnosing impaired glucose metabolism and frank diabetes was published in a report of the WHO and IDF consultation in 2006. In this report both fasting blood glucose (FBG) and two hours venous plasma glucose (PPG), after an oral glucose tolerance test (OGTT), using a 75g oral glucose load, was used. In the impaired fasting blood glucose (IFG) category, the 2h OGTT is important as diabetes cannot be excluded if only fasting blood glucose (FBG) is known [2].

Table 1: Criteria for diagnosing impaired glucose metabolism and diabetes

	Fasting glucose		**2h postprandial glucose**
Impaired fasting glucose (IFG)	6.1–6.9 mmol/L	And	< 7.8 mmol/L
Impaired glucose tolerance (IGT)	< 7.0 mmol/L	And	≥7.8 and <11.0 mmol/L
Diabetes (DM)	≥ 7.0 mmol/L	Or	≥ 11.1 mmol/L

The basis of these cut-off criteria are the increased risk of microvascular complications seen beyond these values. Macrovascular disease is increased to the same degree in IFG and IGT as in frank diabetes.

1.2.1Prevalence

Diabetes is now considered one of the main causes of morbidity and mortality worldwide. Currently an estimated 347 million (314-382) are diagnosed with diabetes. This result was published recently in a report using data from health examiners surveys and epidemiologic studies published between 1980 and 2008. This survey included data from 2.7 million participants over the age of 25 years from 199 countries [3]. It was reported that the highest increase in FBG over the 28 year period was seen in Oceania, where a rise of 0.2-0.3 mmol/L per decade was observed. This trend was attributed to the increase in BMI as a correlation between changes in BMI and increases in FBG was 0.71 for women and 0.57 for men [3]. In Australia >700,000 (3.6%) were diagnosed with diabetes in 2004-05. Newer data from the National health surveys and the national diabetes service scheme (surveys conducted in 2007-08) report the diagnosed number of diabetics to be 818,200 and 945,746 respectively [4, 5]. It was estimated that 1.23 million people in Australia had diabetes in 2010 [6].

Untreated or uncontrolled T2DM can lead to serious complications i.e. cardiovascular disease, neuropathy, retinopathy and nephropathy [7].

In 2003 diabetes and diabetes related complications were responsible for 8% of the total burden of disease in Australia. In 2005 diabetes was directly responsible for 3% of all deaths in Australia and contributed together with the aforementioned complications to 6% of all deaths [8].

1.2.2 Economic implications

The direct cost of diabetes was estimated at $116 billion in the United states in 2007 [9]. In Australia the annual direct cost of T2DM was estimated at $2.2 billion in 2003 [10].

1.3 **Causes of type 2 diabetes**

1.3.1 Non modifiable causes

The genetic predisposition together with age (where people aged above 60 years had the highest prevalence of diabetes (15.1%)) and ethnicity are the main non-modifiable causes of diabetes.

There are strong familial links in T2DM [11]. The National Health and Nutrition examination survey (NHANES) 1999-2002 showed that subjects with a family history of T2DM, defined as having an affected first degree relative (parent or sibling), had an increased prevalence of diabetes of 14.3% compared to subjects with no family history (3.2%). An increased risk was found with an increasing number of relatives with diabetes. In the model adjusted for sex, age, race, income and BMI, the odds ratio (OR) for type 2 DM with one parent affected by diabetes was 3.0. If both parents were affected the OR was 7.0.

Likewise with siblings; if one sibling had diabetes the OR was 3.5. Having three or more relatives affected by diabetes increases the OR to 14.8 [11].

The prevalence of diabetes increases with age. The highest prevalence is found in people between the age of 60 and 80 years old, with a prevalence of 15.1% in subjects >60 years compared with people between 20 and 59 years old [11, 12].

1.3.2 Modifiable causes

Obesity is the single most powerful predictive factor for the development of diabetes. It is estimated that approximately 80 % of all T2DM patients are overweight or obese [12]. The risk of developing T2DM has been found to increase by 20% for each 1 kg/m^2 increase in BMI, in persons with a BMI above 27 kg/m^2, compared to those with normal weight (i.e. BMI of 25 or less). A BMI above 27 but below 29 kg/m^2 is associated with an increased risk of diabetes of 100% and a BMI above 29 kg/m^2 increases the risk by 300% [13].

Numerous intervention studies have shown that intensive intervention with diet high in dietary fibre and low in saturated fat (SAFA) and increased exercise can decrease the risk of diabetes by as much as 58% in high risk groups (subjects with impaired glucose tolerance (IGT)) [14-17]. In the Nurses Health Study it

was concluded that approximately 50% of women with a BMI in the normal to overweight range (BMI 25 -29.9), could have prevented diabetes from occurring by adhering to healthy lifestyle advice (weight loss, increased dietary fibre, SAFA, regular exercise and abstaining from smoking) and it appears that weight reduction exerts the greatest benefit [18].

In the baseline evaluation of food intake in the Action for Health in Diabetes (Look AHEAD) study, the food intake of 2757 T2DM patients was assessed using food frequency questionnaires (FFQ). This survey showed that 93% had a fat intake greater than recommended, 85% had too much SAFA and 92% exceeded the recommendation for sodium. The recommended servings for fruit, vegetables, wholegrain and milk products were met by only 50% of the participants [19]. These data in T2DM is in line with nutrient consumption in the general population where >90% of the studied population (16338 individuals aged 2 years and above) in the NHANES (2001-2004) were found to consume too much solid fat, sugar and alcohol, compared with the estimated maximum energy allowance [20].

These lifestyle choices were shown to increase the risk of chronic kidney disease in a cohort of more than 9000 adults in the NHANES II study. Alcohol consumption did not seem to be associated with CKD in this survey [21]

A modest reduction in salt consumption has been linked to a decrease in blood pressure and a decreased albumin excretion. He et al found a significant 11% reduction in albumin excretion between added salt tablets and placebo in a cross over study in mild hypertensive. Additionally, the albumin to creatinine ratio decreased significantly (0.81 to 0.66 mg/mmol, p=0.001) [22].

Sedentary lifestyle, now common in industrialized countries, is a contributing factor to the increasing prevalence of obesity and T2DM.

In the Da Qing IGT and diabetes study (Da Qing) 577 men and women with IGT were randomized to one of four groups and followed for 6 years. The main outcome was the incidence of T2DM. Participants were divided into the following groups:

1. Diet alone: Dietary recommendations where energy intake was aimed at achieving or maintaining a BMI <25 kg/m^2. The recommendations for the dietary

intake was 105-126 kJ/kg if within normal BMI range, for the overweight and obese kJ were reduced to produce a weight loss of 0.5-1.0 kg/week until a BMI of 23 kg/m^2 was reached. The nutrient composition was: carbohydrate 55-65 % total energy (TE), protein 10-15 %TE and fat 25-30 %TE. Lifestyle advice was given at regular follow up visits.

2. Exercise alone: Recommendations for the exercise group was at least one unit per day added to the usual leisure activities if aged more than 50 years and 2 units if aged below 50 years. One unit of added exercise was 30 minutes of mild exercise (like slow walking, taking the bus, shopping or housekeeping); 20 minutes of moderate exercise (faster walking, walking down stairs, cycling, slow ballroom dancing); 10 minutes of strenuous exercise (jogging, climbing stairs, faster dancing, playing volleyball or tennis) or 5 minutes of very strenuous exercise (jumping rope, playing basketball or swimming). Lifestyle advice was given at regular follow up visits.

3. Diet and exercise: The combined group was recommended both the diet and exercise interventions with regular follow up visits.

4. Control: The control group had general lifestyle recommendations explained and were handed a brochure, but no individual counselling as in the three other groups was given.

At the end of the six year follow up period ≈ 68% in the control group had developed T2DM. The group with combined diet and exercise advice had an incidence of T2DM of ≈ 46%. In the diet alone group ≈44% had developed T2DM. The incidence of diabetes was lower in the exercise alone group (41%). Comparing the intervention groups to the control group there was a significantly lower incidence of T2DM with a 33% reduction in the diet alone group $p=0.03$; 38 % reduction in the diet and exercise group $p<0.01$ and 47% reduction in the exercise alone group $p<0.01$, however, comparing the three intervention groups to each other there was no significant difference. [23]. The exercise requirements were very moderate: participants were asked to perform one to two units of exercise per day depending of age. The number of exercise units performed in the exercise alone group was significantly higher than in the control group (4.0±3.0 vs. 2.5±1.9 units; $p<0.05$). Units of exercise performed in the diet alone group was 1.7±1.9 units, this was not significantly different from

control or baseline. At the end of six years follow up exercise units in the combined diet and exercise group had increased significantly compared to baseline (3.9±2.3 units; $p<0.05$). This study show even very moderate changes in lifestyle have a great effect on the prevention of the progression from IGT to T2DM [17].

Physical exercise have also been linked to a lowering of albumin excretion in non-diabetic women [24] and in patients with hypertension [25]. In the NHANES ll survey it was reported that sedentary lifestyle increased the risk of chronic kidney disease (CKD) by 2.2 and a more moderate exercise habit increased the risk by only 1.2 compared to persons with high level of physical exercise [21].

Smoking has been shown to increase the risk of T2DM [26]. The pooled data from a meta-analysis conducted in 1.2 million non diabetic participants from 25 prospective studies, a total of 45,844 persons were diagnosed with diabetes during follow up ranging from 6-30 years. The risk of developing T2DM increases in a dose dependent manner. In the total sample (pooled data) the relative risk was 1.44 (1.31-1.58). The risk of developing T2DM was highest in persons smoking more the 20 cigarettes per day = 1.61 (1.43-1.80) and lower in people smoking less than 20/day = 1.29 (1.13-1.48). The relative risk of diabetes decreases with smoking cessation to 1.23 (1.14-1.33), however, it was shown in a study investigating the association between smoking and diabetes risk in 10,892 middle aged adults [27] that the relative risk of T2DM rose to 1.73 (1.19-2.53) in the initial period after cessation, the authors attribute this increased risk to the weight gain often seen in “new quitters” [27].

Smoking increases the risk of myocardial infarction (MI) and stroke by 2-5 times compared to non-smokers in both diabetic and non diabetic people [28].

In data from the second national health and nutrition examination survey (NHANES ll) where 9082 individuals were followed for 12-16 years smoking was found to increase the relative risk of chronic kidney disease by 1.2 in persons smoking less than 20 cigarettes per day and by 2.3 in smokers who smoked more than 20 cigarettes per day. This is in agreement with the increased risk of CKD in T2DM smoking more than 20 cigarettes /day (RR= 1.4 (0.3-7.4)) [21].

1.4 **Diabetes complications**

1.4.1 Macrovascular complications

Both micro- and macrovascular complications have been attributed to hyperglycaemia associated with diabetes. In the United Kingdom prospective diabetes study (UKPDS 35) cardiovascular and cerebrovascular complications accounted for 50 to 60% all deaths in this group [29]. In a cross sectional observation analysis the risk of myocardial infarction decreased by 14%, the risk of stroke decreased by 12% and the risk of heart failure decrease by 16% for every 1% lower HbA1c. Furthermore, the decrease in microvascular complications was 37% for every 1% lower HbA1c and finally the risk of amputation is also decreased by 43% with 1% lower HbA1c [29].

1.4.2 Retinopathy

Early retinopathy is caused by an altered autoregulation of the capillary blood pressure, resulting in increased pressure and thereby increased perfusion [30]. The earliest signs are saccular out pouching of the capillary wall. Macular oedema results in thickening of the macula leading to blurred vision. Abnormal formation of new blood vessels poses an increased risk of haemorrhage, scarring, retinal detachment and severely impaired vision [31].

The main causes of diabetic retinopathy are hyperglycaemia, hyperlipidaemia and hypertension, where hyperglycaemia is the major contributor [31]. The progression of diabetic retinopathy depends on diabetes duration. In T2DM approximately 23% have retinopathy after 11-13 years of diabetes and after 16 years the prevalence is 41% [31].

In the action to control cardiovascular risk in diabetes (ACCORD) eye sub-study 2856 participants with retinopathy were followed for four years. The aim of the ACCORD study was to determine the effect of intensive treatment strategies for lowering blood glucose (HbA1c <6% in the treatment group and between 7 to 7.9% in the control group), blood lipids (simvastatin plus fenofibrate vs. simvastatin and placebo in the treatment and control groups respectively) and blood pressure (systolic blood pressure <120 mmHg in treatment group and <140 mmHg in control) in T2DM with cardiovascular risk factors. After four years of intensive treatment, retinopathy progressed by 7.3% in the intensive

glucose treatment group and by 10.4% in the control group. In the group treated with the addition of fenofibrate for lipid reduction the progression was 6.5% vs. 10.2% in control. However, the ACCORD eye study did not find a beneficial effect of lowering systolic blood pressure (progression in treatment group was 8.8% vs. control group of 10.4%). These results show that intensive treatment of hyperglycaemia and hyperlipidaemia reduce the progression of diabetic retinopathy while intensive control of systolic blood pressure does not [32].

1.4.3 Neuropathy

Hyperglycaemia can cause neural damage if it persists. Unlike muscle tissue the neurons are dependent on extracellular glucose level and are independent of insulin action. Neurons are sensitive to the glucose level and depend on a relative stable interstitial glucose. Persistently high glucose levels are neurotoxic and result in the loss of protective fine touch, temperature and pain sensation initially in the feet which can contribute to foot ulcers [33]. The most common types of neuropathy in diabetes are peripheral, autonomic and focal neuropathy. Autonomic neuropathy affects nerves to the internal organs affecting the heart, digestive system, bladder, sexual function and sweat production. Focal neuropathy affects the eyes, limb and facial muscles and hearing [34]. The main treatment for diabetic neuropathy is tight control of blood glucose.

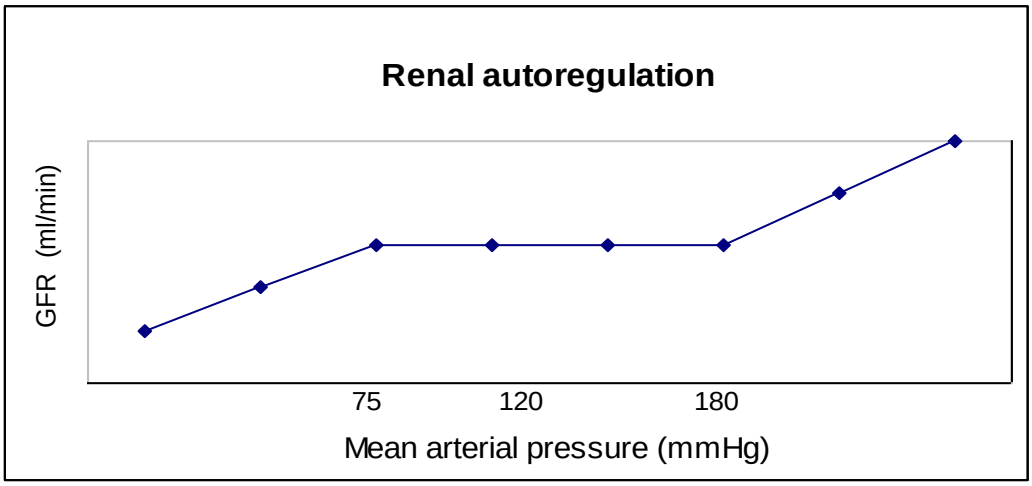
1.4.4 Nephropathy

1.4.4.1 Normal haemodynamic autoregulation of glomerular filtration rate (GFR)

Normal auto-regulation of the renal vascular system is a protective measure whereby the kidney can maintain a stable function in the face of altered blood pressure. Blood flow to the kidney and GFR will be kept relatively stable, by vasoconstriction of the afferent arteriola, when arterial blood pressure is varying between 75 and 180 mmHg [35-37]. The threshold for auto-regulation to fail is not well known in humans and will depend on the suddenness and speed of the BP change [36, 37]

Figure 1: Autoregulation of GFR

The normal autoregulation of GFR under increasing BP. It is shown that GFR remains stable within the range of 80 to 160mmHg, as the upper threshold is reached GFR increases rapidly.



In the kidney hydrostatic pressure is kept within a narrow range. This is regulated by myogenic reflexes on the afferent arteriola and the tubuloglomerular feedback. In the case of increased hydrostatic blood pressure, the afferent arteriola constricts within seconds to limit increased pressure in the glomerular capillaries. As the hydrostatic pressure falls the afferent arteriola relaxes, thus restoring flow through the glomerulus [36].

In the case of decreased hydrostatic blood pressure renin activity increases, and increases sodium reabsorption via the renin-angiotensin aldosterone system (RAAS). Renin is an enzyme excreted from the juxtaglomerular cells at the afferent arteriole. Renin acts on angiotensin to form angiotensin I (AI) a virtually inactive protein which is then deaminated by angiotensin converting enzyme (ACE) produced in the endothelium to form angiotensin II (AII). Angiotensin II acts as a vasoconstrictor supporting the myogenic reflex [36].

1.4.4.2 Major risk factors for nephropathy

1.4.4.2.1 Obesity and renal function

Obesity is an increasing problem affecting millions of people worldwide. Obesity is defined as a body mass index (BMI) >30 kg/m^2, overweight is defined as a

BMI between 25 and 29.99 kg/m^2 and the normal weight range is defined as a BMI between 18.8 and 24.99 kg/m^2 [38]. Obesity is now recognised as an independent risk factor for kidney disease (KD) independently of other risk factors such as age, sex, ethnicity, smoking, alcohol, diabetes mellitus, dyslipidemia and hypertension [39]. In industrialized countries an estimated 13.8% of obese men and 24.9% of obese women, have obesity related kidney disease [39]. Moreover, obesity exacerbates KD from other causes compared to normal weight controls [40].

Additionally obesity is a driving force in the increasing incidence of hypertension and T2DM [39] and significantly increases morbidity and mortality [41]. In a review assessing the epidemiologic literature on the relationship between obesity and KD, Wang et al found a strong association between BMI and KD. Furthermore, they reported a “dose-response” association where a BMI ≥ 25 kg/m^2 was associated with an increased risk of KD of 40 %, whereas a BMI above 30 kg/m^2 predicted an 80 % increased risk of KD [39].

Similarly Hsu et al investigating 320,252 adults who participated in a health check between 1964 and 1985, reported a seven fold increase in relative risk of end stage renal disease (ESRD) between BMI ≤25 and ≥40.Baseline BMI remained an independent risk factor even after adjusting for the occurrence of hypertension and diabetes [42].

The same association was found between microalbuminuria and BMI in T2DM in the Look AHEAD (action for health in diabetes) study [43]. An increase in BMI has been shown to increase filtration rate even in participants with normal BMI [44].

Figure 2 Correlation between increased BMI and the relative risk of ESRD

Hsu, C.Y., et al., *Body mass index and risk for end-stage renal disease.* Ann Intern Med, 2006. **144**(1): p. 21-8. [42]

1.4.4.2.2 Fat mass distribution

Obesity is related to albuminuria in humans and an abdominal fat distribution has been shown to increase the risk [40, 43]. Some studies have shown that there is a difference in the fat distribution and risk of KD between men and women. For men it seems that the hip circumference is an important predictor and in women the general obesity is the important factor [39, 44].

In a sub-analysis of the prevention of renal and vascular end stage disease (PREVEND) study, investigating 8050 subjects from the general population, where 24h urine was collected for analysis of albumin excretion rate (AER), it was reported that 6.6% of the subjects in the “healthy” population without diabetes or hypertension had microalbuminuria. Subjects were divided into BMI groups. Microalbuminuria was present in 9.5% of men and 6.6% of women in the normal weight group, 18.3% of men and 9.2% of women in the overweight group and 29.3% of men and 16.0% of women in the obese group [45]. An interaction between BMI and sex was shown so that men had a steeper rise in AER with increasing BMI compared to women indicating an increased risk of KD in abdominal obesity characteristic for men[46].

1.4.5 Prevention and treatment of diabetes related complications

Large prospective intervention studies have shown that intensive blood glucose treatment and continued diabetes education have a beneficial effect on diabetes related complications. In the Diabetes Control and Complication Trial (DCCT), 1441 T1DM patients were randomly assigned to two groups. One group received intensive insulin therapy with multiple daily injections or continuous insulin pump treatment. The second group acted as a control group, continuing on the conventional therapy (once or twice a day insulin injections). Patients in the intensive treatment group had additional monthly visits at the clinic for education in lifestyle changes. There was a long follow-up period of 6.5 years. The aim of the intervention was to achieve tight overall glycaemic control, with pre-prandial blood glucose between 4 and 7mmol/L and postprandial blood glucose no higher than 10mmol/L. The results of the intensive treatment were convincingly strong and the overall risk of diabetes related complications decreased by between 35% and 70%. The risk of microalbuminuria and albuminuria associated with renal disease was reduced by 54% and 39% respectively [47].

The United Kingdom Prospective Diabetes Study (UKPDS) recruited 3867 newly diagnosed type 2 diabetic patients and investigated the effect of intensive nutritional and medical (tablet or insulin) treatment on the risk of developing diabetes related complications. As in the DCCT, patients were randomly assigned to a treatment group or control group. The treatment aim in the intensively treated group was a pre-prandial blood glucose ≤6 mmol/L. Furthermore, the intensive treated group received additional life style education during monthly visits to the clinic. The control group remained on the conventional treatment (diet and oral agents), together with the usual life style education. The aim for blood glucose control in this group was fasting plasma glucose (FBG) <15 mmol/L. In the control group, FBG and HbA1c increased during the course of the study. In the intensively treated group FBG and HbA1c decreased slightly in the first year of treatment, but increased similarly to the control group thereafter. A mean difference in HbA1c remained over the 10 years of follow-up, with an HbA1c of 7.0mmol/l vs. 7.9mmol/L in the intensively treated group and the control group respectively. After 10 years of follow-up, a significant (12%) reduction in the risk of any diabetes related complication and a

substantial (25%) reduction in urinary albumin excretion was seen in the intensively treated group compared to the control group [48].

3277 participants from the study were followed for an additional 10 years post trial. The first five years the participants visited the UKPDS study clinics, where standard outcome measures were collected the same way as during the study. Participants who were unable to attend the clinic visits, and all surviving participants from the sixth to the tenth year post trial, were asked to fill out a questionnaire. After one year post trial the differences in HbA1c achieved during the study were lost, but the benefits persisted ten years after cessation of the study. The reduction of microvascular complications of 25% seen in the sulfonylurea-Insulin intensively treated group was sustained through the post study follow up period. During the post trial period a significant reduction in myocardial infarction (MI) of 15% was seen (p=0.01) and the risk of death from all causes was reduced by 13% post trial (p=0.007). These outcome measures did not reach statistical significance during the study [49]. In the group of obese participants assigned to treatment with metformin there was a significant reduction in the risk of MI (39%, p=0.01) and for all cause deaths (36%, p=0.01) during the study. This effect was sustained throughout the post study period [49]

It is therefore clear that lowering FBG and HbA1c in diabetic patients is beneficial in lowering the risk of diabetes related complications such as diabetic nephropathy.

Population-based studies have shown a relation between postprandial glucose (PPG) and the incidence of myocardial infarction (MI) and all cause mortality in type 2 diabetic patients [50, 51]. Surprisingly, it was shown that the higher risk of MI in poorly controlled diabetic patients was associated solely with the PPG and had no association with fasting blood glucose (FBG) [50]. Hence, it seems that PPG is a better predictor of the risk of morbidity and all cause mortality in type 2 diabetes patients, independent of the fasting state.

FBG and HbA1c are currently used to diagnose diabetes and to monitor blood glucose control in diabetes.

In patients with HbA1c <7%, PPG is contributed to a greater extent than FBG, whereas FBG is the major contributor with increasing HbA1c [52]. Bonora et al

[53] investigated the relationship of blood glucose levels throughout the day and HbA1c in type 2 diabetic patients. It was shown that subjects in good glycaemic control according to HbA1c (<7%) and FBG <6.6 mmol/L still had high blood glucose excursions (>2.2 mmol/L) and a high PPG (>8.9 mmol/L). These results indicate that monitoring treatment efficacy should not be restricted to FBG and HbA1c alone [53].

1.5 **Renal physiology and pathology and role of dietary protein**

Diabetic nephropathy is linked to hyperglycaemia, hyperinsulinaemia and hypertension and the earliest sign of renal involvement is microalbuminuria [54]. Diabetes is the leading cause of ESRD worldwide [55]. In a recent report from the United States Renal Data System Coordinating Centre (USRDS) it was shown diabetes accounted for approximately 70% of all cases requiring renal replacement treatment in America in 2004 [56].

The pathophysiological changes seen in early diabetic kidney disease are thickening of the glomerular basement membrane, glomerular hypertrophy and increased permeability, resulting in hyperfiltration and microalbuminuria [41, 57]. Hyperglycaemia is the major factor in diabetic nephropathy. In animal models tubuloglomerular feedback is altered due to increased Na+/glucose transport and increased sodium reabsorption. This leads to decreased electrolyte concentration in the macula densa. The decreased electrolyte concentration stimulates afferent vasodilatation and efferent vasoconstriction, increasing the intra-glomerular pressure resulting in glomerulosclerosis.

Blocking the afferent vasodilatation or the RAAS mediated efferent vasoconstriction reduces the risk of albuminuria in animal models and the same effect is believed to occur in human [36, 58].

Abnormalities in diurnal blood pressure seen as a high night to daytime blood pressure (non-dipping), where the nocturnal blood pressure decrease is less than 10% is often seen in DM, and has also been reported to increase hyperfiltration and subsequently lead to renal injury [58].

Sustained hyperfiltration from increased renal blood flow leads to increased GFR and to microalbuminuria progressing to frank proteinuria and eventually to

advanced renal failure. The progression of microalbuminuria to manifest proteinuria is associated with a fall in GFR [39, 59]. Hyperfiltration in obesity is probably due to increased tubular sodium reabsorption resulting from increased post glomerular oncotic pressure and increased systemic arterial pressure [60]. In addition to the obesity related hyperfiltration a cluster of metabolic and hormonal dysfunctions are present, including insulin resistance, endothelial dysfunction, dyslipidemia, oxidative stress, inflammation and sleep apnoea. Lasta et al [61] reviewed the literature on the effect of the cluster of abnormalities associated with obesity and kidney disease. They found that both hyperinsulinaemia and insulin resistance can be found in obese subjects with CKD who do not have diabetes. Likewise, dyslipidemia in the form of increased triglyceride and apolipoprotein lV, in combination with decreased high density lipoprotein (HDL) was found in patients with progressive CKD. Microalbuminuria is a marker of endothelial dysfunction and is associated with low grade inflammation and increased oxidative stress [61]. Adiponectin is active in preventing albuminuria and obesity associated focal segmental glomerulosclerosis. The low adiponectin present in obesity is associated with glomerular oxidative stress and increased microalbuminuria [44, 62].

1.5.1 Dietary protein in diabetic nephropathy

There is evidence that high protein diets have a deleterious effect on renal function in persons with manifest renal disease [63]. Therefore restricting dietary protein to 0.6 g/kg or less per day has been recommended in an effort to slow progression of diabetic renal disease. However, this recommendation is controversial and there is no consensus about specific dietary goals for patients with different degrees of renal Impairment. More recently there has been a shift towards controlling protein intake to 0.75 to 1.0 g/kg per day [64].

1.5.2 How much protein do we need

Protein needs can be measured in various ways e.g. g/kg body weight, g/kg ideal body weight, g/day or percentage of total energy intake (%TE). Most organizations recommend a wide range of %TE as protein e.g. 10- 35 %TE [65-67]. Likewise, the recommended protein intake for diabetics is between 15 and 20 %TE [65, 68].

However, this latter recommendation does not consider an individual's body weight or the risk of not meeting the needs in an energy restricted diet. Looking at the dietary intake in Americans aged 1 year and older, the National Health and Nutrition Examination Survey 2003-2004 (NHANES) found the average protein intake to be 16.0 %TE and the highest protein intake to be 20.8 %TE, corresponding to a median intake of 1.1 - 1.3 g/kg ideal body weight/day (g/kg IBW/d) in adults [67].

Protein needs are dependent on lean body mass, rather than energy intake. Hence in order to keep the protein intake constant around the recommendation of an average of 56 g/day in energy restriction for a female, protein allowance needs to increase by around 1 %TE when energy is decreased by 500 kJ below 8000 kJ/day (e.g. 56 g protein equals 15% TE of a 8000kJ diet, whereas in an energy restricted diet of 5000 kJ, 56 g protein equals 19%TE) [69].

A more accurate way of calculating protein needs might be to use g/kg body weight/day (g/kg/d). According to the National Health and Medical Research Council (2006) the minimum requirement for protein intake is 0.84 g/kg/d in adult men and 0.75 g/kg/d in adult women. Intake at or above this level will minimize the risk of inadequacy [70]. In obese persons with higher lean body mass, an adjusted body weight is sometimes used in order to avoid overestimating the protein needs, e.g. the protein needs are calculated from an average body mass index (BMI) of 24.9 kg/m^2 [67]. There is not enough evidence to recommend an upper level for protein intake [66]. However, obese people may well need a higher absolute amount of protein to maintain their increased lean body mass and an amount based on an ideal BMI of 25 may cause loss of lean mass [71, 72].

1.5.2.1 How can we define a low and a high protein diet?

In healthy persons the average protein need seems to be 0.8 g/kg/d [63, 70] and this does not seem to change with age [73]. Low protein diets can therefore be described as diets containing less than 0.8 g /kg IBW/d. As the evidence for maximum safe protein intake is lacking, no firm level for a high protein diet can be defined. [70]. In energy restriction the aim is to maintain the same protein intake as in energy balance thereby increasing the energy to protein percentage to >20%TE [74].

1.5.3 Dietary protein and renal function

1.5.3.1 Animal studies

Brenner and his group have clearly shown that the adaptive hemodynamic response to renal loss is detrimental to the remaining normal nephrons, with glomerular sclerosis occurring as a result of the functional overload in the normal glomeruli. They convincingly showed that dietary protein was directly associated with the degree of renal injury in rat models with 80% reduction in renal mass [75].

In the study by Hostetter (1981) three groups of rats were investigated: group I had sham surgery and were fed the standard chow containing 24% total energy (TE) protein, group II had their right kidney removed and 5/6 of the left kidney injured by infarction and were fed the standard chow. Group III had the same surgery and kidney damage as group II, but this group was fed a protein restricted chow of 6 %TE protein. In group II single nephron glomerular filtration rate (SNGFR) was increased compared to group I due to increases in glomerular transcapillary hydraulic pressure and glomerular plasma flow rate. In group III however no changes in hemodynamics compared to group I were seen, indicating that low protein diets (LPD) ameliorate the effect of renal injury [76]. However, 6% protein is a protein deficient diet and is not sustainable long-term in rats or humans.

1.5.3.2 Human Studies

1.5.3.2.1 The effect of protein restriction on renal function in subjects without diabetes

The effect of dietary protein restriction on renal function has been extensively researched in type 1 diabetics and non-diabetic subjects [77]. Results from these intervention studies have been inconsistent and no firm conclusions about the effect of protein restriction on renal disease have been made.

In the earlier prospective randomized controlled studies investigating the effect of protein restriction in patients with non-diabetic renal disease over the last three decades, there is very little uniformity in reporting dietary protein intake, renal function measures and trial endpoints.

In 1984 Rosman et al conducted the first long-term single centre prospective randomized controlled trial (RCT) with results from 149 patients with varying degrees of renal disease. Patients were stratified according to sex, age and renal function measured by creatinine clearance (CrCl). Two intervention groups (B + C) and two control groups (A1 + A2) were formed. Intervention group B and control group A1 commenced with a baseline CrCl of 31-60 ml/min/1.73m². Intervention group C and control group A2 commenced with a baseline CrCl of 10-30 ml/min/1.73m². Patients in group B were prescribed a diet containing 0.6 g/kg/d of protein. In group C the dietary protein prescription was 0.4 g/kg/d. Control groups A1 and A2 were asked to continue with their usual protein intake, with no specific protein level stated. The participants in the protein restricted groups had follow-up visits with a dietitian every 3 months. Controls saw the dietitian only as required. All participants were followed for a minimum of 18 months. The main outcome measure was the reciprocal of serum creatinine with time (1/Cr/Mo) and renal survival using 5 – 100 % increase in serum creatinine as criteria. A trend towards a faster progression of renal disease in the younger group was noted. In the severely protein restricted group (C) 24h creatinine excretion decreased significantly compared to baseline ($p<0.01$) reflecting lower meat intake, whereas groups A1, A2 and B showed no significant difference with time. Using a regression equation looking at the reciprocal of serum creatinine with time (1/Cr/Mo) the LPD (B) decreased the progression of renal insufficiency by a factor of 5 and the severe LPD (C) decreased progression 3 fold compared to their respective control groups (A1, A2), but unfortunately the difference between groups was not tested statistically. Looking at the increase in serum creatinine using a 10-30% increase as a renal “non-survival” criteria there was a small but significant difference between intervention and control groups, more pronounced in group C compared to group B [78]. The benefits of the LPD were only seen in the patients with primary glomerulonephritis who had reduced renal mass and not in the study population as a whole. Compliance to the diet was measured by urinary urea excretion and the patients scored the diet as being of “fair” to “bad” acceptability.

In a follow-up of the 1984 study Rosman et al (15) looked at patients with a low or high protein excretion rates (proteinuria >1 or < 1 g/24h). It was shown that only LPD participants with proteinuria less than 1 g/24h had a significant improvement in the slope of CrCl (-0.19 to 0.01, $p<0.005$ over time); whereas participants in the free diet and those with proteinuria greater than 1 g/24h stowed no significant difference. The same was seen in participants with primary glomerulonephritis and proteinuria less than 1 g/24h where the slope in CrCl changed (-0.26 to 0.10, $p<0.01$ over time)[79]. In addition they reported that 25 participants in the control group and 14 participants in the LPD group had reached ESRD [80]. Unfortunately the authors fail to report if this difference was statistically significant.

Another single centre study investigated 128 patients stratified according to the underlying kidney disease, without taking the degree of renal function before randomization into account. Participants were randomized to a LPD of 0.6 g/kg IBW/d or to a control diet (UPD) of 1.0 g/kg IBW/d. Patients were followed for 27.1±21.8 months. The authors used a halving of creatinine clearance as the primary endpoint. This endpoint was reached by 40% of the UPD group and 28% in the LPD group ($p=0.038$)[81]. The limitations in this study were, firstly, the use of creatinine clearance as a marker of renal function. Secondly, compliance to the LPD diet was poor and the achieved decrease in dietary protein was 0.25 g/kg/d not 0.4 g/kg/d as planned which cast doubt on whether the changes seen were actually due to these small differences in protein intake.

In a multi-centre study from Locatelli et al [82] 311 participants were randomly assigned to either a UPD of 1.0 or LPD of 0.6 g/kg IBW/d. In this study participants were followed for 2 years or until the primary endpoint was reached (a doubling of serum creatinine from baseline). Contrary to other studies from this period, the participants were not stratified according to the underlying kidney disease [78, 81] rather they were stratified according to baseline plasma creatinine concentration.

In the UPD group 42 participants reached the endpoint compared to 27 in the LPD group, but this was only borderline significant ($p=0.06$). Only the LPD group with a baseline serum creatinine between 222-442 µmol/L had a significantly better renal survival (i.e. need for dialysis) where 10 in the LPD

group vs. 21 in the UPD group reached the endpoint (p=0.02) [82]. Adherence to diet was poor in the intervention group and there was a protein overconsumption of 39.8% as measured by 24h urea excretion. This resulted in a difference between the two groups of 0.16 g/kg/d instead of the prescribed 0.4 g/kg/d.

In a smaller study by Ihle et al [83] 64 participants with chronic renal disease with an entry serum creatinine of 350-1000 µmol/l were randomized to a UPD containing at least 0.75 g/kg/d or a LPD of 0.4 g/kg/d and they were followed for at least 18 months. In this study GFR was measured by a gold standard method, ^{51}Cr-EDTA clearance. GFR decreased significantly in the UPD group by 60% (0.25±0.03 to 0.10±0.05 ml/sec) compared to a non-significant decrease in the LPD group (0.23±0.04 to 0.20±0.05 ml/sec, p=0.01 for difference between diets). Serum creatinine increased significantly in the UPD group compared to the LPD group (52% vs. 16% respectively, p<0.02) so the authors concluded that a LPD has a beneficial effect in the slowing of progression of renal failure [83]. The limitations in this study were the lack of blinding of the treatments as all participants were treated by the principal investigator. There was a significant decrease in weight, lymphocyte count and serum transferrin level in the LPD group indicating malnutrition. Participants that were not compliant to the protein restriction were discontinued hence the authors did not use intention-to-treat analysis which introduces bias in the results and does not reflect the clinical reality of varying compliance.

In a two centre study of 95 patients with chronic renal failure (CRF), patients were divided into three groups (group A= LPD + low phosphorus (LPh), Group B= LPh, and group C= UPD). The LPD group was prescribed a 0.6 g/kg/d together with a phosphorus restriction of 800 mg/d. The LPh group was asked to restrict phosphorus to 1000 mg/d and protein was not restricted. Protein intake in group C was aimed to be ≥0.8 g/kg/d. For all three diet groups the aim for total energy intake was at least 30 Kcal/kg/d. The patients were followed for 6 months prior to randomization and 19 months follow up after randomization. Compliance to diet, as measured by urinary urea excretion (UUE) was good and the protein intake was 0.69±0.02, 1.02±0.05 and 1.14±0.05 g/kg/d for the LPD, LPh and UPD groups respectively. The progression of renal failure was

assessed by the rate of fall in CrCl (ml/min/1.73m²/month), change in the reciprocal of plasma creatinine over time (1/mmol/year) and dialysis or death. The authors found no significant differences in any of the endpoints. [84]. Limitations to this study, was the use of CrCl as measure of GFR with the well known risk of error. Furthermore, participants that were not compliant to diet were excluded and intention-to-treat analysis was not performed.

In 1994 the largest randomized controlled intervention trial to date, the modification of diet in renal disease study (MDRD) [85], was published. This trial included a total of 840 patients with different degrees of renal disease and hypertension. Patients were randomized according to the degree of renal disease.

The study consisted of two parts; study A included 585 patients with moderate renal impairment (GFR 25-55 ml/min/1.73m^2) stratified to either a usual protein diet 1.3 g/kg/d or a low protein diet of 0.58 g/kg/d. Study B included 255 patients with more severe renal disease (GFR 13-24 ml/min/1.73m^2), where patients were randomized to a LPD (0.58 g/kg/d) or a very low protein diet (0.28 g/kg/d) supplemented by 0.28 g/kg/d keto acids and essential amino acids (sVLPD). The two dietary groups contained the same amount of nitrogen but the nitrogen contained in the keto acids was subtracted from the urinary urea. The mean follow-up of both studies was 2.2 years (range 18-44 months).

In study A the decline in GFR initially increased rapidly during the first four months of the LPD compared to the UPD. After four months the GFR was 28% lower in the LPD group compared to the UPD. As this decline was correlated with the degree of compliance to the LPD, this was attributed to the hemodynamic effect of the dietary changes rather than a progression in the renal disease [86]. At the end of 3 years there was no significant difference in GFR between LPD and UPD, although the mean decline was 1.2 ml/min/year less in the LPD group compared to UPD (p=0.30).

In 2006 Levey et al [87] published a follow-up to the study group A looking at the risk of renal failure or death. During the first 6 years after randomization there was a beneficial effect of LPD compared to UPD (hazard ratio (HR) for renal failure or death was 0.68 (95% CI, 0.51 – 0.93) and 0.66 (95% CI, 0.50-0.87) respectively. However, when follow-up was extended beyond 6 years the

HR increased to 1.27 (95% CI, 0.90 – 1.80) and 1.29 (95% CI, 0.94-1.78) respectively. The difference between diet group and follow-up time did not reach statistical significance (p=0.11).

Cianciaruso et al conducted an 18 months intervention study including 400 patients with basal GFR <30 ml/min/1.73m^2. Patients were randomized to either a LPD (0.55 g/kg) or a UPD (0.75 g/kg) and followed for 18 months. The main outcomes were metabolic control, need for drugs and nutritional status. In this study analysis per protocol rather than intention-to-treat was used due to very poor compliance. In the compliant participants metabolic control was improved resulting in a decrease in drug use (4.13±1.56 - 3.76±1.40 tablets in the LPD vs. 4.97±1.72 – 4.62±1.32 tablets in the UPD). Overall malnutrition was not evident, but more participants in the LPD group lost more than 5% body weight [88]. This study could not show superiority of the LPD at 18 months and a long-term follow up study was commenced. In this long-term follow up study the patients were followed for an additional 30 months. The main outcomes were serum urea nitrogen (SUN), death, time to dialysis and composite measures of both. No difference was found in death rate or time to dialysis or composite measures or change in eGFR. However, the increase in SUN seen in both groups was significantly lower in the LPD compared to UPD (7.2±2.0 mg/dl, p<0.05) [89]. This would be expected given the lower protein intake and says nothing about renal function.

In patients already on peritoneal dialysis Jiang et al found a beneficial effect on proteinuria and residual renal function of a keto acid supplemented LPD (sLPD, 0.6-0.8 g/kg from foods + 0.12 g/kg keto acid) in 60 patients compared to either a high protein diet (1.0-1.2 g/kg) or a non-supplemented LPD (0.6-0.8 g/kg) diet. However, the dietary protein intake was self-reported data (3 day record) and the prescribed protein intake was very similar in all three groups, therefore protein intake may not be responsible for the difference [90].

Conclusion: non-diabetic renal disease

The overall outcome of these prospective RCTs in a non-diabetic population with varying degrees and causes of renal impairment is not clear. Some authors finding a beneficial effect of LPD on some aspect of renal function [78, 81, 83, 90], whereas others found no significant difference [82, 84, 85] and one follow-

up study found a possible deleterious effect [87]. The studies were heterogeneous in design, with some authors using CrCl and serum creatinine as endpoints. Only 2 studies used a direct measure of GFR but the results from these studies were also conflicting [83, 85]. Using CrCl as the endpoint measure has different problems: the use of serum creatinine is problematic as it assumes a steady state, where excretion rate equals production. Serum creatinine is dependent on muscle mass and also some tubular secretion exists and the measurement of serum creatinine is very imprecise. Furthermore the collection of the complete 24h urine sample is paramount to the result [91, 92]. In most of the studies discussed above, weight loss was a factor in the LPD group and would have induced a lower muscle mass and lower serum creatinine; additionally, a vegetarian LPD may lead to an overestimation of GFR [92]. Only the MDRD study has looked at long-term follow-up (≥6 years) and no significant difference between allocated diet group and time of follow-up was seen [87]. Outcome of early prospective LPD studies in non-diabetic patients can be seen in appendix 1, table 1.

1.5.4 Comparing the effect of LPD to very low protein diets (VLPD)

A number of studies have reported on the effect of a LPD compared to a VLPD in severe renal impairment (CrCl ≤ 25 ml/min/1.73 m^2).These studies use protein derived from foods in the LPD group and from foods supplemented by keto acids in the VLPD (sVLPD). The most frequently used protein prescription is 0.6 g/kg/d vs. 0.3 g/kg/d (+ supplement) respectively. In the first published results of the MDRD study group 2 (255 participants with severe renal failure; mean baseline GFR 18.5±3.4 ml/min/1.73 m^2) using an intention-to-treat approach, no significant difference was seen in the decline of GFR between LPD (0.58 g/kg) and sVLPD (0.28 g/kg+ supplement) at the end of three years; the decline in GFR was 0.8 ml/min/year lower in the sVLPD group compared to the LPD group (4.4 vs. 3.6, p=0.07) [85]. In 1996 Levey et al published a secondary analysis of MDRD study group 2: the objective was to determine the effect of achieved protein intake and prescribed protein intake on the progression of advanced renal failure. Although the protein prescription was very strict at 0.56 g/kg/d (0.28 g/kg/d from foods supplemented with 0.28 g/kg/d keto acids) in the sVLPD and 0.58 g/kg/d in LPD, the achieved protein

restriction was more moderate at 0.66 g/kg/d in the sVLPD and 0.76 g/kg/d in the LPD group. The authors looked at the effect on GFR decline dividing participants into groups according to achieved protein intake rather than allocated groups. They found that a 0.2 g/kg/d decrease in protein intake from foods was associated with a risk ratio of 0.52 (95% CI 0.35, 0.78) for renal failure or death at a given follow-up time, likewise for protein from foods and supplements the risk ratio was 0.50 (95% CI 0.34, 0.76) indicating no difference between foods alone or foods and supplements. GFR was slowed by 1.15 ml/min/y with a decrease in protein intake of 0.2 g/kg/d and a prolonged time to renal failure by 49% [93]. In a more recent follow up study of this patient group, Menon et al [94] assessed the long-term (1993-2000) outcome on kidney failure, all cause mortality and a composite outcome of both. They found no significant difference between diets on kidney failure or composite outcome. However, there was a 2 fold higher risk of death in the sVLPD group following the onset of renal failure compared the LPD group (p=0.01). From these results it does not seem feasible to prescribe a sVLPD which is difficult to adhere to in the long-term. Indeed participants in this study showed no difference in protein intake nine months after the end of the intervention [94]. The limitations of secondary analysis from the MDRD study are the lack of dietary information; protein intake was assessed nine months after the end of study and has not been assessed since. However, sVLPD showed a deleterious long-term effect on survival even though current protein intake was not known.

In an early RCT Junkers et al [95] reported results from 14 volunteers with a mean GFR of 8.1±0.9 ml/min/1.73 m^2. The LPD protein prescription was 0.6 g/kg/d vs. 0.4 g/kg/d (food and supplement combined) in the sVLPD. Follow-up ranged between 2 and 18 months. The assessed endpoints were 1/Cr/Mo and renal survival (measured as time to dialysis). Time to dialysis was on average 7.9 months (range: 4-18) in the LPD (n=7, one participant was excluded due to continued diet treatment) group vs. 12.5 months average (range: 8-18) in the sVLPD group (n=7) indicating a possible benefit of the sVLPD. The 1/Cr/Mo decreased more in the LPD group although not statistically significant between groups. The limitations of the study are the small number of patients treated for more than 12 months (n=5: two in LPD and three in sVLPD). One patient (LPD group) was still continuing treatment at the time of analysis and was therefore

excluded from the survival study; this may explain some of the difference in survival rate between the two groups [95]. In a similar study using the same protein restriction 50 patients with a baseline CrCl <19 ml/min/1.73 m^2 were randomised to LPD (0.6 g/kg/d) or sVLPD (0.3 g/kg/d + 0.17 supplement); no significant difference in renal survival (renal failure defined as GFR <5ml/min/1.73m^2) was seen after a follow up period of 40 months. However a significant weight loss ($p<0.01$) was seen in the sVLPD group indicating malnutrition, which led to the conclusion that an sVLPD is not justified in this patient group [96]. Contrary to these findings Di Iorio et al found significantly better renal survival, measured by time to dialysis, in a 2 year follow-up study investigating 20 patients with CrCl ≤25 ml/min/1.73m^2 randomized to either continue their usual LPD (prescribed protein intake 0.6 g/kg/d, actual intake 0.8 g/kg/d) or an sVLPD (prescribed protein intake 0.3 g/kg/d + supplement, actual protein intake 0.5 g/kg/d). The mean time to dialysis was 23.2±1.9 months vs. 19.6±4.0 months in sVLPD and LPD respectively ($p<0.02$) [97]. These findings are supported by a 48 week study in 45 patients with overt renal disease where one participant in the sVLPD group and seven in the LPD group (4% vs. 27%) commenced dialysis after 21 and 26 weeks respectively [98]. However, in a slightly different design comparing a LPD supplemented with keto acids (0.6 g/kg + 100 mg/kg) to a LPD (0.6 g/kg) supplemented with placebo in a randomized, double blind study design. A positive effect was seen on CrCl, inulin clearance and proteinuria with the addition of keto acids but it is difficult to relate these results to LPD to VLPD studies [99].

Conclusion: LPD vs. sVLPD

The results of these studies are inconclusive, with some studies showing a beneficial effect of the sVLPD on renal survival [90, 95, 97, 98] whereas others did not [85, 87, 94, 96, 99] and one study with long-term follow-up found a deleterious effect of sVLPD [94]. Most studies intervening with a sVLPD have a study population of severely renal impaired patients. Either the patients are stage 4 (GFR 15-29 ml/min/1.73m^2) [94] or they have passed the usual point of initiating renal replacement treatment (dialysis or transplantation) according to the KDOQI/guidelines of < 15 ml/min/1.73m^2 [100]. In some studies malnutrition on the sVLPD was evident, represented by weight loss [96]. The benefits

reported in these studies are small but the possible adverse effects are substantial. A VLPD is a major challenge in the patients everyday life and compliance is rarely achieved, therefore it seems questionable to recommend this severe diet to delay dialysis for 3-6 months.

1.5.5 The effect of protein restriction on renal function in patients with type 1 diabetes mellitus (T1DM)

Eight long-term studies (follow-up ≥12 months) looking at protein restriction in T1DM patients were found. Four studies were before and after intervention studies with no control groups, where the participants were followed for a period of up to 29 months on their usual protein diet and then changed to a LPD containing 0.25 – 0.67 g/kg/d and followed for up to 44 months [101-104]. Four randomized controlled trials were also identified; here participants were randomly assigned to either continue their usual diet or a LPD diet. The follow-up period ranged from 12 months to 11 years [105-108]. A total of 162 participants with type 1 diabetes mellitus were included in the randomized trials and an additional 67 participants took part in the uncontrolled trials. The intervention diet consisted of a mix of animal and vegetable protein, except for the two studies by Barsotti et al [103, 104] where the diet was changed from animal protein to vegetable protein. Compliance to diet was assessed by diet recall only in the study by Evanoff et al [102] and by both dietary record and urinary urea nitrogen excretion in the remaining studies [101, 103-108].

Renal function was assessed by CrCl (ml/min) [63, 102-104], ^{51}Cr-EDTA clearance [101, 107], inulin clearance [105], or iothalamate clearance [106, 108].

The dietary protein in the LPD was 0.25 to 0.67 g/kg/d. Two groups used 40 g protein per day in the LPD [101, 102] corresponding to 0.6 g/kg/d. One group calculated the protein restriction as g/kg IBW/d [108]. In two studies animal protein was excluded and all foods were vegetarian [103, 104]. The control diet in all studies was the participant's usual diet [101-108].

In a very small non-randomized study by Evanoff et al [26] retrospective data 12 months before dietary protein restriction and prospective data after 12 months follow up was used to assess renal function in 8 patients with T1DM. The UPD

contained 80 g protein/d and was changed to a LPD containing 40 g protein/d. CrCl and proteinuria was used as endpoints. No significant change in GFR measured by CrCl was found after 12 months whereas proteinuria decreased significantly from 2105±1355 to 142±164 mg/d ($p<0.001$). Furthermore there was a marked decrease in blood pressure from 158/92±21/10 to 130/80±14/18 ($p<0.01$, mean (SEM)) [102].

Barsotti et al conducted another small non-randomized study investigating 8 patients with T1DM with severe renal failure (CrCl 19.2 ±13.4 ml/min). Patient data was available for at least 1 year prior to inclusion in the study during which period protein was unrestricted. Patients were switched to a LPD supplemented by essential amino acids and keto acid analogs but restricted in phosphorus. Animal foods were excluded from the diet in order to maintain either a protein intake of 0.25-0.35 g/kg/d (n=4) or 0.5-0.6 g/kg/d (n=4). Compliance to diet was measured by urinary urea nitrogen. CrCl and proteinuria was used as major endpoints. At the end of the 17.4±5.8 months follow-up, proteinuria had decreased significantly with the LPD from 5.7±1.9 to 3.1±0.6 g/24h ($p<0.001$); the fall in CrCl decreased from 1.38±0.27 to 0.03±0.37 ml/min/month ($p<0.001$) [104].

More recently Barsotti et al [103] conducted a non-randomized study investigating 32 diabetics (22 T1DM and 10 T2DM) looking at the effect of LPD containing (A) 0.3 g vegetable protein/kg/d or (B) 0.7 g vegetable protein/kg/d. The rate of decline in the pre-study period was 0.9±0.62 ml/min/month and significantly less (0.22±0.21 ml/min/month) after 3.7 years of follow-up ($p=0.001$). Results with all participants combined, regardless of type of diabetes or allocated protein restriction.

In the last non-randomized study, Walker et al [101] investigated 19 patients with T1DM with moderate renal impairment (GFR 23-125 ml/min/1.73m^2 measured by ^{51}Cr-EDTA). They were observed for a pre-intervention period of 12-39 months on their UPD, after which they were switched to a LPD of 0.67 g/kg/d equally divided between vegetable and animal protein. The rate of decline of GFR was -0.61±0.14 ml/mi/1.73m^2 during the UPD vs. -0.14±0.08 ml/mi/1.73m^2 ($P=0.001$) while on the LPD; but the effects were highly variable ranging from a reduction in 8 patients to an accelerated decline in 1 patient.

Four patients started angiotensin converting enzyme inhibitor (ACE) while on the LPD, which is also known to positively affect renal function [109].

In three relatively small randomized, controlled trials with a follow up period of at least 12 months (range 12-37.1 months), using more precise methods to estimate GFR, variable results have been reported. Brouhard and LaGrove undertook a RCT looking at the 12 month effect of a LPD (n=8) on GFR measured by inulin clearance and comparing that to the UPD (n=7). The GFR decreased in both groups but more so in the UPD group (0.68±0.4 in the UPD vs. 0.28±0.15 in the LPD group; p=0.005) [105]. Similarly Zeller et al found a decrease in Iothalamate clearance in both study groups but the decrease was less in the LPD (n=20) group compared to the UPD (n=15) group (-0.0042 ml/sec/m vs. -0.0177 ml/sec/m; p=0.03) [108]. The third study by Dullaard et al included 30 patients with T1DM and found GFR decreased in both groups. The decrease in GFR in the LPD (n=14) group was significant compared to baseline (131±34 vs. 113±24 p=0.03); in comparison the decrease in UPD (n=16) was only borderline significant (122±26 vs. 112±21; p=0.05) but the two arms were not different statistically [106].

In a more recent, larger (n=82) RCT with a mean follow up of 4 years, Hansen et al likewise found no significant difference in decline in GFR between the two groups (-3.9 ml/min/year UPD vs. -3.8ml/min/year LPD). However, the main endpoints of ESRD or death occurred in 27% of patients in the UPD group compared to 10% in the LPD group (p=0.042).

Conclusion: type 1 diabetes

Compliance to the diet was poor in most studies. The total number of participants in the 8 studies combined was only 229 patients. There was a beneficial effect of LPD in three out of the four non-randomized trials but the studies varied widely in design and the total number of participants was only 67. In the RCT trials two studies [101, 108] show a significant decrease in the fall in GFR with a LPD but the remaining larger studies showed no significant effect of the LPD.
Outcome of non-randomized and randomized trials including type 1 diabetic patients are shown in appendix 1, table 2.

1.5.6 RCT trials in type 2 diabetes

Pijls et al conducted a 12 months study of 121 patients with T2DM and microalbuminuria. Patients were randomly assigned to a LPD (0.8 g/kg/d) or to continue on UPD (1.2 g/kg/d). Albuminuria in the LPD group decreased by 12 % compared to control in the intention-to-treat analysis [110]. In 2002 Pijls et al published a second study. Patients were followed for 28 ± 7 months. In this study the endpoints were albuminuria and GFR. To avoid the hemodynamic changes where GFR initially decreases on a LPD [111], the first 6 months of intervention were omitted from this analysis and data are presented from 6 months onwards.

Achieved protein intake after the first 6 months was 1.1 g/kg body weight/day vs. 1.19 g/kg body weight/day in the control group. At 12 months this difference had decreased to 1.1 g/kg body weight/day vs. 1.14 g/ kg body weight/day respectively. Both intention-to-treat and best-case analysis (where the result is analysed based on actual protein intake regardless of allocated diet) were used. Both analyses failed to show significant beneficial effect of protein restriction on renal damage. The researchers concluded that protein restriction was ineffective in preventing the progression of diabetic nephropathy in T2DM; however a significant difference between diets was never achieved.

Similar effects were seen in a further three studies comparing LPD to UPD in type 1 and type 2 diabetes patients [112-114]. The effect of a LPD (0.6-0.8 g/kg/d) compared to a control group receiving an UPD (1.2 g/kg/d) was investigated in two studies by Meloni et al and one study by Dussoll et al. Follow-up was 12-24 months. No significant changes were seen in proteinuria, microalbuminuria or isotope GFR. Compliance was either poor or absent. In the study by Meloni [113] there was a decrease in serum pre-albumin and serum albumin indicating a tendency towards malnutrition in the LPD group. Energy intake decreased by approximately 600 Kcal within the first 3 months of intervention and stayed low throughout the study duration in this group and the patients lost weight. In contrast, energy intake in the UPD stayed constant throughout the study. Recently the effect of a LPD (0.8 g/kg) compared to an UPD (1.2 g/kg) was investigated in a multicentre study including 112 Japanese T2DM patients. Follow-up was 5 years and main outcome was mean annual

change in estimated GFR, CrCl and a doubling of serum creatinine. No significant difference was detected in any of the outcome measures. Compliance was low, in fact at the end of five years there was no difference in protein intake (p=0.16) [115]. Using another approach, in 170 T2DM patients with normal to moderate renal impairment (GFR 15-75 ml/min) Facchini and Saylor [116] compared an iron modified, HPD (25-30% of energy from protein where red meat was exchanged with white meat, dairy, egg and fish, CHO was reduced to 50% of energy, tea and wine was controlled and polyphenol enriched olive oil was used) to the “standard care LPD” of 10 % TE from protein (0.8 g/kg). Serum creatinine doubled in significantly more patients on the LPD compared to the iron modified diet (31 vs. 19, $p<0.01$), Renal replacement treatment and death occurred more often in the LPD group (31 vs. 18, $p<0.01$). The major limitation of this study is the lack of dietary information, compliance to the dietary regimes was not measured and the only measure of diet was serum ferritin which decreased in the iron modified diet. However the study suggests protein restriction per se is not required to obtain a benefit and that other dietary strategies may be useful.

Conclusions for studies including both type 1 and type 2 diabetes patients:

Seven RCTs which included 664 T2DM and 66 T1DM participants found no significant difference after up to five years follow-up. One study found that protein restriction may promote malnutrition [113]. One study differed in design where an iron modified HPD was used as intervention compared to the LPD. In this study a beneficial effect on renal survival was seen in the iron modified HPD [116]. Outcome of RCT studies including type 2 diabetic patients can be seen in appendix 1, table 3.

1.5.7 Major reviews of low protein diets

After publication of the MDRD study, Pedrini et al published a meta-analysis of randomized controlled trials (RCT) from the period between 1966-1994 on LPD and renal disease. These included 5 studies in T1DM patients (n=108) and 5 studies in non-diabetics (n=1413) including the MDRD study. The endpoints were ESRD or death and GFR, albumin excretion rate and creatinine clearance. The prescribed protein intake in the non-diabetic studies was 0.4-0.6 g/kg/d in the LPD group and in diabetes the prescribed protein intake ranged between

0.25-0.6 g/kg/d. The results of these studies on GFR in the non-diabetic studies were inconsistent: 2 studies showed a positive effect of protein restriction, 2 studies showed no effect and the MDRD study did not show a beneficial effect of LPD in the 585 participants with moderate renal impairment. In the 255 participants with advanced renal disease treated with LPD and strict BP control a beneficial effect was seen in the secondary analysis.

Contrary to the results in the non-diabetic individuals a beneficial effect was seen in the T1DM patients. The prescribed protein restriction was generally not adhered to. The achieved protein intake was 0.7-0.8 g/kg/d. However, the pooled results of these studies showed a decreased risk of ESRD or death of 0.67 in non-diabetics and 0.56 in diabetic patients. The authors found there was a decreased relative risk, 0.67 [CI, 0.50 to 0.89]; p=0.007 of renal failure or death. This was used as evidence to recommend a low protein diet containing 0.6 g/kg/d to delay the need for dialysis [77].

Kasiske et al found in their selective meta-analysis which included 13 RCT (n=1919, in which 102 were patients with diabetes) a small 0.53 ml/min/y (95% CI, 0.08 to 0.98 ml/min/Y, p<0.05) reduction in the decline of renal function [117] with LPD. However, in a recent Cochrane review by Robertson et al based on 9 RCT and 3 before and after studies (n=585, 322 T1DM and 236 T2DM patients) a small but insignificant (p=0.18) benefit of LPD of 0.1 ml/min/month was found [118]. In 8 RCTs Pan et al found a small but not statistically significant beneficial effect of LPD in diabetic nephropathy in both T1DM and T2DM patients [119].

The latest systematic review was published from the Cochrane library by Fouque and Laville in 2009; in this review they updated earlier reviews by Fouquet et al 1992 and Pedrini et al [120]. Ten RCT comparing UPD to a LPD, excluding diabetics and children were included. A total of 2000 participants (range 19 to 585) with moderate to severe renal disease participated. The outcome was renal death (ie need for dialysis) during follow-up. In the UPD group 168 participants died or commenced dialysis vs. 113 in the LPD group. A reduction of the relative risk in the LPD of 32% was reported (p=0.0002).The number of patients who needed to be treated in order to prevent one case of renal death during one year of intervention ranged between 2 and 56, with the

larger studies showing less effect of protein restriction. It is not clear how the analysis was performed as one of the studies in the meta analysis from Malvy et al [96] showed that 100% of patients were on dialysis after 36 months with only 1 month difference in reaching this end point between the 2 groups. The meta analysis claims that in this study only 8 patients would need to be treated to avoid one “renal death” -this is true only at 12 months.

Conclusion from major reviews

A major problem with the published studies so far is the lack of homogeneity in study designs. Endpoints range from ESRD to death, or changes in renal function like GRF, CrCl and proteinuria. The dietary prescription for LPD is very often not adhered to, the prescribed protein restriction range from 0.25-0.8 g/kg/d, whereas achieved protein intake range from 0.7-1.1 g/kg/d. When comparing the achieved protein intake in the LPD groups, to the protein intake in the UPD groups (0.8 – 1.4 g/kg/d) there is no significant difference. However, Fouque and Laville reported a highly significant reduction in the relative risk of renal death of 32%, with the smaller studies showing the strongest effect; but as noted above this conclusion is suspect. Delaying the need for dialysis does not have any long-term benefit on mortality and use of a LPD may be harmful under some circumstances.

1.5.8 Epidemiologic studies

The effect of different levels of protein intake in humans with normal or moderately impaired renal function has been assessed in a number of epidemiologic studies. Knight et al [121] in a subgroup of women from The Nurses Health Study assessed the changes in renal function in1624 women with normal (GFR ≥ 80 ml/min /1.73 m^2) or impaired renal function (GFR ≥ 55 but ≤80 ml/min /1.73 m^2) over an 11 year period based on serum creatinine only. After adjusting for measurement error they demonstrated a fall in CrCl of 7.72 ml/min /1.73 m^2 per 10 g protein over the 11 year period in the subgroup with impaired renal function with borderline statistical significance (95% CI, -15.52 to -0.08 ml/min /1.73 m^2). In the subgroup of women with normal renal function there was a non-significant decrease in GFR of 1.14 ml/min /1.73 m^2 per 10 g (CI, -3.63 to 5.92 ml/min /1.73 m^2) [121]. The third national health and nutrition examination survey (NHANES III) found no association between

protein intake and microalbuminuria in participants without renal impairment, hypertension or diabetes. However, in participants with both hypertension and diabetes a high intake of protein was associated with an increased prevalence of microalbuminuria [122]. In the more recent PREVEND study investigating the effect of protein intake (which ranged between 0.3 – 3.33 g/kg/d, as assessed by urinary urea excretion) on renal function in 8461 participants who did not have renal disease, no significant difference was seen in the change in GFR over a follow up period of 7.2 years [123]. In participants with renal impairment there seems to be a u-shaped association between protein intake and the deterioration of GFR. A low protein intake (<90% of recommended intake (K/DQOI 0.6-0.75 g/kg)) and a high protein intake (≥110% recommended intake) was associated with deterioration whereas intake in the recommended range had no deleterious effect. The same effect was seen in participants with low energy intake [124].

1.5.9 Conclusion

The evidence of a beneficial effect of LPD in delaying the progression of renal disease is still lacking. In the studies focusing on renal function in rodents with extensive renal mass loss there is a significantly better outcome when low protein diets are used. However, this is not so clear in humans. The relatively few long-term, RCT published so far are inconclusive. Some meta-analyses show beneficial effect of LPD and VLPD on uremic symptoms, resulting in a delayed time to renal replacement therapy which may be of benefit to some patients [77, 120]. It is however a concern that the long-term effect of these diets may result in increased mortality as seen in the secondary analysis of the MDRD study [94]. The cause of renal disease in people with type 2 diabetes is multi factorial and hypertension, hyperglycaemia, obesity, and cigarette smoking all play major roles. Long-term RCT investigating the effect of protein intake on renal function in T2DM patients who are matched for these confounding factors are needed.

1.6 **Weight loss diets**

1.6.1 Weight loss and renal function

It is well established that proteinuria increases with obesity [42, 125]. In the Prevention of Renal and Vascular End stage Disease (PREVEND) study, a population based longitudinal study, 6894 participants were followed for 4.2 years to determine the effect of weight loss on albuminuria. The participants were divided into three groups according to weight change; 1) significant weight gain (≥10 kg from baseline), 2) significant weight loss (≥10 kg from baseline) and 3) stable weight (<10 kg weight gain or loss from baseline). There were significant changes in albuminuria in the two groups with significant weight change compared to the weight stable group. Among the participants who lost weight, 27% had a halving of albuminuria compared to 10% in the weight stable group (p<0.01). Among the participants who gained weight, 14% doubled albumin excretion compared to 12% in the weight stable group (p<0.01) [126].

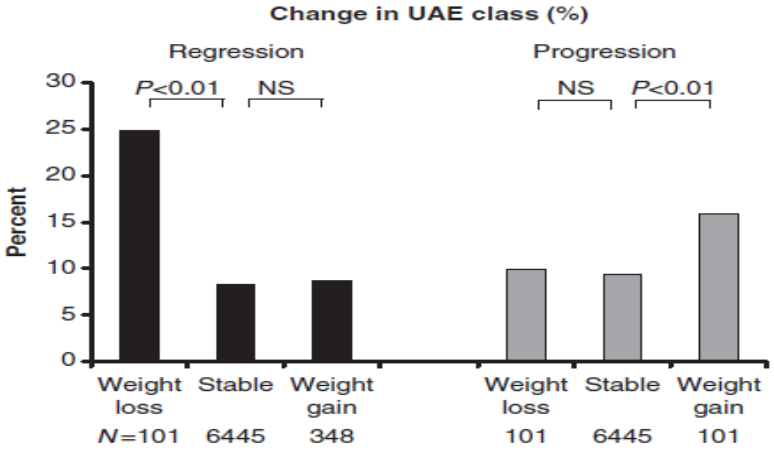


Fig. 1. Changes in weight related to changes in class of urinary albumin excretion (UAE), with albuminuria classes defined as normoalbuminuria (<15 mg/24 h), high-normal albuminuria (15–30 mg/24 h), microalbuminuria (30–300 mg/24 h), and macroalbuminuria (>300 mg/24 h). On the left albuminuria class regression (black bars), and on the right albuminuria class progression (grey bars).

Adapted from:

Bello et al, Impact of weight change on albuminuria in the general population. Nephrol Dial Transplant, 2007. 22 (6): p. 1619-1627

Similar effects of weight loss on urinary protein excretion in obese participants were reported in a systematic review by Afshinnia et al. The review included five controlled trials and eight uncontrolled trials including 522 participants. Urinary protein excretion was assessed both before and after weight loss. The meta regression in these studies revealed that for every 1 kg body weight lost, a reduction in proteinuria of 110 mg and in albuminuria of 1.1 mg was seen [125].

1.6.2 The traditional high carbohydrate, low fat, energy restricted diet

The traditional high carbohydrate diet was used in many of the studies investigating the effect of weight loss on albumin excretion [127-129]. This diet has been recommended for many years to the general population [130] and to people with T2DM [65, 68].

In energy restricted diets kJ intake is usually decreased by 30% or 2500 to 4200 kJ compared to estimated energy need for the individual. The aim is to produce a weight loss of 5 to 10% of total body weight [65]. The recommended nutrient composition of a weight loss diet widely used during the past decades is CHO 50-60% TE (≤10%TE sugar, 3.5 g dietary fibre/100kJ), fat ≤30%TE (<10%TE SAFA, ≥10%TE PUFA, ≥10%TE MUFA) and protein 15-20%TE. These recommendations have been indorsed by, among others, the American Diabetes Association (ADA) and the European Diabetes Nutrition Study Group (DNSG) [65, 68].

High carbohydrate, low fat diets have been found to be effective in reducing weight by 3-4 kg in ad libitum energy intake diets [131]. For a spontaneous decrease in energy intake an ad libitum low fat, high complex CHO diet is recommended [132], However, for weight loss, an energy restricted low fat diet is recommended [133]. Calorie restricted diets may result in a 6-7 kg weight loss over 4 years, depending on participant compliance [134]. Furthermore, a high fibre (cereal, vegetable and fruit), low saturated fat (SAFA less than 10%TE) and low trans-fatty acid, weight loss diet in combination with increased exercise have been shown to reduce the risk of advancing from IGT to T2DM in two large intervention studies with long-term follow up (the Finnish diabetes prevention study and the Diabetes Prevention program) [135, 136].

1.6.3 High protein, weight loss diets

Diets with an increased ratio of protein to carbohydrate have become popular. Research has shown high-protein diets sometimes promote favourable changes in body composition compared with high carbohydrate diets in healthy obese participants [137], in women with increased triglyceride level >1.5 mmol/L [138] and in T2DM [139].

Total weight-loss was increased in healthy [137, 138], insulin resistant participants [140] and in women with type 2 diabetes [139]. Furthermore, improved satiety and decreased overall hunger have been reported [74, 137].

1.6.3.1 Definition of a high protein weight loss diet

Most studies have used a low fat (20 to 30 %TE), moderate to high protein (20 to 43 %TE) and moderate to low CHO (20 to 40 %TE) nutrient composition [137-139, 141-146]. Protein in g/kg/day ranged between 0.5-0.8 in the low/standard protein groups and 1.1-1.8 g/kg/day in the high protein groups. Total protein intake ranged between of 55 to 80 g in the low protein group and in the high protein group the reported intake was 87 to 202 g protein /day.

Some researchers have looked at ad libitum energy intake in HPD [137, 144]. Most studies have looked at the effect of high protein diets with energy restriction (decreased by 30% of total energy need) [138, 139, 141-143, 145-147]

1.6.3.2 Energy expenditure and high-protein diets

Diet induced thermic effect (DITE) is one component of total daily energy expenditure (TEE), accounting for approximately 10% of TEE for persons in energy balance [148]. Additional components of TEE are resting metabolic rate (RMR), usually measured in the morning after an overnight fast, which accounts for 60-70% and physical activity (PA) which makes up the remaining 15-30% TEE. [149]^{pp23}

The increases in energy expenditure after a meal varies with the macronutrient composition, where dietary fat increases basal metabolic rate (BMR) by 0-3%, CHO increase BMR by 5-10% and protein increase BMR by 20-30% [150]. Alcohol produces a similar thermic effect to protein (10-30%); but is usually ingested as an additive source of kJ [148].

The energy cost of protein oxidation is dependent of the number of amino groups that are converted to urea. This can range between 99 kJ/mol of Adenosine Triphosphate (ATP) for glutamate to 153 kJ/mol of ATP for cysteine compared to 91 kJ/mol of ATP and 96 kJ/mol of ATP for CHO and fat respectively [150].

Protein oxidation measured by indirect calorimetry has been shown to almost triple after a protein rich compared to a fat rich meal in both lean and obese women ($p<0.01$). Diet induced thermic effect of the protein rich meal was 13.4% compared to 4.3% in the fat rich meal. If sustained this difference translates into 2 kg weight loss over one year. Importantly, no difference in DITE was seen between lean or obese women [151].

The higher thermic effect of protein might be responsible for the increased satiety shown in multiple intervention studies as summarized in a review by Halton and Frank 2004. They looked at 15 randomized controlled trials (RCT), reporting the effect of high-protein vs. lower protein diets on thermogenesis. This effect is seen in both lean and obese persons [152].

The decrease in energy expenditure with energy restriction and weight loss may be less pronounced in HPD compared to conventional diet. Baba et al found resting energy expenditure (REE) decreased less in the high-protein group (protein 45%TE) compared to the conventional diet group (protein 12%TE) when adjusted for weight loss differences (-22.09±2.16 kcal/kg weight loss in HPD vs. -58.79±5.21 kcal/kg weight loss SPD; $p<0.05$) [140].

Protein intake has an increased thermal effect mainly due to glucogeneogenesis and oxidation as amino acids are not stored in the body but have to be metabolized or synthesized immediately [152].

1.6.3.3 Dietary protein induced satiety

In a comprehensive review by Westerterp-Plantenga et al of the literature reporting data on high protein diets in energy balance, energy deficiency and weight maintenance, it was shown that in energy balance a high protein diet (25-30 %TE) given for one to several days produced sustained higher satiety compared to a normal protein diet (15-30%TE) [150]. High protein diets result in reduced appetite and increased satiety in ad libitum energy intake [153, 154]. Moreover, a hierarchy has been observed where protein is the most satiating nutrient, then carbohydrate and least satiating was fat [150].

Skov et al found energy intake in the HPD group to be 2 MJ lower than in the high CHO group under ad libitum energy intake. This is probably explained by the higher satiety experienced with the HPD [137]. In energy restriction the total

amount of protein should be kept constant in order to avoid negative nitrogen balance and this usually means that the total energy percentage (%TE) from protein is between 25 and 30 %TE [150]. High protein energy restricted diets have been reported to produce weight loss equal to high CHO diets; but the high protein diet group reported less hunger and more satiety in the first four weeks of a six week weight loss intervention [150]. The increased satiety of the HPD may result in sustained weight loss or less weight regain in the follow-up after weight reduction [150].

1.6.3.3.1 Acute protein induced satiety

In studies looking at different changes in single meal protein intake and the effect on satiety, using visual analogue scale (VAS), results have consistently shown a significantly higher satiety score when other macronutrients are exchanged for protein.

In preload studies an increased concentration of anorexigenic hormones glucagon-like peptide 1 (GLP1), cholesystokinin (CCK), insulin response and slowing of gastric emptying associated with subsequent energy intake reduction have been reported [155-160].

Ghrelin a gastric hormone which has been shown to increase appetite in rodents and humans [161], was decreased equally by different macronutrient preloads. However, the decrease after protein preloads was prolonged compared to glucose and fat preloads [155, 162]. Subsequent ad libitum energy intake was lower after high protein breakfasts (25%TE from protein) compared to lower protein (10%TE) and CHO preloads [155-160] and after high protein lunch preloads [163]. Although Blom et al found postprandial ghrelin to decrease, cholecystokinin (CCK) and glucagon-like peptide 1 (GLP1) to increase, causing slower gastric emptying, they found no significant difference in satiety scores comparing a high protein breakfast to a high carbohydrate breakfast in young men and there was no significant difference in energy intake at a subsequent ad libitum lunch [164].

The research in this area is heterogeneous in design, choice of pre-load and study population. High protein diets are more satiating as shown in pre-load

studies with subsequent ad libitum feedings and short term high protein diets, but this may not be the case for long-term consumption.

1.6.4 Diets with decreased carbohydrate content

Low CHO diets, where CHO has been exchanged with protein and fat, have a beneficial effect on the rate of body fat (BF) loss, body weight (BW) loss and retention of fat free mass (FFM) compared to high CHO, low fat diets [165]. Krieger et al [165] performed a meta regression of 87 studies, where dietary intake was measured by self-reported data, either verified by biological measures (urinary nitrogen excretion, blood urea nitrogen, urinary or plasma ketones or plasma fatty acids) or In the case of no biological markers of compliance, the subjects had to be supplied with at least 60% of their daily energy needs for the study to be included in the meta analysis.

A total of 325 studies where the investigators reported poor compliance were excluded. Krieger et al found diets in the lowest quartile of CHO intake (≤35%TE, defined as the first quartile below the median) were associated with a 1.6-1.7 kg greater BW loss compared to the high CHO diets (the highest three quartiles above the median) in studies of < 12 weeks duration. In studies of more than 12 weeks duration (n=7), the low CHO diet produced a 6.56 kg greater BW loss compared to high CHO diets (>35%TE).

There was a trend towards a greater %BF loss with low CHO diets, so that a diet containing >1.05 g protein/kg increased %BF loss by 0.96% (however this was not statistically significant, p=0.21). In studies of >12 weeks duration this difference in %BF rose to 3.55% (p=0.27). This corresponds to an increased fat mass change of 5.57 kg in the low CHO diets.

There was a tendency of a higher retention of lean body mass (LBM) in the two highest quartiles of protein intake (>1.05g protein/kg body weight) compared to the low quartile of protein intake (0.7g/kg) and the effect was greater with increased length of intervention [165] .

Increasing the ratio of high quality dietary protein to CHO intake in low fat, energy restricted diets has shown a tendency to spare lean body mass under conditions of weight loss. This effect is usually achieved by unchanged or minor decreases in LBM in the HPD compared to a larger decrease in LBM in the

SPD diet groups [166-168]. The effect of HPD on body composition will be discussed in the section 1.7

1.6.5 Very low carbohydrate diets

1.6.5.1 Ketogenic diets

The very low carbohydrate (ketogenic) diet usually contains 20 g CHO (often increasing over time to approximately 50g), with no restriction in energy, fat or protein intake [169-173]. These ketogenic diets are often compared to a low fat, energy restricted diet (a deficit of 2100-4200 kJ, ≤30%TE from fat, 55-60%TE from CHO and 15%TE from protein).

Studies investigating the effect of weight loss using a ketogenic diet have found an increased weight loss, increased loss of fat mass and abdominal fat mass (AFM). A meta-analysis of five trials including 447 participants (222 in the ketogenic diet and 225 in the low fat diet) found a beneficial effect on TG and HDL cholesterol; but an adverse effect on total cholesterol and LDL cholesterol in the ketogenic diet. It was concluded that the ketogenic diet was as effective as the low fat diet in reducing weight; but cautions the unfavourable changes in LDL and total cholesterol may outweigh the favourable changes in TG and HDL cholesterol [174].

The effect of the low CHO diet is usually seen within the first 2 to 4 months of intervention after which the effect seemed to level off and be the same as the higher CHO diet after 12 months [173, 174]. Very few studies report longer term effect (52 weeks) [171, 175-177].

In 307 obese participants Foster et al [176] found no significant treatment effect on weight loss after 6 months intervention and 24 months follow up. The diets were designed to give either 20g/day CHO (increasing after 6 months by 5g/day until desired weight was stabilized ≈BMI 23 kg/m2) in an ad libitum energy intake diet or a low fat, energy restricted diet (energy reduction of 1.2 to 1.8 MJ and fat intake ≤30%TE). After 12 months there was a mean weight loss of 11% in both groups and this decreased to 7% at 24 months.

In a study looking at 322 obese participants, with 24 months follow up, three different diet allocations were investigated: a low fat, energy restricted diet; a Mediterranean (high fibre and high MUFA intake), energy restricted diet; and

the low CHO, ad libitum energy intake diet. Weight loss occurred within the first six months and a maintenance phase was initiated from seven months to the end of 24 months. A significantly greater weight loss was observed after 24 months in the Mediterranean and the low CHO diets (-4.4±6.0 and -4.7±6.5 kg in the two groups respectively, compared to -2.9±4.2 kg in the low fat group; $p<0.01$ for the treatment*time interaction) [177].

In summary, the ketogenic diet has a beneficial effect on weight loss in the short term; but this effect is usually not sustained long-term. Triglyceride is decreased and HDL cholesterol is increased in the ketogenic diet. However, LDL cholesterol and total cholesterol are increased, and this increase may counter the positive effect seen on TG and HDL.

1.6.6 High-protein, ad libitum energy intake, weight loss diets

Halton et al [152] reviewed five studies of long duration (≥6 months) and 10 studies of shorter duration (7 days to 16 weeks) and concluded that there was a slight trend towards a beneficial effect of HPD on total weight loss, when the diets used ad libitum energy consumption. No difference was seen in isocaloric HPD when compared to the conventional high-carbohydrate, low fat, weight reduction diet (HCLF) [152].

In ad libitum energy intake weight loss diets comparing HPD to HCLF, Skov et al found weight loss at 6 months to be greater in the HPD (8.9 kg vs. 5.1 kg respectively). Furthermore they found that more people in the HPD group (19 of 23 =79%) lost in excess of 5 kg compared to the HCLF group (12 of 23 = 52%; $p<0.05$) during the first 3 months and at 6 months more people in the HPD group (8 of 23 =35%) had lost in excess of 10 kg (2 of 23 = 9% HCLF; $p=0.02$) [137]. Fat mass had decreased by 7.6 kg in the HPD group compared to 4.3 kg in the HCLF group, a highly significant difference of 3.3 kg body fat mass lost ($p<0.0001$). Of the loss in fat mass, intra-abdominal fat mass measured by DXA scan had decreased 2 fold in the HPD group when compared to HCLF (33 cm^2 vs. 16.8 cm^2; $p<0.0001$) [137]. A follow up study was conducted looking at attrition rate, body weight, body composition and metabolic markers after another 6 and 12 months [178]. At twelve months 7 of 23 (28%) had dropped out of the HCLF group compared to 2 of 23 (8%) in HPD ($p<0.07$). At 24 months follow up attrition rate was high; 19 of 23 had dropped out of the HCLF group

and 14 of 23 in the HPD group (76% and 56% respectively; p>0.05). At 12 months weight loss was no longer significantly different between the two intervention groups (6.2 vs. 4.3 kg in the HPD and HCLF groups respectively; p>0.05) and at 24 months the weight had remained stable in both groups (6.4 vs. 3.2 in HPD and HCLF respectively). Weight loss of more than 5 kg was equal in the two groups at 24 months (6/11 HPD and 3/6 HCLF; p>0.05). Weight loss of more than 10 kg was achieved by 2 subjects in the HPD (18%) and none in the HCLF group. The increased loss of abdominal fat mass seen in the HPD group after six months intervention remained significant at 12 and 24 months (p<0.05) [178].

More recent studies investigating the effect of a moderately higher protein intake compared to the traditional diet have also reported greater weight loss and decreased waist circumference under ad libitum energy intake [175, 179]

1.6.7 High protein, low fat, energy restricted diets

The energy restriction in these diets is usually around 30% compared to estimated or measured energy needs. The recommended fat intake is usually ≤30 %TE, with saturated fatty acids limited to ≤10%TE. The main difference in the high protein, energy restricted diet is the ratio between CHO and protein. Protein allocation in these studies usually range from 25 – 35%TE, carbohydrate level is usually in the range of 40-45 %TE.

In a recent short term (8 weeks) weight loss intervention study where energy intake was reduced by 30% of estimated energy needs measured by indirect calorimetry Abete et al [141] was able to show that weight loss was significantly higher in the HPD group (-8.3±1.2% vs. -5.5±2.5%, p=0.01). Similarly waist circumference decreased more in the HPD indicating a greater loss of visceral fat mass (-9.8±2.4% vs. -6.1±2.9%, p=0.03) [141]. Protein intake in the HPD was planned to be 30%TE (30 %TE from fat and 40%TE from CHO) compared to 15 %TE (55%TE CHO and 30 %TE from fat) in the control diet.

This confirms the findings from Due et al, who found a decrease in waist circumference of 5.7 cm (p<0.001) in the HPD compared to the HCLF [144]. However, a part of the weight lost in the study by Abete et al was due to decrease in body water which was significantly higher in the HPD compared to

the HCLF (-2.1±0.2% vs. -1.3±0.6%, p=0.008) [141]. In a study looking at HPD in type 2 diabetic volunteers, a weak sex by diet interaction has been shown such that men lost more weight on a HCLF and women lost more weight on a HPD. The same trend was seen in the rate of fat mass loss and abdominal fat [139]. This trend was also reported in the follow up study by Due et al, where men tended to lose more abdominal fat mass compared to women. However, due to the low number of men remaining in the study at 24 months, this did not reach statistical significance [178].

Noakes et al investigated 100 women for 12 weeks. They were randomly allocated to one of two 5.6 MJ diets (HPD 34 %TE protein 97.8 g protein, or 1.2 g/kg/day 20 %TE fat and 46 %TE CHO and the HCLF 17 %TE protein 54.6 g protein or 0.6 g/kg/day, 20 %TE fat and 64 %TE CHO); the mean weight loss in the HPD group was 7.6±0.4 kg and 6.2±kg in the HCLF group (p=0.29). Interestingly they found a 25% higher weight loss with high protein in the group of women with increased triacylglycerol level (TG >1.5 mmol/L) compared to women with low TG levels (p=0.005) and in this group total weight loss was 6.4±0.7 kg vs. 3.4±0.7 kg in the HCLF group, p=0.035 [138]. In a long-term follow up of this study the achieved weight loss for the two groups was similar (4.6±5.5 vs. 4.4±6.1) but diet was not adhered to [143]. However, when the researchers analyzed diet data based on reported protein intake, verified by urinary urea, weight loss was greater in the participants who reported a high protein intake compared to reported lower protein intake (6.3±7.9 vs. 3.6±4.2 kg, p=0.05) [143].

In insulin resistant and T2DM participants, the weight loss achieved from following a high protein, weight loss diet is equally effective as a HCLF diet [139, 180-183].

1.7 **Beneficial changes in body composition of high protein, weight loss diets**

1.7.1 Lean body mass / fat mass

A number of studies have found significant benefit of HPD on body weight, body fat, and abdominal fat mass compared with HCLF under both ad libitum and

isocaloric conditions [137, 139, 141, 175, 179]. Retention of lean body mass was found in some studies [145, 167].

Most studies reviewed by Halton and Hu found an increased loss of fat mass with the high protein diet, but in most cases the difference was not significant [152].

Retention of FFM was found to be dependent on the protein intake: in the review by Krieger et al an additional retention of 0.78 kg FFM was seen in the group who consumed > 1.05 ≤ 1.2 g protein/kg compared to the group consuming the recommended 0.8 g protein/kg/d. In the highest protein intake group (>1.2 g protein/kg) an additional retention of 0.96 kg was seen, however, this was only significant in studies of more than 12 weeks duration [165]. Other studies have reported no significant difference in the loss or retention of FFM in energy restricted, high protein diets when measured by DXA or bioelectrical impedance [139, 179, 180, 182, 184, 185].

Adding resistance training to the diet regime was studied over 16 weeks in 83 volunteers with T2DM, randomly assigned to either an energy restricted, HPD (33%TE protein, 43%TE CHO, 22%TE fat) or a conventional high CHO, low fat diet (19%TE protein, 53%TE CHO, 26%TE fat), with both diets restricted to 6 – 7 MJ. In addition to the diets, volunteers in both groups were randomly assigned to resistance training or no resistance training (creating four intervention groups). An overall decrease in weight, FFM (measured by DXA) and waist circumference was reported with a significant group effect favoring the HPD + resistance training group [186].

1.7.2 Long-term maintenance of weight loss

In a large multicentre, randomized, controlled trial investigating the maintenance of weight loss (11.0 kg) achieved with a VLCD in 773 participants from eight European countries, Larsen et al reported greater success in the high-protein, low-glycaemic diet group compared to the low-protein, high-glycaemic diet groups and controls. Weight regain was 1.62 kg in the low-protein group compared to 0.69 kg in the high-protein group (intention-to-treat analysis).

Although the participants did not reach the planned target for difference between the protein intake (5% increase achieved with the target set as 12%)

and the target for difference in glycaemic index (GI; 4.7 U increase achieved with a target of 15 U) the study show that even small increases in protein contend and a small decrease in GI resulted in less weight regain during the follow-up period after weight loss [187].

1.8 **Effect of high dietary protein on serum lipids and glycaemic control**

1.8.1 Serum lipids

Clifton et al found an ongoing benefit with decreased blood glucose, C-reactive protein (CRP), total and LDL cholesterol and increased HDL cholesterol, regardless of diet allocation, with sustained weight loss [143]. Similarly, Due et al found no beneficial effect of the high protein diet on inflammation markers in a 6 months weight loss study.[144]. Likewise no significant difference in the change in cholesterol, LDL, HDL or triacylglycerol was detected in the study by Abete et al [141].

In a HPD weight loss study, triglycerides (TG) decreased by 23% at 16 weeks compared to 10% in SPD diet in hyperinsulinaemic overweight subjects [166]. A similar effect was also seen in a 10 week intervention study in obese women [145]. Other studies have shown a significant decrease in TG with no treatment effect [137, 140, 180, 188].

High density lipoproteins (HDL) increase more in the HPD compared to SPD in some studies under conditions of weight loss [167, 189, 190]. Other studies have reported decreases in HDL cholesterol following a HPD compared with a SPD [140, 191]. Some studies have reported decreased HDL with no differences between diets [137, 138, 180]. However, numerous studies have reported an increase in HDL after weight loss regardless of diet allocation [139, 143, 145, 166, 188, 192, 193].

In energy restricted high protein diets, a significant decrease in LDL has been reported in T2DM (5.7 vs. 2.7%, $p<0.001$) [139]. Many studies show a decrease in LDL from baseline, with no difference between groups [143, 145, 171], however others report increased LDL in both groups, with no difference between groups [173, 194]. In one study using the very low CHO diet LDL increased in the HPD by 1.6 mg/dl and decreased in the high CHO diet by 7.4

mg/dl (p>0.2). This low CHO diet was also high in fat, predominately animal fat [173]. The second study reported an initial decrease in LDL by 6 months, but this was not sustained to 12 months and the authors attribute this to a greater than recommended intake of saturated fatty acids in the HPD and high fat groups [194].

In very low CHO diets (ketogenic diets) mixed effects on serum lipids have been seen with a significant decrease in TG and a significant increase in HDL cholesterol. However, in most studies a significant increase in LDL cholesterol have also been observed [169, 171, 173, 175].
The effect of low CHO/high protein diets on serum lipids are inconclusive and most studies have shown similar effects with decreased total cholesterol, TG and LDL cholesterol and increased HDL cholesterol under conditions of weight loss regardless of nutrient composition. However, in the very low CHO diet an increased LDL cholesterol level has been reported. Caution should therefore be taken to monitor cardiovascular risk factors when low CHO diets are chosen for weight loss.

1.8.2 Glycaemic control

For participants with impaired glucose metabolism, the HPD have shown beneficial decreases in FBG and PPG. In a five week weight maintenance study, investigating eight male T2DM patients, a doubling of protein intake, from 15 % to 30 %TE, reduced the 24h mean glucose concentration by 36% ($p<0.001$) and mean 24h insulin concentration decreased by 25%. These changes resulted in a decrease in HbA1c of 22% [195]. After five weeks consuming a HPD, mean peak blood glucose was lower compared to a SPD after every meal, the mean 24h glucose response decreased significantly from baseline in the HPD ($p<0.02$). [196]. This study, however, was a highly controlled feeding study where all foods were prepared and eaten at the research clinic on the day of the collection of samples. Other studies have shown diverse results for the changes in glycaemic control.

Layman et al found fasting blood glucose to be lower in the SPD group after 10 weeks intervention with either a SPD (68g protein/day) or a HPD (125 g protein/day) in 24 overweight women consuming isocaloric diets. This difference

was attributed to an increased serum insulin 2h post meal test in the SPD compared to the HPD [145].

In a recent study, including 49 volunteers with obesity and T2DM, the effect of a very low CHO diet (20 g CHO) vs. a low fat low GI energy restricted diet (energy deficit 2.1 MJ) on HbA1c was tested over 24 weeks [197]. HbA1c decreased significantly more in the low CHO diet compared to the low fat diet (1.5% and 0.5% respectively, p=0.03). However, when the change in HbA1c was adjusted for baseline values the difference was only borderline significant (p=0.06). More volunteers in the low CHO diet group were able to reduce anti diabetic medication (95.2% in the low CHO diet vs. 62.1% in the low fat low GI diet). In this study no association between the changes in weight and HbA1c was found [197].

Numerous studies have looked at the effect of HPD compared to SPD on FBG and most studies have found a significant decrease with weight loss regardless of macronutrient composition [138, 139, 143, 144, 166, 179, 188-190]. However, these studies did not include T2DM volunteers.

In a recent study including 99 T2DM volunteers, two diets were given for 12 months. The diets were designed to give ≤30%TE from fat in both diets with HPD (30%TE from protein) and SPD (55%TE from CHO). The intervention was a randomized parallel study. The primary endpoint was changes in HbA1c over 12 Mo [188]. There was a significant decrease in HbA1c in both groups with time (HPD -0.23% and SPD – 0.28%; p<0.001); but there was no treatment effect. The change in HbA1c was associated with the decrease in energy intake (r=0.31; p=0.01) and to waist circumference (r=0.34; p=0.008). There was a clear association between the perception of self-management of the diet and the changes in HbA1c. Participants with the highest score for self-management had a decrease in HbA1c of 0.87% compared to 0.03% in participants with the lowest self-monitoring scores [188].

In contrast to the highly controlled weight maintenance study by Gannon et al, where a clear benefit on glycaemic control in the HPD was seen, this is not repeated long-term under weight loss conditions in “free living volunteers”. Most studies report similar effect on glycaemic control when comparing a HPD to a SPD with concurrent weight loss.

1.8.2.1 Postprandial blood glucose

Acute increases in blood glucose are harmful and there does not seem to be a lower threshold under which diabetes complications will not occur [198]. Restoring blood glucose to normal range is the aim of diabetes treatment. In both the DCCT and the UKPDS trials it was shown that achieving HbA1c in the lower range (≤6.5% and ≤7% respectively) substantially decreased the morbidity rate [48, 199]. However, decreasing acute surges in postprandial blood glucose (PPG), which are present even in patients with good glycaemic control with HbA1c levels ≤7% [53], may further reduce the risk of micro- and macro vascular complications. In studies designed to target FBG and HbA1c the benefit on CVD risk was minimal; however in studies also targeting PPG CVD events were reduced, indicating a strong independent effect of PPG (reviewed by Bonora [200]). In these reviewed studies, the two hour BG after an oral glucose tolerance test was used (OGTT). The OGTT, a surrogate measure for PPG may not be able to be directly extrapolated to post meal conditions. However, in an eleven year follow up study by Hanefeld et al, the 1h PPG after the patient's normal breakfast was used. This study showed that patients who died from myocardial infarct had higher PPG but similar FBG to the group without events [50]. Cavalot et al studied 529 T2DM in whom blood glucose profiles were available (blood profile was FBG, 2h post breakfast, 2h post lunch and before dinner) and they found blood glucose after lunch to predict the incidence of CVD more strongly than FBG [201].

Distributing CHO evenly across the day has been recommended for people with diabetes, in order to maintain a stable BG throughout the day and thereby minimizing postprandial peaks [202]. However, there are only very few studies investigating the effect of CHO timing [203]. The CGMS was used to determine glycaemic response in 23 adults with T2DM, when comparing four different meal patterns. The meal sequences were equal in total CHO, but with CHO loaded at different meals. Even distribution with equal amount of CHO and glycaemic load at all three meals of the day was compared to meals with CHO loaded at breakfast, lunch or dinner and at the same time minimizing CHO at the two other meals of the day. This study showed that minimizing CHO at breakfast and dinner, and shifting it to lunch time provided lower 24h blood glucose excursions measured as AUC, peak BG and time spent with a BG

above 12 mmol/L [203]. Contrary to this a study looking at equal nutrient composition at breakfast and lunch meals using CGMS, found no significant difference in AUC and peak BG [204]

1.9 **Adverse effect of high dietary protein**

To date there is no evidence that high protein diets adversely affects kidney function in healthy persons [205]. Dietary protein has been linked to adverse effects on renal function, and a protein intake above the recommended level has been discouraged [206]. However, evidence in this field is lacking. Martin et al reviewed the literature reporting the role of dietary protein in chronic kidney disease, normal kidney function and kidney stone formation. They found that protein restriction may be warranted in patients with existing kidney disease, but there was no evidence to suggest that high protein diets have a detrimental effect in healthy persons [205]. It has been assumed that the HPD will have a deleterious effect on renal function in the diseased kidney in humans as has been shown in animal studies investigating the effect of high protein diets in rodents with 80% renal ablation [207].

High protein intake result in increased renal plasma flow and increased GFR, but this may be a normal autoregulation designed to enable the kidney to excrete nitrogenous waste products at a higher rate [208]. In the study by Skov et al increased renal size and volume was reported. However, no deleterious effect on albuminuria was found after 6 months intervention with high protein diet in 65 obese participants [137]. The same results were reached in the study by Preis et al looking at protein intake in ≈ 44,000 healthy men. IHD was reported in 2959 men over a period of 18 years. The relative risk in the fully adjusted highest quartile of protein intake was 1.08 (95% CI: 0.95, 1.23) [209].

The fear that high protein diets may increase the risk of coronary heart disease (CHD) has recently been the focus of a 26 year follow up study from the Nurses Health Study [210]. In the 26 year follow up analysis, more than 84,000 women were surveyed. 2210 cases of CHD and 952 cases of death from CHD were reported. The study looked at the association of different sources of protein (red meat, poultry, fish, dairy and nuts) on the relative risk of CHD (data was analyzed using the fully adjusted model including age, BMI, total energy intake, cereal fibre intake, alcohol, trans fat, multivitamin supplementation,

supplements of E vitamin, cigarette smoking, menopausal status, aspirin use, physical exercise, and follow up period). An increased risk of CHD was found in the group consuming most red meat, processed meat and high fat dairy, whereas consuming fish, poultry, low-fat dairy and nuts exchanged serving by serving for red meat was associated with lower risk of CHD. It was therefore cautioned that high red meat consumers might substantially decrease their risk of CHD by shifting to other protein sources [210]

In a recent population study, including half a million participants from the National Institutes of Health (AARP) diet and health study, an elevated risk of CHD (HR= 1.17 for men and 1.50 for women) was found in the highest quintile (68.1g/1000 Kcal) of red meat intake compared to the lowest quintile (9.3 g/1000Kcal). Consumption of processed meat increased the relative risk of CHD by 1.09 in men and 1.26 in women. It was concluded that high intake of red meat and processed meat was associated with a moderate higher risk of death from all causes, death from cancer and death from CHD [211].

Recently the association between the consumption of unprocessed and processed red meat and the incidence of T2DM has been examined. In a study looking at results from the Health Professional Follow-up Study, the Nurses Health Study1 and the Nurses Health Study ll a HR of 1.12 was found per serving of unprocessed red meat, 1.32 for processed meat and 1.14 for total red meat consumption was found. These results were confirmed by an updated meta analysis of studies looking at the risk of T2DM when consuming red meat [212].

1.10 **Gastric emptying**

Meal composition has a direct impact on gastric emptying (GE) in T2DM patients [213]. In preload studies dietary fat and protein have been shown to slow gastric emptying in T2DM [214, 215]. Hyperglycaemia and hypoglycaemia has also been shown to modulate GE [213], so that a high BG results in delayed and a low BG results in increased GE in order to regulate BG towards normal. Gastric emptying lag time (the initial gastric emptying speed, 5% GE) is longer in poorly controlled T2DM patients compared with patients with good glycaemic control [213].

1.11 **Conclusion on dietary intervention**

A diet that is deficient in energy will result in weight loss as has been reported in multiple intervention studies [216]. High protein diets are as successful in producing weight loss as high CHO diets, under some circumstances more successful. Ad libitum high protein diets have shown good short term effects but this is not sustained long-term. Energy restricted high protein diets have a beneficial effect on weight loss and loss of fat mass [137]. The assumed sparing of lean body mass has not been consistently shown. No adverse effect has been documented for high protein diets on renal function in T2DM. However, in very low CHO diets a significant increase in total and LDL cholesterol have been shown and this may cancel out the beneficial decrease in TG and increase in HDL cholesterol also reported from these studies [170]. In HPD with a high proportion of the meat consumed as red meat or processed meat an increased risk of T2DM, mortality of all cause and of CHD in particular have been reported [210, 211].

1.12 **Scope of this thesis**

Main study:

Because of the doubt about the effect of HPD on renal function in T2DM, there is a need to show that high protein diets are safe when accompanied with weight loss. The primary aim of the main study was to explore whether a weight-loss diet with a high protein to carbohydrate ratio has a beneficial effect on renal function in subjects with type 2 diabetes and with microalbuminuria and/or renal impairment.

We hypothesize that a high-protein weight-loss diet will be as effective as a standard protein weight loss diet in achieving weight loss without negatively affecting renal function measured by GFR and cystatin C.

Sub study:

Diverse effects on blood glucose have been shown when comparing a HPD to a SPD. The relationships between BG and microvascular complications are well established. We wanted to investigate, using CGMS, differences in postprandial glucose excursions in patients on two diets: a high-protein weight-loss diet and a high-carbohydrate weight-loss diet.

We hypothesize that a high-protein weight-loss diet will result in a reduced glycaemic response compared to a high-carbohydrate weight-loss diet in both fasting and postprandial conditions.

Study 3:

Currently an even distribution of CHO over all meals is recommended in the diabetes diet. However there are very few studies showing this pattern to be optimal in preventing hyperglycaemia. As the purpose of this thesis is to improve renal function, we explored other ways of improving renal function by reducing hyperglycemia without weight loss. The aim of study 3 was to explore the effect of withholding carbohydrates at the first meal after an overnight fast on the impact of glucose excursions at lunch time and overall 24 hr glycaemia.

We hypothesize that withholding CHO at breakfast will result in higher blood glucose excursions at lunch time, but with lower overall glycaemia.

Chapter 2: Method

2.1 **Method**

2.2 **Method for the Main study and CGMS sub-study**

2.2.1 Study design

"Weight Loss, Protein and Renal Health" was a randomized, parallel group, dietary intervention study. Two weight loss diet groups studied for a minimum of 12 months, after a three month run in period.

2.2.2 Recruitment

Participants were recruited by advertisement in local papers, radio and television. They were included if they met the following inclusion criteria:

- Male or female aged between 18 and 75 years,
- BMI >27 kg/m^2
- Type 2 diabetes verified by a FBG >7.0 mmol/L and/or a two hour BG >11.1 mmol/L or if they were on treatment for diabetes,
- Had microalbuminuria (30-300 mg/24h) or an albumin to creatinine ratio of 3.0-30.0 mg/mmol, with or without moderately impaired renal function (e-GFR 40-60 ml/min/1.73m^2).

Participants were excluded if they met any of the following exclusion criteria:

- BMI less than 27.0 kg/m^2
- a history of malignancy, or a history of metabolic disease such as liver unstable cardiovascular, respiratory or gastrointestinal disease
- pregnant or breastfeeding
- impaired kidney function due to unrelated disease e.g. glomerulonephritis (this was verified by medical history and urine samples measured for blood at the screening visit)
- uncontrolled hypertension (resting recumbent BP>160/100 mmHg)
- a history of alcohol abuse (>5 standard drinks /day)
- known hypoglycaemia unawareness

- unwilling to be randomized to either diet group

Three hundred fifteen volunteers with type 2 diabetes were screened for eligibility and of these 241 were ineligible for the study, mainly through absence of persistent microalbuminuria. Eleven refused to participate and 7 withdrew consent prior to randomization. Fifty-six participants were included in the study and of these eight withdrew consent before commencing the dietary intervention. Twelve participants (7 HPD, 5 SPD) discontinued during the study due to unrelated illness, social/financial problems with the study regime or because they were not satisfied with achieved weight loss. Thirty six participants (27 men and 9 women) completed the one year dietary intervention (19 HPD and 18 SPD).

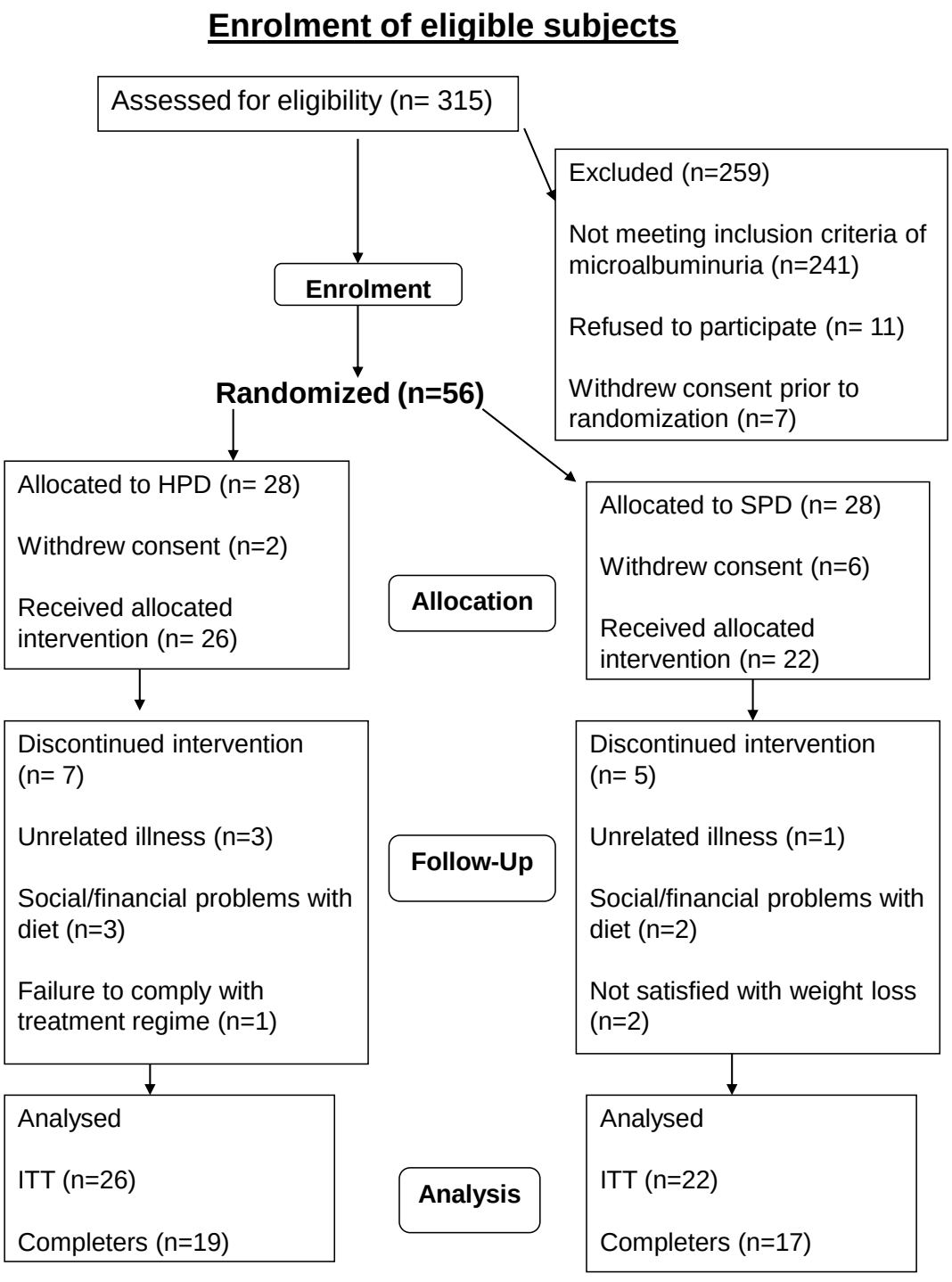
2.2.2.1 Run in period and safety measures

As this population is considered at a higher risk for macrovascular disease, a run in period was added to secure optimal diabetes and blood pressure control prior to the study start. All potential volunteers underwent a physical examination conducted by an endocrinologist. The endocrinologist monitored the volunteer's diabetes and blood pressure control throughout the study and adjusted medication accordingly. The endocrinologist was blinded to the diet allocation. The target BP was a systolic BP of 120 and a diastolic BP of 80 mmHg. The aim for HbA1c was <7% as suggested by the current guidelines [217]. Insulin was initiated if HbA1c was consistently above 8% despite maximum oral hypoglycaemic treatment (two consecutive visits).

Participants were withdrawn from the study if there was a 10% or greater decline in renal function at any time confirmed on two consecutive occasions or a 20% decline in renal function at any time (no volunteer was excluded due to deterioration of renal function). There were no restrictions on medication used (including insulin) but doses were reduced to avoid hypoglycaemic episodes with weight loss and energy restriction.

All potential volunteers (n=56) were approached to take part in study 2. Eight volunteers declined participation. A total of thirty nine volunteers (21HPD and 18 SPD) agreed to participate.

Figure 3: The enrolment of eligible subjects.



2.2.3 Randomization:

Volunteers were randomized into 2 diet groups blocks matched on Sex, BMI and HbA1c. Due to the long inclusion period (2008-2010) the randomization was done using the minimization method assigning participants to the two groups while attempting to minimize imbalances between treatment groups. If for example average BMI was substantially higher in group A compared to group B, the next participant with a lower BMI would be allocated group A. The randomization was performed by a trial manager not directly involved in the intervention.

2.2.4 Dietary intervention

A high protein diet (HPD) was compared to a standard protein diet (SPD). The aim for the nutrient composition was for protein to fat to carbohydrate was 30:30:40 %TE for the HPD and 20:30:50 %TE for the SPD. The planned range of protein intake was 90-120 g/day in the HPD vs. 55-70g/day in the SPD. Saturated fat was planned to be similar in both diets around 10%TE. Alcohol was limited to 2 standard drinks per week (4g or 2%TE). Fibre intake was high in both diet plans (31g/day in HPD and 36g/day in SPD). As both diet regimes aimed at reducing body weight, the kJ content was 6000 kJ subject to change according to body size (energy intake ranging from 6000-7000 kJ was used and %TE for protein changed accordingly).
The two diets differed only in the proportion of protein and carbohydrate; total fat and saturated fat (SAFA) was the same in both diets.

A breakdown of the food choice template is presented in table 2.

An example of the diet allocation can be found in appendix 2

Table 2: Food choice template:

Foods used in template	6 MJ HP	6 MJ LP
Food	**Amount**	**Amount**
High fibre Breakfast cereal	40g	40g
Wholegrain bread (min. 5g fibre/100g)	80g	120g
Savoury crisp bread		1 biscuit
Milk (Fat 1-2%)	250 ml	250 ml
Low Fat yoghurt	200g	
Cheese (full fat)	25g	25g
Fruit	300g	450g
Lean cooked meat, poultry or fish for lunch	50g	0
Lean meat, poultry or fish for dinner (raw weight)	200g	100g *
Legumes		100g *
Potato or sweet potato / rice or pasta (raw weight)		200g/50g
Vegetables and salad	2½ cups (300g)	2½ cups (300g)
Oil or margarine	3 tsp	5 tsp
Alcohol	2 standard drinks/week	2 standard drinks/week

Table 3: Nutrient composition

Comparing the HP to the LP diets.	6 MJ HP	6 MJ LP
Nutrients	**Amount**	**Amount**
Energy (DF) kJ	**5785**	**5915**
Protein (g)	**100**	**68**
Fat (g)	**45**	**44**
SAFA (g)	**16**	**14**
Carbohydrate (g)	**121**	**161**
Sugar (g)	**72**	**61**
Starch (g)	**44**	**99**
Alcohol (g)	**4**	**4**
Dietary Fibre (g)	**31**	**36**
Protein %TE	**29**	**19**
Fat %TE	**29**	**28**
SAFA %TE	**11**	**9**
Carbohydrate(+DF) %TE	**36(+4)**	**46(+5)**
Alcohol %TE	**2**	**2**

In the upper panel, the food choice template is outlined with total amount of the individual foods needed. In the lower panel nutrients in the template are given in g/day and %TE/day. * meat, poultry and fish are allowed as 100g six times per week in addition a vegetarian meal containing 100g of legumes was recommended for the seventh day.

2.2.5 Compliance measures

Participants were asked to weigh all foods eaten for the duration of the intervention (twelve months). If weighing the foods was not possible (e.g. when eating out) participants were asked to estimate portion size using household measurements (e.g. teaspoon, tablespoon, cup, etc.).

Digital kitchen scales with precision down to 1g were provided.

2.2.5.1 Diet information booklets:

Diet information booklets specifying the foods needed for the two diets, a food selection guide for the different energy levels and general advice in how to

comply with the diet regime were handed out. A sample daily meal plan and a selection of diet specific recipes were also included (appendix 2).

2.2.5.2 Checklists:

For the duration of the study, starting at day one of the diet treatment, participants completed daily weighed records of all foods and liquids consumed. A pre-printed checklist was provided (an example of the checklist for the two diets is given in appendix 3).

2.2.5.3 Food frequency questionnaire

Dietary intake was measured by the food frequency questionnaire (FFQ) developed by the Cancer Council Victoria specifically for use in Australian adults [218] and validated by Hodges et al [219]. The questionnaire is a 74 item four page questionnaire, with questions linked for cross referencing intake for completeness. As an example the first questions asks about the amount of fruits eaten per day, with answers ranging from none to four or more. This question is linked with a question on page three where the respondent is asked to specify the type of fruits eaten and the frequency in the range of never to 3 or more times per day. From these responses portion sizes are calculated. To calculate nutrient composition the NUTTAB95 [220] database was used. The output includes total kJ, protein, CHO (total, starch, sugars, dextrin, and dietary fibre), fat (total, saturated, polyunsaturated, monounsaturated and cholesterol), alcohol, some vitamins and minerals.

The questionnaire was administered at baseline on the day of randomization and was filled in before the volunteer was informed of the diet allocation. Participants were instructed in the importance of filling in all the questions and only record one response per line unless otherwise instructed. Questionnaires were completed using a pencil so that errors could be erased completely. The FFQ was repeated at four months where the volunteers were asked to report dietary intake during the last four months from randomization. Capturing the dietary changes during the entire intervention period of 12 months the FFQ was repeated at the last visit.

2.2.5.4 Three day diet diary

Additionally a three day diet diary was completed before randomization (before instruction of the diet had commenced), four months and at the end of the study in conjunction with the continuous blood glucose measuring study. The diaries were open ended with a column for time, a column for food/ drink taken, a column for preparation method and a column for amount in weight or household measures. The diary book contained pages for registering daily exercise performed and a page for tracking blood glucose. An example of a diet diary is provided in appendix 4.

Dietary intake was reported using the three day diet records at four and eight months and at the end of the study. The three day diet record was chosen because of better correlation between reported and measured protein intake (FFQ and daily checklists were also assessed; but the reported protein intake correlated less with measured intake).

2.2.6 Height and weight

Body height was measured at baseline (day of randomization), with the volunteers not wearing shoes, using a stadiometer to the nearest 0.1 cm (SECA, Hamburg, Germany).

Volunteers were weighed at all visits to the clinic, wearing light clothing without shoes, using calibrated electronic digital scales to the nearest 0.05 kg (Weight range 0-220kg with a deviation at 220 kg = 0.06; Mercury, AMZ 14, Tokyo, Japan).

2.2.7 Blood pressure

The volunteers were resting in a seated position for at least five minutes before measurement. The cuff was placed on the bare arm so that the bladder of the cuff was applied over the brachial artery of the upper arm. The arm was supported on the arm rest of the clinical chair, which ensures that the arm was always at the level with the heart. The same arm was used for each visit. An average of three measurements taken at least two minutes apart, was used. The measurements needed to be consistent so that systolic blood pressure was within the range of 10 mmHg and diastolic blood pressure was within the range of 5 mmHg and more measurements were taken if there was inconsistency. The

very first measurement was discarded. When possible, the clinic visits were arranged at similar times of the day for consistency.

(Philips SureSigns VS3 Patient Monitor, with Philips cuffs small, medium, & large).

2.2.8 Body composition

Body composition was assessed at the Endocrine and Metabolic unit at the Royal Adelaide hospital by dual-energy X-ray absorptiometry (DXA), (Norland DXA bone densitometer medical systems).The DXA machine sends a thin, invisible beam of low-dose x-rays with two distinct energy peaks through the body. One peak is absorbed mainly by soft tissue and the other by bone. The accuracy of the whole body scan was within 2% for total soft tissue.

For the whole body scan a 6.5x13mm resolution was used together with a scan speed of 260mm/sec. in the table below the coefficient of variation based on 3 scans of the same 14 subjects are outlined (manufacturer data).

Table 4 Variation DXA

The coefficient of variation as given by the manufacturer (Norland DXA, operators guide 434D142 rev.F)

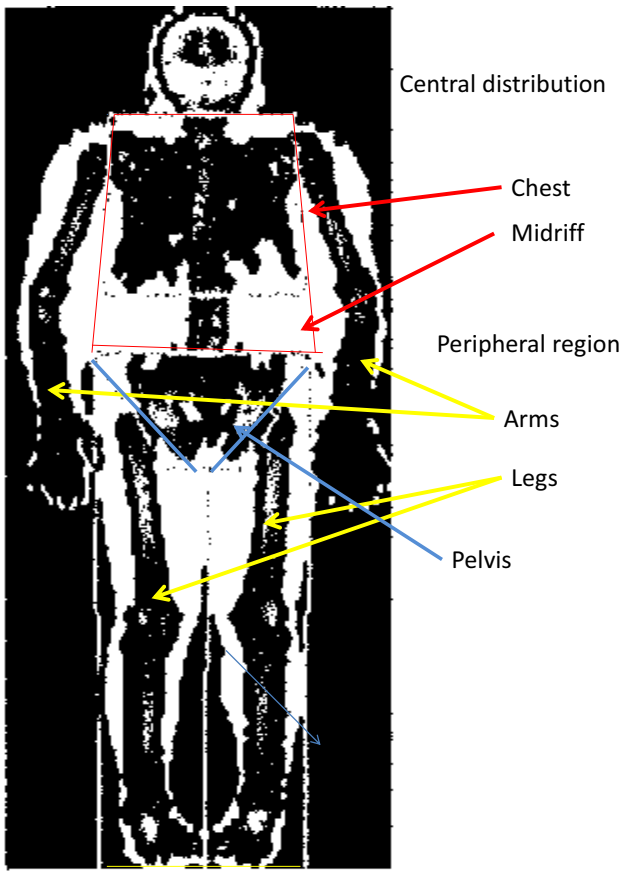
	Total body C.V.	Head C.V.	Trunk C.V.	Abdomen C.V.	Arms C.V.	Legs C.V.
Soft tissue mass	0.1%	1.4%	0.81%	2.3%	1.8%	0.57%
Lean body mass	0.93%	1.4%	1.6%	2.6%	2.3%	1.5%
Fat mass	1.4%	1.9%	1.9%	3.7%	4.8%	2.1%
Percent Fat	1.4%	0.91 %	1.7%	2.2%	3.5%	2.0%

Volunteers were placed on the table in a supine position. They were wearing hospital gowns to avoid any metal clasps or zippers. Arms and legs were strapped to the side to minimize movement during the scan. The operator

stayed in close proximity of the DXA machine to insure that there was minimal subject movement.

Figure 4: Fat distribution

In order to determine the distribution of fat mass as visceral or peripheral fat two indexes were computed.



The central fat mass:

This area includes the chest and midriff.

Peripheral Fat mass:

This area includes the arms and legs

The pelvis area was not included because it contains part abdominal fat and part gluteal fat mass, these areas of fat mass has been shown to have opposite effect on insulin resistance and arterial stiffness leading to increased risk of diabetes and CVD [221, 222].

2.2.9 Biochemical measurements

The following urine and blood samples were sent to the Institute of Medical and Veterinary Science (IMVS), a certified commercial laboratory, for processing.

The Olympus system analyzer was used to analyze creatinine, albumin, urea, lipids, FBG

2.2.9.1 24h Urine Save

Urine was collected 24-hour prior to clinic visit. Urine was kept cold in a cooler bag with ice bricks until it could be processed– no preservative was added. The urine samples were processed in the Clinic; participants were asked if the sample was complete. Weight and volume was recorded. Date & times of commencement & completion as reported by volunteer were entered into 24hour urine record book. The urine sample was thoroughly mixed. Two 8 ml (10 ml tube) aliquots of urine were taken for analysis of creatinine, albumin, urea, protein, Na, K, phosphate. One aliquot was sent to the IMVS for analysis. The other aliquot was stored at -20°C at the CSIRO as a back-up sample.

2.2.9.2 Spot urine sample

A fresh urine sample was collected at the Clinic at screening, baseline, four weeks, four months and at the end of the study for the analysis of the albumin to creatinine ratio. Two aliquots were saved. One was sent to the IMVS for analysis of albumin level and albumin to creatinine ratio.

2.2.9.3 Urinary creatinine

24h Urine was collected without the use of preservatives. The sample was analyzed using the kinetic colour test (Jaffé). In an alkaline medium, creatinine forms a yellow/orange compound when combined with picric acid. At 520/800nm the change in absorbance is proportional to creatinine concentration. (Olympus analyzer, Biorad liquicheck urine chemistry controls cat. No. 397 or 398)

2.2.9.4 Urinary albumin

Urinary albumin was collected in a 24h urine collection without preservatives. Anti-human albumin antibodies were added to yield an insoluble aggregate. Albumin concentration is directly proportional with the absorbance of this aggregate. (Olympus System Reagent Microalbumin OSR6167)

2.2.10 Blood samples

2.2.10.1 HbA1c

Whole blood samples were collected in vacuum collection tubes containing EDTA. Analysis of HbA1c was done by ion-exchange high-performance liquid

chromatography in a hydrolyzed whole blood sample (Bio-Rad variant II haemoglobin A1c pack)

2.2.10.2 FBG

Fasting samples were collected in sodium fluoride/EDTA tubes, and stored on ice. “Glucose was phosphorylated by hexokinase (HK) in the presence of adenosine triphosphate (ATP) and magnesium ions to produce glucose-6-Phosphate and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G6P-DH) specifically oxidises glucose-6-phosphate to gluconate-6-phosphate with the concurrent reduction of NAD^+ to NADH. The increase in absorbance at 340nm is proportional to the glucose concentration in the sample”. (Olympus System Reagent Glucose OSR 6121 and OSR 6221).

2.2.10.3 Total cholesterol

Cholesterol esters are hydrolyzed by cholesterol esterase. The free cholesterol is then oxidized with cholesterol oxidase to form cholesterol-3-one and hydrogen peroxide. This couples with 4-aminoantipyrine and phenol in the presence of peroxidase to form a chromophore. The red dye formed (quinoneimine) is measured spectrophotometrically as increased absorbance at 540/600 nm. (Olympus analyzer, Cholesterol reagent OSR6516).

2.2.10.4 Low Density Lipoproteins

LDL cholesterol was calculated for each person in fasting samples free of chylomicrons using the Friedewald formula: $C\text{-}_{LDL} = C\text{-}_{plasma} - C\text{-}_{HDL} - TG*0.45$

2.2.10.5 High Density Lipoproteins

HDL cholesterol was measured using an enzymatic colour test in human serum. R1 which is an anti human-β-lipoprotein antibody that binds to lipoproteins other than HDL (LDL, VLDL and chylomicrons) is added to the sample. R2 is added (PEG-modified enzymes/4-amino-antipyrine/buffer) which forms a purple-blue-dye. The colour intensity of the dye is directly proportional to the HDL concentration and is measured photometrically at 600/700 nm wavelength (HDL-Cholesterol reagent OSR6587 for use on the AU2700 and AU5400 systems only).

2.2.10.6 Triglycerides

Triglycerides (TG) are hydrolyzed using microbial lipase to form glycerol and fatty acids. Glycerol is phosphorylated by adenosine triphosphate to give glycerol-3-phosphate. Glycerol-3-phosphate is then oxidized by glycerol-3-phosphate oxidase to dihydroxyacteone phosphate and hydrogen peroxide to produce a chromophore which is read at 660/800 nm. The increase in absorbance at 600/800 nm is proportional to the triglyceride content of the sample (Olympus Triglyceride Test System is an *in vitro* diagnostic reagent).

2.2.10.7 Serum Creatinine

Blood was collected in tubes containing a serum clot activator. The sample was analyzed using the kinetic colour test (Jaffé) where in an alkaline medium; creatinine forms a yellow/orange compound when combined with picric acid. At 520/800nm the change in absorbance is proportional to creatinine concentration (Olympus analyzer, control no. ODC0003 or ODC0004).

2.2.11 Glomerular Filtration Rate (GFR)

GFR was measured directly by ^{99m}Tc-diethylenetriamine-pentraacetic acid (^{99m}Tc-DTPA) at baseline and at the end of the study. Upon admission to the Department of Nuclear Medicine at the RAH a dose of 50 - 60 MBq ^{99m}Tc-DTPA was injected. Blood samples were taken exactly one and three hours after the radiopharmaceutical injection. 5-10 ml whole blood was collected in tubes containing anti-coagulant and centrifuged at 3000 rpm. 200µl plasma and 200 µl of the standard together with 4 ml water was added into a labelled tube and counted in a multi sample counter. The rate of clearance of ^{99m}Tc-DTPA corresponds with GFR.

This method of measuring renal function is considered the gold standard. However, it is not practicable in the everyday clinic because it is time consuming, more expensive, invasive and requires radioactive tracer. Therefore numerous equations for estimating GFR (eGFR) have been developed.

Today the most widely used equations are the Cockroft and Gault (CG) with and without adjusting for body surface area (BSA), the modification of diet in renal disease (MDRD) and the chronic kidney disease- epidemiology collaboration (CKD-EPI). Numerous studies have looked at the bias between

the different equations and the isotope measured GFR, and no clear consensus has been reached regarding the equation best suited for estimating GFR (eGFR) [223]. As participant's renal safety was paramount, GFR was estimated at regular intervals using an eGFR based on the MDRD equation during the diet intervention period.

At the end of the study the four most popular equations were assessed to establish which equation performed better in this study population. To assess the agreement between methods a linear regression was performed with each estimated GFR method and the isotope GFR. The tables show the results of the linear regressions and estimated group mean using each eGFR method. The bias of an eGFR method is the difference between the isotope mean and the eGFR mean. The significance and R^2 value of each regression is shown. The group-level precision of each eGFR method is indicated by the 95% confidence interval for the predicted mean. The precision of a eGFR method to estimate individual level values is shown by the prediction intervals in the figures, showing the range of isotope GFR values that may be observed for a specific eGFR value with 95% confidence. Prediction intervals are non-linear, being narrower at values around the eGFR mean and wider at outer edges of the eGFR range.

The abbreviated MDRD equation was better at predicting the change in GFR compared to the other three equations. The CG equations, both the adjusted and the unadjusted for BSA versions, overestimated and the CKD-EPI equation underestimated true GFR in this sample.

The following equations were tested against iGFR to determine the equation with the least bias. The test showed the MDRD4 equation to predict GFR with least bias.

Abbreviated MDRD equation 4 variables (MDRD4):

GFR= 186*(S-Cr)-1.154*(age) -0.203*(0.742 if female)*(1.212 if black).

The Cockroft and Gault equation unadjusted (CG):

Ccr= (140-age(y))*(wt (kg))/72*S-Cr (mg/dl)

The CG adjusted for body surface area (CG-BSA):

Ccr= (140-age(y))*(wt (kg)) / (72*S-Cr (mg/dl))*(1.73/BSA)

(BSA calculated as: BSA (m2) =0.0235*kg*0.51456*cm*0.42246)

The CKD-EPI equation:

eGFR = 141 x min(Scr/k, 1)a x max(Scr/k, 1)-1.209 x 0.993Age x 1.018 [if female]

Table 5 Predicted bias of eGFR compared to iGFR.

	Actual mean for the group	***r^2***	***t***	***p***	***Predicted Mean for the group***	**95% CI for the group**		**95% CI for the individual**	
^{99m}Tc-DTPA	99.2±35.4								
CG	139.8±59.7	0.46	6.2	<0.01	99	92	107	49	150
CG-BSA	109.0±37.6	0.51	6.9	<0.01	98	91	106	51	147
MDRD 4	**93.3±27.5**	**0.57**	**7.5**	**<0.01**	**98**	**92**	**105**	53	144
CKD-EPI	86.9±19.8	0.56	7.5	<0.01	99	92	106	53	145

Predicted bias of eGFR compared to iGFR was assessed at baseline

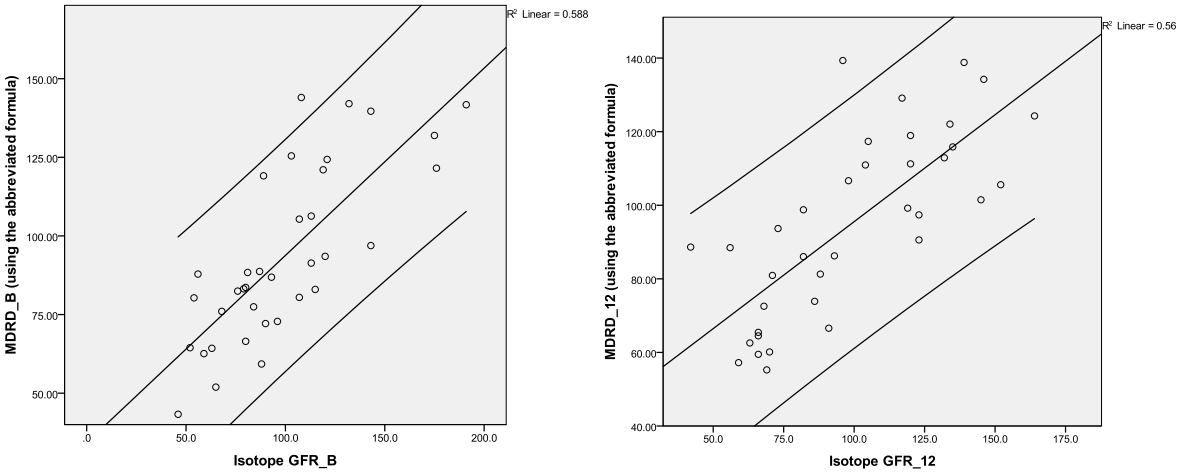
Table 6 b: Level of bias between iGFR and eGFR

	Actual mean for the group	***r^2***	***t***	***p***	***Predicted Mean for the group***	**95% CI for the group**		**95% CI for the individual**	
^{99m}Tc-DTPA	99.0±32.0								
CG	141.6±55.9	0.50	6.0	<0.01	100	93	108	54	147
CG-BSA	115.4±39.3	0.50	6.0	<0.01	99	92	107	53	146
MDRD 4	**94.9±24.8**	**0.58**	**7.2**	**<0.01**	**99**	**92**	**106**	57	141
CKD-EPI	88.4±18.6	0.57	6.9	<0.01	98	91	105	55	141

Predicted bias of eGFR compared to iGFR was assessed again at the end of the study.

Figure 5: GFR regression scatter plot

The MDRD equation still remained the formula with the least bias compared to iGFR at the end of the study.



The mean difference between measured and estimated GFR using the MDRD equation was 5.9±22.7 (-36.0, 54.5). As can be seen the mean absolute difference is small but the variation is large.

The abbreviated MDRD equation was used to estimate GFR in this study.

2.2.12 The following samples were analyzed at the CSIRO

2.2.12.1 CRP

An antigen/antibody complex is formed by coupling anti-CRP antibodies to latex micro particles which reacts with antigen in the sample. This complex is measured turbidimetrically. (Particle enhanced immunoturbidimetric assay, Roche diagnostics, Hitachi 902, wavelengths - main 546nm/secondary 800nm).

2.2.12.2 Cystatin C

Cystatin C is determined turbidimetrically at 546nm with a particle enhanced assay from Randox. Human cystatin C agglutinates with latex particles coated with antibodies against cystatin C (Randox kit, Hitachi 902).

2.3 **Continuous blood glucose measurements (CGMS)**

(CGMS sub-study and study 3)

The Continuous Glucose Monitoring System (CGMS) Medtronic MiniMed, Gold standard system (Northridge, CA) was used in both the sub study and in study 3. CGMS is a well-recognized tool currently used to gain a complete picture of the diurnal blood glucose profiles in diabetic patients. This system has been validated in studies including T1DM patients and is now also used in T2DM patients and in research. The CGMS provides a complete picture of the diurnal BG with minimal inconvenience to the patient [224].

A glucose oxidase–based sensor is inserted into the skin of the abdomen or upper buttock subcutaneous tissue, using a spring loaded insertion device. Insertion is quick and virtually painless. After insertion, the sensor is secured in place with tape and attached via a cable to a pocket size monitor, which is worn on a belt or placed in a pocket, like a pager, while the volunteer continues with normal everyday activities for up to 72 hours.

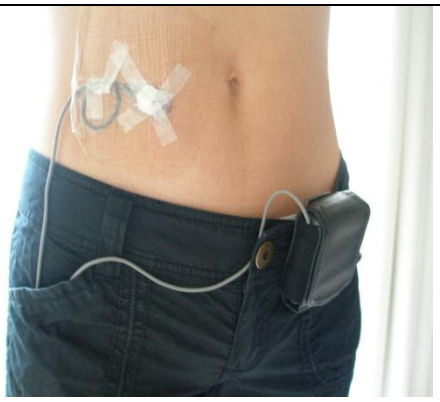
Extracellular glucose is measured every 10 seconds and averaged every five minutes. The measurements are stored in the monitor yielding 288 measurements / 24 hours.

The CGMS monitor was calibrated against corresponding blood glucose levels determined by using finger prick measurements. The participants were blinded to the actual blood glucose measurements which was first revealed when data was downloaded via the Minimed computer program (MEDTRONIC MINIMED software 3.0C program). For calibration purposes and to prevent unsuspected sustained periods of hyper or hypo-glycaemia at least four self-measured blood glucose (SMBG; Medisense, Optimum; Abbott Laboratories, Abbott Park, IL) measurements were used every day starting at initiation of the system and then before breakfast, lunch and dinner and bedtime. Participants were encouraged to use more SMGB measurements if the BG was low or high or if symptoms arose.

The accuracy of measurements using either SMBG or the glucose oxidized sensor have been assessed using time difference between interstitial glucose and SMBG measured blood glucose in 24 sets of data. Results showed a lag

time between the two glucose measurements of 4-10 minutes. In all instances the interstitial glucose lagged behind the measured blood glucose. Therefore significant differences between interstitial and blood glucose may occur in rapid increases or decreases of BG [225].

Figure 6 CGMS monitor and position of the sensor.

NOTE: This figure is included on page 73 of the print copy of the thesis held in the University of Adelaide Library.	
CGMS monitor (Medtronic 2005) http://www.minimed.com/professionals/products(cgms/	Sensor insert beneath the skin of the abdomen.

2.3.1 Gastric emptying breath test (study 3)

Gastric emptying time was measured using ^{13}C-Octanoic acid. For testing solid meals ^{13}C-Octanoic Acid is used, the advantage is that ^{13}C-Octanoic acid is retained in a solid meal while passing through the stomach, followed by a rapid absorption of the ^{13}C-Octanoic acid and subsequent oxidation to $^{13}CO_2$ and elimination through the lungs [226]. The limiting factor for the rate of $_{13}CO^2$ in the breath is gastric emptying [227].

For estimating gastric emptying time two mathematical formulas are fitted [228]. The first formula calculate the excretion of the percentage of the dose given

$$y=at^b c^{-ct}$$

Where y represent the % cumulative excretion of $^{13}CO_2$ /hour; a, b and c are constants and t is the time in hours.

The second formula is derived from the fact that the breath test curve of the cumulative dose eliminated over time is inversely analogous to the curve produced by the radioscintigraphy method.

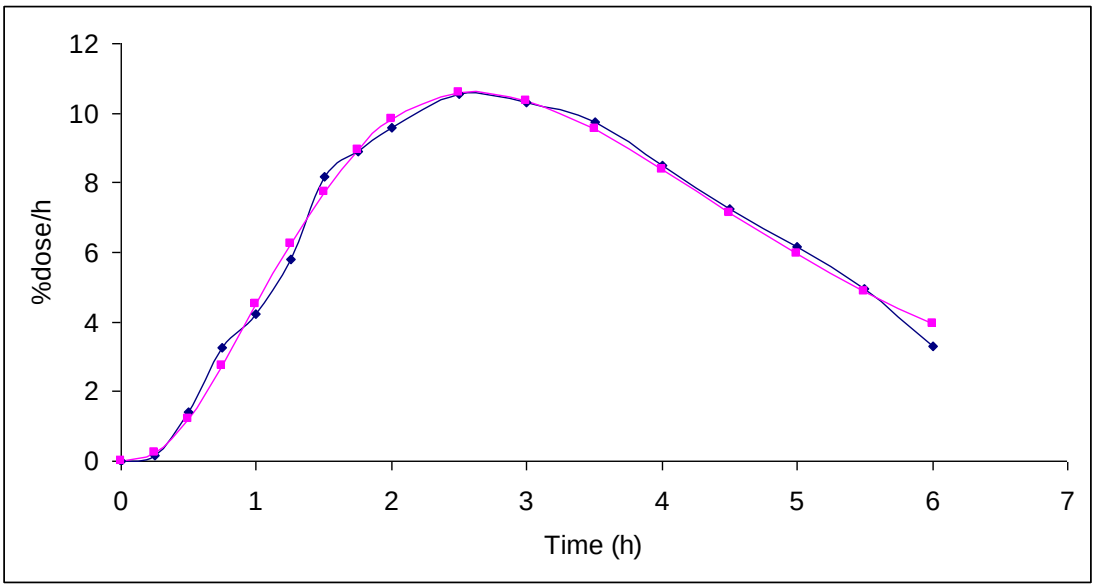
$$y = mk\beta e^{-kt} (1-e^{-kt})^{\beta-1}$$

A nonlinear regression is applied to estimate the constants m, k and β using the least square method. The nonlinear regression analysis is performed using the solver program in excel (Microsoft Corp., Redmond, WA) is then used to allow three variables of gastric emptying [226].

From this analysis three variables are derived T½, Tlag and Tmax

Where T½ is the time elapsed for one half of the meal to be emptied from the stomach, Tlag is the time for 5% of the meal emptying and Tmax is the gastric peak time which is the maximum percentage ^{13}C-Octanoic Acid dose recovered per hour, it is measured at the point of inflection on the recovery curve. All variables are expressed in minutes.

Figure 7: Typical curve for gastric emptying in this study

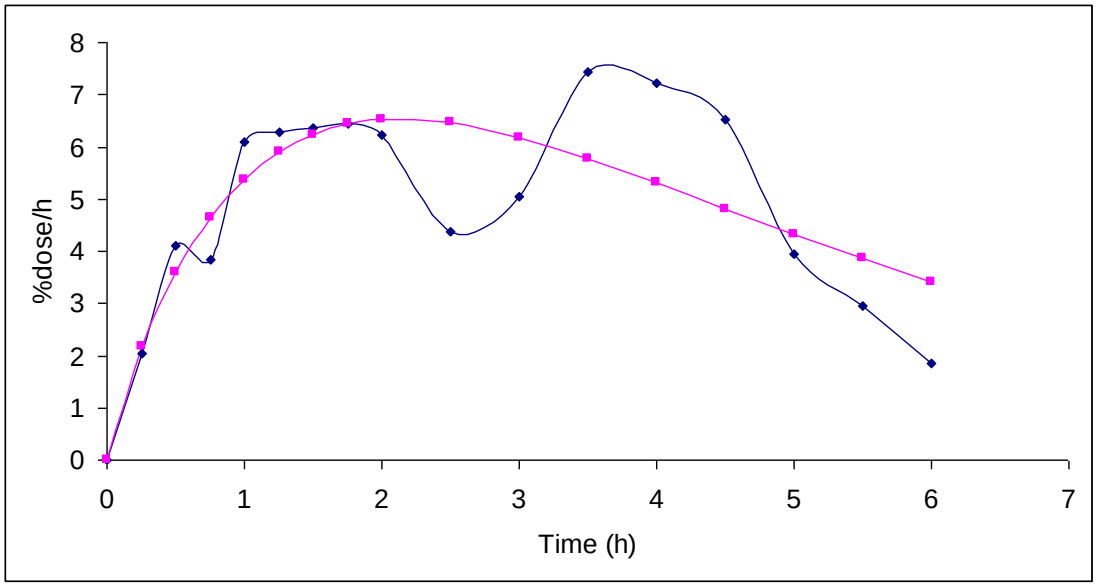


Typical GE curve, the ■ represent the theoretical GE curve. The ♦ show the actual GE time when 100 mg ^{13}C-Octanoic Acid is given in a solid meal.

For correcting the analysis of abnormal gastric emptying with a double peaked emptying curve a mathematical fitting formula has been developed to classify the type of curve and calculate the gastric emptying variables [229].

This formula includes fitting two curves, using the point of the first curve and the starting point of the second curve as nadir. One single curve and one double curve was applied, the correlation between the two curves were compared using the measured data and the fitted line. Correlation had to be >95% for the model to be accepted.

Figure 8 Typical example of a double peaked gastric emptying curve



Typical example of a double peaked GE curve, the ■ represent the theoretical GE curve. The ♦ show the actual GE time when 100 mg 13C-Octanoic Acid is given in a solid meal.

The rate of gastric emptying was assessed using 100 mg of ^{13}C-Octanoic Acid mixed into a one egg “omelette” and included as a part of the sandwich meal given at lunch time.

The breath test continued for 6 hours after the lunch meal on the first day of the test in both weeks. The breath tests were collected in non-sterile 13ml Exetainer tubes (Labco limited, High Wycombe, England) by exhaling through a straw, the tubes were immediately closed tight.

Test schedule: T0 (immediately before the meal for baseline value), T15, T30, T45, T60, T75, T90, T105, T120, T150, T180, T210, T240, T270, T300, T330 and T360

Volunteers were asked to consume the meal within 20 minutes. All foods allocated for the meal were be consumed together, with no snacks or drink other than coffee, tea, water and diet soft drinks were allowed between meals.

All tubes were labelled with subject number, breakfast meal code and date. Volunteers were asked to write the actual time of the test on the tubes. Each breath test was taken in duplicate at every time point.

One breath test tube was sent to the Women's and Children's Hospital, Adelaide, for analysis. The other test tube was stored at CSIRO for back up.

2.4 **Statistics**

All data was tested for normal distribution using the residuals for the model used in the specific analysis (Residual Q-Q plots, histogram, scatter plot)

Descriptive statistics (means, crosstabs and graphs)

Independent samples T-test was used to test for differences between treatments at baseline.

Paired sample T-Test was used to test for changes over time.

A one-tailed, non-inferiority approach (ie HPD was not inferior to a SPD weight loss diet) was used to analyse the main outcome of renal function (iGFR, eGFR, cystatin C and S-creatinine). Results are given as baseline adjusted values.

To answer the question "Are there differences between groups at the end of the study, controlling for baseline pre-test values?" and "did the groups change differently?" an analysis of covariance (ANCOVA) was used. The post test results were used as the dependent variable. The pre-test scores obtained before the treatment started were used as covariates. Group used as a between subject variable. The ANCOVA using pretest as covariate is more powerful than the ANOVA using pretest as a linear component to the dependent variable. When participants have been randomized and a pretest have been collected prior to treatment, the ANCOVA will have higher statistical power [230]

Logarithmic transformation (log10) was used when data was not normally distributed. In the case of log transformation, the descriptive data was given in the non-logged form but all analysis was done in the logged form.

Pearson's product moment was used to determine correlations and multiple linear regression was used to assess associations.

All data are given as means with standard error (mean (SEM)) unless otherwise stated.

For the analysis of the completer's data SPSS 18 for windows, IBM, was used.

For the intention-to-treat (ITT) analysis a full information maximum likelihood (FIML) model was used. The software SPSS AMOS, student version, was used. All randomized volunteers who completed the baseline visit and had all measurements at this visit done were included in the analysis. This method of analysing missing data has been reported to be superior to the standard statistical method using either list-wise exclusion (where all records with missing data at any point are excluded) or pair-wise exclusion (where all reports with missing pairs of variables (one or both variables) are excluded from the analysis) [231].

This is a modified ITT analysis because the volunteers who dropped out of the study were not contacted at the end of the 12 Mo intervention.

Significant level was set at $p<0.05$.

For study 3 differences in between and within groups were analyzed using repeated measures ANOVA. Differences between meals were assessed using paired sample T-Tests. Treatment was used as a within subject factor (CHO = breakfast containing carbohydrate and NoCHO = breakfast without carbohydrate). The groups were divided by glycaemic control according to baseline HbA1c into good control (HbA1c ≤7%) and poorly controlled (HbA1c ≥8%).

2.4.1 Power calculation

The power of the study was based on the paper by Knight et al 2003 who assessed women with impaired renal function over an 11 year period based on serum creatinine only. Essentially they demonstrated a fall in creatinine clearance of 7.72 ml/min /1.73 m2 per 10g protein over the 11 year period. The

diets were planned to give a difference of approximately 50g protein between the diet groups. Therefore after one year, a difference between diets in the change in GFR of about 5% may be expected. Using a non-inferiority approach and using the standard deviation of 13.4 ml/min from this paper we would have 80% power ($p<0.05$) to see this change at one year with 89 people in 2 groups completing the study. An additional 25% were planned to be recruited to account for dropouts. A difference in GFR between treatments of 0-5% will not be considered clinically significant.

Based on other similar weight loss studies [143, 188], a drop-out rate was 25% was expected. That meant that 120 participants would have to be included in each group.

2.4.2 Ethics

All experimental procedures were approved by Human Ethics Committees of the Commonwealth Scientific Industrial Research Organization (CSIRO) and the University of Adelaide. Participants were instructed in all study procedures in both oral and written form and all participants provided written informed consent.

ANZCTR Registration number:
ACTRN 12608000045314 for the main study,
ACTRN 12609000331235 for the carbohydrate timing study (study 3)

Chapter 3: Renal function

3.1 **Main study**

Weight loss is one of the cornerstones in the treatment and prevention of Type 2 diabetes (T2DM) and a weight loss of 5 – 10 % of total body weight has been reported to result in improved glycaemic control, improved insulin sensitivity and improvement in micro and macrovascular risk markers [232]

Long-term randomized intervention studies looking at the effect of high protein weight loss diets in T2DM are scarce. Long-term randomized intervention studies designed to analyse the effect of high protein weight loss diets compared to the standard diabetes diet in T2DM participants with impaired renal function, are lacking.

In this study, we evaluated the effect of two energy restricted diets, a high protein diet (≈30%TE) vs. a standard protein diet (≈20%TE), on renal function in type 2 diabetes participants with microalbuminuria. This was a randomized, parallel, controlled study with participants stratified according to HbA1c, BMI and sex before randomization.

3.2 **Aim and hypothesis**

The aim of this study was to explore whether a weight-loss diet, high in protein in place of carbohydrate, has a beneficial effect on renal function, measured by GFR, in subjects with T2DM and with microalbuminuria. We hypothesized that a high-protein weight-loss diet, by virtue of the weight loss, would have a beneficial effect on markers of renal function in type 2 diabetes subjects with microalbuminuria and/or renal impairment

3.3 **Main outcome**

- Renal function measured by:
- Isotope glomerular filtration rate (iGFR)
- Estimated GFR (eGFR)
- serum cystatin C
- Serum creatinine (S-Cr)
- Microalbuminuria (AER)
- Albumin to creatinine ratio (alb/cr)

3.3.1 Secondary outcome

- Weight loss (WL)
- Blood pressure (BP)
- Serum lipids
- Glycaemic control

3.4 **Results**

Forty eight volunteers were randomized for this study; twelve volunteers discontinued after randomization. Four discontinued due to unrelated illness (3 HPD and 1 SPD), and five due to social or economic problems with diet (3 HPD and 2 SPD). One did not comply with treatment regime (did not attend clinic visits, HPD) and two were unsatisfied with weight loss results (SPD). Thirty six volunteers completed the twelve months intervention and are included in the analysis.

Each group has been stratified according to sex, HbA1c and BMI before being randomly assigned to either the HPD or the SPD diet.

There were no significant differences between randomized groups at baseline.

Table 6 Baseline characteristics

	HPD	SPD	p value
Sex (M / F)	14 / 5	13 / 4	
age at study start	59.5±2.4	61.4±1.7	0.53
Diabetes duration (years)	12.8±2.7	8.5±1.1	0.17
Height (m)	1.7±0.0	1.7±0.0	0.84
Weight (kg)	106.9±5.4	107.6±4.9	0.92
BMI (kg/m2)	36.4±1.6	36.3±1.1	0.95
FBG (mmol/L)	8.0±0.4	8.1±0.5	0.94
HBA1C (%)	7.4±0.2	7.1±0.2	0.32
IGFR	108.3±9.2	89.1±6.5	0.10
eGFR	93.9±6.1	92.7±7.1	0.89
S-Cr (µmol/l)	77.4±4.3	80.6±6.4	0.68
Cystatin C (mg/L)	0.9±0.04	0.9±0.05	0.67
AER (µg/min)	41.8±10.6	71.8±13.9	0.09
Alb/cr	5.9±1.6	8.4±1.9	0.33
SBP	126.3±3.0	125.1±2.9	0.78
DBP	74.2±1.6	69.0±2.3	0.06

HPD is the high protein diet and SPD is the standard protein diet. Data are given as means (SEM). Differences in baseline values were tested using independent samples t-test. Significance is set at $p<0.05$.

3.4.1 Diet

At baseline the self-reported energy intake did not differ significantly between groups. Participants consumed similar amounts of protein at baseline, but there was a significant difference in protein intake at the end of the study ($p<0.01$). There was no significant difference in percentage protein to energy ingested at baseline ($p=0.42$). At the end of the study the percentage protein to energy ingested was significantly higher in the HPD ($p<0.01$).

Carbohydrate intake did not differ between groups in the self-reported data when expressed as gram of CHO. However, when the data was expressed as %TE there was a significant difference between diets at four and 12 months ($p<0.01$) but no difference at baseline ($p=0.80$).

There were no significant differences in the fat, alcohol or fibre intake at any point.

For nutrient breakdown refer to table 7 and 8.

3.4.1.1 Measured protein intake calculated from urinary urea excretion.

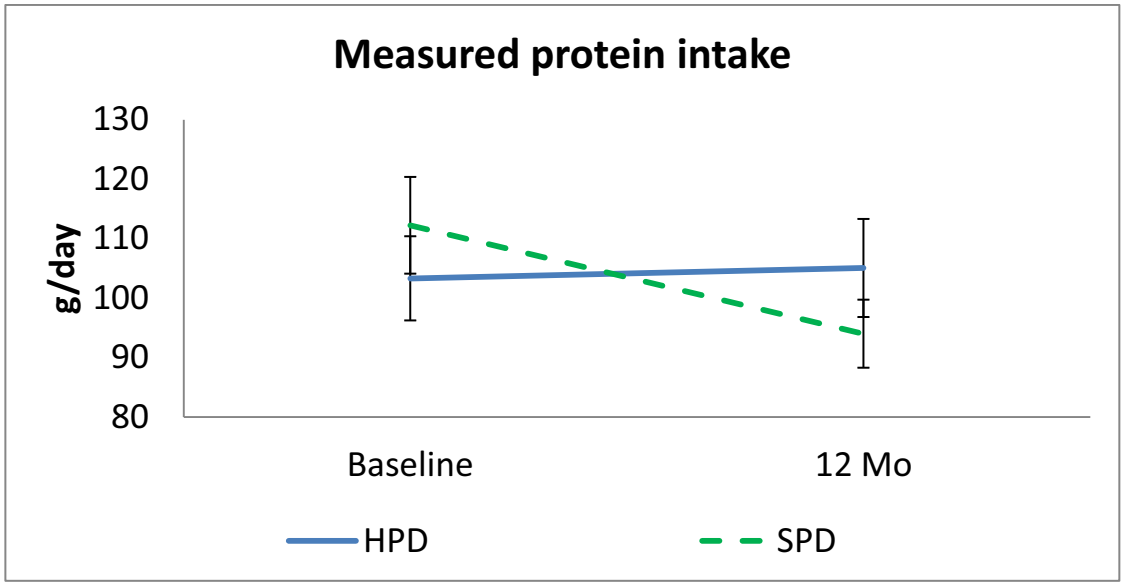
Compliance with protein prescription was measured by 24h urine urea excretion (UUE). At baseline UUE did not differ between groups (486.3±33.3 and 528.2±38. 3 mmol/24h in HPD and SPD respectively; $p=0.41$). There was a significant treatment effect at four months (estimated marginal means adjusted for baseline: 480.1± 22.2, 95% CI 435.6, 528.9 for the HPD and 368.8 ± 23.5, 95% CI 321.1, 416.5; $p=0.002$). At 12 Mo the adjusted difference in UUE was still significant (estimated marginal means adjusted for baseline: 507.8± 24.1, 95% CI 458.8, 556.8 for the HPD and 427.6 ± 25.5, 95% CI 375.7, 479.7; $p=0.029$).

Conversion from UUE to protein intake in gram per day using the equation: Protein intake = ((UUE * 0.034) * 6.25)) [233]

Converted to grams of protein ingested per day the UUE values are equal to 103.3±7.1 and 112.3±8.1 g dietary protein/ day at baseline in the HPD and SPD groups respectively ($p=0.41$). At the end of the study the protein intake in the

HPD group was 105.1±8.2 and in the SPD 94.0±5.7 g protein/day. This difference in protein intakes at 12 months did not reach statistical significance. However, because of the different protein intakes at baseline (SPD had a higher intake), there was a significant difference in protein intake adjusted for baseline value between treatment groups at 12 Mo (p=0.03).

Figure 9: Protein intake measured by urinary urea excretion.



Data represented as mean (SEM).

3.4.1.2 Nutrient composition

The 3 day diet record was used to assess changes in nutrient intake between baseline, four months and 12 months. There was a significant decrease in energy intake over time ($p<0.001$) with no significant difference between treatments at 12 Mo. Protein intake changed significantly with time ($p=0.04$) with a significant treatment effect ($p<0.001$). Fat intake decreased with time ($p<0.001$) with no significant treatment effect. CHO decreased significantly with time ($p=0.001$) with no treatment effect. Alcohol and dietary fibre did not change significantly with time .The saturated fatty acids (SAFA) decreased significantly with time with no difference between treatments (table 7).

Table 7: Nutrient intake measured by 3 day diet record

	Treat	Baseline	4Mo	12 Mo	Baseline to 4Mo		Baseline to 12 Mo	
					Time	Treat	Time	Treat
kJ	HPD	8860±477	7214±430	7238±376	<0.001	0.17	<0.001	0.31
	SPD	8800±570	6448±384	6553±561				
Protein (g)	HPD	110±9	111±8	113±6	**0.005**	**<0.001**	**0.035**	**<0.001**
	SPD	102±5	75±6	71±6				
Fat (g)	HPD	80±6	59±4	60±4	**<0.001**	**0.09**	**<0.001**	0.50
	SPD	81±4	48±4	55±7				
SAFA (g).	HPD	32±3	23±2	24±2	**<0.001**	0.23	**0.001**	0.66
	SPD	31±3	19±2	22±3				
CHO (g)	HPD	210±13	176±12	161±13	**0.011**	0.71	**<0.001**	0.48
	SPD	219±16	185±12	177±14				
Alcohol (g)	HPD	11±5	0.2±0.2	7±3	**0.007**	0.11	0.434	0.59
	SPD	5±3	1.2±0.5	6±3				
Fibre (g)	HPD	26±2	27±2	24±2	0.251	0.47	0.336	0.49
	SPD	28±2	30±3	27±2				

The data in the table represents the self-reported nutrient intake at baseline, four and 12 months, using the 3 day diet record. Treat is treatment, HPD is the high protein diet and SPD is the standard protein diet. SAFA is saturated fatty acids. There were significant changes over time in all nutrients except fibre at 4 Mo and all nutrients except alcohol and fibre at 12Mo compared to baseline. Adjusted for baseline protein and fat was significantly different between treatments at 4 Mo. At 12Mo only protein differed significantly when adjusting for baseline.

Nutrient composition measured as percentage of energy intake changed significantly with time. Protein %TE increased in the HPD and remained stable in the SPD. %TE for fat decreased significantly with time, with no significant difference between groups. %TE for CHO increased in the SPD and remained stable in the HPD, indicating that the change in energy intake was due to decreased fat. Alcohol decreased in both groups with time with no significant treatment effect. There was a significant change in g fibre/MJ over time and between diets, however the actual difference was only 1g/MJ (table 8).

Table 8: Nutrient intake expressed in percentage of total energy

					Baseline to 4Mo		Baseline to 12Mo	
					Time	Treat	Time	Treat
Protein (%TE)	HPD	22	26	27	0.08	**<0.001**	0.049	**0.003**
	SPD	20	20	19				
Fat(%TE)	HPD	33	30	30	<0.001	0.196	0.001	0.71
	SPD	34	27	30				
CHO(%TE)	HPD	41	41	38	0.008	**0.001**	0.799	**0.001**
	SPD	42	49	46				
Alcohol (%TE)	HPD	3	0.1	3	0.006	0.115	0.008	0.701
	SPD	1	0.5	2				
Fibre(g/MJ)	HPD	3	4	3	<0.001	0.026	0.003	0.011
	SPD	3	5	4				

Nutrients expressed in %TE, fibre is expressed as g/MJ (all numbers rounded to the nearest whole number).

3.5 **Renal Function**

The aim of this study was to determine if weight loss had a beneficial effect on markers of renal function. There was a significant weight loss in both groups over time (HPD -10.5 kg and SPD -7.5 kg) with no significant difference between groups (weight change and body composition will be discussed in chapter 4).

Renal function was measured by iGFR, eGFR, S-Cr, serum creatinine to lean body mass ratio (S-Cr/LBM), AER and alb/cr.

3.5.1 Glomerular filtration rate

3.5.1.1 Isotope glomerular filtration rate (iGFR, ml/min/1.73 m^2)

iGFR showed no significant difference between groups at baseline 108.3±9.2 and 88.9±6.5 ml/min/1.73 m^2 in the HPD and SPD groups respectively (p=0.10). At the end of the 12 months intervention iGFR had decreased in the HPD to 101.8±8.1 ml/min/1.73 m^2 and the SPD had increased to 95.9±6.9 ml/min/1.73 m^2 (p=0.94 for time). Adjusting for baseline values there was no significant difference between groups (p=0.095).

The change in iGFR was significantly negatively correlated with diabetes duration (r= -.39, p=0.04) and the change in mean arterial blood pressure (r= -.40, p=0.02). There were borderline negative correlations between the change in SBP, DBP and the change in iGFR (r= -.32 and r= -.32, p=0.06 respectively).

Using multiple linear regression with change in iGFR as the dependent variable and change in protein intake, weight loss and change in SBP as independent variables revealed no significant associations to the change in protein intake r^2 = 0.04, p=0.84 or weight loss r^2 = 0.13, p=0.49 but there was a borderline significant positive association to SBP r^2 = 0.33, p=0.06.

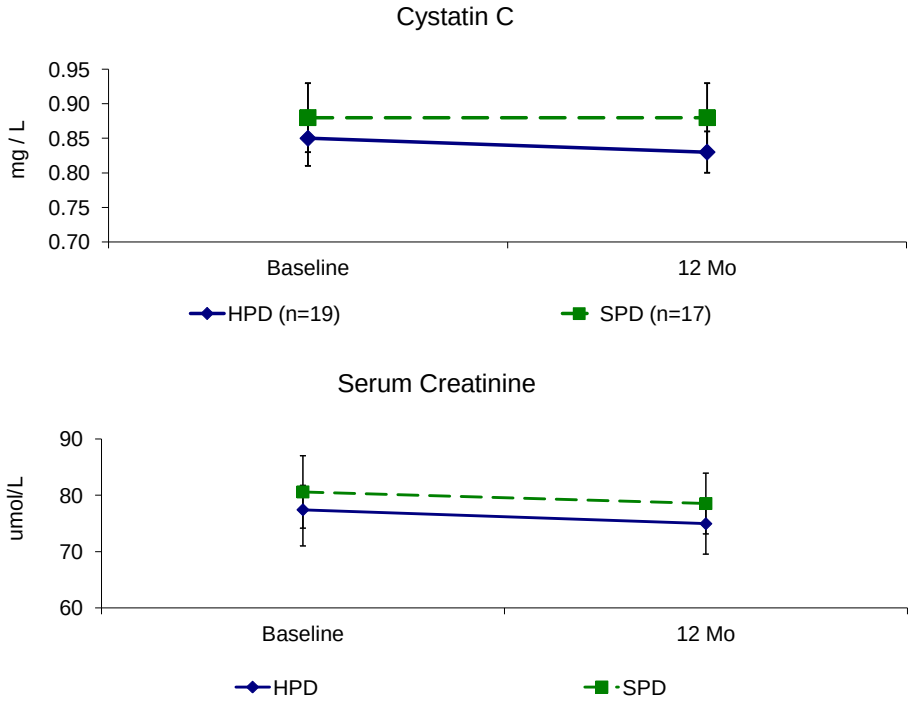
3.5.2 Serum creatinine (µmol/L)

Serum creatinine (S-Cr) as a marker of renal function is the simplest measure available. In steady state production and excretion of creatinine will be identical.

There was no significant difference in S-Cr at baseline (p=0.68). During the intervention there was a non-significant decrease (p=0.21).

Adjusting for baseline values, there was a non-significant difference in S-Cr between randomized groups at twelve months (0.34).

Figure 10: Changes in Cystatin C and Serum Creatinine over time.



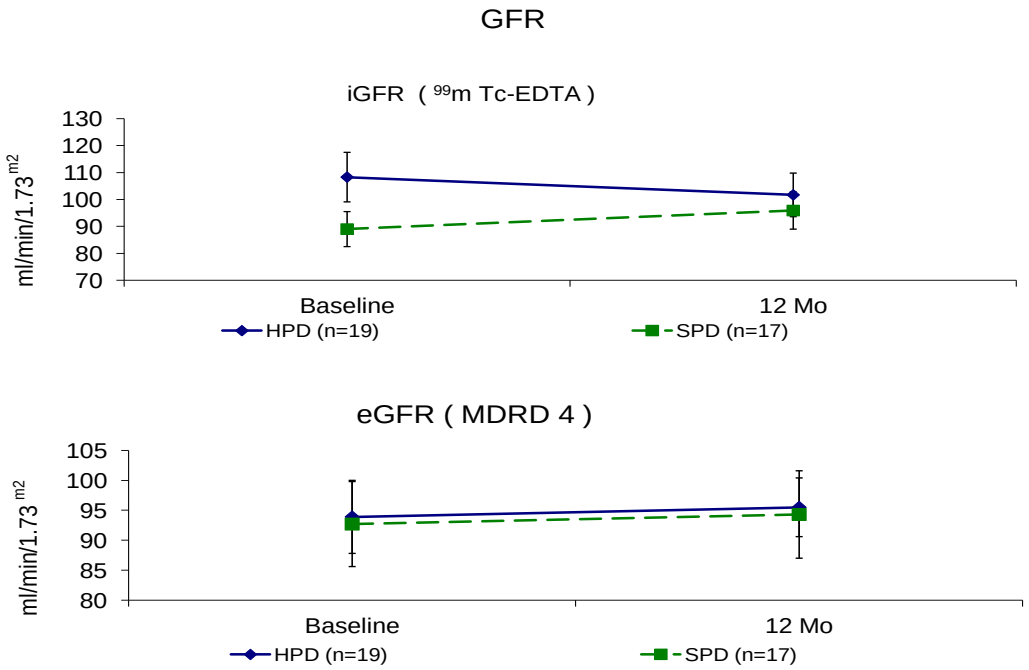
HPD (blue solid line) and SPD (green broken line). The bars represent mean (SEM). There were no significant differences between treatments at twelve months.

3.5.2.1 Estimated GFR by MDRD4 equation (eGFR, ml/min/1.73 m^2)

At baseline the eGFR was similar between the two groups and did not change significantly over time. Adjusting for baseline values, there was no significant difference between groups.

The change in eGFR was negatively correlated with the change in Cystatin C (r=- 0.33; p=0.047), no significant correlations were found between the change in eGFR and sex, protein intake, glycaemic control, BP or AER.

Figure 11: iGFR measured by ^{99m}Tc-DTPA and estimated using the abbreviated MDRD formula at baseline and at the end of the study.



HPD (blue solid line) and SPD (green broken line). The bars represent mean (SEM). There were no significant differences in either iGFR or eGFR over time or between groups.

3.5.3 Cystatin C (ml/L)

At baseline there were no significant differences in cystatin C between the randomized groups (p=0.85). There was no significant change in Cystatin C over time (p=0.26). No significant treatment effect was found at twelve months (p=0.14).

All measures of renal function were highly significantly correlated with iGFR at baseline, with the eGFR showing the strongest correlation (r= 0.77; p<0.01); both Cystatin C (r= -0.69) and S-Cr (r = -0.58) were strongly negatively correlated with iGFR (p<0.01). At twelve months the correlation was still strong eGFR (r = 0.75); Cystatin C (r = -0.68) and S-Cr (r = -0.52) p<0.01.

Furthermore there was a strong correlation between S-Cr and Cystatin C at both baseline and twelve months (r = 0.75, p<0.01 and r=0.73, p<0.01 respectively).

3.5.4 Serum creatinine to lean body mass ratio

The S-Cr/LBM (µmol/L/kg) did not differ between groups at baseline (p=0.83) and there were no significant changes with time (Change in the HPD= 0.03±0.04 and SPD 0.0003±0.05 respectively, p=0.64) or between groups at the end of the study.

3.5.5 Microalbuminuria

Albumin excretion rate (AER) was tested for normal distribution using the residuals of the outcome model, a violation was found and the AER was log10 transformed. Descriptive data are presented using the un-logged data, all analysis are done using the logged data.

3.5.6 Albumin excretion rate (µg/min)

AER measured in a 24h urine collection, was significantly different at baseline (41.7±10.6 vs. 71.8±13.9 µg/min in HPD (n=19) and SPD (n=17) respectively; p=0.01). There was a non-significant change with time (to 29.8±9.0 vs. 72.8±16.4 µg/min in HPD and SPD, p=0.20).

Adjusting for baseline values there was a borderline significant difference between groups at the end of the study (p=0.06), where the HPD had decreased and the SPD had increased slightly. Adding WL, change in SBP and average protein intake at 12Mo to the model did not have a major effect on the outcome (p=0.066).

3.5.7 Albumin to creatinine ratio (alb/cr)

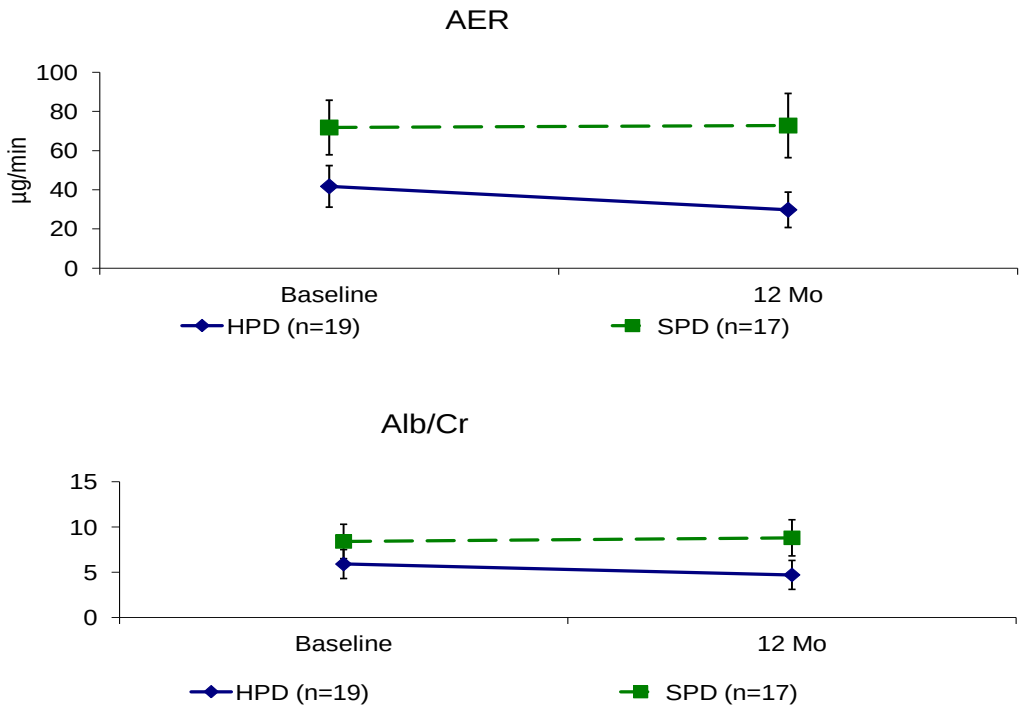
Alb/cr did not differ between randomized groups at baseline (HPD = 5.9±1.6 and SPD 8.4±1.9; p=0.70). There was no significant change with time. The HPD decreased by 1.2 and the SPD increased by 0.4. Adjusting for baseline values there was a borderline significant difference between groups at twelve months (p=0.06). Adding WL, change in SBP and protein intake to the model as covariates did not change the outcome (p=0.061)

Using multiple regression with the change in AER as dependent variable and the known major contributors to increased AER (HbA1c and SBP) and weight loss as independent variables revealed a significant model (r^2= 0.28, p=0.02). In this model WL showed the strongest association with the change (r^2= 0.48,

p=0.01). SBP was borderline significantly negatively associated (r^2= -0.34, p=0.06), there was no significant association with HbA1c (r^2= 0.22, p=0.28).

Using the same model with alb/cr change as dependent variable showed a strong negative association with SBP (r^2= -0.56, p=0.003).but no significant association with HbA1c or WL. (Changes in BP are described in chapter 5).

Figure 12: Microalbuminuria



Microalbuminuria measured as Albumin excretion rate and albumin to creatinine ratio. The bars represent mean (SEM). HPD (blue solid line) and SPD (green broken line). There were borderline significant differences in AER (p=0.059) and alb/cr (p=0.055) at the end of the study.

3.6 **Medication**

Of the 36 subjects who completed the study, three managed their diabetes with diet alone, 33 were treated with metformin, 11 with sulfonylurea, seven with glitazones and six were treated with insulin.

Metformin in mono therapy was given to 15 volunteers, nine were treated with a combination of metformin and sulfonylurea, one with metformin and glitazones, three with metformin, sulfonylurea and glitazones and four were treated with a

combination of metformin and insulin glargine and two volunteers were treated with metformin, sulfonylurea and insulin Novomix and mixtard.

During the study there were some medication changes, where three participants in HPD stopped medication, two increased medication dose and four changed to other medication vs. three volunteers increasing and two changed to other medication in the SPD group.

Blood pressure was treated with ACE inhibitors (n=16), beta blockers (n=6), angiotensin II receptor blocker (n=9), calcium channel blockers (n=9), diuretics (n=10) and an alpha blockers (n=1). Eight volunteers used no BP medication, 13 were on mono-therapy, eight were on dual treatment, six were on triple therapy and one volunteer received four different BP medications.

Other medication included lipid lowering medication and antidepressants. Medication was optimized as indicated by an endocrinologist or the participant's general practitioner before the start of the study and monitored throughout the study. All participants reported a moderate to low physical exercise level and they were asked to maintain this level throughout the study.

Table 9: Changes in renal function markers between baseline and 12 month

	Treatment		baseline	12 Mo	Change	P= Time	P adjusted for baseline
Weight (kg)	HPD	n=19	106.9±5.4	96.4±5.1	-10.5±3.2	**<0.01**	0.41
	SPD	n=17	107.6±4.9	100.1±4.8	-7.5±1.9		
iGFR (ml/min/1.73 m2)	HPD	n=19	108.3±9.2	101.8±8.1	-6.6±4.9	0.94	0.095
	SPD	n=17	88.9±6.5	95.9±6.9	+6.8±4.7		
eGFR (ml/min/1.73 m2)	HPD	n=19	93.9±6.1	95.5±4.9	+1.5±3.2	0.49	0.49
	SPD	n=17	92.7±7.1	94.3±7.3	+1.7±3.4		
Cystatin C (ml/L)	HPD	n=19	0.85±0.04	0.83±0.03	-0.02±0.01	0.26	0.14
	SPD	n=17	0.88±0.05	0.88±0.05	0.0±0.0		
S-Creatinine (µmol/L)	HPD	n=19	77.4±4.3	75.0±3.5	-2.5±2.2	0.21	0.34
	SPD	n=17	80.6±6.4	78.5±5.4	-2.1±2.9		
AER (µg/min)	HPD	n=19	41.7±10.6	29.8±9.3	-12.0±9.1	0.20	0.059
	SPD	n=17	71.8±10.6	72.8±16.4	+1.0±17.0		
Alb/cr	HPD	n=19	5.9±1.6	4.7±1.6	-1.2±1.9	0.24	0.055
	SPD	n=17	8.4±1.9	8.8±2.0	+0.4±2.4		

Data are given as mean (SEM) for markers of renal function, iGFR, eGFR, Cystatin C and S-Creatinine, the non-inferiority approach was used and a one tailed test used. For weight, AER and Alb/cr the adjusted ANCOVA with a two side p value was used.

3.7 **Discussion**

The main finding of this study was that a high protein weight loss diet did not exert a negative effect on renal function in type 2 diabetes participants with microalbuminuria or renal impairment, compared to a standard protein, weight loss diet. Although the number of people randomised was a lot lower than planned in the statistical analysis, the fact that there was no correlation at all between protein intake and change in renal function provides confidence that the negative result was not a result of inadequate power. Changes in eGFR and cystatin C clearly show no differences between diets.

Renal function was measured by a number of different methods requiring blood and urine collection:

1. Serum creatinine (S-Cr) is used as a simple and easy measure of renal function, this measure was used as one of the primary outcomes in many of the early studies looking at the effect of decreasing dietary protein intake on renal function [78, 83, 84]. The benefit of using S-Cr is the relative ease of obtaining the measure and interpretation can be done quickly at bedside. However, there are major limitations in the use S-Cr as diagnostic measure of renal dysfunction, S-Cr is dependent on muscle mass and if the volunteer is not in steady state, i.e. in conditions of weight loss, S-Cr will decrease. The major determinants of the total creatinine pool are age and sex [234]. It has been shown that an estimated 50% decrease in GFR is evident by the time S-Cr moves outside the normal range [91]. The Jaffé colour reaction assay lacks precision in determining S-Cr within the normal range which makes interpretation of any change difficult [234]. Therefore S-Cr should not be used alone as the measure of renal function in early renal disease [235]. No cut-off point has been established which will effectively determine renal disease. The reference values used in this study were between 72-127 µmol/L for male and 58-96 µmol/L for female volunteers, the level of S-Cr where patients are often misdiagnosed as having normal kidney function is within the normal range of 80-120 µmol/L [235]. In the present study there was a non-significant decrease of S-Cr in both groups indicating loss of muscle mass due to weight loss. When S-Cr was measured per kg LBM the change was equal in the two randomized groups indicating no difference in renal function.

2. Serum cystatin C is a more reliable predictor of early kidney disease compared to S-Cr [236]. Cystatin C is a low molecular amino acid which is produced at a constant rate in all nucleated cells and is unaffected by age, sex, or muscle mass. Cystatin C is freely filtered and metabolized by the tubules [237]. Testing the predictive value of cystatin C compared to S-Cr and estimated GFR using the MDRD formula with measured GFR using ^{51}CrEDTA clearance in 164 participants with mildly impaired renal function (GFR 30-80 ml/min/1.73m^2) as reference, showed cystatin C to have higher accuracy in diagnosing renal impairment than both S-Cr and eGFR (MDRD) [236]. The same results were reported in 125 participants with T1DM and 163 participants with T2DM with impaired renal function (GFR 4-222 ml/min/1.73m^2), in this population iohexol clearance, S-Cr and MDRD estimated GFR were compared. Cystatin C correlated better with iohexol clearance (r = 0.86) compared to S-Cr (r = 0.77) and eGFR (MDRD, r = 0.81) p<0.01 [237].

We found no significant change in Cystatin C over time or between groups, indicating no harmful effect of the higher protein intake during the 12 months study in the randomized group.

3. To obtain a more precise measure, renal function was measured using the gold standard isotope method (^{99m}Tc-DTPA) which has been shown to be a precise measure of GFR [238]. The ^{99m}Tc-diethylenetriamine-pentraacetic acid (^{99m}Tc-DTPA) isotope tracer clearance has been shown to correlate well with inulin clearance and is one of the most commonly used methods for directly assessing GFR [239]. The dual blood sampling technique has shown the closest correlation with the multiple blood sampling Inulin clearance technique previously used as the gold standard [238]. iGFR was measured by ^{99m}Tc-DTPA at baseline and at the end of the study using the dual blood sampling technique (blood sample taken at one and four hours post injection) There were no significant differences in iGFR with time and adjusting for baseline there was no significant treatment effect (p=0.095).

In the clinical setting an estimated GFR is used as a non-invasive estimate of renal function. In this study GFR was estimated using the MDRD abbreviated equation (using serum creatinine adjusting for age, sex and race). Testing for bias the MDRD equation was more accurate in predicting eGFR compared to CG and CKD-EPI equations (method pp. 67-71) however the MDRD

underestimated GFR compared to iGFR. In this study there was no significant change in eGFR over time or adjusted for baseline values between groups.

4. Microalbuminuria assessed as increased AER and increased albumin to creatinine ratio are the earliest signs of renal involvement and a powerful independent risk factor for the development of diabetic nephropathy [55]. Obesity is associated with increased renal plasma flow, hyperfiltration and increased albumin excretion [240, 241]. Ribstein et al found GFR and effective renal plasma flow to be increased in obese, compared to lean, participants regardless of hypertensive status. They found an increased albumin excretion (40%) in the obese, compared to the lean, participants (19%). Albumin excretion was positively correlated with BP and mean arterial pressure, with the regression line steeper in the overweight group (0<0.5) [240]. Obese hypertensive subjects had increased renal plasma flow, greater blood flow and increased cardiac output together with decreased peripheral and renovascular resistance compared with lean hypertensive subjects. This suggests that lean and obese subjects have different haemodynamic characteristics and the effect on target organs may differ [241]. AER decreases after weight loss; Chagnac et al found a highly significant decrease in AER from 16 µg/min (4-152 µg/min) to 5 µg/min (3-37 µg/min) in 17 severely obese subjects after substantial weight loss [242]. Similar results have been reported in several weight loss studies. A 52% reduction in AER from baseline has been reported in a meta analysis of both surgical and non surgical weight loss, with surgical weight loss showing the greatest benefit [243]

In this study microalbuminuria was measured at four monthly intervals to monitor renal function. AER did not change significantly with time and adjusted for baseline values there was no significant treatment effect. Adding WL, change in SBP and averaged protein intake did not change the significance.

The same trend was seen for alb/cr rate where no significant change was seen with time and adjusted for baseline values no significant treatment effect was observed.

In this study AER decreased in the HPD group between baseline and twelve months; but remained relatively stable in the SPD group and this difference showed a trend toward significance (p=0.06) This can probably be ascribed to the slightly higher (NS) weight loss in the HPD group. There are no

physiological reasons why a high protein diet should be more effective in reducing microalbuminuria. Although not statistically significant this may be of some clinical importance.

Weight loss plays a major role in the decrease in microalbuminuria it has been shown that weight loss per se will reduce microalbuminuria regardless of dietary treatment [125]. In this study absolute weight loss in kg was higher in the HPD group (although not significantly) compared to the SPD group indicating that weight loss may play a role in the changes in microalbuminuria.

Weight loss has a beneficial effect on AER with some participants returning to normoalbuminuria after a relatively modest weight loss [125]. A meta regression of 522 participants in 13 intervention studies (five controlled and eight uncontrolled studies) showed that for every 1 kg weight loss achieved was associated with a 1.1 mg decrease in microalbuminuria (95% CI= 0.5, 2.4; p=0.01) [125].

In a secondary analysis of the PREVEND study, investigating the association between BMI and albumin excretion rate in 8050 participants from the general population, it was found that BMI was associated with microalbuminuria. The prevalence of microalbuminuria in men was 9.5% in those with a BMI <25 kg/m^2 increasing to 18.5% in the overweight (BMI 25-29.9) and to 29.3% in the obese (BMI >30kg/m^2) group. The same trend was seen in women with 6.6% in the normal weight, 9.2% in the overweight and 16.0% in the obese group [45].

This study had a low number of participants (19 HPD and 17 SPD). In the initial power calculation we expected a change GFR of 5 ml/min (5%) with a difference in protein intake of 50g/day. Using the non-inferiority approach and using the change in the variable at 12 months this study was estimated to be powered with 80% confidence ($p<0.05$) to detect a significant difference between groups of 15.9 ml/min for iGFR, 11.8 ml/min for eGFR, 0.04 mg/L for cystatin C, 9.3 µmol/L for serum creatinine, 49.4 µg/l for AER and 7.8 for alb/cr.

We conclude that under conditions of weight loss and good glycaemic control, a HPD does not exert a deleterious effect on renal function in T2DM participants with microalbuminuria or mild renal dysfunction.

Caution should be taken in interpreting these results as the sample size was small and follow-up duration was short, but the results are relatively clear.

Chapter 4: Change in weight and body composition

4.1 **Weight loss and body composition**

High protein, weight loss diets have been shown to produce a greater weight and fat mass loss in settings of ad libitum energy intake, compared to a lower protein diet [137, 152]. Skov et al found fat mass to be 3.3 kg lower in the HPD compared to the SPD after a six months intervention. Total weight loss was higher in the HPD compared to SPD after six months (35% of the participants in the HPD lost more than 10kg body weight compared to 9% in the SPD) [137]. In a quantitative review of 50 studies, looking at the effect of a HPD compared to a low protein diet on weight loss, Halton and Hu concluded HPDs have a short term beneficial effect on weight loss. However, the studies were small and short-term [152]. In energy restricted, isocaloric diets the difference in weight loss between the two groups is not so clear [139, 244]. HPD have been shown to decrease waist circumference and produce a greater loss of visceral fat mass in men [141]. Retention of lean body mass has also been reported using a HPD compared to a SPD, often in the form of a greater decrease in LBM in the SPD group and minor decreases in the HPD if any.

For this study the aim was to have a similar weight loss in both the HPD and the SPD groups. The planned reduction in energy intake was equal (6 MJ) in both groups. I wanted to investigate the effect of a HPD and a SPD, under standardized conditions, on weight loss and body composition using DXA data.

It was hypothesized that an HPD would result in increased loss of visceral fat mass with minimal to no loss of LBM compared to the SPD under conditions of equal total weight loss.

4.2 **Results**

4.2.1 Weight loss

Of the 48 participants who started the diet intervention, 36 completed the study (HPD 14 men & 5 women and SPD 13 men & 4 women). At baseline weight in the two groups were similar. There was a significant decrease in weight in both groups over time ($p<0.01$). At 4 months weight loss was 8.9±1.6 kg and 8.3±1.5 kg in the HPD and SPD respectively. By the end of the study the HPD group had lost 10.5±3.2 kg and was still losing weight (weight loss between four

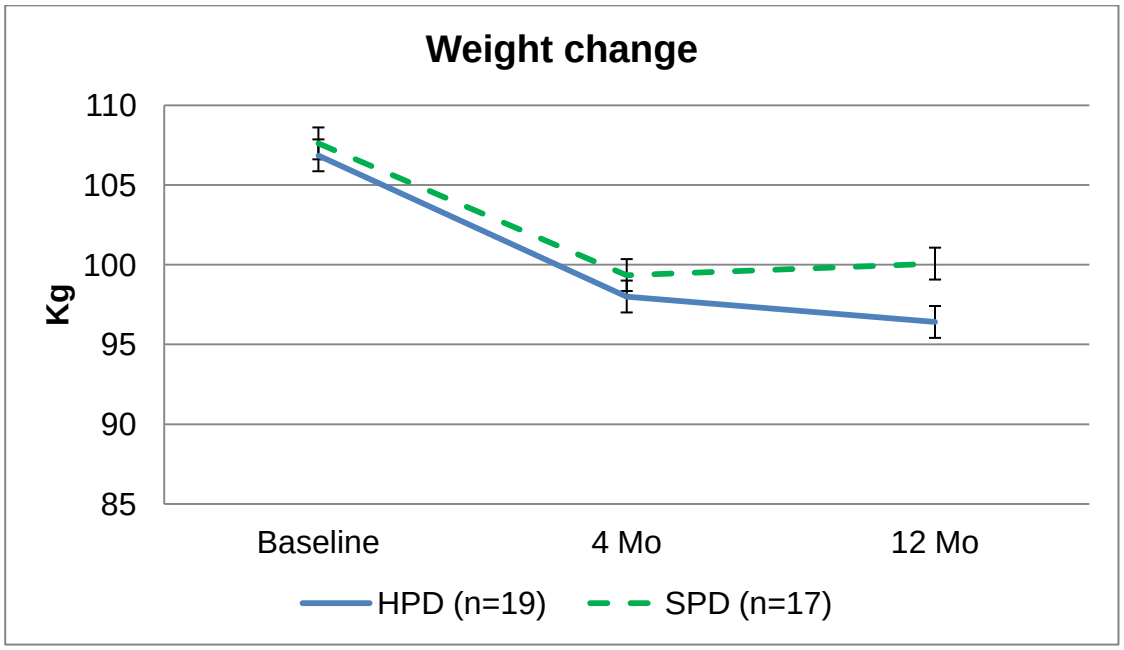
months and the end of the study was 1.6±1.8 kg), whereas the SPD group had lost 7.5±1.9 kg but had a slight weight regain of +0.7±0.9 kg between four months and the end of the study (ns). Although the HPD group was still losing weight at the end of the study and the SPD group had regained some weight, there was no significant difference between the groups adjusted for baseline values at 12 months.

Table 10: Weights at baseline, 4 and 12 months

Treatment	Baseline	4 months	12 months
HPD (n=19)	106.8±5.5	98.0±5.0	96.4±5.1
SPD (n=17)	107.6±4.9	99.3±4.3	100.1±4.8

The mean weights at baseline, four months and at the end of the study for the 36 volunteers who completed the study (Completers only, no missing data). Results are shown as means (SEM).

Figure 13: Weight change over the 12 months diet intervention.



The lines describe the mean weight change over time, the bars represent the mean (SEM). The blue solid line is the HPD and the green broken line is the SPD.

4.2.2 Body mass index

Body mass index was 36.4±1.6 kg/m^2 in the HPD group and 36.3±1.1 kg/m^2 in the SPD group at baseline. There was a significant decrease in BMI at four months to 33.4±1.6 kg/m^2 and 33.6±1.1 kg/m^2 in the HPD and SPD groups respectively ($p<0.01$ for time effect). At the end of the study the BMI in the two groups had not changed significantly from the four months values (32.9±1.6 kg/m^2 and 33.8±1.3 kg/m^2 respectively) and adjusting for baseline values there was no treatment effect at either four or 12 months.

4.2.3 Percentage body weight lost

The average percentage body weight lost at 12 Mo was 9% in the HPD and 7% in the SPD (ns). At four months 26 participants had lost more than 5% body weight (16 HPD and 10 SPD) and by the end of the study a total of 21 participants lost more the 5% body weight (11 HPD and 10 SPD).

At four months a 10% weight loss was seen in 13 volunteers (8 HPD and 5 SPD) and by the end of the study 14 volunteers had lost more than 10% body weight (8 HPD and 6 SPD).

In absolute amounts, men lost more weight than women (10.5±2.4 and 4.7±2.2 kg) at 12 months; however adjusting for baseline weights this difference was not statistically significant.

At four months men had lost 9.9±2.0 and 9.6±1.7 kg on the HPD and SPD respectively and at 12 months the weight loss was 12.1±4.1 kg on the HPD and 8.9±2.1 kg on the SPD (ns).

Women achieved all their weight loss during the first four months of the diet intervention. Weight loss in the HPD group was 5.8±2.0 kg and in the SPD group weight loss was 4.0±2.2 kg at four months. The total weight loss at 12 months was 5.8±3.1 kg in the HPD and 3.3±3.7 in the SPD. There were no significant differences in weight lost between groups.

At 12 months' nine men in each treatment group had lost more than 5% body weight and of these men six in HPD and five in SPD had lost more than 10% total body weight. For the women two in the HPD and one in SPD had lost more than 5% in fact all three had lost more than 10% of total body weight.

The relationships between treatment, age, sex and weight loss were analyzed using Pearson product-moment correlations. There was no correlation between weight loss and treatment at 12 months ($r= -0.13$, $n=36$, $p=0.45$) or between sex and weight loss ($r= -0.23$, $n=36$, $p=0.18$). There was a small negative correlation between age and change in body weight at the end of the study ($r= -0.35$, $n=36$, $p=0.04$). The average age was 60 years, the younger group (age <60 years) lost more weight (13.6 kg or 10.9% vs. 6.2 kg or 6.4% in the older group (≥60 years) ($p=0.05$).

4.2.4 Body composition

Body composition was assessed at the endocrine and metabolic unit at the Royal Adelaide Hospital by dual-energy X-ray absorptiometry (DXA), [Norland medical systems] at baseline and at 12 months.

Of the 36 volunteers who completed the study 32 (23 men (12 HPD and 11 SPD) and 9 women (5 HPD and 4 SPD)) had DXA measurements at baseline. Four volunteers (all men) exceeded the weight limit of 140 kg allowed by the manufacturer and were not scanned.

At baseline the mean weight for this sub-group ($n=32$) was 100.6±3.6 kg and 102.2±3.7 kg for the HPD ($n=17$) and SPD ($n=15$) groups respectively with no difference between groups.

4.2.5 Fat mass change

Total fat mass decreased significantly with time ($p<0.01$) with no significant difference between groups when adjusting for baseline values.

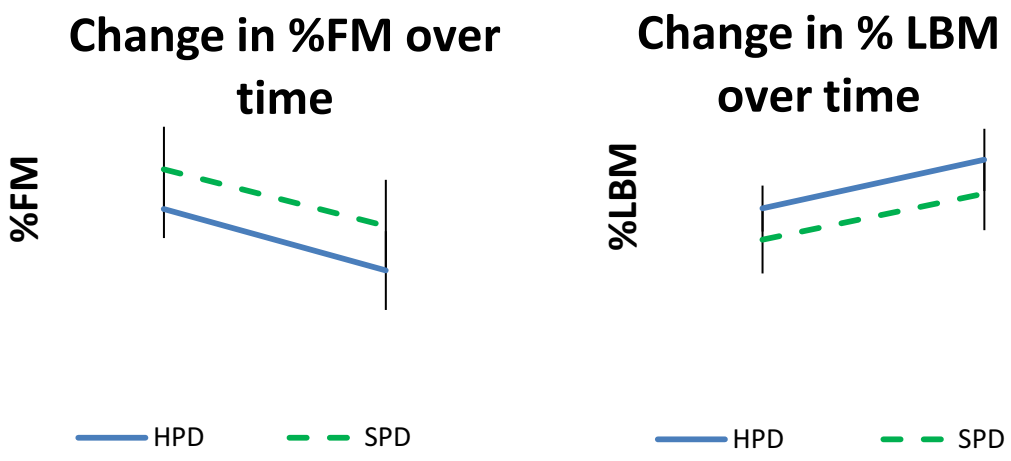
At baseline the percentage body fat mass (%FM) was similar in the two groups, 41.0±1.7 in the HPD and 43.2±2.5 in the SPD. At the end of the study %FM had decreased significantly in both groups ($p<0.01$ for time). There was no significant treatment effect at 12 months.

4.2.6 Lean body mass

There was a significant decrease in lean body mass over time ($p<0.01$). The HPD ($n=17$) lost 2.5±0.7 kg and the SPD ($n=15$) lost 1.2±0.7kg, however there was no significant treatment effect. The %LBM increased in both groups with time ($p<0.01$) with no significant treatment effect at 12.

Figure 14: Changes in body composition as a % of total body mass

Changes in %FM between baseline and 12 months (Left) and the change in %LBM between baseline and 12 months (right).



The data are given as mean (SEM). HPD is the blue solid line and SPD is the green broken line. There were no differences at baseline. The change over time was equal in both groups, with no significant difference between groups when adjusting for baseline values.

Table 11: Changes in body weight and body composition

	Baseline	12 Mo	Change	*Difference at baseline.*	*Difference over time*	ANCOVA group effect at 12 Mo
	Body weight (kg)					
HPD (n=17)	100.7±3.7	91.2±3.8	-9.5±2.6	p=0.92	p<0.01	p=0.41
SPD (n=15)	102.2±3.7	95.1±3.8	-7.1±1.9			
	FM (kg)					
HPD (n=17)	41.2±2.4	34.3±2.9	-6.9±2.1	p=0.47	p<0.01	p=0.20
SPD (n=15)	43.6±2.3	37.8±2.8	-5.8±1.4			
	FM (%)					
HPD (n=17)	40.9±1.7	37.3±2.3	-9.4±3.3	p=0.45	p=0.01	p=0.95
SPD (n=15)	43.2±2.5	39.9±2.7	-8.3±1.7			
	LBM (kg)					
HPD (n=17)	59.4±2.6	56.8±2.7	-2.6±0.7	p=0.83	p<0.01	p=0.65
SPD (n=15)	58.5±3.6	57.3±3.4	-1.2±0.7			
	LBM (%)					
HPD (n=17)	59.1±1.7	62.7±2.3	+6.0±2.2	p=0.45	p=0.01	p=0.95
SPD (n=15)	56.8±2.5	60.2±2.7	+5.8±1.4			

Data are presented as means (SEM). Significance was reached with p<0.05.

4.2.7 Fat distribution

Fat distribution was estimated using two areas: the central area including chest and midriff fat mass and the peripheral fat mass including fat from arms and legs.

At baseline the central body fat mass was equal in both groups. There was a significant decrease in central fat mass with time ($p<0.001$) with no difference between treatments at 12 months.

Likewise peripheral fat mass was similar at baseline. There was a significant decrease in peripheral FM with time ($p<0.001$). There was no significant difference between treatments at 12.

The same two areas were used to assess the distribution of lean body mass. Central LBM including chest and midriff, was similar at baseline. There was no significant change in central LBM over time and there was no treatment effect between groups at 12 Mo.

Peripheral LBM including arms and legs was similar at baseline. There was a significant decrease with time ($p<0.01$) with no significant difference between treatments at the end of the study.

Table 12: Changes in central, peripheral fat mass and lean body mass

		Baseline	12 Mo	Change	*Difference at baseline.*	*Difference over time*	ANCOVA group effect at 12 Mo
Central FM (kg)	HPD (n=17)	16.9±0.7	13.9±1.1	3.0±1.0	p=0.42	p<0.01	p=0.80
	SPD (n=15)	17.9±0.9	15.0±1.6	2.8±0.5			
Peripheral FM (kg)	HPD (n=17)	15.9±1.3	13.4±1.3	2.5±0.8	p=0.53	p<0.01	p=0.61
	SPD (n=15)	17.1±1.5	15.0±1.6	2.1±0.6			
Central LBM (kg)	HPD (n=17)	18.9±1.0	18.6±1.0	0.2±0.3	p=0.91	p=0.12	p=0.82
	SPD (n=15)	18.7±1.5	18.5±1.3	0.1±0.3			
Peripheral LBM (kg)	HPD (n=17)	28.0±1.3	26.0±1.3	1.8±0.8	p=0.84	p<0.01	p=0.29
	SPD (n=15)	27.6±1.7	26.4±1.7	1.0±0.4			

Data are given as mean (SEM), significance was set at p<0.05.

As shown above there was a positive effect of weight loss on body composition with a significant decrease in body fat in both central and peripheral fat distribution with time. Lean body mass decreased over time in the limbs but remained stable in the central body distribution. There was no between treatment effect when adjusting for baseline.

An opposite effect on disease risk of fat mass situated on the lower abdomen and the buttocks have been reported. Where fat situated on the buttocks has shown a beneficial effect on insulin sensitivity and markers of CVD risk, whereas fat situated on the lower abdomen have a deleterious effect increasing the risk of T2DM and CVD [245-247]. Because it is not possible to distinguish between these two fat mass areas using the DXA scan, the pelvis area was omitted from the analysis; thus creating the difference between total mass and the combined central and peripheral mass.

There was no significant difference between groups in pelvis area for LBM or FM at baseline. LBM did not differ over time and there was no treatment effect. FM decreased significantly with time (by 1.1±0.4 and 0.7±0.3 kg in the HPD and SPD groups respectively; $p<0.01$) with no significant difference between treatments.

4.2.8 Central to peripheral fat distribution ratio

In order to determine the total fat distribution as central or peripheral, a ratio was calculated dividing central fat mass by peripheral fat mass (Central/peripheral) [211]. A ratio of central to peripheral FM above 1.0 signifies abdominal fat distribution and a value less than 1.0 signifies a peripheral fat distribution.

At baseline the ratio was 1.12±0.06 in the HPD and 1.12±0.07 in the SPD indicating a central fat distribution at baseline. The ratio decreased with time in both groups to 1.07±0.05 and 1.08±0.07 (HPD and SPD respectively; $p=0.047$ for difference with time). There was no significant difference between treatments.

4.2.8.1 Fat mass distribution divided by sex

At baseline the males had a central to peripheral fat mass ratio of 1.25 indicating central obesity. This ratio decreased to 1.17 ($p=0.01$) by the end of the study. The males carried their excess weight around the abdominal area at baseline and even though they lost a significant amount of weight during the study, the distribution did remain predominantly abdominal.

The women had a central to peripheral fat mass ratio of 0.80 the ratio increasing to 0.83 by the end of the study ($p=0.19$). The women had their fat

distributed mainly as peripheral fat at baseline. With weight loss this distribution did not change.

Males lost significantly more central fat mass (2.8 kg) compared to women (p=0.02 between sex).

Peripheral fat mass loss was equal between the sexes (p=0.77).

Males lost twice as much LBM compared to women (especially on the limbs) but this was not statistically significant.

Table 13: Changes in body composition divided by sex

Sex	Male Mean (n=23)	Female Mean (n=9)	Sex Difference
Loss of fat body mass (kg)			
Total FM loss	7.5±1.5	3.6±2.0	p=0.17
Central FM loss	3.6±0.6	0.8±0.8	p=0.02
Peripheral FM loss	2.3±0.6	2.0±1.0	p=0.77
Loss of lean body mass (kg)			
Total LBM loss	2.2±0.7	1.1±1.7	p=0.34
Central LBM loss	0.2±0.2	0.2±0.2	p=0.10
Peripheral LBM loss	1.8±0.4	0.5±0.5	p=0.87

Data are the mean differences in body composition at 12 Mo. The statistical difference is analyzed using independent samples T-test with sex as the independent factor.

4.3 **Discussion**

There was a significant decrease in weight and BMI between baseline and 12 months with no significant difference between treatments. At 12 months 58% (21) of the volunteers had lost more than 5% body weight and of these participants 66% (14) had lost more than 10% body weight.

In studies of shorter duration (≤6 months) an increased weight loss has been reported in the HPD compared to a SPD [137, 169, 171-173, 179]. However, during follow up the weight regain is greater in the HPD, abolishing the initial benefit of the HPD compared to the SPD [144, 171]. These studies used ad libitum energy intake exchanging CHO for protein and it has been speculated that the satiating effect of protein is the cause of decreased energy intake in the HPD. However, adherence to diet is usually not sustained long-term, resulting in increased weight regained in the HPD group [144].

In energy restriction, comparable weight loss is most often reported when comparing weight loss diets with increased protein to CHO ratio. In the present study there was a trend of a greater weight loss in the HPD group; but this did not reach statistical significance. With the observed SD`s in this study (13.9 and 7.7 for the HPD and SPD respectively) and the sample of 19 in the HPD and 17 in the SPD group, this study was estimated to be powered to detect a difference of 10.2 kg between groups.

However, greater weight loss have been shown using HPD in different groups. Parker et al showed a sex difference where women lost more weight and abdominal fat mass on the HPD compared to women randomized to the SPD; whereas this was not the case in male participants [139]. In the study by Noakes et al women with increased TG lost more weight on the HPD compared to women without elevated TG.

In the present study men lost more weight compared to women (ns), however the sample size for women was very small which may have influenced the outcome. For males the loss of abdominal fat mass was highly significant over time (3.6 kg; $p<0.001$). Men lost significantly more abdominal fat mass compared to women (3.6 vs. 0.8 kg; $p=0.002$).

This difference has previously been shown. In a study looking at body composition, glycaemic and lipid control in hyperinsulinaemic men and women

after a 16 week intervention with a HPD (27%TE) compared to a SPD (16%TE), men lost more abdominal fat compared to women. As in our study, there was no effect of diet allocation [166].

In a meta-regression assessing the effect of 102 treatment groups in 51 studies looking at low CHO diets a greater retention of FFM was found in the highest quartiles of protein intake (protein intake >1.05 g/kg) compared to the lowest quartile (protein intake <1.05 g/kg) [165]. The protein intake at 12 Mo in our study was 1.1 g/kg in the HPD vs. 0.9 g/kg in the SPD group (p=0.03). Although this difference was significant, the actual difference was not sufficient to produce a LBM sparing effect in one group compared to the other.

In low CHO diets greater loss of FFM due to greater loss of body water has been documented with a CHO intake below 100g (ketogenic diets) [248]. In our study there was a significant decrease in LBM in both groups with no significant treatment effect. The lack of effect on retention of LBM using the HPD may be explained by the CHO content of the diets, where the average intake was 161±13 g in the HPD group and 168±9 in the SPD group reported by three day diet record (ns).

We conclude that a HPD is equally effective in producing weight loss and loss of fat mass compared to a SPD. There was no retention of fat free mass in either diet group.

The limitations to this study include the problems with precision of the DXA as some of the participants were too large to fit the scanner bed and others were too heavy to have the examination at baseline. This meant that the sample size for the body composition changes was small and may not be sufficient to show significant differences.

Chapter 5: Changes in glycaemic control, serum lipids and blood pressure

5.1 **Glycaemic control**

Glycaemic control has been shown to improve significantly with HPD. In a weight maintenance study investigating the effect of a doubling of protein from 15%TE to 30%TE in T2DM patients a 36% decrease in 24h BG was reported. This resulted in a decreased HbA1c from 8.1 to 7.3% after five weeks consumption of the HPD [195]. Weight loss per se has a beneficial effect on glycaemic control in T2DM [181]. A 58% decrease in the incidence of T2DM has been demonstrated in a group of men and women with increased risk of T2DM (IGT). The weight loss achieved during the first year, of a 3.2 year follow up study, was 4.5 kg in the intervention (energy restricted diet and exercise) group compared to 0.8 kg in the control group. The significant difference in weight lost remained through the follow up period [249].

In the present study, glycaemic control was measured at four monthly intervals with FBG and HbA1c.

At baseline the two measures were similar in the two randomized groups (FBG = 8.0±0.4 and 8.1±0.5 in the HPD and SPD respectively and HbA1c = 7.4±0.2 and 7.1±0.2 in the HPD and SPD respectively (n=36)).

There was a significant decrease in FBG at four months in both groups by 1.3 and 1.6 mmol/L in HPD and SPD respectively (p=0.001). By the end of the study the difference in FBG compared to baseline was no longer significant.

There was no significant treatment effect in the change in FBG at the end of the study.

Similarly, HbA1c decreased significantly between baseline and four months in both groups by 0.9 and 0.5 % in the HPD and SPD groups respectively ($p<0.01$). At the end of the study HbA1c was not different compared to baseline values (p=0.46). There was no treatment effect in the difference at four months or at the end of the study when adjusting for baseline values.

Table 14: Blood glucose changes over time

FBG (mmol/L)	Baseline	4 Mo	12 Mo	Treatment (12 Mo)
HPD (n=19)	8.0±0.4	6.7±0.3	7.0±0.4	p=0.41
SPD (n=17)	8.1±0.5	6.5±0.4	6.6±0.4	

HBA1C (%)	Baseline	4 Mo	12 Mo	Treatment (12 Mo)
HPD (n=19)	7.4±0.2	6.4±0.2	7.0±0.3	p=0.46
SPD (n=17)	7.1±0.2	6.6±0.2	7.0±0.3	

Data are given as mean (SEM). There was a significant change in both variables over time $p<0.01$.

When adjusting for medication changes there was still no significant difference in HbA1c at 12 Mo.

The multiple regression showed a significant association between HbA1c and weight loss ($r = 0.38$; $p=0.03$). There was no significant association between HbA1c and FBG ($r = 0.21$; $p=0.22$).

5.2 **Lipids**

Differential effects of a HPD on changes in serum lipids have been reported. We aimed to investigate the effect of a high protein, weight loss diet on serum lipid. We hypothesize a HPD will be as effective in reducing lipids as a SPD.

At baseline all volunteers were well treated for dyslipidaemia. Total cholesterol (Tot-chol), LDL, HDL and TG were all in the recommended range (Tot-chol <5.5, TG <2.0, LDL < 3.7 and HDL >0.9; all measured as mmol/L). There were no significant differences in serum lipids at baseline (Table 1)

There was a non-significant decrease in Tot-chol over time in both groups with no significant difference between randomized treatments at 12 Mo.

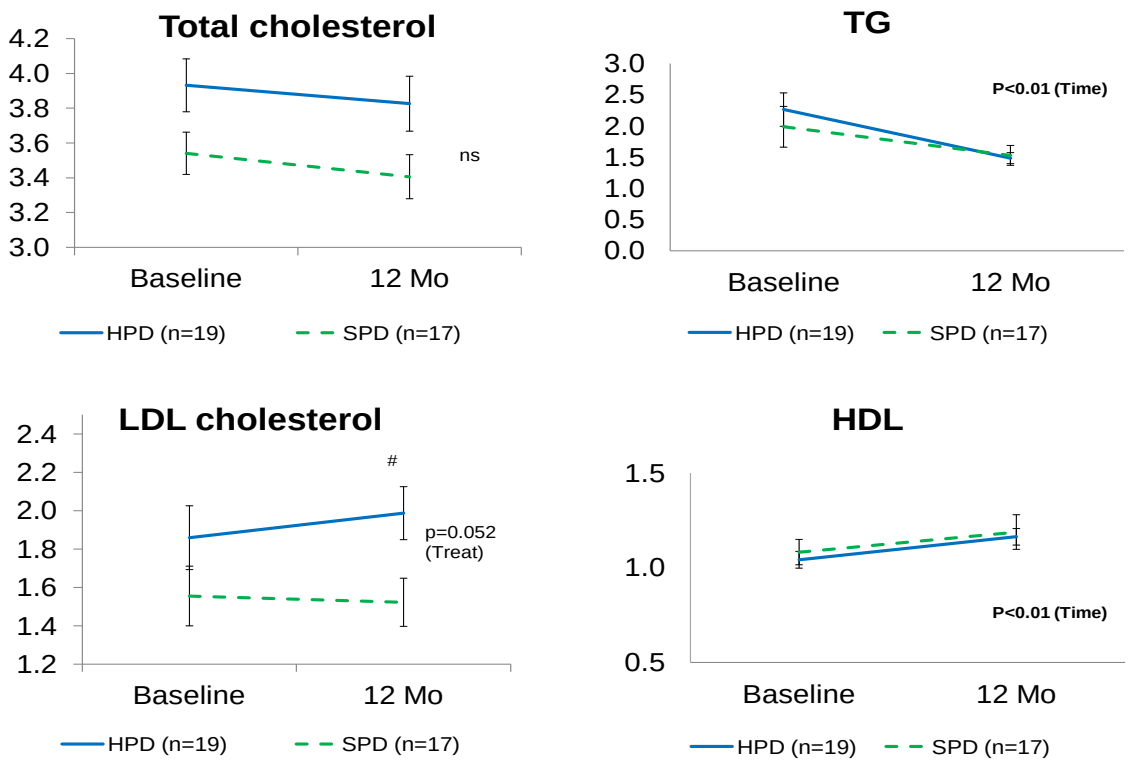
TG decreased significantly with time ($p<0.01$); There was no significant effect of treatment at 12 months when adjusting for baseline.

HDL increased with time in both groups ($p<0.01$). There was no significant treatment effect at 12 months when adjusting for baseline values.

There were no significant changes in LDL over time. Adjusting for baseline values, there was a borderline significant difference of 0.4 mmol/L in LDL cholesterol between the randomized groups (estimated marginal means for HPD = 1.9 and estimated marginal means for SPD = 1.6; $p=0.052$.

(For detailed changes in serum lipids refer to Table 15).

Figure 15: Changes in serum lipids (mmol/L)



There were no significant treatment effect at 12 months in total cholesterol, TG or HDL, there was a trend towards a treatment effect in LDL with HPD increasing by 0.1 mmol/L and SPD decreasing by 0.03 mmol/L between baseline and 12 months (p=0.052).

Table 15: Lipids

		Baseline	***12 Mo***	***Change***	***Baseline***	***Time***	***Group***
Chol.	HPD	3.9±0.2	3.8±0.2	-0.1±0.2	p=0.06	p=0.45	p=0.12
	SPD	3.5±0.1	3.4±0.1	-0.1±0.1			
TG	HPD	2.3±0.3	1.5±0.1	-0.8±0.3	p=0.52	**p<0.01**	p=0.54
	SPD	2.0±0.3	1.5±0.2	-0.5±0.3			
HDL	HPD	1.0±0.0	1.2±0.0	+0.1±0.0	p=0.61	**p<0.01**	p=0.84
	SPD	1.1±0.1	1.2±0.1	+0.1±0.1			
LDL	HPD	1.9±0.2	2.0±0.1	+0.1±0.2	p=0.19	p=0.79	*p=0.052*
	SPD	1.6±0.2	1.5±0.1	-0.03±0.1			

Data are given as means (SEM), Significance level was set at p<0.05. Number of participants in each group: HPD (n=19), SPD (n=17), All variables are measured in mmol/L.

5.2.1 Lipid lowering medication

All participants except for two were on statin treatment at baseline (34 on mono therapy and 5 on treatment with both statin and ezetimibe), one took ezetimibe on mono therapy and one participant did not take any lipid lowering medication.

Statin dose was decreased in one (HPD) and increased in three participants (1 HPD and 2 SPD), stopped in four (2 HPD and 2 SPD) and changed to other medication in three (2 HPD and 1 SPD) participants. Given changes in statin dose in both treatment groups it is likely that about a 0.1 mmol/L difference between the groups could be explained.

5.3 **Blood pressure**

Hypertension is an independent risk factor renal disease. Reduction in blood pressure to ≈130/80 has been recommended in T2DM [250]

5.3.1 SBP

There was a significant 5.7 mmHg decrease in SBP between baseline and four months ($p<0.01$). The change in SBP between baseline and twelve months was not significant (-2.3 mmHg; $p=0.29$). Adjusting for baseline values the difference between treatments was insignificant at both four months ($p=0.09$) and twelve months ($p=0.21$).

5.3.2 DBP

Diastolic blood pressure decreased between baseline and four months (-2.3 mmHg; but the decrease was not statistically significant. Between baseline and twelve months there was no change in DBP ($p=0.47$).

When adjusting for baseline values there was a significant difference between treatments at four months (EMM = 66.8±1.7 (63.4, 70.2) in the HPD and 72.4±1.8 (68.7, 76.0) in the SPD; $p=0.03$) and at twelve months (EMM = 69.8±1.9 (65.9, 73.6) in the HPD and 76.3±2.0 (72.2, 80.4) in the SPD; $p=0.03$).

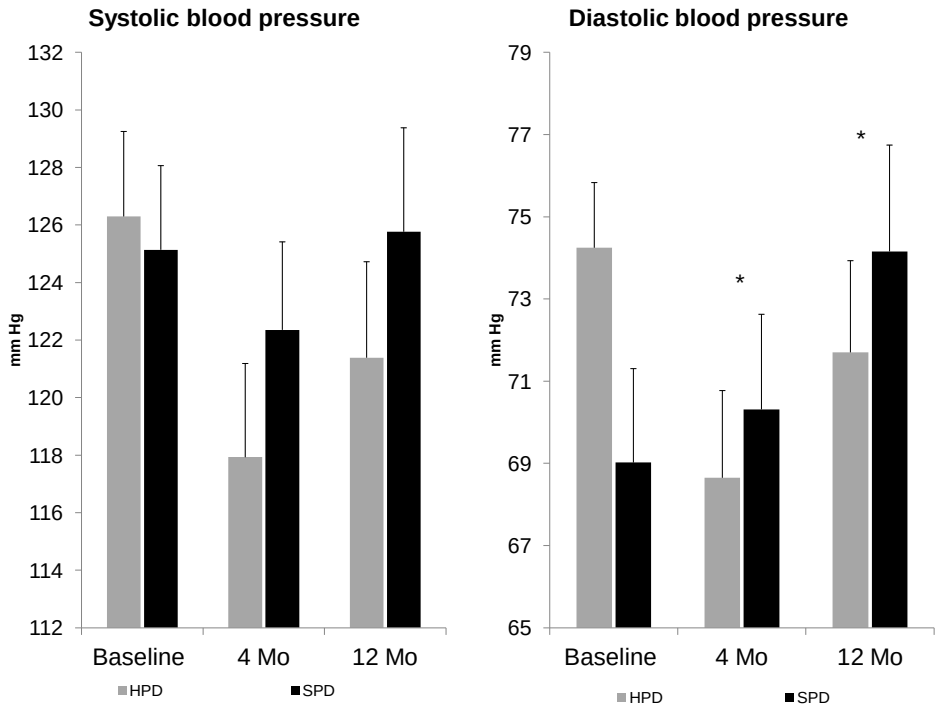
Adding weight loss as covariate did not change the significance ($p=0.03$); adding sex and age to the model did not change the significance between groups ($p=0.046$).

Table 16: Blood pressure changes over time (mmHg)

Treatment	SBP Baseline	SBP 4 Mo	SBP 12 Mo	DBP Baseline	DBP 4 Mo	DBP 12 Mo
HPD (19)	126.3±3.0	117.9±3.3	121.4±3.3	74.2±1.6	68.6±2.1	71.7±2.2
SPD (17)	125.1±2.9	122.4±3.1	125.8±3.6	69.0±2.3	70.3±2.3	74.2±2.6

Data are presented as mean (SEM).

Figure 16: Blood pressure change



Data are given as mean (SEM) for completers only (n=36). * =p<0.05 for treatment effect.

5.4 **Intention-to-treat analysis**

Forty eight volunteers started the randomized treatment and had at least baseline data. Twelve volunteers discontinued (7 in the HPD and 5 in the SPD). There were no significant differences between the completers and the non-completers at baseline.

Table 17: Baseline characteristics of the completers and the discontinued participants

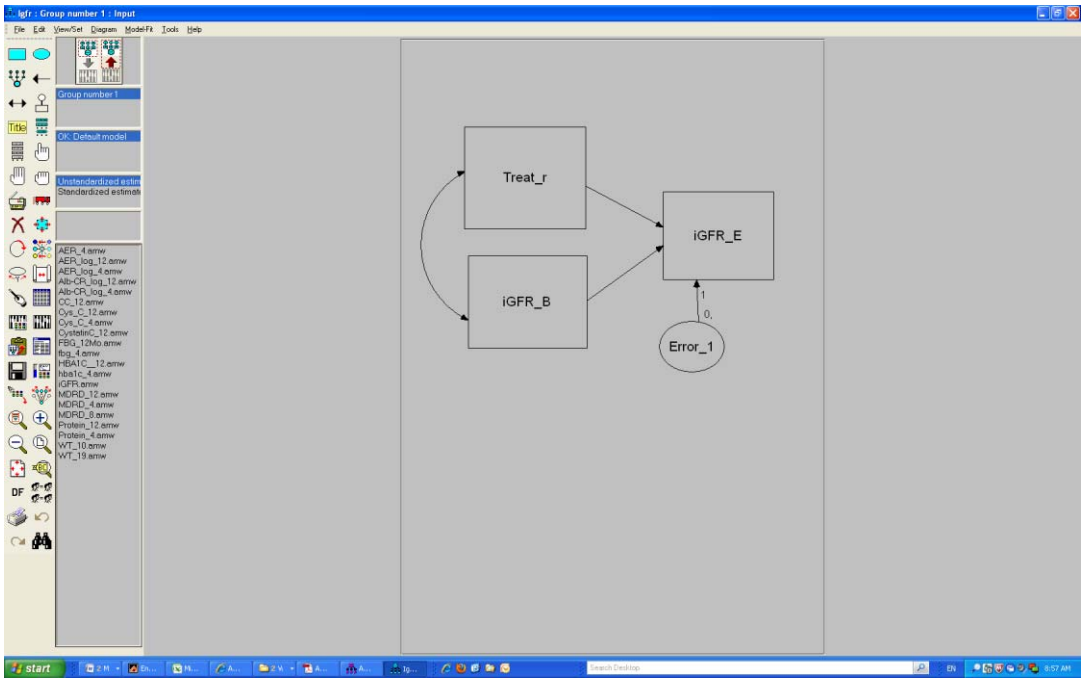
	Completers (n=36)	Discontinued (n=12)	Difference
age	60.4±1.5	56.4±2.9	p=0.20
Diabetes duration (years)	10.8±1.6	8.9±1.7	p=0.51
Height	1.7±0.02	1.7±0.02	p=0.11
BMI	36.3±1.0	35.7±1.2	p=0.74
Weight	107.2±3.6	99.4±5.3	p=0.26
FBG (mmol/L)	8.1±0.3	7.0±0.4	p=0.09
HBA1C (%)	7.2±0.1	7.3±0.3	p=0.91
AER (µg/min)	55.9±8.9	80.1±25.6	p=0.26
Alb/cr ratio	7.1±1.2	5.7±1.3	p=0.53
SBP (mmHg)	125.8±2.1	127.4±3.8	p=0.69
DBP (mmHg)	71.8±1.4	72.5±2.8	p=0.81

Data are given as mean (SEM).

A modified intention-to-treat analysis (volunteers who dropped out were not re-examined at the end of the study) was done to evaluate the outcomes for the complete population randomized to this study. The software SPSS AMOS (a structural equation modelling program that includes full information maximum likelihood estimation, student version 16) was used to perform the ANCOVA model in the full dataset, including only volunteers who had all measurements done at baseline (n=48).

This model will estimate the outcome of the variable using trends from all data obtained in the data set. Data are adjusted for baseline values.

Figure 17 Example of the AMOS analysis of missing data.



This analysis contains information on all 48 volunteers who had measurements taken at baseline (26 in HPD and 22 in SPD). Of interest was the outcome on renal function. Measures of: iGFR, eGFR, Cystatin C, AER and alb/cr were tested. We wanted to test if, using all randomized volunteers, renal function differed between treatments after 12 Mo intervention.

For the measures of GFR there was no significant treatment effect in the ITT analysis at twelve months (p=0.16 for IGFR and p=0.98 for eGFR) Or for Cystatin C (p=0.29).

However, when the AER was analyzed, there was a significant difference between treatments at 12 Mo, indicating that AER for the SPD would be 60% higher compared to HPD. Alb/cr would be 56% higher in SPD compared to HPD p=0.039 and for alb/cr p=0.036 (adjusted for baseline).

A more conventional method of analysing missing data is “Last Obtained Value Carried Forward” (LOVCF). To assess the effect of weight loss on renal function this method was also utilised. The same trend of no significant difference in analysing iGFR, eGFR and Cystatin C was found when LOVCF was used and adjusted for baseline values (p=0.13, p=0.43, p=0.54 respectively). Log transformed AER and Alb/cr did not differ significantly between treatments when the LOVCF was used (p=0.10 and p=0.19 respectively).

5.5 **Discussion**

In this chapter, measures of metabolic control were assessed. As expected, glycaemic control measured as FBG and HbA1c improved significantly with weight loss in the short term. However, with slowing of weight loss and decreasing compliance with diet, the effect was no longer evident at 12 months. Diastolic BG decreased significantly in the HPD group, and increased in the SPD group. Adjusted for baseline this difference was significant. Although not significant, the decrease in TG and the increase in HDL cholesterol remained at the end of the study. Systolic BG remained decreased in the HPD and unchanged in the SPD at 12 months. The intention-to-treat analysis showed the same trend as the main analysis, with no significant difference in renal function measured as GFR and cystatin C (true for both full-information missing data and LOVCF). For AER and alb/cr the ITT analysis showed significant benefit in the HPD when the full-information missing data method was used; but this was not found in the LOVCF analysis.

Numerous studies have found a similar decrease in FBG when comparing a HPD to a SPD. However, these studies did not include T2DM participants. In our study we found a significant decrease in FBG over time by 16% in the HPD and 21% in the SPD at four months and at 12 Mo by 12 and 21% (HPD and SPD respectively; ns for treatment). This is in agreement with multiple studies looking at overweight to obese men and women, where a significant decrease in FBG was found in both diet groups regardless of treatment allocation [138, 139, 143, 144], and in T2DM [179].

Glycaemic control measured as HbA1c was optimized and recommended levels [217] were reached at four months (6.4% in the HPD and 6.6% in the SPD groups respectively), with no significant difference between treatments.

By 12 Mo HbA1c had increased in both groups and were no longer significantly different from baseline. This was also reported in a study looking at 99 overweight to obese T2DM participants after 12 Mo on al low fat HPD (30%TE protein) or a high CHO (55%TE CHO) diet. The drop in HbA1c was seen in the initial weight loss period of three months, then it increased to just below baseline. Like in the present study, the authors in this intervention study report

decreases in anti-diabetic medication in the HPD, however when adjusting for baseline values the change was not significantly different between groups [188]

In studies with a protein intake ranging between 100-130g protein/day (CHO ≤140g/day and fat ≤30%TE) a reduction of TG of 20-55% has been reported (reviewed by Layman in [251]). The magnitude of the decrease depends on subject compliance; but seems to be maintained at a lower level, long-term.

In a similar study to the present looking at a high CHO (58%TE CHO, 30%TE fat and 12%TE protein) compared to a HPD (25%TE protein and 30%TE fat and 45%TE CHO) with ad libitum energy intake, Skov et al also reported a significant treatment effect in TG after 3 months with a decrease in HPD and an increase in the high CHO diet. This difference was not sustained long-term (12 Mo) [137]

Total and LDL cholesterol have been reported to improve in high CHO, low fat diets compared to HP low fat diets, whereas HDL and TG improved more in the HP low fat diet. Usually these changes occur during the first 6 months of the weight loss period, levels out and the significant treatment effect is lost. This trend has been reported in reviews reporting on low CHO diets [174, 252]

In the present study TG decreased significantly between baseline and four months. At 12 Mo TG had increased slightly; but was still significantly lower compared to baseline. There was no significant effect of treatment in the change in TG.

In this study a significant decreases in TG and increases in HDL over time with no treatment effect at 12 months was found. There was a non-significant change in total cholesterol over time with no difference between groups. LDL did not change significantly with time; however a borderline group difference was seen at 12 Mo. This difference is probably due to medication changes during the study.

Although TG and HDL are related in the metabolic syndrome to microalbuminuria the mechanism is likely to be obesity and insulin resistance rather than lipids. The changes in lipids in this study were relatively small and were unrelated to changes in microalbuminuria. This was tested using Persons correlation in the full participant set (n=36) and in volunteers with persistent microalbuminuria at 12 months (n=22).

The serum lipids were already very well controlled at study start and only minor changes were seen over the 12 months period.

In this study there was a significant decrease in DBP in the HPD compared to the SPD. Similar results were found in a study by Sargrad et al, they found both SBP and DBP to decrease significantly more in the HPD (Protein: CHO: fat = 30:40:30%TE) compared to the SPD (15:55:30) after 8 weeks intervention.
In a study looking at the effect of two low fat diets differing in protein content, 66 obese T2DM participants were followed for eight weeks in energy restriction, followed by four weeks energy balance. This study showed a significant decrease in both SBP and DBP after the 12 weeks intervention. Participants were asked to return to the research clinic for a follow-up visit after another 12 Mo on the intervention diet. At 12 Mo follow-up BP had increased in both groups; but the increase was significantly higher in the low protein group [185]. In the present study only the adjusted DBP differed significantly between groups at 12 months. As stated before it has previously been shown that a HPD decreases BP. Usually both SBP and DBP decrease simultaneously, it is therefore surprising that only DBP differed significantly between groups. In a study assessing the effect of protein intake on BP in 10020 men and women in the INTERSALT study [253], it was reported that a protein intake 30% above the mean, resulted in a decrease of SBP of 3.0 mmHg and 2.5 mmHg for DBP: These changes are in line with the present study, where SBP decreased ~5 mmHg and DPB ~2.5 mmHg. There was a wide variation in the measures with SD of 14.5 in HPD and 14.9 in SPD. It is possible that a larger sample showing the differences in both SBD and DBD would have reached statistical significance.

Limitations of this study include a small sample size. This study was powered to detect a difference in GFR and thereby not designed to detect differences in glycaemic control, serum lipids or BP. The power to detect a significant difference of the individual variables reported, using the two sided approach and aiming for 80% confidence ($p<0.005$) with 19 participants in HPD and 17 participants in SPD was calculated. This revealed that the study was powered to detect a difference in glycaemic control of 1.2 mmol/L for FBG and 1.2 and in HbA1c of 0.8%. For serum lipids the study was powered to see a difference of 1.0 mmol/L for total cholesterol, 1.1 mmol/L for TG, 0.2 mmol/L for HDL, 0.6

mmol/L for LDL cholesterol. For changes in blood pressure this study was powerd to detect a difference between groups of 12.1mmHg for SBP and 8.1 mmHg for DBP.

There were conflicting results in the ITT analysis depending on the method used. The full-information analysis predicted a significant decrease in AER in the HPD group compared to the SPD group, whereas the LOVCF showed no significant difference in this variable.The problem with the LOVCF test is that it assumes no further benefit occurs after a participant discontinues. It thereby underestimates the possible benefit, had the person continued the treatment but also disregards the possible deteriation when treatment is ceased. The full-information missing data analysis on the other hand predicts the outcome using baseline values and the effect seen in comparable participants.

It has previously been shown that weight loss and improved metabolic control decreases AER, therefore the slightly higher weight loss in the HPD, although not significant, may explain the difference in AER and alb/cr seen in the completers and the full-information missing data analysis (AMOS). Additionally, there was a trend towards a decrease in GFR, due to decreased hyperfiltration with weight loss, which would also account for the decreased AER in this group [243].

The strengths of this study include the use of CGMS which obtains a comprehensive and dynamic measurement of the 24h fluctuations in BG. This would not be possible using self monitoring finger pricks.

Concusion

Both diets were effective in improving metabolic control measured as FBG, HbA1c, serum lipids and BP. There was borderline significant difference in LDL cholesterol between groups at 12 months, which may be explained by changes in lipid lowering medication in the HPD group. Diastolic BP showed a significant treatment effect at 12 months adjusted for baseline, whereas differences in SPD were not significant. This is unexpected as improvements in both SBP and DBP is most commonly reported. The study was not powered to show significant differences in these variables and it is possible that a larger sample size would show a significant trend in these measures

Chapter 6: Changes in glycaemic control using CGMS (Sub-study)

In this study we wanted to investigate the effect on microalbuminuria of BG excursions. Diabetes nephropathy is caused by a combination of hyperglycaemia and hypertension [254]. It is well known that hyperglycaemia increases the risk of microvascular complications in both type 1 and type 2 diabetes patients [48, 199]. Fasting blood glucose (FBG) and HbA1c are currently used to assess diabetes control [2]. However, the increased mortality risk reported in the ACCORD study which targeted strict control of FBG and HBA1c may warrant the use of postprandial BG as a better predictor of glycaemic control.

We hypothesise that a protein rich, weight loss diet will result in reduced fasting, postprandial and total 24h glycaemic response, compared to the standard protein weight-loss diet when weight loss is equal in the two groups and will exert a beneficial effect on the progression of microalbuminuria.

6.1 **Subjects and method**

All volunteers recruited for the main study were approached for this additional sub-study investigating the glucose excursion for a period of 24 hours on 3 separate occasions (baseline, four and twelve months).

As an additional “arm” to the main study, volunteers were asked to wear a Continuous Glucose Measuring System (CGMS), MiniMed from Medtronic, for at least 48 hours at the 3 sample points.

Additional exclusion criteria were added for the CGMS sub-study. (1)Not willing to measure finger prick blood glucose, at least four times per day for a period of 72 hour period during the CGMS data collection (at baseline, four and twelve months). (2) They had to be willing to refrain from submersing into water (bathtub, swimming pool or spa) and (3) they were excluded if they were deemed not able to comply with the regime (assessed by the investigator).

Of the 48 participants who started the diet intervention in the main study (described previously), 39 agreed to participate in the sub-study. Participants were examined at baseline, before the start of the diet intervention, at four months after intervention start and at the end of the intervention (12 months). A total of 39 participants completed baseline (21 HPD and 18 SPD), 32 completed

four months CGMS (16 in each group) and 29 volunteers completed all three time points (15 HPD and 14 SPD).

6.1.1 Main outcome

Blood glucose measured by CGMS analyzed as peak blood glucose (Gmax), percentage time spent with a BG above 10 mmol/L (%T>10) and area under the blood glucose curve (AUC) computed as mmol/L per min over a 24 hour period ((mmol/L)/min).

6.1.2 Secondary outcome

Fasting blood glucose, HbA1c and microalbuminuria measured as albumin excretion (µg/min) derived from a 24 hour urine collection and albumin to creatinine ratio based on separate spot urine (Described in chapter 3 “Renal function”).

6.2 **Results**

6.2.1 Subjects

A total of 39 volunteers (26 male and 13 female) were recruited to this study. Participants with only baseline CGMS data are not included in the analysis because there are no data to compare at other time points.

Baseline characteristics for all participants who completed both baseline and 12Mo CGMS measurements are shown in table 1.

Table 18 Baseline characteristics of the 29 participants completing the CGMS study.

Baseline	Randomized groups		
Completed . 12 Mo	HPD (n=15)	SPD (n=14)	T-Test (p=)
Sex (M/F)	11 / 4	11 / 3	0.75
Age (years)	61.5±2.7	62.4±1.9	0.79
Height (m)	1.72±0.0	1.73±0.0	0.62
Weight (kg)	101.9±3.8	108.9±5.9	0.51
BMI (kg/m2)	34.7±1.5	36.0±1.2	0.32
FBG (mmol/L)	8.1±0.5	7.8±0.5	0.72
HbA1c (%)	7.4±0.2	7.1±0.2	0.32
AER (ug/min)	48.0±13.0	75.2±16.5	0.20
Alb/cr	7.0±1.9	8.6±2.3	0.60
SBP (mmHg)	128.0±3.2	125.2±3.3	p=0.55
DBP (mmHg)	74.0±1.6	68.8±2.8	p=0.11

Data are given as means (SEM). Significance level is set at $p<0.05$.

This sub-group of volunteers did not differ significantly from the main group.

6.3 **Glycaemic control:**

6.3.1 FBG

Adjusting for baseline values, there was no significant effect of treatment at 4 or at 12 months. At baseline mean FBG was similar in both groups 8.1±0.5 and 7.7±0.5 mmol/L respectively (ns). FBG decreased to 6.9±0.3 in HPD and to 6.2±0.4 mmol/l in the SPD by four months ($p<0.01$ compared to baseline). Between four and twelve months mean FBG increased slightly in both groups (7.1±0.4 and 6.3±0.5 mmol/L in HPD and SPD respectively ($p<0.05$ compared to baseline).

6.3.2 HbA1c

When adjusting for baseline values there was no significant effect of treatment on HbA1c at four months ($p=0.07$) or at twelve months ($p=0.34$). HbA1c decreased significantly with time ($p<0.01$). At four months HbA1c had decreased in the HPD group by 0.98% and in the SPD group by 0.43% ($p<0.05$ compared to baseline). At the end of the study there was a mean decrease of 0.2 % ($p>0.05$ compared to baseline) in the SPD and 0.4% ($p=0.02$ compared to baseline) in the HPD groups respectively.

HbA1c at four months was significantly negatively correlated with weight loss ($r = -0.49$, $p<0.01$), and positively correlated with FBG ($r = 0.62$, $p<0.01$), AUC ($r = .58$, $p<0.01$), %T>10 ($r = 0.58$, $p<0.01$) and Gmax ($r = 0.50$, $p<0.01$).

At twelve months HbA1c was strongly correlated with %T>10 ($r = 0.70$, $p<0.01$) and AUC ($r = 0.69$, $p<0.01$). There was a significant correlation with Gmax ($r = 0.57$, $p<0.01$), FBG ($r = 0.44$, $p=0.02$), and BMI ($r = 0.42$, $p=0.02$). The negative correlation with WL was still significant ($r = -0.54$, $p<0.01$).

Using multiple regression with change in HbA1c at 4 Mo as the dependent factor and change in FBG, change in %T>10 and change in AUC as independent variables revealed a highly significant total model (adjusted $r^2=0.31$, $p=0.007$); the strongest association was the change in %T>10 (adjusted $r^2 = -0.48$, $p=0.008$) and AUC (adjusted $r^2= 0.29$, $p=0.077$). Change in FBG (adjusted $r^2= 0.20$; $p=0.23$) was not significantly associated with change in HbA1c at this time point

This model was not significant at 12 months.

6.3.3 24h blood glucose

The 24h BG decreased significantly with time (by 1.7 vs. 0.7 mmol/L in HPD and SPD respectively; $p<0.01$). The difference at 4 months was borderline significant for treatment effect ($p=0.050$).

By the end of the 12 months intervention period the decrease in 24h BG in the HPD had diminished to 0.9 mmol/L and the SPD had increased above baseline by 0.1 mmol/L, but there was no significant treatment effect.

6.3.4 T>10

When adjusting for baseline values there was a non-significant treatment effect in %T>10 at four months ($p=0.12$) and at twelve months ($p=0.29$).

There was a significant decrease in %T>10 over time ($p<0.01$).

6.3.5 Gmax

At four months G_{max} had decreased in the HPD group by 2.1 mmol/L ($p=0.05$ compared to baseline) and in the SPD by 1.1 mmol/L ($p=0.31$ compared to baseline). Adjusting for baseline values a significant effect of treatment was found at four months ($p=0.02$). There was no significant differences in overall mean G_{max} at the end of the study ($p=0.10$).

6.3.6 AUC

Adjusting for baseline values there was no significant effect of treatment at any time point ($p=0.06$ at four months and $p=0.19$ at twelve months). AUC decreased significantly with time ($p<0.01$) with a decrease in the HPD group at four months of ≈2200 (mmol/L)/min and a decrease in the SPD group of ≈1000 (mmol/L)/min. There was an increase in both groups by twelve months.

6.4 Correlations with change in microalbuminuria

At four months there was only small non-significant correlations between AER change and changes in weight, FBG, HbA1c, AUC, T>10, Gmax, 24hBG or SBP.

At 12 Mo using the same model with 12Mo results the only significant correlations was total weight loss. There was a small non-significant correlation

with AUC, T>10; Gmax, 24h mean BG and SBP. There was no correlation with HbA1c or FBG.

Table 19: AER Correlations

Change in AER at 12 Mo	Pearson Correlation	Sig. (2-tailed)
Total weight loss	**0.439**	**0.017**
HbA1C	0.066	0.733
FBG	0.066	0.735
AUC	0.196	0.308
T>10	0.165	0.391
Gmax	0.329	0.082
BG24h	0.168	0.384
SBP	0.256	0.179

* Correlation is significant at the 0.05 level (2-tailed).

Using multiple regression with the change in AER at 12 Mo as the dependent variable and WL, change in SBP and change in Gmax as independent variables showed a highly significant model (adjusted r^2=0.43; p=0.002). The strongest associations were total WL (adjusted r^2=0.51; p=0.003) and change in Gmax (adjusted r^2= -0.44; =0.008). There was a borderline significant association between change in AER and change in SBP (adjusted r^2= -0.26; p=0.099). No significant association between the change in AER and the change in AUC, 24h BG, HbA1c, FBG or DBP was seen.

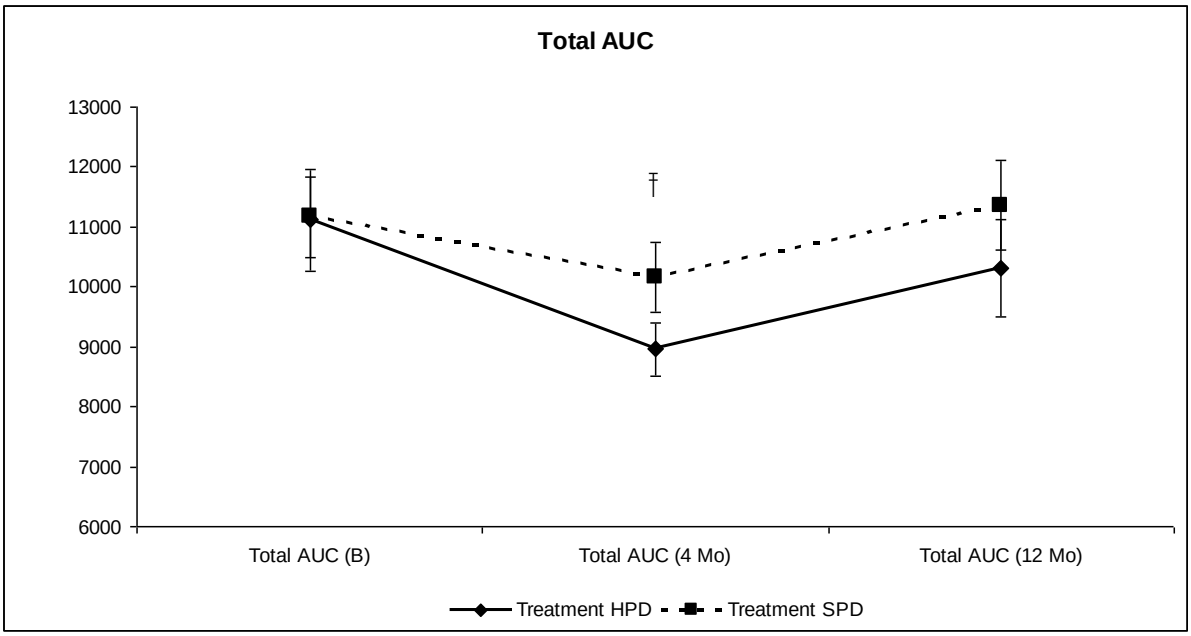
The change in alb/cr showed no significant correlation with markers of glycaemic control (AUC, Gmax, T>10, FBG or HbA1c). There was a significant correlation with weight loss (r= -0.48; p=0.008), SBP (r= 0.45; p=0.014), DBP (r= 0.47; p=0.010) and borderline correlation with HbA1c (r= 0.35; p=0.065) at 4 Mo:

At 12 Mo only alb/cr was positively correlated with total weight loss (r= 0.44; p=0.018) and DBP (r= 0.47; p=0.011).

Using the regression model as above, looking at the change in alb/cr ratio revealed borderline significant associations with total weight loss (adjusted r^2= -

0,41; p=0.053) when using alb/cr change at 12 Mo as dependent variable and SBP and AUC as independent variables.

Figure 18: The total AUC (24 hour blood glucose; mmol/L/24h)



Data are given as mean (SEM). At four months there was a significant decrease in AUC with time † (p<0.01). There was no significant difference between groups at any time point.

Table 20**:** Changes in glycaemic control

	Group	Baseline (n=29)	4 Mo (n=29)	12 Mo (n=29)	Significance Time	Group 4 Mo	Group 12 Mo
HbA1c (%)	HPD	7.4±0.2	6.4±0.3	7.0±0.3	**<0.01**	0.73	0.34
	SPD	7.1±0.2	6.6±0.3	6.9±0.3			
FBG (mmol/L)	HPD	8.1±0.5	6.8±0.3	7.1±0.4	**<0.01**	0.23	0.29
	SPD	7.8±0.5	6.2±0.3	6.3±0.5			
Mean 24h BG (mmol/l)	HPD	8.2±0.6	6.4±0.3	7.2±0.6	**<0.01**	**0.05**	0.12
	SPD	7.7±0.4	7.1±0.4	7.9±0.5			
Gmax (mmol/l)	HPD	13.5±0.8	11.1±0.8	11.5±1.0	0.06	0.52	0.10
	SPD	12.6±0.8	11.6±0.8	13.5±0.8			
T>10 (%)	HPD	22.1±5.1	6.2±1.9	16.0±7.0	**<0.01**	0.12	0.29
	SPD	16.1±4.7	10.3±4.0	19.9±5.8			
AUC (mmol/L)/24h)	HPD	11165±792.6	8958±439.3	10308±818.6	**<0.01**	0.06	0.19
	SPD	11046±593.4	10206±517.7	11356±749.3			

This table describes the changes in glycaemic control for the 29 completers. Data are given as means (SEM). Significance level is set at 0<0.05. (HPD n= 15, SPD n=14.

Figure 19: Changes in mean BG in the HPD at baseline, 4 Mo and 12 Mo

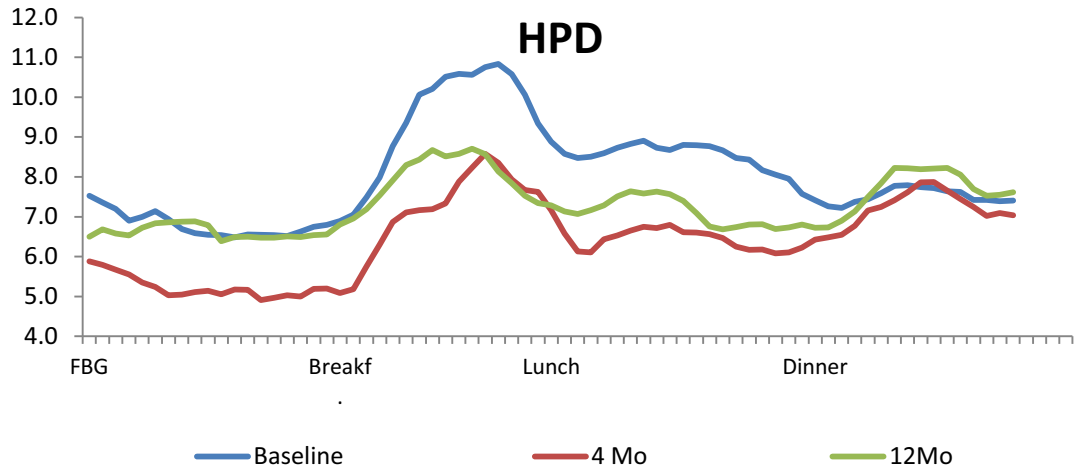
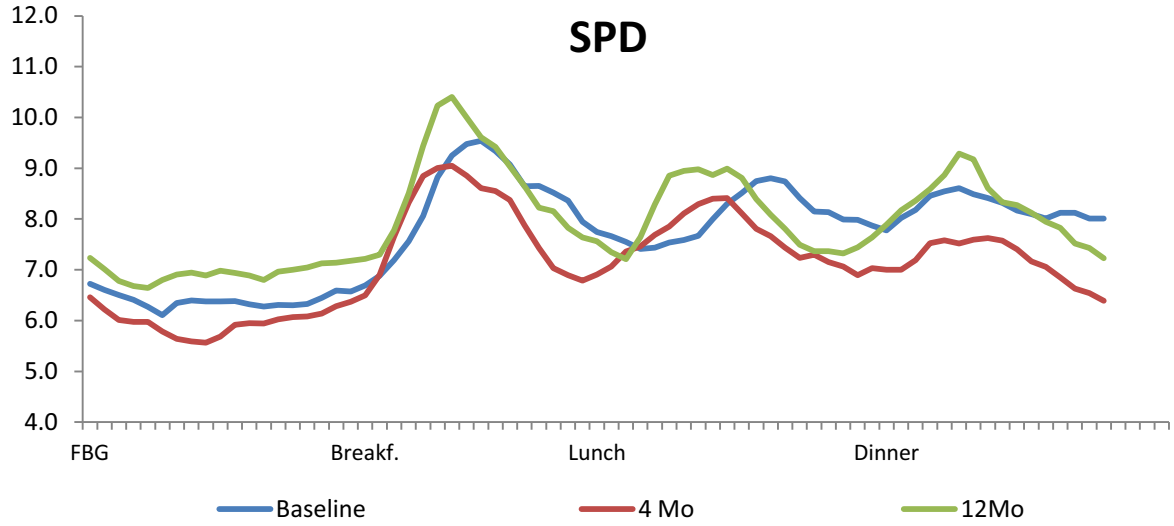


Figure 20: Changes in mean BG in the SPD at baseline, 4 Mo and 12 Mo



Mean 24h blood glucose for the SPD and HPD. Mean 24h BG decreased significantly in HPD and non-significantly In SPD at four months. At twelve months mean 24h BG was lower in the HPD group; but the difference was not statistically significant.

6.5 **Discussion**

Early detection and treatment of microalbuminuria is important as the presence of microalbuminuria predict the progression to proteinuria and the increased risk of micro- and macrovascular complications [54, 255]. The main risk factors for microalbuminuria are hyperglycaemia and hypertension [54].

In this study BG measured as Gmax ($p=0.06$), %T>10 and AUC ($p<0.01$) decreased in both groups by four months but reverted back to slightly above baseline in the SPD group by the end of the study. The decreased Gmax at four months was maintained in the HPD group but did not reach the recommended levels of PPG <8 mmol/L [217]. In the HPD group time spend with a BG above 10 mmol/L was reduced by 6%. As in the main study group, HbA1c and FBG changed significantly with time ($p<0.01$) with no significant treatment effect.

Results from the UKPDS clearly showed a beneficial effect of decreasing overall blood glucose. It was shown cross-sectionally that a 1% lower HbA1c was related to a 21% decrease in any endpoint or death related to diabetes or all cause mortality [29]. No threshold for HbA1c was observed under which the complication risk did not decrease or above which the complication risk no longer increased. The greatest benefit was seen in persons with HbA1c near to or in the normal range (≤6%) [29]. However, results from the ACCORD study suggests that aggressive lowering of HbA1c may result in increased mortality and the study was discontinued early after 3.5 years. The findings from the ACCORD study showed that the intensively treated “arm”, where the aim was a normalization of HbA1c to <6%, resulted in an increased mortality risk of 1.41% compared to the conventionally treated group (HbA1c 7-7.9%) of 1.14%; or 257 vs. 203 deaths respectively [256]. More recently an investigation studied the effect of a lower HbA1c on mortality risk in 21155 participants with diabetes and renal impairment (GFR 15-59.9 ml/min/1.73kg^2). They found a U-shaped association between HbA1c and mortality, such that an HbA1c higher than 9% and lower than 6.5% resulted in excess mortality in participants with non-haemodialysis-dependent CKD. This excess risk was more pronounced in people with better kidney function. The study did not distinguish between T1DM and T2DM and no data on postprandial glycaemic control was obtained [257].

In the DECODE study, which investigated the ability of FBG vs. 2h post challenge BG to predict all cause mortality in newly diagnosed T2DM, it was found that the 2h post load BG was an independent predictor of an increased risk of death, whereas FBG did not independently predict the risk of death [51].

Postprandial blood glucose has been shown to predict cardiovascular risk in line with known risk factors such as hypertension, hyperlipidaemia and smoking [200, 258]. As many as 80% of T2DM patients have been shown to have postprandial BG above 8.9 mmol/L in spite of an HbA1c<7% [53]. It is therefore reasonable to target postprandial BG in the aim of decreasing the risk of diabetes related complications.

There was a non-significant decrease in AER over time with a trend towards a significant treatment effect at 12 months. The change in AER was associated with a combination of weight loss, change in SBP and postprandial blood glucose spikes (Gmax). It is well known that obesity results in increased AER and GFR [60, 240]. Weight loss has been shown to decrease both hyperfiltration and albumin excretion [243]. The albumin to creatinine ratio improved by four months in both groups; but the improvement was only maintained in the HPD group at twelve months. Using the same model in multiple regression showed changes in SBP to be strongly associated with the change in alb/cr.

Glycaemic control and especially postprandial BG control is essential in preventing and decreasing albuminuria in T2DM [51]. In the present study postprandial blood glucose was improved in both groups with borderline significant decrease in mean 24h BG at four months; however with time BG control reverted back to near baseline values. The same trend was seen in AER, with a mean decrease of 18µg/min at 4 months but only 5µg/min at 12 months. The lack of effect on AER long-term may be explained by baseline BG control. HbA1c was close to 7% at baseline, with a significant improvement at four months, and a subsequent return to close to 7% at the end of the study; meaning the participants were relatively well controlled according to the recommended levels of HbA1c at baseline [217], Major improvements would demand high level of compliance to diet which in most studies reported, and in this study was not maintained.

A major limitation of this study was the low number of participants (15 HPD and 14 SPD). Using the SD`s from the change in the individual variables, this study was powered with 80% confidence ($p<0.05$) to show a difference between groups of 0.8% for HbA1c, 1.5 mmol/L for FBG, 1.6 mmol/L for mean 24h BG, 3.9 mmol/L for Gmax, 18% for time spend with a BG >10% and 2263 ((mmol/L)/24h). None of these differences were reached in this study and a larger sample size is needed to show significance.

Participants in this study lost weight which may help explain changes in GFR and AER. This study was based on a subset of participants from the main study, and therefore not specifically powered for changes in glycaemic control.

Chapter 7: Study 3

Effect of carbohydrate timing and previous meal on glycaemic control in type 2 diabetics

During the course of T2DM the insulin response to a CHO load is compromised, with a loss of first phase insulin response and impaired second phase insulin response, leading to hyperglycaemia [259, 260].

Population-based studies have shown a relationship between postprandial glucose (PPG) and the incidence of myocardial infarction (MI) and all cause mortality in patients with type 2 diabetes [50, 51].

Currently an even distribution of CHO over all meals is recommended in the diabetes diet. However there are very few studies showing this pattern to be optimal in preventing hyperglycaemia.

Gannon and Nuttal in 2004 reported large changes in HbA1c with an absolute fall of 2.2% over 5 weeks with a reduction in carbohydrate (CHO) from 388g to 142g. [195]. Data on the effect of carbohydrate distribution on glucose control is limited. In order to determine the daily occurrence of glucose spikes, 24 hour continuous glucose monitoring (CGMS) is a useful tool that has been little used in type 2 diabetes [261]. Using CGMS our lab have recently shown that CHO at breakfast leads to higher glucose spikes than CHO in meals at other times and the best overall profile was achieved by CHO loaded at lunchtime [203]. Interpretation of this study was complicated by the fact that meals at each time point had slightly different composition and glycemic index (GI) and that lunch and evening meals were preceded by some CHO at both breakfast and lunch. It is not clear if the augmented response to CHO at breakfast occurs because it comes after a 12 hour fast, or because there is no prior exposure to CHO during this time. Thus it is possible that the lunchtime CHO-rich meal could lead to large spikes in glucose if CHO is not eaten at breakfast. Alternatively calorie exposure from any source may be all that is required to mute the glycemic response at the second and third meal. Trovati et al (2002) found that although pre-prandial blood glucose is dependent on FBG, they also showed that FBG is higher than both pre-lunch and pre-dinner blood glucose. Furthermore, the fall in pre-lunch blood glucose compared to FBG, is greater than the fall in pre-

dinner blood glucose compared to pre-lunch glucose. This effect is greater with increasing FBG levels [262].

A sustained decrease in the risk of microvascular disease has been reported with tighter glycaemic control in T2DM [49, 263] Thus strategies that minimize postprandial spikes and overall glycaemia are important to prevent diabetic complications and in particular diabetic nephropathy.

This study examined the BG surges following lunch time CHO ingestion, after withholding CHO at the first meal after an overnight fast. It was hypothesized that withholding CHO at breakfast would result in higher blood glucose excursions at lunch time, but in lower overall 24 h glycaemia.

7.1 **Study protocol**

57 participants with type 2 diabetes, aged between 35 and 75 years, were recruited to the study. Participants were included if they had been weight stable (within ± 2kg) and on stable medication during the last three months.

Participants were excluded if they had type 1 diabetes, had any malignancy, or a history of metabolic disease such as liver, kidney, or gastrointestinal disease.

Twelve participants did not meet the inclusion criteria (HbA1c between 7 and 8%) and seven participants withdrew consent prior to study start.

Volunteers were divided into two groups matched on blood glucose control before randomization. One group had an HbA1c of ≤7% and the other group had an HbA1c of ≥8%. Randomization was done by block random number, sequence was predetermined and volunteers were allocated treatment sequence as they were recruited.

Of the 38 participants randomized to the study, 20 were in good glycaemic control and 18 were poorly controlled (HbA1c ≤7% or ≥8% respectively). Of these 31 completed both weeks of diet intervention. Three participants, who had stated they had T2DM on the screening questionnaire, showed normal glucose levels when assessing the peak and fasting blood glucose values despite the participants not taking any hypoglycaemic drugs. Consequently they were excluded from the analysis. The remaining 28 participants (18 male and 10 female) were included in the analysis. The Good control group consisted of 10

men and 4 women and the poorly controlled group consisted of 8 men and 6 women.

Of the 28 participants included in the analysis, six were treated by diet alone, fourteen were treated with metformin alone, five were treated with metformin and glimepiride or arcabose or rosiglitazone (dual treatment), one with metformin, glimepiride and pioglitazone, (triple therapy) two with metformin, glimepiride or gliclazide and insulin glargine (triple therapy with insulin). Other medication included anti hypertensives, lipid lowering agents and antidepressants. Participants were asked to maintain medication dose and timing unchanged throughout the study period.

All participants reported low physical activity and they were asked to maintain this level.

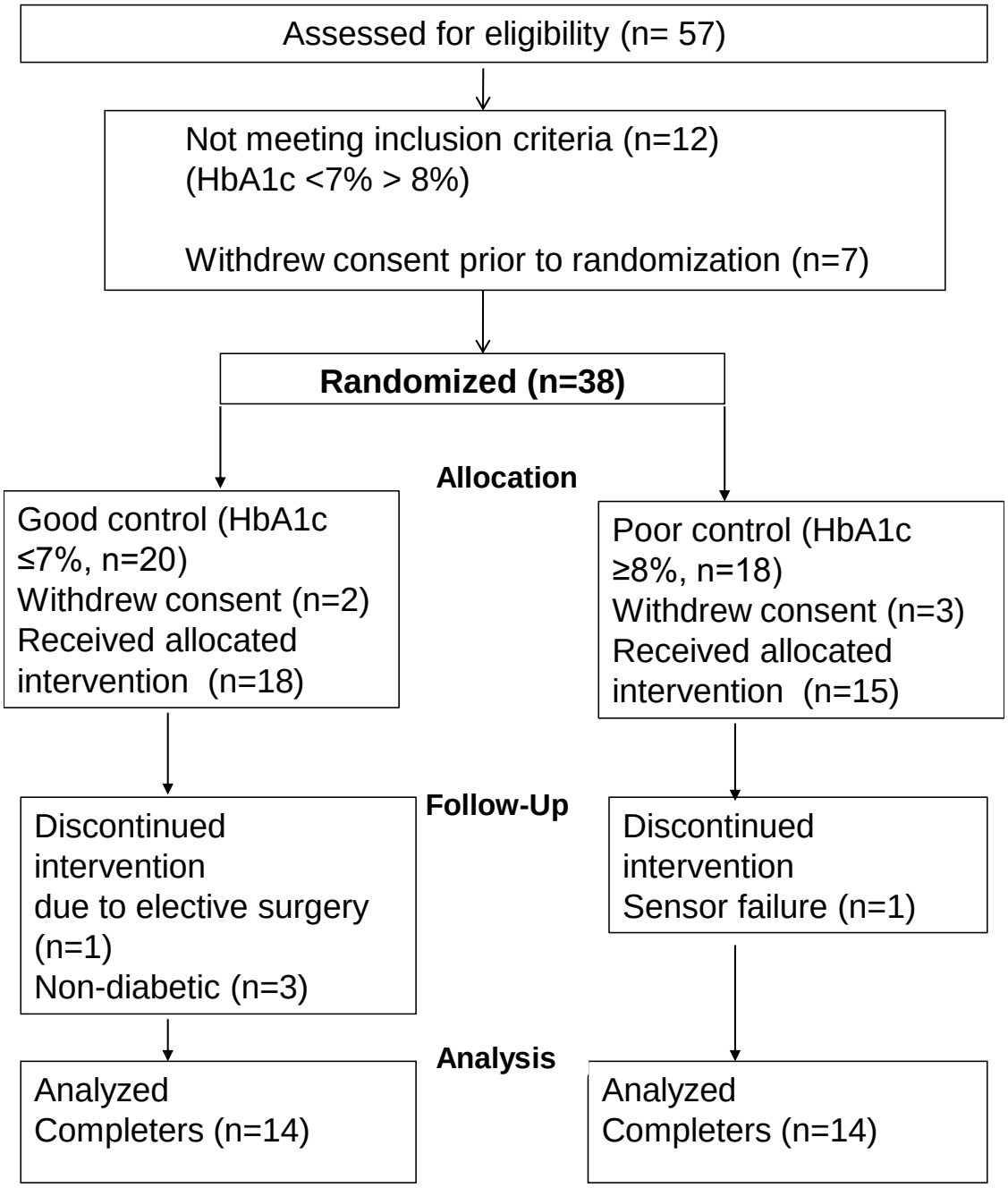
Randomization into dietary sequence was done by block random number.

Blood glucose excursions were measured using the Continuous Glucose Monitoring System (CGMS) Medtronic MiniMed, Gold standard system (Northridge, CA) (method pp 72-73).

Rate of gastric emptying (GE) was measured using ^{13}C-Octanoic acid mixed into the lunch meal. GE was measured for a 6 hour period between lunch and dinner (method pp 73-76).

Baseline characteristics are shown in table 23.

Enrolment of eligible subjects



Diet protocol:

The meal tests provided 8000 kJ for men and 7000 kJ for women. These energy levels were chosen to enable the volunteers to eat all the foods provided. Due to the short term nature of the study weight change was not expected to occur.

Participants were divided into two groups according to HbA1c.

Group 1: good control HbA1c ≤ 7.0 mmol/L (n=14, ten men and four women)

Group 2: poorly controlled HbA1c ≥8.0 mmol/L (n=14, eight men and six women).

They were asked to consume their usual diet until dinner the night before the meal test, where a standardised dinner meal was provided. Participants were asked to fast for twelve hours prior to the meal test comencement. A randomized two day treatment model with a cross over design was used. Participants were asked to eat only the foods provided and to eat all foods provided. The food was pre-weighed and individually wrapped meal by meal. Participants were asked to space the three meals six hours apart; accordingly they were encouraged to start the breakfast meal early (6:00 am) in order to obtain a twelve hour fast before the second meal test.

Participants acted as their own control and were randomly allocated to the two meal tests.

Treatment A: carbohydrate (CHO): Three identical meals (even distribution).
Treatment B: no carbohydrate (NoCHO): Breakfast without carbohydrates, lunch and dinner identical to CHO meals.

These treatments were repeated on two separate study weeks with different orders of treatment, to rule out the effect of meal sequence.

The evening prior to diet intervention participants were instructed to eat a standard dinner consisting of 3000 kJ (Carbohydrate 44 %TE, Protein 33 %TE, fat 23 %TE). The only difference between the two test days were the breakfast meals. The remaining two daily meals (lunch and dinner) were identical on both occasions and were identical to breakfast in the even distribution meal test day.

All foods for the intervention were provided. The individual meals were packed in bags clearly marked with meal time and date. All foods were weighed and individually wrapped. The three pre-weighed individually wrapped meals were then packed into bags marked with meal sequence and date. A diet information booklet was issued showing the individual meals detailing the amount of food and giving suggested recipes. The participants were instructed to eat all foods provided in the sequence described. If the volunteer did not eat all foods or added foods, they were asked to note the discrepancies in the diet information booklet. Kitchen scales were provided (DZC 5000A; Procon Technology, Brisbane, Australia). All experimental procedures were done on an outpatient basis.

Table 21: Nutrient composition of the two diets over 24 hours

Nutrient distribution	CHO	NoCHO
kJ	7887	7827
Protein (g)	96	118
Fat (g)	59	82
SAFA (g)	15	26
PUFA (g)	11	12
MUFA (g)	25	35
Cholesterol (mg)	657	1271
Carbohydrate (g)	229	155
sugars (g)	117	80
starch (g)	112	75
dietary-fibre (g)	35	25
Alcohol (g)	0	0

The nutrient distribution as a percentage of total energy:

% total energy (%TE)	CHO	NoCHO
Protein %TE	21	26
Total fat %TE	28	40
Carbohydrate %TE	51	34
Alcohol %TE	0	0

The only difference between the two meal test days was breakfast meal.

Lunch and dinner were identical at all occasions and were identical to breakfast in the CHO meal test.

Table 22: Breakfast meals:

Nutrient distribution	CHO	NoCHO
kJ	2629	2569
Protein (g)	32	54
Fat (g)	20	43
SAFA (g)	5	16
PUFA (g)	4	4
MUFA (g)	8	18
Cholesterol (mg)	219	832
Carbohydrate (g)	76	2
sugars (g)	39	2
starch (g)	37	0
dietary-fibre (g)	12	2
Alcohol (g)	0	0

Percentage of total energy

	CHO	NoCHO
Protein %TE	21	36
Total fat %TE	28	63
Carbohydrate %TE	51	1
Alcohol %TE	0	0

7.2 **Results**

7.2.1 Baseline characteristics

The participants were stratified by diabetes control prior to randomization.

Table 23: Baseline charactaristics of the 28 completers

	Good control	Poorly controlled	T-Test
Sex (M/F)	10/4	8/6	
Age (years)	63.07±2.1	64.71±2.2	p=0.51
Height (m)	1.7±0.0	1.7±0.0	p=0.85
Weight (kg)	86.81±2.4	102.2±4.6	p=0.007
BMI	30.6±1.3	35.5±1.2	p<0.001
Waist circumference	101.8±2.0	114.0±2.7	p=0.001
HbA1c	6.3±0.2	8.8±0.3	p<0.001

Data are given as means (SEM). There were significant differences in weight, BMI, waist circumference and HbA1c at baseline.

7.2.2 Test meals

The volunteers were instructed to consume their three daily meals six hours apart. However, the minimum time between two meals was five hours; therefore the blood glucose traces were divided into five hour time periods, starting at the time of meal initiation for breakfast, lunch and dinner.

One volunteer disliked corn and omitted this ingredient at all meals. All other participants reported no discrepancies between planned food intake and food consumed.

7.2.3 Blood glucose excursions

7.2.3.1 Peak blood glucose

There was a significant treatment effect for the Gmax over the 24h period (Estimated marginal mean for CHO was 12.1 mmol/L and for NoCHO 11.0 mmol/L). The difference between the two treatments in 24h peak blood glucose of 1.1 mmol/L was independent of diabetes control (p=0.004).

There was a significant effect of treatment on breakfast Gmax (estimated marginal means were 12.2 mmol/L and 9.3mmol/L for CHO and NoCHO groups respectively). The Gmax was 2.95 higher after the CHO meal compared to the NoCHO meal (p<0.001). The differences in Gmax at lunch and dinner were not significant. There were no significant correlations between mean Gmax and sex, age, BMI and weight at baseline.

There was a significant difference in Gmax between the two diabetes control groups. For 24h Gmax the difference was 1.8 mmol/L higher in the poorly controlled group compared to the good control group with estimated marginal means of 12.4 mmol/L vs. 10.7 mmol/L respectively (p=0.02). At breakfast, lunch and dinner the difference between diabetes control groups was not significant (p=0.06 for breakfast, p=0.16 for lunch and p=0.35 for dinner) suggesting the Gmax occurred with different meals in different subjects.

7.2.3.2 Blood glucose above 10 mmol/L

Time spent with a blood glucose above 10 mmol/L (T>10) was assessed by averaging the two days on each diet treatment. There was a non-significant treatment effect in minutes of T>10 (min T>10) for the total 24h period estimated marginal means for CHO was 180.7 min and for NoCHO 144.3 min.

Withholding CHO (NoCHO) in the first meal of the day resulted in 36 minutes less spent with a BG above 10 mmol/L compared to the CHO group (p=0.16). The treatment by diabetes control interaction was not significant; p=0.93).

There was a significant difference in min T>10 between diabetes control groups of 133.6 min between the good and poorly controlled volunteers in 24h T>10; p=0.03. With estimated marginal means for HbA1C<7% group of 95.7 min and 229.3 min for the HbA1c ≥8% group.

After the breakfast meal there was a significant treatment effect, where the CHO meal resulted in longer time spent with BG above 10 mmol/L, estimated marginal means 76.4 min compared to 32.9 min in the NoCHO treatment, a difference of 25 minutes or 15% (p=0.001).

Min T>10 for lunch and dinner was not significantly different by treatment (p=0.82 for lunch and 0.64 for dinner).

Table 24: Blood glucose excursions

	CHO	**NoCHO**	**CHO**	**NoCHO**
	HbA1c<7%		**HbA1c>8%**	
24h Gmax * #	11.2±0.6	10.2±0.4	13.0±0.5	11.8±0.6
Gmax Breakfast *	10.9±0.6	8.0±0.5	12.3±0.5	10.8±0.6
Gmax Lunch	9.4±0.5	9.4±0.4	10.5±0.5	10.8±0.6
Gmax Dinner	9.2±0.5	10.0±0.4	10.7±0.6	10.5±0.5
24h T>10 average min)#	118.0±37.7	56.4±19.6	223.8±35.9	184.6±43.5
min. T>10 Breakfast*#	55.5±16.0	11.4±7.3	97.3±20.2	54.3±18.9
min. T>10 Lunch	29.6±11.6	20. 9±9.8	58.8±13.0	63.6±18.3
min. T>10 Dinner	32.0±14.4	23.0±7.2	65.0±15.1	66.1±15. 6

Data are given as mean (SEM). * Significantly different treatment effect p<0.05, # Significant difference between DM_control groups p<0.05

7.2.3.3 Total area under the blood glucose curve (AUC in mmol/L*5h)

There was a significant treatment effect for AUC after breakfast. Estimated marginal means 2800 and 2215 for CHO and NoCHO respectively, with a lower AUC in the NoCHO group of 586 mmol/L*5h (p<0.001).

There was a non-significant treatment by DM_Control effect in AUC.

AUC for lunch and dinner was not significantly different for treatment effect or for treatment by DM_Control interaction.

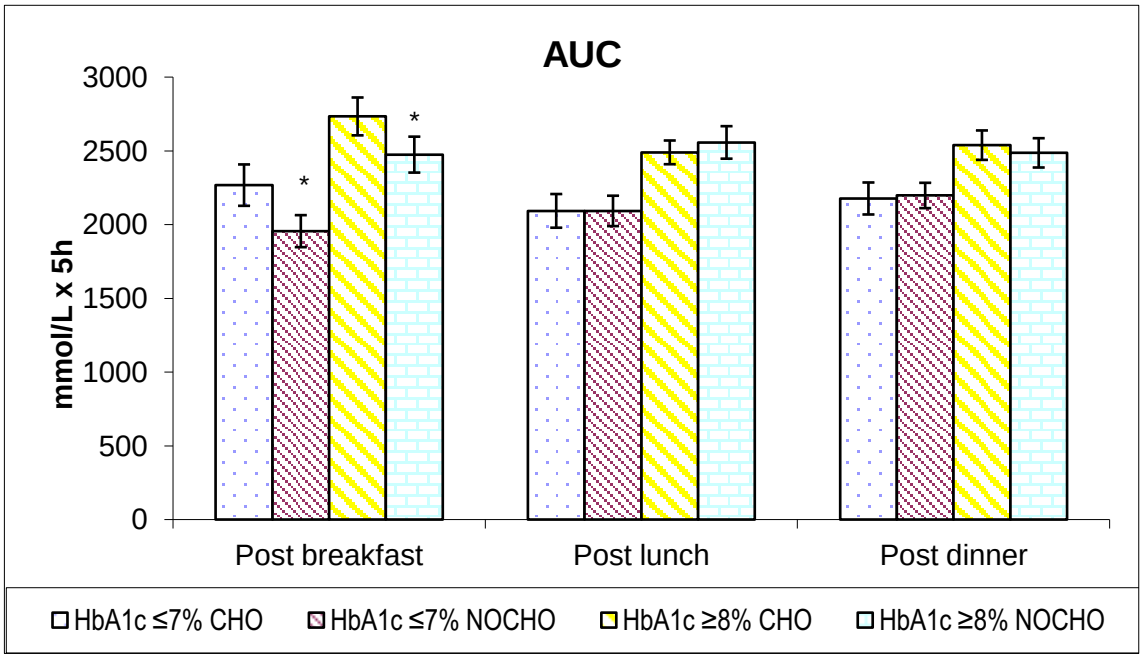
For all time points the AUC was significantly higher in the poorly controlled group, regardless of treatment (p=0.006, p=0.003 and p=0.015 for breakfast, lunch and dinner respectively).

Table 25: Total AUC for the 5h post meal period

		Good control	**Poor control**
CHO	Post breakfast	2268±140.3	2733±128.4[ab]
	Post lunch	2093±114.1	2490±80.4[a]
	Post dinner	2177±108.5	2538±99.8[a]
NoCHO	Post breakfast	1955±108.5	2474±121.8[ab]
	Post lunch	2093±103.0	2557±110.2[a]
	Post dinner	2197±85.8	2486±99.3[a]

The data is analyzed as the mean of two test days with either CHO or NoCHO meals, two volunteers had missing sensor data for one of the meal tests. All volunteers had data for at least one 24h period of the individual test meals. Data is presented as mean (SEM). [a] AUC was significantly higher in the poorly controlled volunteers compared to good control $p<0.05$. [b] AUC was significantly lower in the NoCHO breakfast compared to the CHO breakfast $p<0.001$ independently of DM_Control group.

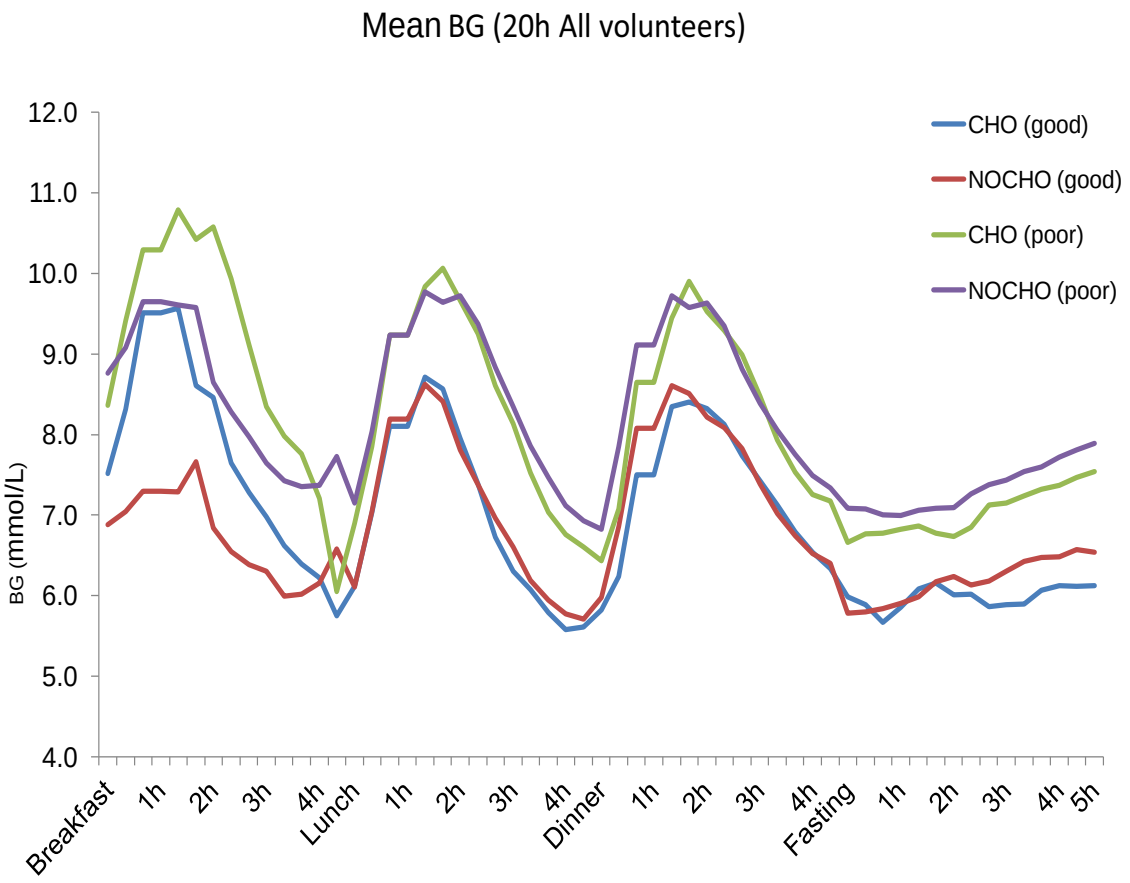
Figure 21: Total area under the blood glucose curve.



AUC was calculated for the individual meals using the trapezoid method. The time periods are presented with time zero as baseline and lasting for 300 minutes (5h), within this timeframe all volunteers had at least one complete day of meal test. Data from the two weeks were averaged meal by meal the error bars illustrate standard error of the mean AUC.

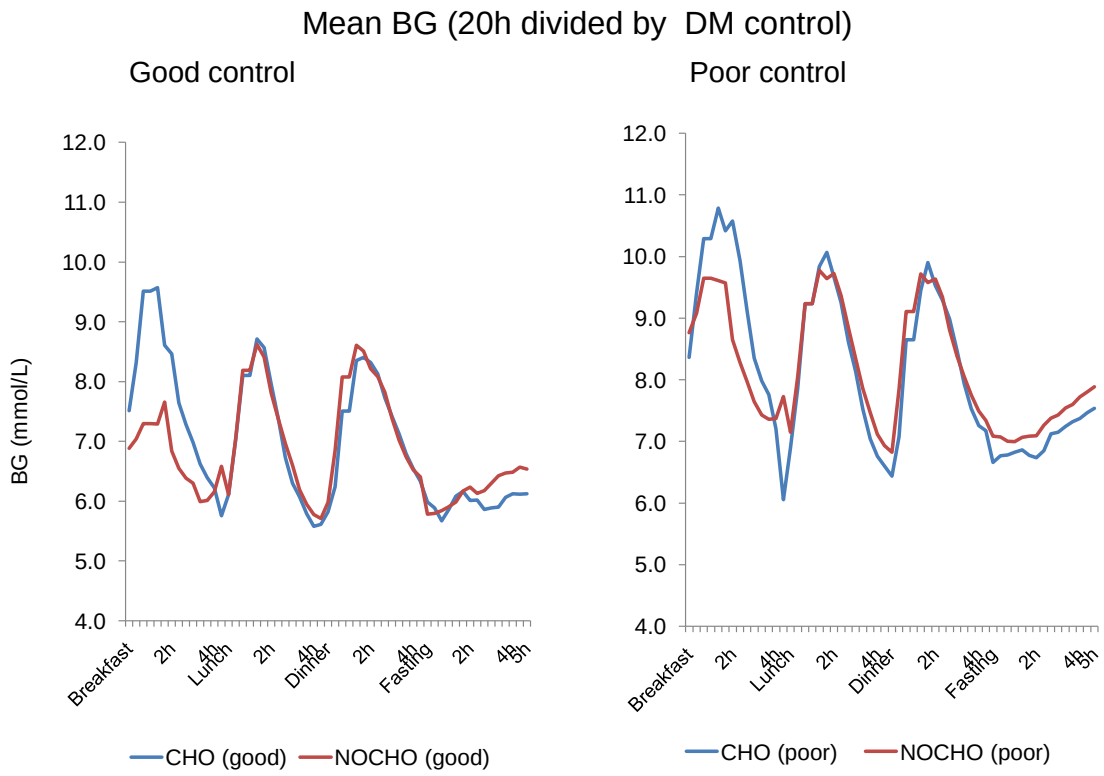
*AUC was significantly lower after NoCHO breakfast compared to CHO breakfast regardless of diabetes control group<0.05.

Figure 22: Cumulative blood glucose excursions



This figure represents the differences in meal composition and diabetes control. As expected the major differences were seen in the breakfast meals, where NOCHO diet in good control patients (red line) experienced the lowest blood glucose excursions compared to CHO good control (blue line), CHO poor control (green line) and NoCHO poor control (purple line) ($p<0.001$ for treatment effect). Lunch and dinner excursions were not significantly different between treatments. There was an overall difference between good and poor control ($p<0.05$).

Figure 23: Mean BG for the entire 24h period divided by DM control groups



This figure depicts the two control groups separately. It is shown that the main difference is in the breakfast meal, with lunch and dinner similar in both groups.

7.2.4 Gastric emptying at lunch time

In the test meals the macronutrients differed in the breakfast meal, resulting in higher fat and protein content and decreased CHO content in the NoCHO meal (table 3 gives the nutrient composition).

Three measures were compared: the time elapsed for one half of the meal to be emptied from the stomach (T½), the time for 5% of the meal emptying (Tlag) and gastric peak time which is the maximum percentage 13C-Octanoic Acid dose recovered per hour, it is measured at the point of inflection on the recovery curve (Tmax), all variables are expressed in minutes.

There were no significant differences in the gastric emptying rate between treatments or between groups after the standard lunch ($p>0.05$).

Table 26: Gastric emptying

Gastric emptying times

		$T½$	T_{lag}	T_{max}
Good control (n=13)	CHO	190.8±34.5	74.6±12.1	180.5±26.5
Poor control (n=14)		187.4±30.4	70.1±9.8	173.2±22.6
Good control (n=13)	NoCHO	162.0±17.6	73.7±5.5	171.3±11.4
Poor control (n=14)		151.3±18.7	63.5±5.6	153.3±12.4

7.3 **Pre-prandial blood glucose**

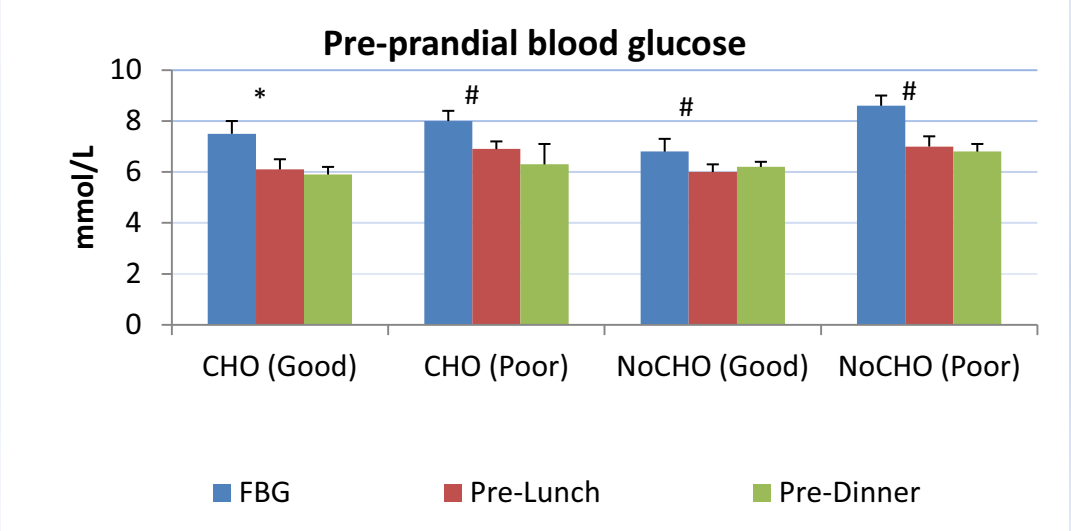
The FBG in all treatment and diabetes control groups was significantly higher than pre-lunch and pre dinner BG (*p<0.05 and #p<0.01). Pre-lunch BG was higher than pre-dinner BG, except in the good control group when consuming NoCHO treatment, however, the difference was not significant.

Table 27: Pre-prandial BG divided into treatment and DM_Control groups.

	FBG	Pre-Lunch	Pre-Dinner
CHO (Good)	7.5±0.5	6.1±0.4*	5.9±0.3#
CHO (Poor)	8.0±0.5	6.9±0.3#	6.3±0.2#
NoCHO (Good)	6.8±0.4	6.0±0.3#	6.2±0.8#
NoCHO (Poor)	8.6±0.4	7.0±0.4#	6.8±0.3#

Data are mean (SEM) *p<0.05 compared to baseline. # p<0.01 compared to baseline.

Figure 24: Blood glucose excursions after identical meals eaten at different times of the day.



The three meals were identical in the CHO treatment "arm" however the BG fluctuations differed with different time of day. Overall the mean BG differed between breakfast and lunch (p<0.01), there were no significant differences between lunch and dinner mean BG (p=0.39).

7.4 **Discussion**

The main findings of this study were that withholding CHO in the breakfast meal and only consuming fat and protein did not alter the response to CHO at the lunch time meal and as expected resulted in a significantly decreased blood glucose excursions in the 5h post meal period, where Gmax, T>10mmol/L, and AUC were significantly lower in the NoCHO compared to the CHO group ($p<0.05$). Surprisingly there was still a considerable rise in glucose after breakfast especially in the poor control group. There was as expected a significant difference in blood glucose excursions between diabetes control groups, such that the poorly controlled diabetics had consistently higher Gmax, T>10 and AUC values compared to the good control volunteers; but there was no interaction with treatment.

Data on the effect of carbohydrate distribution on glucose control is limited. Pearce et al investigated the effect of CHO distribution, when CHO was loaded at different meals. They found that CHO loaded at lunch resulted in the lowest BG excurtion, measured as Gmax, T>12, and AUC_{20h}; compared to CHO loaded at breakfast or dinner and also when compared to the recommended even CHO distribution. In this study AUC adjusted for FBG was significantly lower when CHO was loaded at lunch compared to all other distributions tested ($p=0.006$). Peak BG (Gmax) was significantly lower after the CHO loaded lunch ($p=0.003$) with CHO loaded at breakfast showing the highest Gmax [203] .

Post meal peaks in glucose probably contribute more to the complications of diabetes than high steady levels even though under both circumstances HbA1c may be equally high [264].

Population-based studies have shown a relationship between postprandial glucose (PPG) and the incidence of myocardial infarction (MI) and all cause mortality in patients with type 2 diabetic [50, 51]. Restoring blood glucose to normal range is the aim of diabetes treatment. In both the DCCT and the UKPDS trials it was shown that achieving HbA1c in the lower range (≤6.5% and ≤7% respectively) substantially decreased the morbidity rate [48, 199].

7.4.1.1 Second meal effect

Looking at blood glucose excursions at lunch time Jovancovic et al found a 95% higher rise in BG following the lunch meal if no breakfast had been given compared to a lunch following a standard breakfast and a 40% lower rise in BG if intravenous arginine was given prior to the lunch meal [265]. Also when using a protein preload two hours prior to the breakfast meal, a reduction of 40% in AUC was seen after the subsequent breakfast [266].

In acute meal tests comparing a breakfast with a high glycaemic index (GI) to a breakfast with added psyllium fibre resulting in a lower GI, lower postprandial BG and AUC was observed in the low GI group in T2DM. More relevant to this study BG excursions at the subsequent lunch meal were decreased. AUC was decreased at both breakfast and lunch after a low GI breakfast meal [267]. Nilsson et al found significantly decreased incremental AUC after breakfast and lunch, when a barley kernel breakfast (low GI) was given compared to a whole wheat meal (high GI) [268]. An overnight second meal effect has been shown in healthy volunteers when a low GI evening meal was consumed. The altered substrate oxidation (higher fat oxidation and lower CHO oxidation) was noted up to but not beyond the second meal [269, 270]. A decrease in AUC of 14% in the good controlled and 9% in the poorly controlled participants after NoCHO breakfast and a substantial decrease in T>10 in the period between breakfast and lunch (80% and 44% in good and poorly controlled participants respectively) was found. Likewise Gmax decreased significantly after a NoCHO breakfast by 27% and 17% in the good control and poorly controlled diabetes respectively. Our results are similar to those of Clark et al they investigated the effect of a high GI breakfast compared to a low GI breakfast, on glucose excursion in the period between breakfast and lunch. They showed significantly lower AUC following a low GI breakfast; but no second meal effect at the lunch meal was seen. The marked improvement in AUC after the breakfast meal can probably be attributed to improvement in insulin action and pancreatic function [267].

7.4.1.2 Circadian rhythm or dawn phenomenon

Abnormal high fasting blood glucose has been repeatedly shown in T2DM [271, 272]. Glucose tolerance increases progressively during the daytime in T2DM,

this effect is opposite to healthy subjects, where insulin sensitivity is higher in the morning [273]. In glucose tolerance tests (50 g liquid glucose tested over 120 min) performed at different times of the day (09.00, 15.00 and 20.00 in random order) the morning BG was found to be lower whereas afternoon and evening BG were higher, but not different from each other in healthy volunteers [274]. Using hyperglycaemic insulinaemic clamp technique Boden et al showed that there is an increased insulin sensitivity in the early morning peaking in the early afternoon and declining throughout the evening and night in healthy volunteers [275].

It has been shown that BG increases after the breakfast meal and remains elevated for the extended period between breakfast and lunch, whereas there is a progressive improvement in insulin sensitivity during the afternoon and evening period, resulting in a suppression of endogenous glucose production in T2DM with lower BG and AUC [268, 276]. In comparison, healthy persons have insulin excursions defined with an early morning increase with a peak at midday and declining at night time [272].

This was shown by Trovati et al (2002). In 337 T2DM participants they found that although pre-prandial blood glucose is dependent on FBG, they also showed that FBG is higher than both pre-lunch and pre-dinner blood glucose. Furthermore, the fall in pre-lunch blood glucose compared to FBG, is greater than the fall in pre-dinner blood glucose compared to pre-lunch glucose. This effect is greater with increasing FBG levels [262].

Our study confirms these latter findings where pre-prandial BG decrease over the course of the day, with FBG being higher than pre-lunch BG and pre-dinner BG was lower than pre-lunch BG, Interestingly, even with the exact same nutrients served at different times of the day (CHO treatment) this effect was still evident indicating improved insulin sensitivity as the day progresses.

Limitations of this study are the lack of control implicit in free living conditions. Medication was very diverse ranging from no medical treatment to multiple medications including insulin. The aimed recruitment was 20 volunteers in each group. This recruitment was not met and a number of volunteers were excluded

or dropped out of the study. Using the original values of the SDs that were estimated for the design of the study (2.5-3.5), sample sizes of 14 in each group meant that the study was estimated to be powered at between 51%-79% for the 2 mmol/L change in 24 hour Gmax over time.

Strengths of this study include the use of CGMS to obtain comprehensive and dynamic fluctuations in BG which would not have been possible with SMBG in free living volunteers. The meals in the study were identical except for the breakfast meal tested which rule out changes due to slightly different nutrient composition. Gastric emptying rate was measured after lunch to rule out effects on the GE of different breakfasts. This was a cross over design where the volunteers acted as their own control with no changes in medical treatment and physical exercise during the study period.

7.5 **Conclusion**

Withholding CHO in the first meal results in significantly decreased blood glucose excursions after this meal, with lower Gmax, T>10 and AUC. There was no residual effect beyond this meal.

The substantial decrease in T>10 may lead to a decreased risk of complications, especially in the poorly controlled T2DM already on multiple anti-diabetic medication.

Chapter 8: General discussion and conclusion

High protein diets have become increasingly popular with the aim of reducing body weight in the general population and in type 2 diabetes patients. However, the safety of utilizing this dietary pattern in type 2 diabetes patients with renal impairment has been questioned [65].

An increasing body of evidence reporting added benefit on cardiovascular risk markers such as decreased triglyceride [137, 139, 145, 175, 244], increased high density lipoproteins [167], retention of lean body mass [189], increased satiety [155], increased weight loss [175] and increased loss of abdominal fat mass [175] of the HPD makes this pattern attractive in a group of patients with increased risk of CVD.

The main aim of this thesis was to investigate the effect of an energy restricted diet with a high protein to carbohydrate ratio, compared to the standard protein weight loss diet, currently recommended for T2DM patients, on renal function, HbA1c and lipids in T2DM participants with microalbuminuria [65].

Renal function

The main finding was that renal function was unaffected by allocation to a high protein weight loss diet, although the dietary intake goals were not achieved and the numbers fell short of the planned size. There were no significant changes in GFR measured as iGFR or as estimated by the MDRD equation or serum cystatin C and changes in GFR were unrelated to protein intake.

The earliest measure of renal dysfunction in T2DM is AER and spot urine alb/cr ratio. There was a significant decrease in AER between baseline and 4 Mo ($p=0.018$); but the change between baseline and 12 Mo was not significant.

AER decreased in the HPD whereas it remained stable in the SPD group and this difference was borderline significant at the end of the study, when adjusting for baseline differences ($p=0.06$). The same results were seen for alb/cr.

In a study looking at the effect of 6 months HPD compared to a SPD weight loss study; in 65 healthy overweight to obese participants a similar effect was seen regarding changes in AER. In this study the SPD group decreased protein intake by 21g and the HPD increased protein intake by 16g while there were no restrictions in energy intake. Although adaptive changes in kidney volume and GFR occurred, no change in AER was seen. Baseline AER in this study was in

the normal range except for one volunteer in each group who had microalbuminuria. Excluding these volunteers from the analysis did not change the outcome; both became normoalbuminuric during the intervention [277].

In a more recent study, 99 T2DM participants with microalbuminuria were randomized to a HPD (30%TE protein) or a SPD (15%TE protein) 3 months energy restricted, weight loss dietary intervention, followed by nine months energy balance. The difference in protein intake between the two groups was comparable to our study, with a difference between groups of ≈25 g (ns at 12 Mo). In this study no difference was found for AER or GFR between groups at either 3 or 12 months. No association between renal outcome and protein intake was reported [188]. In our study protein intake changes only marginally (+2g/d) in the HPD but decreased by 18g in the SPD. It seems protein intake is unrelated to the change in AER and other factors may play a role.

Weight loss

Weight loss of 5-10% of total body weight is recommended for most T2DM patients [66]. This level of weight loss has been reported to improve measures of renal function (predominately albuminuria) [278-280]. In the weight loss study conducted in for this thesis, mean weight loss was 9% in the HPD group and 7% in the SPD group (ns).

The effect of weight loss on BP and AER was tested in 19 obese subjects who followed either an energy restricted diet (1000-1400 Kcal; ad libitum protein intake) or were given an ACEi inhibitor (captopril 78±36 mg/d). This showed weight loss to be as effective as treatment with ACEi in reducing proteinuria. After 12 Mo the decrease in proteinuria was 83% after treatment with the energy restricted diet and 80% in the ACEi treated group, while GFR remained stable in both groups [279].

Proteinuria has also been shown to be associated with change in BMI. Fifty six volunteers with biopsy proven obesity related glomerulopathy, who completed a six month weight loss (low fat, energy restricted diet) and exercise intervention (60 min moderate exercise three times a week), were divided into three groups according to changes in BMI: group 1. BMI decreased more than 3% (n=27), group 2: weight stable (n=21) and group3: BMI increased (n=8). A decrease in

BMI of 8% resulted in a ≈ 35% lower proteinuria after six months. When BMI increased, a 29% increase in proteinuria followed. In 27 volunteers who still had a decreased BMI after 24 months of follow up, the beneficial effect of weight loss was maintained. In this group the decrease in BMI was 9% and the decrease in proteinuria was 51% at 24 Mo [278].

In a meta regression by Afshinnia et al [125] it was shown that weight loss is associated with a decrease in AER of 1.1 mg/24h for every 1 kg lost. This effect was seen regardless of intervention type. The studies in this meta-regression were highly heterogeneous ranging from short term dietary intervention to bariatric surgery. This led the authors to speculate that the effect on AER was due to weight loss but the evidence was moderate. In this meta-regression a mean of 14mg/24h decrease in AER was reported as a result of weight loss.

Decreased AER have been reported in participants with proteinuria (AER >200µg/min) as a result of weight loss. This, however, is not so clear in volunteers who are normoalbuminuric [277] or microalbuminuric [188]. In these studies no significant change in renal function and AER, at the end of 6 and 12 months, has been reported, indicating that weight loss per se may not result in improvements in AER and other factors such as blood pressure and glycaemic control also play a role.

In this study a decrease in AER of 16.8mg/24h in the HPD group (1.6 mg/kg WL) and an increase of 1.4 mg/24h in the SPD group was found. The decrease in the HPD group is comparable to the overall decrease in AER found in the meta-regression discussed above. However, the lack of change in the SPD group is puzzling. Using the results reported by Afshinna, given the total weight loss of 7.5 kg a decrease in AER by around 8 mg/24h in the SPD group could be expected. However, between eight and twelve months of intervention SBP and DBP increased, with both variables exceeding baseline values in the SPD at the end. The change in AER was significantly associated with the change in BP (SBP: r= .54; p=0.001, DBP: r= .49; p=0.003).

Blood Pressure

Systolic blood pressure decreased significantly between baseline and four months in both groups, but at 12 months the decrease was not statistically

significant and the SPD had returned to baseline values. Between eight and 12 Mo BP increased in SPD (+3.8 SBP and 4.0 DBP) and decreased (-2.9 SBP and +1.0 DBP) in the HPD. The decrease in BP over the entire 12 Mo period was -4.9/-2.5 in the HPD and +0.7/+5.2 mmHg in the SPD for SBP/DBP respectively; this difference was significant only for DBP.

These results are similar to findings in a 12 Mo follow up study of 66 T2DM volunteers. The participants were randomly assigned to a HPD (30%TE) or a SPD (15%TE) energy restricted diet, for eight weeks. Both groups lost weight with no significant difference between groups. In this study a decrease in SBP of 6 mmHg was seen in both groups after the weight loss period (8 weeks). After 12 Mo follow up the SBP was still 4 mmHg lower than baseline in the HPD and 2 mmHg higher than baseline in the SPD. The change in DBP showed the same trend with an increase in SPD against a decrease in HPD [185]. A similar effect of changing CHO for protein was also seen in a study including 60 healthy volunteers. It was shown that increasing the usual diet with 35-40 g protein in place of CHO resulted in lower BP in the HPD [281].

Diastolic blood pressure decreased over time in the HPD group and increased in the SPD group and there was a significant treatment effect at both four and 12 months. Delbridge et al [282] investigated the effect of two weight maintenance diets, a HPD vs. a high CHO diet after 12 months following a weight loss of 16.5 kg obtained using a VLCD for three months. Participants maintained a weight loss of 14 kg with no significant difference between treatments. Both SBP and DBP decreased significantly during the first phase (VLCD). At the end of phase 2 the return toward baseline values for both SBP and DBP were significantly higher in the high CHO group compared to the HPD group. The sample size in this study was higher compared to the present study; but the trend is comparable.

This decrease in DBP with a high protein diet has been reported previously. In a recent study 83 overweight and obese women were randomized to either a HPD (protein 30%TE and CHO 40%TE) or a high CHO, high dietary fibre diet (CHO 50%TE, fibre 35g and protein 20%TE). Both diets were energy restricted by 2 to 4 MJ. After eight weeks intervention DBP had decreased more in the HPD (-3.7 mmHg; $p = 0.005$) compared to the high CHO diet. The change in

SBP was not significantly different between groups [283]. Other weight stable studies have shown an effect of high protein diets on both SBP and DBP [183, 185].

Glycaemic control

High protein diets may result in greater benefit in glycaemic control compared to SPD. In very short term studies a low carbohydrate isocaloric diet have shown greater benefit in improving glycaemic control compared to a high CHO, low fat diet [195, 284]. A decrease in 24h AUC was seen after 5 weeks on the HPD and HbA1c was decreased by 0.8% compared to 0.3% in the SPD [196, 285].

However, in energy restricted diets, equal effects on glycaemic control, regardless of macronutrient intake, have been most frequently reported [139, 286].

We found significant decreases in FBG and HbA1c in the randomized completers group at 12 months, with no significant difference between groups. In the CGMS sub study, significant changes were seen in AUC, %T>10 and Gmax over time, with no significant difference between treatment groups. Although not statistically significant (p=0.26) there was a 6% decrease in the time spent with a BG above 10 mmol/L in the HPD, which may translate into health benefits over time. The HPD had an absolute weight loss ≈3kg more than the SPD (ns) and this may explain the difference in %T>10. This would indicate that weight loss, and probably the loss of abdominal fat mass is responsible for the glycaemic benefits achieved.

Withholding CHO in the breakfast meal after an overnight fast resulted in a decreased glucose excursion at that meal, with no carryover effect at the subsequent lunch meal. Overall 24h blood glucose, T>10 and Gmax were lower in the NOCHO diet allocation as a consequence of the lower CHO intake in the breakfast meal. Further studies are needed to investigate the effect of withholding CHO in the first meal translate into long-term benefit if sustained for a longer period.

Serum lipids

A decrease in TG has been reported in HPD compared to SPD in high protein diets [138, 145, 196], usually attributed to the decrease in carbohydrate intake.

Increases in HDL have also been reported but usually only in the ketogenic low CHO diets, where fat intake is higher [134].

The main finding of this study was a significant decrease in TG and a significant increase in HDL over time in both groups with no difference between treatments. There were no significant differences in total cholesterol and LDL over time.
LDL increased in the HPD by 0.1mmol/l and decreased by 0.03 mmol/L in the SPD, resulting in a net difference between groups of 0.4mmol/L (p=0.052). However, the increase in LDL in the HPD group may be explained by the termination of lipid lowering drugs in two volunteers from this group.

Dietary fat

Other dietary factors may affect the outcome of this study. One study (265) looked at the effect of a diet high in MUFA compared to a diet high in SAFA on blood pressure in 162 healthy volunteers. The volunteers were randomly assigned to isocaloric diets containing 37 %TE total fat; the SAFA diet consisted of 17%TE SAFA, 14%TE MUFA and 6%TE polyunsaturated fatty acids (PUFA) and the MUFA diet consisted of 8%TE SAFA, 23%TE MUFA and 6%TE PUFA. A significant decrease in BP was found in the MUFA diet with no significant difference in the SAFA diet. The decrease in SBP in the MUFA diet was -2.2% whereas the decrease in DBP was -3.8% compared to the SAFA diet ($p<0.01$). Diastolic blood pressure was significantly lower in the MUFA diet compared to the SAFA diet, but this effect was only evident in the volunteers who consumed less than 37%TE from total fat [287].

We found a significantly higher MUFA intake in the HPD at four months (35g/day vs. 24g/day in the HPD and SPD respectively (p=0.01)) and higher but not significantly higher MUFA intake at the end of the study (29g/day vs. 22g/day, (p=0.07)). Total fat intake at 12 Mo was 30%TE in both groups (reported in 3 day diet diary). These differences in MUFA as well as the differences in protein and weight loss may explain the differences in DBP at the end of the study (the MUFA intake did not differ between groups at baseline; 37g/day vs. 31 g/day, p=0.23).

Limitations / strengths

The power calculation was based on the study by Knight et al [121] who demonstrated a fall in creatinine clearance of 7.7 ml/min/1.73 m^2 per 10 g protein over a period of 11 years (SD 13.4 ml/min). We updated the power calculation using the actual SD (14.1ml/min) from the first 12 volunteers. Using these calculations with the current sample size of 19 volunteers in HPD and 17 volunteers in SPD meant that this study was estimated to be powered to detect a difference of 13.6 ml/min between groups in the eGFR change scores which is a very large effect and much greater than that observed by Knight over 11 years.

However, in this study the SD for the eGFR were large, at baseline SD was 26.4 and 29.5 in the HPD and SPD respectively and at the end of the study 21.2 and 29.0 for the HPD and SPD respectively (for the iGFR the SD's were larger, at baseline were 40.3 and 26.8 for the HPD and SPD respectively and 35.3 and 28.4 at the end of the study). We based our power calculation on much lower SD's (14.1), meaning this study was under powered to detect even a large difference of 13.6 ml/min/1.73m^2. However changes in eGFR and cystatin C were exactly the same in both groups and protein intake was unrelated to changes in renal function.

The duration of the study (12 Mo) might not be sufficient to see major changes in renal function in patients with mild renal impairment. The 2-3 year intervention in the MDRD study was inconclusive after 10 years of follow up and the authors recommend longer intervention and follow up time [87]

Dietary intake was based on self-reported data. It is well known that underreporting of energy intake is common and misreporting of protein intake is also frequent [288]. It has been shown that obese people tend to underreport energy intake to a larger extent than non-obese people. There is a tendency to over report protein intake and underreport fat and CHO intake in obese people, indicating differential reporting of different food groups [289]. Protein intake was overestimated in the FFQ, more so in the HPD. The HPD reported an increase of 16 g protein/d but the measured increase based on urinary urea was 1.8 g/d. In the SPD the reported decrease in protein intake was 18g/d and the measured decrease was 20 g/day. In comparison, the reported protein increase in the

HPD using 3 day diet record was 3g and the reported decrease in the SPD group was 30g, accordingly, more in line with measured protein intake (+2g/d in HPD and – 18g/d in SPD).

Compliance to diet was achieved in the randomized sample although the difference in protein intake was at the lowest planned level of 20g (achieved difference between diets at 12 Mo was 20g/d) and this may be a significant limitation of the study. However, this difference is comparable to ad libitum HPD weight loss studies [277] and energy restricted HPD weight loss diets [188] reported.

There are major strengths in this study. The use of an isotope tracer to measure GFR is considered the gold standard measurement technique [239]. This measure was used together with measurement of Cystatin C, AER and Alb/cr ratio, yielding a very comprehensive assessment of renal function during the study.

For assessment of glycaemic control CGMS was used. This method yields a very comprehensive glycaemic profile with 288 blood glucose measurements over 24 h.

Conclusion

In conclusion both diets were effective in producing weight loss, with 58% volunteers losing more than 5% and 39 % volunteers losing more than 10% of their body weight. Renal function was unaffected by treatment. Serum lipids were already very well controlled at study start and only minor changes were seen in the 12 months period. Glycaemic control was significantly improved at 4 months but returned to near baseline values by 12 months in both groups. BP decreased in the HPD but only DBP was significantly different.

Differences in composition of the weight loss diet, although relatively small at the end, did not adversely affect any outcome. It is therefore possible to recommend a dietary treatment suited to the individual’s preference, either high protein, low fat or high complex CHO, low fat diets, to maintain long-term compliance.

There was a possible increase in microalbuminuria in the SPD secondary analysis (ITT), but caution should be taken in interpreting this given baseline differences in diabetes control.

These results should be viewed with caution. Because of the low number of participants and consequent low power to see a small change, further longer term (2-3 years) studies are recommended.
This study is the first to examine the long-term efficacy and safety of higher protein diets in T2DM renal impaired individuals. Both diets had positive effects on cardiovascular risk factors with no changes in renal function.

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Appendices

Appendix 1: Tables of low protein diets

Table 1; Outcome of prospective LPD studies in non-diabetic patients

Study (year)	**Mo**[1]	**Subject (no)** [2]	**Baseline** **GFR**[3] **ml/min/1.73 m²**	**Baseline** **S-Cr** [8] **(µmol/L)**	**Control diet (UPD)**[4] **(achieved intake g/kg/day)**	**Protein restriction (LPD)**[5] **(achieved intake g/kg/day)**	**Endpoint**	**Control Follow-up** **before**	**Control Follow-up** **after**	**LPD Follow-up** **Before**	**LPD Follow-up** **after**	**Comment**
Rosman et al (1984) RCT	18	149 (228)	CrCl[7] ml/min/1.73 m² 31-60 (B) 10-30 (C)		NA (Compliance measured by Urine urea)	0.6 0,4	serum creatinine (1/Cr/Mo) Renal survival				Progression renal insufficiency decreased by a factor 5 in B and a factor 3 in A compared to control Non-survival = persistent 10% increase in serum creatinine	P not tested P=0.01
Ihle et al (1989) RCT	18	64 (72)	^{51}Cr-EDTA-Cl LPD 0,23±0.04 UPD 0,25±0.03	350-1000	≥0.75 (Compliance measured by Urine urea)	0.4	^{51}Cr-EDTA-Cl (ml/sec) S- Cr (µmol/L) ESRD	0,25±0.03 610±180	0,10±0.05 (P<0.01) 930±250 (+52%) 27%	0.23±0.04 680±270	0.20±0.05 (NS) 790±210 (+16%) 6%	mean (SEM) P<0.02 P=0.05

Outcome of prospective LPD studies in non-diabetic patients continued

Study (year)	**Mo**[1]	**Subject (no)** [2]	**Baseline** **GFR**[3] **ml/min/1.73 m²**	**Baseline** **S-Cr** [8] **(µmol/L)**	**Control diet (UPD)**[4] **(achieved intake g/kg/day)**	**Protein restriction (LPD)**[5] **(achieved intake g/kg/day)**	**Endpoint**	**Control Follow-up** **before**	**Control Follow-up** **after**	**LPD Follow-up** **Before**	**LPD Follow-up** **after**	**Comment**
MDRD (1994) RCT	2,2 Y	Study 1 n=585 Study 2 n=255	^{125}I-iothalamate Clearance ml/min/1.73 m² 25-55 13-24 ml/min/1.73 m²		(Compliance measured by Urine urea) 1.3 0.58	0.58 0.28	Decline in GFR		0-4 months ml/min/4 months -1.8 (1.1-2.6) 4-12 months ml/min/year -3.9 (3.3-4.4) 12-36 months ml/min/3 years -12.1 (10.5-13.8)		0-4 months ml/min/4 months -3.4 (2.7-4.2) 4-12 months ml/min/year -2.8 (2.2-3.4) 12-36 months ml/min/3 years -10.9 (9.2-12.5)	Initial rapid decline in LPD the first 4 months From 4-12 months the decline in GFR was slower in LPD (NS) At the end of follow up the difference in GFR between groups was NS.

[1]Mo=months, [2]no=total number of subjects eligible, [3]GFR= glomerular filtration rate, [4]UPD= usual protein diet, [5]LPD = Low protein diet, [6]LPh=Low Phosphorous diet, [7]CrCl= Creatinine Clearance, [8]S-Cr = Serum Creatinine.

Table 2; Non randomized and randomized trials including type 1 diabetic patients

Study (year)	Duration (Mo)[1]	Subject (no) [2]	Mean GFR[3] Baseline	UAER[3] or UPER[4] Baseline	Control diet (UPD)[5] (achieved intake g/kg/day)	Protein restriction (LPD)[6] (achieved intake g/kg/day)	Endpoint	Control Follow-up before	Control Follow-up after	LPD Follow-up before	LPD Follow-up after	Comments
Evaloff et al (1987) UPD followed by LPD Non randomized intervention	12	8	CrCl (ml/min) 55±27	UPER 2105±1355 mg/24h	80 g/d	40 g/d (0.6 g/kg/d) (Compliance measured by diet recall)	CrCl UPER			55±27 ml/min 2105±1355 mg/d	56±26 ml/min 147±164 mg/d	(NS) Mean (SEM) (p<0.001)
Barsotti et al (1988) UPD followed by LPD Non randomized intervention	15.9 UPD 17.4 LPD	8	CrCl (ml/min) 19.2 ±13.4	UPER 5700±1.9 mg/24h	1.2-1.4	0.25-0.35 [b] 0.5-0.6 [b] + wheat flour (Compliance measured by Urine urea)	UPER mg/24h Rate of decline GFR ml/min/Mo		-1.38±0.27	5.7±1.9	3.07±0.6 -0.03±0.37	Mean±SD (P=0.001) Mean±SD (P=0.001)
Walker et al (1989) UPD followed by LPD Non-randomized intervention	29 UPD 33 LPD	19	^{51}Cr-EDTA Cl ml/min/1.73m^2 23-125	UAER ≥ 300 mg/24h	1.13	40g/d (0.67 g/kg/d) (Compliance measured by Urine urea)	UAER mg/24h Rate of decline ^{51}Cr-EDTA GFR ml/min/1.73m^2		-0.61±0.14	467 (234-895)	340 (138-719) -0.14±0.08	95% CI (P=0.01) Mean (SEM) (P=0.001)

Non randomized and randomized trials including type 1 diabetic patients **continued**

Study (year)	**Duration (Mo)[1]**	**Subject (no) [2]**	**Mean GFR[3]** **Baseline**	**UAER[3] or UPER[4]** **Baseline**	**Control diet (UPD)[5]** **(achieved intake g/kg/day)**	**Protein restriction (LPD)[6]** **(achieved intake g/kg/day)**	**Endpoint**	**Control** **Follow-up** **before**	**Control** **Follow-up** **after**	**LPD** **Follow-up** **before**	**LPD** **Follow-up** **after**	**Comments**
Barsotti et al (1998) Non-randomized	3.7 y	32 (22 T1DM+ 10 T2DM)		1.5-9 g/24h	Free diet	A 0.3 [b] B 0.7 [b]	CrCl (ml/min)		-0.9±0.6 (ml/min/Mo)		-0.22±0.21 (ml/min/Mo)	P=0.001 Difference between diets
Brouhard et al (1990) RCT	12	15	Inulin Cl ml/min/1.73m^2 UPD 89 ±24 LPD 72 ±40	UAER ≥30 µg/min	1.0	0.6 (Compliance measured by Urine urea)	Rate of decline GFR Inulin Cl ml/min/1.73 m$^{2/}$Mo		-0.68±0.4		-0.28±0.15	Mean±SD (P<0.05)
Zeller et al (1991) RCT		35	Iothalamate Cl. ml/sec/1.73m^2 UPD 0.813±0.118 LPD 0.772±0.08	UPER mg/24h UPD 4266±715 LPD 3144±417	≥1.0 (1.08±0.10) (Ideal body weight)	0.6 (0.72±0.06) (Ideal body weight) Low phosphorous (Compliance measured by Urine urea)	UPER mg/24h Rate of decline GFR Iothalamate Cl. ml/sec/1.73 m^2		+1024 -0.0177±0.0053		-196 -0.0042±0.0015	No mentioning of significance Mean (SEM) (P<0.03)

Non randomized and randomized trials including type 1 diabetic patients **continued**

Study (year)	**Duration (Mo)[1]**	**Subject (no) [2]**	**Mean GFR[3]** **Baseline**	**UAER[3] or UPER[4]** **Baseline**	**Control diet (UPD)[5]** **(achieved intake g/kg/day)**	**Protein restriction (LPD)[6]** **(achieved intake g/kg/day)**	**Endpoint**	**Control Follow-up** **before**	**Control Follow-up** **after**	**LPD Follow-up** **before**	**LPD Follow-up** **after**	**Comments**
Dullaart et al (1993) RCT	24	30	Iothalamate Cl. ml/min/1.73m^2 ≥90	UAER 10-200 g/min	UPD (1.09±0.21)	0.6 (0.79±0.16) (Compliance measured by Urine urea)	UAER µg/min GFR ml/min/1.73m^2 ERPF[8]	 122±26 520±149	-5% 112±21* p=0.05 496±132	 131±34 537±150	-26% 113±24$^{\#}$ p=0.001 482±95	Δ group (p=0.05) mean±SD (NS)
Hansen et al (2002) RCT	4 Y (1-11)	82	^{51}Cr-EDTA Cl ml/min/1.73m^2 UPD 67 ±32 LPD 69 ±30	UAER mg/24h UPD 737 ±1.2 LPD 681 ±1.2	UPD (1.02)	0.6 (0.89) (Compliance measured by Urine urea)	Rate of decline GFR ml/min/1.73m^2 Albuminuria ESRD/death	 737±1.2	-3.9 (2.7-5.2) 614 (389-969) -27%	 681±1.2	-3.8 (2.8-4.8) 542 (382-769) -10%	(NS) (NS) Log rank test (P=0.042)

[1]Mo=months, [2]no=total number of subjects, [3]GFR= glomerular filtration rate, [4]UAER= urine albumin excretion rate, [5]UPER= Urinary protein excretion rate, CrCl= Creatinine Clearance. [6]UPD= usual protein diet, [7]LPD = Low protein diet, [b] All vegetarian food, [8]ERPF= Estimated renal plasma flow

Table 3; Studies reporting on LPD in T2DM

Study (year)	**Duration (Mo)[1]**	**Subject (no) [2]**	**Mean [3]GFR (ml/min/1.73m^2)**	**Albuminuria (mg/24h)**	**Control diet (UPD)[4] (achieved intake g/kg/day)**	**Protein restriction (LPD)[5] (achieved intake g/kg/day)**	**Endpoint**	**Control Follow-up change**	**LPD Follow-up change**	**Comments**
Pijls et al (1999) RCT	12	T 2 = 121	83	20-300	UPD (1.15±0.30)	0.8 (1.12±0.23)	[6]UAER (mg/24h) [7]CrCl (ml/min/1.73m^2)	+ 4 (-23,44) -1.5±14	-8 (-35,19) -2.8±16	(NS) (NS)
Pijls et al (2002) RCT	28	T 2 = 131	83	20-300	UPD (1.14±0.18)	0.8 (1.10±0.24)	UAER (mg/24h) CrCl (ml/min/1.73m^2)	+0.1 (-2.5,6.1) -6.4 ±14	+1.2 (-0.8,10.8) -4.8±12	(NS) (NS)
Meloni et al (2002) RCT	12	T 1 = 32 T 2 = 37	45.0±5.1	>40 Hypertension	UPD = Free diet (1.39)	0.6 (0.68)	Proteinuria (g/24h) Isotope GFR	-0.2 -6.26±1.84	-1.1 -6.15±1.61	(NS) regardless of diabetes type (NS)
Meloni et al (2004) RCT	12	T 1 = 24 T 2 = 56	46.8±5.8	>30 Hypertension	UPD = Free diet (1.39)	LPD 0.8 (0.86)	Isotope GFR	-6.03±1.3	-5.78±1.5	(NS)
Dussol et al (2005) RCT	24	T 1 = 10 T 2 = 37	>80	>30 >300	1.2 (1.03±0.15)	0.8 (1.10±0.02)	Micro-albuminuria Isotope GFR	+156±486 -7±11	+114±364 -5±15	(NS) (NS)

[1]M=months, [2]no=total number of subjects, [3]GFR= glomerular filtration rate, [4]UPD= usual protein diet, [5]LPD = Low protein diet, [a] analyzed excluding the first 6 months, [b] All vegetarian food, [6]UAER= urine albumin excretion rate, [7]CrCL= Creatinine Clearance.

Table 4; Studies reporting a positive effect of HPD on weight loss and body composition

Author	Type of diet	Measurements	Higher protein	Higher CHO	P
Skov et al 1999 [137]	HCH (CHO 58%TE)		HPD	HCD/LFD	P<0.01
RCT	HPD (P 25%TE)	BW (kg)	-8.7	-5.0	P<0.01
		FM (kg)	-7.6	-4.3	P<0.01
65 overweight to obese	Ad libitum energy intake	AFM (cm^2)	-33.0	-16.8	P<0.05
	6 Mo intervention	WL>10kg	35%	9%	
		FFA (µmol/l)	-30%	-	P<0.05
		T-chol, MEAN (SEM), HDL			ns
Due et al 2004 [178]	HCH (CHO 58%TE)	**6 Mo follow up**	HPD	HCD/LFD	
RCT	HPD (P 25%TE)	BW (kg)	-9.4	-5.9	P<0.01
		FM (kg)	-7.6	-4.3	P<0.01
50 Overweight to obese	Ad libitum energy intake	BMI (kg/m^2)	-3.3	-2.1	P<0.01
Follow up study of the 6 Mo	12 and 24 Mo	AFM (cm^2)	-34.9	-18.3	P<0.01
intervention study [137].		WCir (cm)	-9.9	-4.2	P<0.01
At 12 Mo and 24 Mo		W/H	-0.04	-0.0	P<0.01
		12 Mo follow up			
		BW (kg)	-6.2	-4.3	ns
		FM (kg)	-4.6	-3.1	ns
		BMI (kg/m^2)	-2.2	-1.2	ns
		AFM (cm^2)	-22.0	-10.5	ns
		WCir (cm)	-8.4	-1.8	p<0.01
		W/H	-0.04	+0.01	p<0.01
		Inflammatory markers & lipids			ns

Table of studies reporting a positive effect of HPD on weight loss and body composition (continued).

Author	Type of diet	Measurements	Higher protein	Higher CHO	P
Foster et al 2003	LCD (CHO 20 g ↗) (Atkins)		LCD/HPD/HFD	HCD/LFD	
[171]		BW (kg)			
	RCD/HCD/LFD (60,15,25 %TE)	3Mo	-6.8±5.0	-2.7±3.7	P<0.01
RCT		6Mo	-7.0±6.5	-3.2±5.6	P=0.02
	Including T2DM	12Mo	-4.4±6.7	-2.5±6.3	ns
63 Obese		T-Chol (mg/dl)			
		3Mo	+1.7±15.0	-5.4±10.1	P=0.03
Results in intension to treat analysis	Energy restricted:	6Mo	2.4±9.3	-2.4±9.5	P=0.06
	5-7 MJ	12Mo	0.1±9.8	-2.9±8.0	P=0.27
		TAG (mg/dl)			
	1 year	3Mo	-18.7±25.7	1.1±34.6	P=0.01
		6Mo	15.0±29.4	-7.6±19.3	P=0.13
	Low drop-out rate	12Mo	-17.0±23.0	0.7±37.7	P<0.04
		LDL (mg/dl)			
		3Mo	5.4±19.2	-7.4±16.6	P<0.01
		6Mo	2.7±12.8	-1.5±15.8	P=0.34
		12Mo	0.31±16.6	-1.6±11.1	P=0.04
		HDL (mg/dl)			
		3Mo	9.6±19.1	1.4±16.1	P=0.04
		6Mo	14.7±20.5	2.5±12.0	P<0.01
		12Mo			

Table of studies reporting a positive effect of HPD on weight loss and body composition (continued).

Author	Type of diet	Measurements	Higher protein	Higher CHO	P
Foster et al 2010 [176]	Low CHO	12 Mo	Low CHO	HCD/LFD	
RCT	(20g/d for 6 Mo then increasing by 5g/d)	BW, FM, LBM (kg)			ns
		MEAN (SEM) (mg/dl)	-17.9	-31.5	P=0.04
307 obese		VLDL (mg/dl)	-3.6	-8.2	P<0.01
	Low fat	LDL (mg/dl)	-8.7	-8.6	ns
	Energy restricted (5-7 MJ)	HDL(mg/dl)	+3.9	+8.0	P<0.01
		T-Chol (mg/dl)	-0.6	-0.9	P<0.02
	6 MO intervention and 2 years follow up	24 Mo			
		BW, FM, LBM (kg)			ns
		MEAN (SEM) (mg/dl)	-14.6	-12-2	ns
		VLDL (mg/dl)	-2.2	-2.2	ns
		LDL (mg/dl)	-8.0	-4.8	ns
		HDL(mg/dl)	+4.6	+7.8	p<0.01
		T-Chol (mg/dl)	-0.6	-0.7	ns
Samaha et al 2003	LCD		LCD	HCD/LFD	Mean±SD
[172]	(CHO <30g/d)	BW (kg)	-5.8±8.6	-1.9±4.2	P<0.01
	(Atkins)	WL≥10% (%)	14	3	P=0.02
RCT		TAG (%)	-20±43	-4±31	P<0.01
	RCD/LFD	T-chol			ns
64 severely obese	Energy restricted: (-500Kcal/d)	HDL			ns
		LDL			ns
68 severely obese	(F ≤30%TE)	FBG (%)	-9±19	-2±17	p=0.02 (T2DM)
		Insulin sensitivity (%)	6±9	-3±8	
39% T2DM	26 weeks	BP			p=0.01 (T2DM)
43% MetS		High dropout rate			

Table of studies reporting a positive effect of HPD on weight loss and body composition (continued).

Author	Type of diet	Measurements	Higher protein	Higher CHO	P
Brehm et al 2003	LCD (Atkins)		LCD/HPD/HFD	HCD/LFD	Mean±SD
[169]	Ad libitum	BW (kg)			
	(CHO ≤20g/d)	3Mo	-7.6±0.7	-4.2±0.8	P<0.01
RCT		6Mo	-8.5±1.0	-3.9±1.0	P<0.01
	RCD/LFD	Lipids, BP,BG, Insulin			ns
53 obese women	Energy restricted: (-450Kcal/d)				
	(F ≤30%TE)	Low drop-out rate			
	26 weeks				
Yancy et al 2004	LCKD (CHO <20g/d)		LCD	HCD/LFD	
[173]	(Atkins, ad libitum)	BW (%)	-12.9	-6.7	P<0.01
		FM (kg)	-9.4	-4.8	ns
RCT	LFD (F <30%TE, <300mg cholesterol)	TAG (mmol/l)	-0.84	-0.31	P<0.01
		HDL(mmol/l)	+0.14	- 0.04	P<0.01
120 overweight	Energy restricted:		+0.04	-0.19	P=0.2
	- 500-1000 Kcal/d	LDL(mmol/l)			
	24 weeks (High drop- out rate)				

Table of studies reporting a positive effect of HPD on weight loss and body composition (continued).

Author	Type of diet	Measurements	Higher protein	Higher CHO		P
McAuley et al 2005	HCD (CHO 55%TE)	Change 6 to 12 Mo	HPD	HCD	HFD	a difference from baseline P<0.05
[194]	LPD (CHO 20g ↗50g)	BW (%)	1.5 a	1.1	2.9a,b	
		BMI (kg/m2)	0.6 a	0.4	1.1a,b	
	HPD (P 30%TE)	WCir (cm)	1.3	3.0a	4.4a,b,c	
RCT	8 WL	FM (kg)	0.9a	0.5	2.3a,b,c	b difference between HCD &HFD P<0.05
93 Insulin resistant women		FFM (kg)	0.6a	0.8a	0.8a	
	8 WB	T-chol (mmol/l)	0.06	0.21	0.13	
		TAG (mmol/l)	-0.07	0.09	0.28a	
(76 follow up)	36 Follow-up	HDL(mmol/l)	0.04	0.02	-0.02	c difference between HPD & HFD
		LDL(mmol/l)	0.06	0.14	0.02	
		Insulin (pmol/l)	5.47	5.73	6.81	
		FBG (mmol/l)	-0.04	0.20a	0.14	
		2g Glucose (mmol/L)	-0.01	0.09	0.70a	

Table of studies reporting a positive effect of HPD on weight loss and body composition (continued).

Author	Type of diet	Measurements	Higher protein	Higher CHO	P
Keogh et al 2008	LCD		LCD	HCD	
[146]	(CHO 4%TE, P 35%TE)	BW (kg)	-7.6±2.6	-6.2±2.9	P<0.05[2,3]
		BMI (kg/m^2)	-2.7±0.8	-2.2±1.0	P<0.01[3]
RCT	HCD (CHO 46 %TE)	AFM (kg)	-0.6±0.4	-0.4±0.3	P<0.05[2]
		FM (kg)	-5.3±2.5	-4.9±3.6	P<0.01[3]
117 overweight and obese	Energy restricted : to 6-7 MJ	FFM (kg)	-2.1±2.6	-1.5±2.0	P<0.01[3]
0.20[a]		Fat%	-2.6±2.6	-2.4±2.5	P<0.01[3]
	8 weeks	T-chol (mmol/l)	↓	↓↓	P<0.05[2]
P values		TAG (mmol/l)	↓	↓↓	P<0.05[2]
[2]between diets		HDL(mmol/l)	↑	-	P<0.01[3]
[3] change with time		LDL(mmol/l)	-0.15±0.56	-0.0.36±0.71	P<0.05[2]
Clifton et al 2009	Energy restricted (5-6 MJ).		HPD	SPD	
[244]		Weight (kg)[1]	-7.8±0.4	-7.7±0.4	[1]P<0.01 for change over time no diet effect
	12 weeks intervention.	Total fat mass (kg)[1]	-6.8±0.4	-5.2±0.4	
Pooled data from three intervention studies.		Total lean mass (kg)[1]	-1.7±0.2	-2.3±0.2	
		Abdominal fat mass (kg)[1]	-1.7±0.1	-1.6±0.1	
		Insulin (mU/L)[1]	-4.30 ±0.58	-3.90 ±0.83	[2]p<0.01 for differences between treatment
215 volunteers (108 HPD and 107 SPD)		Glucose (mmol/L)[1]	-0.14 ±0.04	-0.21 ±0.08	
[138, 147, 166]		TC (mmol/L)[1]	-0.53 ±0.07	-0.39 ±0.06	
		TAG (mmol/L)[2]	-0.48 ±0.07	-0.27 ±0.06	
		HDL-C (mmol/L)	-0.02 ± 0.01	-0.02 ± 0.02	
		LDL-C (mmol/L)[1]	-0.2 ±0.1	-0.2 ±0.1	

Table of studies reporting a positive effect of HPD on weight loss and body composition (continued).

Author	Type of diet	Measurements	Higher protein	Higher CHO	P
Parker et al 2002	MCD/HPD		MCD/HPD	LPD	Mean (SEM)
[139]	(P 28%TE)	BW (kg)			
		Men	-4.7	-5.8	P=0.04
66 T2DM	LPD	Women	-6.0	-4.2	
	(p=16%TE)	FM (kg)			
		Men	-3.8	-5.1	P=0.01
	6.5 MJ	Women	-5.3	-2.8	
		AFM (kg)			
	8 WL	Men	-1.4	-1.7	P=0.02
	4 WB	Women	-1.3	-0.7	
		Lipids, BP, FBG, Insulin			ns
Shai et al 2008 [177]	Low CHO	24 Mo	LCHO	Control	
	(20 g CHO gradually increasing to	BW (kg)	-4.7±6.5	-2.9±4.2	P<0.01
322 obese (36 T2DM)	120 g, ad libitum energy)	MEAN (SEM) (mg/dl)	-23.7	-0-3	P=0.03
		LDL (mg/dl)			ns
	Low fat (control)	HDL(mg/dl)	+8.4	+6.3	P<0.01
	(30%TE fat, <10%TE SAFA, energy restricted 6.7 MJ)				

Table of studies reporting a positive effect of HPD on weight loss and body composition (continued).

Author	Type of diet	Measurements	Higher protein	Higher CHO	P
Abete et al 2009 [141]	MCD/HPD		LCD/HPD	HCD/LFD	Mean±SD
	(P 30%E)	BMI (kg/m2)	-9.3±1.6	-4.9±3.0	P=0.001
		FM (kg)*	-18.4±3.4	-12.7±7.2	P=0.08
No data on stratification	RCD/LFD	WCir (cm)	-9.8±2.4	-6.1±2.9	P=0.03
	(F ≤30%TE)	BP (mmHg)			ns
19 obese men		T-chol, TG, LDL (mg/dl)			ns
	Energy intake – 30%	HDL(mg/dl)*			ns
*** statistically different from baseline**		Glucose(mg/dl)			ns
	8 weeks	Insulin (µIU/ml)*			p=0.05
		REE (Kcal/d)*	-37.3±24.7	-11.3±70.5	ns

LCKD=Low carbohydrate ketogenic diet, T-chol=total cholesterol, TAG=triacylglycerol, LFD=Low fat diet, HDL=high density lipoprotein cholesterol, LCD=Low carbohydrate diet, HPD= high protein diet, HFD=high fat diet, HCD=high carbohydrate diet, RCD=reduced calorie diet, BP=blood pressure, BG=blood glucose, T2DM=type 2 diabetes mellitus, MetS=Metabolic syndrome, FBG=fasting blood glucose, EE=energy expenditure, BMI=body mass index, WCir=waist circumference, REE=resting energy expenditure, MCD=moderate carbohydrate diet, WL=weight loss, WB=weight balance, AFM=abdominal fat mass, W/H=waist hip ratio, P=protein, F=fat, CHO=carbohydrate

Appendix 2: Diet allocation template

DAILY FOOD SELECTION GUIDE

(Food's that you will need to eat each day 6000kJ High protein diet)

Cereal: 40g High-fibre cereal
eg All Bran, Weet-bix

Bread: 2 slices wholegrain bread
eg Vogel's, Bürgen

Dairy: 3 serves
1 serve = 250ml skim milk,
or 200g diet yoghurt (eg Yoplait No Fat & Nestle Diet yoghurt),
or 25g full-fat cheese (eg Mainland Tasty or Bega tasty),
or 50g reduced fat cheese (eg Bega so light & tasty or Kraft light)

Fruit: 2 serves
1 serve = 150g fresh or tinned, unsweetened fruit,
or 150 ml unsweetened fruit juice,
or 30g dried fruit.

Protein foods: Lunch: 50 g. cooked meat, poultry or fish.
eg Ham, tuna, chicken, turkey
Dinner: 200 g. lean meat, poultry or fish (raw weight)
Red meat 4 times per week (eg Beef, lamb & veal)
White meat 1 time per week (eg pork & poultry)
Fish 2 times per week.

Vegetables: 2½ cups - see free list (not including potato/sweet potato)
½ cup salad greens
and
2 cups mixed vegetables

Fats & oils: 15g (3 tsp) poly- or mono-unsaturated oil or spread
eg olive, canola, sunflower

DAILY FOOD SELECTION GUIDE

(foods that you will need to eat each day 6000kJ Standard protein diet)

Cereal: 40g High-fibre cereal
eg All Bran, Weet-bix

Bread: 3 slices wholegrain bread
eg Vogel's, Bürgen
1 serve high-fibre crispbread
eg 1 Ryvita multigrain or 2 Cruskits rye or 2 Vita-wheats 9-grain.

Dairy: 250ml skim milk or 200g diet yoghurt
eg Yoplait No Fat & Nestle Diet yoghurt
and
25g full-fat cheese
eg Mainland Tasty or Bega Tasty

Fruit: 2 serves
1 serve = 150g fresh or tinned, unsweetened fruit,
or 150 ml unsweetened fruit juice,
or 30g dried fruit.

Protein foods: 100g lean (raw) meat, poultry, fish 6 times a week
100g legumes once a week, eg chickpeas, 4-bean mix

Carbohydrate foods: 200g potato/sweet potato or
50g rice/pasta (raw weight)

Vegetables: 2½ cups - see free list (not including potato/sweet potato)
½ cup salad greens and
2 cups mixed vegetables

Fats & oils: 25g (5 tsp) poly- or mono-unsaturated oil or spread
eg olive, canola, sunflower

Appendix 3: Examples of checklists

Example of a completed checklist for 7 days HPD

FOOD CHECKLIST HP 6000kJ WEEK: DATE:	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
High fibre Cereal (40g)	Burgen	Burgen	Burgen	Burgen	Burgen	Burgen	Burgen
Bread, wholegrain (2 slices)	Seedy Roll	Burgen	Burgen	Burgen	Burgen	Bran Bread	
Dairy 1 eg 250ml skim milk, 25g full-fat cheese, 200g low fat yoghurt	Cheese	Yoghurt	Cheese	Cheese	Yoghurt	Yoghurt	Cheese
Dairy 2	Yoghurt	Cheese	Cheese	Yoghurt	Cheese	Cheese	Cheese
Dairy 3	Cheese	Cheese	Yoghurt	Cheese	Cheese	Cheese	Yoghurt
Fruit 1 (150g)	Apple	Pear	Apple	Pear	Pear	Pear	Pear
Fruit 2 (150g)	Mandarine	Apple	Pear	Mandarine	Apple	Pear	Apple
Salad veg (½ cup)	Mixed Salad	✓	✓	✓	✓	✓	✓
Lunch protein (50 g) eg. tuna, ham, poultry	Smoked Salmon	Smoked Salmon	Ham	Smoked Salmon	Chicken	Beef	Smoked Salmon
Lean protein (200g raw weight) eg. meat, poultry, fish	Chicken	Steak	Fish	Chicken 100g	Beef	Lamb	Steak
Veg 1 (½ cup) (75g)	Mixed Salad	Mixed	Mixed	Coleslaw	Peas	Broccoli	Peas
Veg 2 (½ cup) (75g)	"	Salad	Salad		Carrots	Peas	B/Beans
Veg 3 (½ cup) (75g)	"	"	"		Broccoli	Beans	Green Beans
Veg 4 (½ cup) (75g)	"	"	"		Cauliflower	"	Sweet Potato
Oil/margarine, 3 tsp (15g)	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓✓
Alcohol (2 STD drinks/wk) OPTIONAL							
OTHER – list			Raw Salted mixed nuts 30gms	3 slices pizza	1 cup rice		

Example of a completed checklist for 7 days SPD

FOOD CHECKLIST LP 6000kJ WEEK: DATE:	DAY 1 29/1/23	DAY 2 30/1/23	DAY 3 31/1/23	DAY 4 1/2/23	DAY 5 2/2/23	DAY 6 3/2/23	DAY 7 4/2/23
High fibre Cereal (40g)	Weetbix 2	Weetbix 2	Weetbix 2	Weetbix 2	Weetbix 2	Weetbix 2	Weetbix 2
Bread, wholegrain (3 slices)	✓✓✓	✓✓✓	✓✓✓	✓✓	✓✓✓	✓✓✓	✓✓
Crisp bread (1 crispbread)	✗	✗	✗	1 SAO BISCUIT	1 SAO BISCUIT	✗	✗
Milk, skim (250ml)	250 ml	250 ml	250 ml	250 ml	250 ml	250 ml	250 ml
Cheese, cheddar (25g full fat)	✗	150g Yoghurt	150g Yoghurt	✗	150g Yoghurt	Cheese	Cheese
Fruit 1 (150g)	Mango	Banana	Banana	Apple	Banana	Banana	Mango
Fruit 2 (150g)	Banana	Apple	Mango	Banana	✗	✗	Banana
Salad veg (½ cup)	✗	✓	✗	✗	✗	✗	✗
Lean meat, poultry, fish (100g raw weight) **6 times a week**	100g Beef	50g Beef	100g Beef	100g Lamb	100g Lamb	~~50g~~ Ham	100g Fish
Legumes (100g cooked) **once a week**	✗	✗	✗	✗	✗	✗	✗
Carbohydrate food (50g raw rice/pasta OR 200g cooked potato/sweet potato)	200g Potato	50g Pasta	200g Potato	150g Potato	100g Potato	50g Rice	150g Potato
Veg 1 (½ cup) (75g)	Tomato Juice	Tomato Juice	Tomato Juice	Tomato Juice	Tomato Juice	Tomato Juice	Tomato Juice
Veg 2 (½ cup) (75g)	Carrot	Beans	Peas	Broccoli	Carrot	Tomato	Beans
Veg 3 (½ cup) (75g)	Peas	Pumpkin	Carrot	Carrot	Beans	✗	✗
Veg 4 (½ cup) (75g)	Pumpkin	✓ ✗	Cabbage	Beans	✗	✓ ✗	✗
Oil/margarine, 5 tsp (25g) OLIVE OIL SPRAY	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓
Alcohol (2 STD drinks/wk) OPTIONAL	1 Light Beer		1 Red Wine		1 Red Wine	1 Light Beer 1 Home made Pizza	1 Light Beer
OTHER – list							

FOOD CHECKLIST HPD 6000kJ WEEK: DATE:	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
High fibre Cereal (40g)						
Bread, wholegrain (2 slices)						
Dairy 1 eg 250ml skim milk, 25g full-fat cheese, 200g low fat yoghurt						
Dairy 2						
Dairy 3						
Fruit 1 (150g)						
Fruit 2 (150g)						
Salad veg (½ cup)						
Lunch protein (50 g) eg. tuna, ham, poultry Lean protein (200g raw weight) eg. meat, poultry, fish						
Veg 1 (½ cup) (75g)						
Veg 2 (½ cup) (75g)						
Veg 3 (½ cup) (75g)						
Veg 4 (½ cup) (75g)						
Oil/margarine, 3 tsp (15g)						
Alcohol (2 STD drinks/wk) OPTIONAL						
OTHER – list						

Example of HPD checklist

Example of SPD checklist

FOOD CHECKLIST SPD 6000kJ WEEK: DATE:	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
High fibre Cereal (40g)						
Bread, wholegrain (3 slices)						
Crisp bread (1 crispbread) Milk, skim (250ml)						
Cheese, cheddar (25g full fat)						
Fruit 1 (150g)						
Fruit 2 (150g)						
Salad veg (½ cup)						
Lean meat, poultry, fish (100g raw weight) **6 times a week**				x		
Legumes (100g cooked) **once a week**	x	x	x		x	x
Carbohydrate food (50g raw rice/pasta OR 200g cooked potato/sweet potato)						
Veg 1 (½ cup) (75g)						
Veg 2 (½ cup) (75g)						
Veg 3 (½ cup) (75g)						
Veg 4 (½ cup) (75g)						
Oil/margarine, 5 tsp (25g)						
Alcohol (2 STD drinks/wk) OPTIONAL						

Appendix 4: Three day diet diary

Example of the information and diary page for the three day diet diary, used in the CGMS sub study.

Record food and liquid

Record all the **food and liquid** you consume during the monitoring period using the **SWAT** method.

- **S - BE SPECIFIC** – eg note type of bread, type of cheese, type of oil, brand of cereal, meat fatty or lean etc

- **W- WEIGH FOODS** – use scales for best accuracy. You can use metric cups, spoons for some items like fluids or sugar, oil.

- **A - ALL FOODS EATEN** – record as you go, not forgetting snacks, fluids, alcoholic drinks. Have a note pad with you to record on the go.

- **T - TYPICAL** – do not change your eating pattern or eat less just because you are recording!

- **Please start a new food/drink item on a new line and use a new page for each day.

Diet diary

Date:_____

Time	Food consumed	Brand name	Weight/volume	cooking

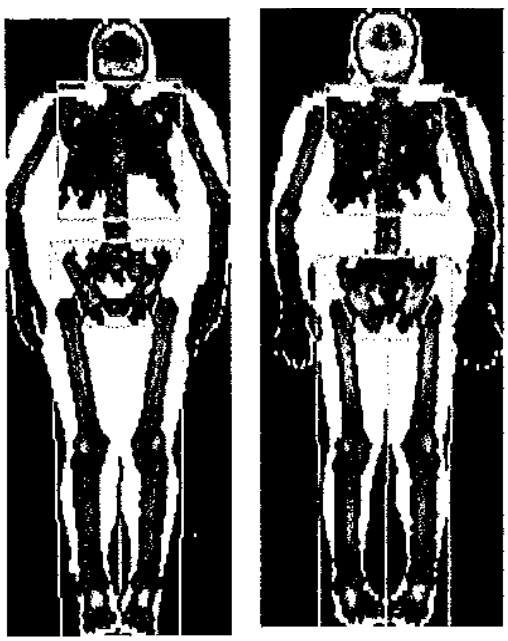
Appendix 5: Position of the volunteers on the DXA bed

All volunteers were obese with a mean BMI of 35 kg/m^2. There are two problems to consider when an obese person is placed on the table:

[1)] The arms can fall outside the scan area due to extended abdominal area and/or

[2)] Because the arms have to be strapped tightly to the body to fit the scan area, overlap of body areas is possible making distinction between the arms and the trunk difficult [290].

In this study a number of participants seemed to gain lean body mass even with a total weight loss after 12 months. Because the participants had to be strapped in to keep the body still during the scan, it cannot be excluded that an overlap of lean mass from the arms or the legs contributed to the problem.



Scan 1. Baseline *Scan 2. 12 months*

An example of a DXA scan with inconsistent weight loss to change in LBM measure. This male participant lost a total of 2.4 kg over the 12 months but was analyzed as having a gain in LBM of 2.59 kg. As can be seen in the two pictures, the midriff area is slightly different due to the different colour density in the bone scan representing the rib cage and the legs are not tied together as tight in the second scan thereby avoiding the thigh overlap seen in scan 1. Additionally the arms were outside the scan area on scan 1 but inside the area on scan 2.