DIETARY APPROACHES FOR THE REDUCTION OF CARDIOVASCULAR DISEASE RISK IN TYPE 2 DIABETES MELLITUS AND OBESITY

By

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A thesis submitted in accordance with the requirements of the University of Surrey for the degree of Doctor of Philosophy

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August 2007

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ACKNOWLEDGEMENTS

In the name of Allah, the most gracious, the most merciful

I wish to express my gratitude to all those who made it possible for me to complete this thesis. I owe my deepest gratitude to my supervisors, Prof. Linda Morgan and Dr. Bruce Griffin, for their continuous encouragement, enlightening comments, and invaluable guidance throughout. Their wide knowledge and outstanding abilities inspired and motivated me. They cannot realize how much I have learned from them.

I extend my gratitude to Dr. Ian Davies for his pivotal role and assistance in the lab, and to Dr. Shelagh Hampton and Ms. Christen Roberts for substantially contributing to the LGI study.

The collaboration of Dr. Helen Truby, Dr. Kath Hart, and Mrs. Rebecca Hiscutt in the Atkins study is highly acknowledged. I thank them all for making me part of such an interesting study.

During the course of the work in Kuwait, I was supported by several individuals without whom the carrying out of the study in the Amiri hospital would not have been possible. I am indebted to the tremendous support and supervision of Dr. A. AlAttar, Dr. A. Ben Nakhi, and Dr. M. AlArooj. I am grateful for the time, effort, attention and space they provided in the hospital during their busiest hours of the day. I am also grateful to Mr. A. Ahmed for his technical support in the Amiri lab. I thank Dr. J. Chakraborty, Dr. B. Akanji and Dr. O. Mojiminiyi from the centre of health sciences at Kuwait University for their many discussions, advisements, and tips that helped keep me on the right track. I also wish to thank all the clinicians at Dasma and Qadiyya clinics for helping with recruitment of the diabetic patients. Especially I am obliged to Dr. T. AlNeama, Dr. A Muqaddas, and Dr. K. Mustafa. I shall not forget here to thank all my diabetic volunteers in Kuwait.

Let me say thank you to my friends, Rabia, Muna, Fade, Mariam, Farah, Sue, Lana, Khulood, Ghada, Sama, maha, and Raja, for making me feel at home and for their wonderful friendship.

The chain of my gratitude would be definitely incomplete if I forgot to thank My husband, for his love and patience during the PhD study period. We lived through one of the best experiences during this time; building our dream home. I thank him for that. My children, without whom this effort would have been worth nothing. My brother Ahmad and his wife Muna for taking such good care of the boys while I was away. My mother and my sisters for their continuous prayers. My father and brothers for their support, especially financially. Alia, Ameera, and Iman for their help remotely, my in-laws and my wonderful friends in Kuwait for always surrounding me with love and support.

Undertaking to complete my PhD was a scary task but definitely one of the best decisions of my life.

Thank you all.
ABSTRACT

This work investigated the effects of different lifestyle strategies for reversing some of the lipid and lipoprotein abnormalities commonly known as an atherogenic lipoprotein phenotype (ALP) in groups at increased risk for CVD, such as type 2 diabetes mellitus (T2 DM), the overweight and obese. These strategies included a low glycaemic index (GI) diet, energy restriction, a low carbohydrate Atkins diet, and increased exercise level. The diets were tested either alone or in combination to examine the potential for additive effects on outcome measures.

The effects of a low GI diet, alone and in combination with energy restriction, were investigated in overweight/obese T2 DM patients in Kuwait (Chapter 4). For this purpose, the glycaemic and insulinaemic indices of some staple foods in the Kuwaiti diets were determined to provide data from which low GI foods could be selected (Chapter 3). A standard WHO procedure was used for determining the glycaemic index (GI) and insulinaemic index (II) of seven types of bread and a type of white rice that are consumed extensively by the Kuwaiti population. Six of the breads in the Kuwaiti diet were shown to have GI values that fell within an intermediate GI range, which was comparable to the GI values of similar foods reported in the literature. The exception to this was a brown pita bread which gave a high GI value and a relatively higher II value compared with glucose. Iranian bread also had a high II value compared to that of the glucose standard.

In Chapter 4, the effects of two low GI diets, with and without energy restriction, were examined on markers of CVD risk in overweight/obese T2 DM patients and compared with a control diet, in a 6 week, randomised controlled trial. The average GI of the diet was reduced by 12 units in the intervention groups. An additive effect of weight loss in the energy-restricted low GI diet group was not shown because of the limited amount of weight loss in this group (3% reduction from baseline weight); a direct result of poor compliance. Both intervention groups produced similar effects that included a decrease in the concentration of plasma triacylglycerol (TAG) and increase in high density lipoprotein cholesterol (HDL-C). Glycaemic control, as measured by fructosamine levels, improved significantly in the low GI group compared to the control. Lowering the
GI of the diet also resulted in a significant decrease in the proportion of small, dense LDL (sdLDL) and an increase in HDL₂.

Chapter 5 compared the effects of a low-carbohydrate, ‘Atkins’ diet either alone, or in combination with an exercise programme, to provide evidence for the potential of exercise to modify the effects of this type of diet over 6-months in a randomised trial. Participants all together in this study had a 6.4% reduction in body weight. The low-carbohydrate diet had some significant beneficial effects on the components of an ALP, in particular, decreasing plasma TAG, increasing both HDL-C and HDL size, and decreasing the proportion of sdLDL. Hyperinsulinaemia was unaffected by the consumption of a low carbohydrate diet. The favourable changes in lipids with the low carbohydrate diet were associated with weight loss as a result of decreased energy intake. This study was however unable to differentiate between the effect of exercise and the low carbohydrate diet on an ALP, probably because of poor compliance to the exercise program.

Taken together, both of the intervention studies resulted in reductions in metabolic risk factors for CVD, including a decrease in plasma TAG and increase in HDL-C across the two studies. Poor compliance to the energy-restricted diet in the low GI study in Kuwait, and to exercise regimens in the Atkins study did not allow any further interpretation of these experimental approaches. The promotion of the dietary approaches used in this thesis should be considered for the management of obesity and/or T2 DM and their associated CVD risk factors.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP</td>
<td>Atherogenic lipoprotein phenotype</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>Apolipoprotein A-I</td>
</tr>
<tr>
<td>Apo B</td>
<td>Apolipoprotein B</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under curve</td>
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<tr>
<td>B.P</td>
<td>Blood pressure</td>
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<tr>
<td>BB</td>
<td>Brown bread</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>BP</td>
<td>Brown pita</td>
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<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
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<tr>
<td>CETP</td>
<td>Cholesterol ester transfer protein</td>
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<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>DGUC</td>
<td>Density gradient ultracentrifugation</td>
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<tr>
<td>EGIR</td>
<td>European Group for the Study of Insulin Resistance</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
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<tr>
<td>GI</td>
<td>Glycaemic index</td>
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<td>GL</td>
<td>Glycaemic load</td>
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<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
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<tr>
<td>HDL-C</td>
<td>High density lipoprotein cholesterol</td>
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<tr>
<td>HGI</td>
<td>High glycaemic index</td>
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<tr>
<td>HL</td>
<td>Hepatic lipase</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>Homeostasis model assessment of insulin resistance</td>
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<tr>
<td>IB</td>
<td>Iranian bread</td>
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<tr>
<td>IDL</td>
<td>Intermediate density lipoprotein</td>
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<tr>
<td>LBP</td>
<td>Light brown pita</td>
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<tr>
<td>LC</td>
<td>Low carbohydrate</td>
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<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>LDL-III</td>
<td>Low density lipoprotein subclass 3</td>
</tr>
<tr>
<td>LDL-C</td>
<td>Low density lipoprotein cholesterol</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>LGI</td>
<td>Low glycaemic index</td>
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<tr>
<td>LPL</td>
<td>Lipoprotein lipase</td>
</tr>
<tr>
<td>LWP</td>
<td>Light white pita</td>
</tr>
<tr>
<td>MUFA</td>
<td>Monounsaturated fatty acids</td>
</tr>
<tr>
<td>NCEP ATP -III</td>
<td>National cholesterol education program adult treatment panel III</td>
</tr>
<tr>
<td>NEFA</td>
<td>None-estrified fatty acids</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acids</td>
</tr>
<tr>
<td>RO</td>
<td>Reverse osmosis</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>sdLDL</td>
<td>Small dense LDL</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of mean</td>
</tr>
<tr>
<td>T.Cholesterol</td>
<td>Total cholesterol</td>
</tr>
<tr>
<td>T.Fat</td>
<td>Total fat</td>
</tr>
<tr>
<td>T2 DM</td>
<td>Type 2 diabetes mellitus</td>
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<tr>
<td>TAG</td>
<td>Triacylglycerol</td>
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<tr>
<td>VAS</td>
<td>Visual analogue scale</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very low density lipoprotein</td>
</tr>
<tr>
<td>WB</td>
<td>White bread</td>
</tr>
<tr>
<td>WC</td>
<td>Waist circumference</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WP</td>
<td>White pita</td>
</tr>
<tr>
<td>WR</td>
<td>White rice</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

CHAPTER 1 INTRODUCTION

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.1</td>
<td>Structure of a lipoprotein</td>
<td>7</td>
</tr>
<tr>
<td>Figure 1.2</td>
<td>Exogenous and endogenous lipid synthesis.</td>
<td>8</td>
</tr>
<tr>
<td>Figure 1.3</td>
<td>Overall trends in obesity in the world from 1980-1998</td>
<td>13</td>
</tr>
<tr>
<td>Figure 1.4</td>
<td>Mechanism of generation of sdLDL and sdHDL in obesity and T2 DM</td>
<td>17</td>
</tr>
<tr>
<td>Figure 1.5</td>
<td>Plasma LDL subclasses of normal and ALP subjects</td>
<td>18</td>
</tr>
<tr>
<td>Figure 1.6</td>
<td>Glycaemic response after ingestion of pasta, wholemeal bread, and white bread</td>
<td>24</td>
</tr>
<tr>
<td>Figure 1.7</td>
<td>Structure of A; amylose and B; amylopectin</td>
<td>25</td>
</tr>
<tr>
<td>Figure 1.8</td>
<td>Potential mechanism for the relationship between high GI foods and insulin resistance</td>
<td>32</td>
</tr>
</tbody>
</table>

CHAPTER 2 MATERIALS AND GENERAL METHODS

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 2.1</td>
<td>Example of a computer generated densitometric scan of electrophoresed plasma from one participant in the low glycaemic index diet study showing a peak magnitude and $R_f$ for HDL subclasses analyses</td>
<td>56</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td>Example of a computer generated densitometric scan of electrophoresed plasma from one participant in the Atkins trial showing a peak magnitude and $R_f$ for LDL subclasses analyses.</td>
<td>58</td>
</tr>
</tbody>
</table>

CHAPTER 3 DETERMINATION OF THE GLYCAEMIC AND INSULINAEMIC INDICES OF EIGHT STAPLES IN THE KUWAITI DIET

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 3.1</td>
<td>Schematic presentation of the GI study day protocol</td>
<td>70</td>
</tr>
<tr>
<td>Figure 3.2</td>
<td>Glycaemic and insulinaemic index for the 8 staples</td>
<td>75</td>
</tr>
<tr>
<td>Figure 3.3</td>
<td>Mean plasma glucose and insulin levels in 10 healthy volunteers after the ingestion of 25g glucose load and the ingestion of pita breads (white, brown, light white, and light brown).</td>
<td>77</td>
</tr>
<tr>
<td>Figure 3.4</td>
<td>Mean plasma glucose and insulin levels in 10 healthy volunteers after the ingestion of 25g glucose load and the ingestion of white, brown, and Iranian breads and white rice.</td>
<td>78</td>
</tr>
<tr>
<td>Figure 3.5</td>
<td>Mean plasma glucose and insulin increments of food types vs. time for pita breads (white, brown, light white, and light brown).</td>
<td>79</td>
</tr>
</tbody>
</table>
CHAPTER 4 INVESTIGATION INTO THE EFFECTS OF LOW GLYCAEMIC INDEX DIET IN ENERGY BALANCE AND AS COMPARED TO ENERGY RESTRICTION ON MODIFYING ALP IN OVERWEIGHT/OBESE T2 DM PATIENTS IN KUWAIT.

Figure 4.1 Low GI study design
Figure 4.2 A schematic presentation of the flow of subjects through the study.
Figure 4.3 Weight changes during the trial. Changes from baseline with different diet and absolute weight loss in the LGI plus weight loss diet group.
Figure 4.4 Changes in BMI (kg/m2) and WC (cm) between groups over 6 weeks.
Figure 4.5 Changes in total energy (kcal/d) between groups over 6 weeks.
Figure 4.6 Changes in %CHO and % protein as total energy/day over 6 weeks for the different groups.
Figure 4.7 Changes in % of total energy from T. Fat, S. Fat, PUFA, and MUFA from baseline in each group.
Figure 4.8 Changes in GI and GL of the diet.
Figure 4.9 Changes in glucose (mmol/l) and fructosamine (µmol/l) between groups over the intervention period.
Figure 4.10 Changes in plasma TAG levels (mmol/l), HDL-C (mmol/l) and LDL-C (mmol/l) concentrations between groups over 6 weeks.
Figure 4.11 Correlation between changes in sdLDL, %HDL2, NEFA and %GI with changes in plasma TAG.
Figure 4.12 Changes in % HDL2 and sdLDL.
Figure 4.13 Changes in sdLDL (%AUCB) in one participant before and after 6 weeks of dietary intervention with LGI diet.
Figure 4.14 Changes in HDL2 before and after 6 weeks of dietary intervention with LGI diet.
Figure 4.15 VAS scores for hunger, desire to eat, fullness, and prospective consumption at baseline and after 6 weeks.

CHAPTER 5 A STUDY OF THE EFFECTS OF A LOW CARBOHYDRATE ATKINS DIET, WITH AND WITHOUT MODERATE EXERCISE, ON THE EXPRESSION OF AN ATHEROGENIC LIPOPROTEIN PHENOTYPE.

Figure 5.1 Study design
Figure 5.2 Flow of participants through the trial.
Figure 5.3 Average ± SEM of total energy/d, percentage CHO and protein from total daily energy.
Figure 5.4 Average ± SEM of T. Fat, S. Fat, PUFA, and MUFA at
baseline and after 6 months of intervention.

Figure 5.5 Absolute changes in weight after 6 months of intervention with low-carbohydrate diet and low carbohydrate with moderate exercise

Figure 5.6 Average ± SEM of weight, BMI, and waist circumference for study participants as a whole at 0 (baseline), 2, 4, and 6 months of intervention

Figure 5.7 Average ± SEM of weight, BMI, and waist circumference at 0 (baseline), 2, 4, and 6 months of intervention in the LC diet and the LC plus exercise group.

Figure 5.8 Average ± SEM of weight, BMI, and waist circumference at 0 (baseline), 2, 4, and 6 months of intervention in the LC diet and the LC diet plus exercise group.

Figure 5.9 Average ± SEM of fitness at 0 (baseline), 2, 4, and 6 months of intervention in the LC diet and the LC diet plus exercise group.

Figure 5.10 Average ± SEM of glucose, insulin, and HOMA-IR for the study participants as a whole at 0 (baseline), 2, 4, and 6 months of intervention.

Figure 5.11 Average ± SEM of glucose, insulin, and HOMA-IR at 0 (baseline), 2, 4, and 6 months of intervention in the diet and the LC diet plus exercise group.

Figure 5.12 Average ± SEM of glucose, insulin, and HOMA-IR at 0 (baseline), 2, 4, and 6 months of intervention in the diet and the diet plus exercise group.

Figure 5.13 Average ± SEM of glucose, insulin, and HOMA-IR for the study participants as a whole at 0 (baseline), 2, 4, and 6 months of intervention with low-carbohydrate diet.

Figure 5.14 Changes in sdLDL and HDL2 in the LC and the LC plus exercise group.

Figure 5.15 Changes in plasma TAG, HDL-C, and C: LDL-C at 0 (baseline), 2, 4, and 6 months of intervention in the diet and the diet plus exercise group.
LIST OF TABLES

CHAPTER 1 INTRODUCTION

Table 1.1  Modifiable and non modifiable risks of CVD 4
Table 1.2  The different definitions of the metabolic syndrome by different organizations 5
Table 1.3  Obesity associated health risks 14
Table 1.4  Characteristics of ALP 16
Table 1.5  Factors affecting the GI of a carbohydrate rich food 26
Table 1.6  Effect of LGI foods on glycosylated proteins in healthy, overweight, impaired glucose tolerance, T1 DM and T2 DM subjects. 29
Table 1.7  Studies against the role of GI in weight loss 31
Table 1.8  Effect of LGI diet on markers of ALP. 36
Table 1.9  Effect of low-carbohydrate diets on weight loss, lipid, and lipoprotein concentrations. 41

CHAPTER 3 DETERMINATION OF THE GLYCAEMIC AND INSULINAEMIC INDICES OF EIGHT STAPLES IN THE KUWAITI DIET

Table 3.1  Weight and composition of bread portions containing 25g available carbohydrate 68
Table 3.2  Characteristics of participants in the GI study 74
Table 3.3  Comparison between GI values derived from the current study and published values in the international tables of GI. 81

CHAPTER 4 INVESTIGATION INTO THE EFFECTS OF LOW GLYCAEMIC INDEX DIET IN ENERGY BALANCE AND AS COMPARED TO ENERGY RESTRICTION ON MODIFYING ALP IN OVERWEIGHT/OBESE T2 DM PATIENTS IN KUWAIT

Table 4.1  Characteristics and dietary composition (per day) of patients with T2 DM in Kuwait (pilot study). 96
Table 4.2  Characteristics of participants in each dietary group at baseline. 108
Table 4.3  Anthropometric measurements at baseline and at week 6. 110
Table 4.4  Macronutrient intakes (g) in each group at baseline and week 6. 115
Table 4.5  Changes in glycaemia and insulinaemia over the intervention period 122
Table 4.6  Chi square test of independence for HOMA-IR. 123
Table 4.7  Changes in plasma lipids and lipoproteins in each group. 127
CHAPTER 5 A STUDY OF THE EFFECTS OF LOW CARBOHYDRATE ATKINS DIET, WITH AND WITHOUT MODERATE EXERCISE, ON THE EXPRESSION OF THE ATHEROGENIC LIPOPROTEIN PHENOTYPE.

Table 5.1 Baseline characteristics of study participants. 156
Table 5.2 Prevalence of the metabolic syndrome in study participants at baseline and at 6 months based on the NCEP ATP-III criteria. 157
Table 5.3 Changes in macronutrient in grams over the intervention period. 158
Table 5.4 Changes in lipids and lipoprotein values over the intervention period. 174
LIST OF APPENDICES

CHAPTER 3 DETERMINATION OF THE GLYCAEMIC AND INSULINAEMIC INDICES OF EIGHT STAPLES IN THE KUWAITI DIET

Appendix 1       Subject randomisation form. 235
Appendix 2       Mean (SEM) of glycaemic and insulinaemic indices for tested foods 236
Appendix 3       Mean plasma glucose at 0, 15, 30, 45, 60, 90, 120 min elicited by eight Kuwaiti foods in 10 healthy subjects. 237

CHAPTER 4 INVESTIGATION INTO THE EFFECTS OF LOW GLYCAEMIC INDEX DIET IN ENERGY BALANCE AND AS COMPARED TO ENERGY RESTRICTION ON MODIFYING AN ALP IN OVERWEIGHT/OBESE T2 DM PATIENTS IN KUWAIT

Appendix 4       Ethical approval form 238
Appendix 5       Letter of approval to conduct the study at the Amiri hospital 239
Appendix 6       24-hour dietary recall form 240
Appendix 7       Low GI diet sheet 241
Appendix 8       Visual analogue scale form 260
Appendix 9       GI knowledge questionnaire 262
Appendix 10      Standard interview form for collection of 24-hour dietary recall 265
Appendix 11      Approval letter from sponsors to conduct the study in Kuwait 266
Appendix 12      Suitability checklist 267
Appendix 13      Patient information sheet 268
Appendix 14      Consent form 273
Appendix 15      Demographic and anthropometric questionnaire 275

CHAPTER 5 A STUDY OF THE EFFECTS OF LOW CARBOHYDRATE ATKINS DIET, WITH AND WITHOUT MODERATE EXERCISE, ON THE EXPRESSION OF AN ATHEROGENIC LIPOPROTEIN PHENOTYPE

Appendix 16      Changes in lipids and lipoprotein values over the intervention period 276
Appendix 17      Correlations between BMI, macronutrient composition for the diet and lipids and lipoproteins 277
# CHAPTER 1. GENERAL INTRODUCTION

1.1 General introduction 2
1.2 Cardiovascular disease 3
  1.2.1 Prevalence 3
  1.2.2 Risk factors 3
  1.2.3 Metabolic syndrome 4
1.3 Lipoproteins; structure and metabolism in relation to metabolic syndrome 6
1.4 Type 2 diabetes mellitus 9
  1.4.1 Prevalence and incidence 10
  1.4.2 Kuwait: background and T2 DM 10
  1.4.3 Pathophysiology of T2 DM 11
1.5 Obesity 12
  1.5.1 Prevalence and health consequences 12
  1.5.2 Abdominal obesity 14
1.6 Dyslipidaemia in T2 DM and obesity 15
1.6.1 Pathophysiology of an ALP
1.6.1.1 Increased TAG rich lipoproteins
1.6.1.2 Role of sdLDL in CVD
1.6.1.3 Low HDL cholesterol level

1.7 Dietary management of CVD risk in T2 DM and obesity
1.7.1 Glycaemic index of carbohydrates
1.7.1.1 Definition of glycaemic index (GI) and load (GL)
1.7.1.2 Factors affecting GI
1.7.1.3 Aspects of the practical measurement of GI
1.7.1.4 Effect of GI on glycaemic control
1.7.1.5 Effect of LGi diet on obesity
1.7.1.6 Effect of GI on insulin sensitivity
1.7.1.7 Effect of GI on an ALP
1.7.2 Effects of weight loss on CVD risk
1.7.2.1 Energy-restricted diets
1.7.2.2 Low carbohydrate diets
1.7.3 Effect of exercise on CVD risk
1.7.4 Effect of exercise combined with LC diet on CVD risk

1.8 Statement of the problem
1.9 Overall aim

CHAPTER 2. MATERIALS AND GENERAL METHODS

2.1 Materials
2.1.1 Equipment
2.1.2 Chemicals and reagents
2.1.3 Electrophoresis buffer

2.2 Methods
2.2.1 Anthropometric and blood pressure
2.2.2 Collection of blood
2.2.3 Biochemical analyses

2.2.3.1 Analyses using the SPACE autoanalyser

2.2.3.2 Assay principle: plasma T. Cholesterol

2.2.3.3 Assay principle: plasma TAG

2.2.3.4 Assay principle: HDL-C

2.2.3.5 Assay principle: LDL-C

2.2.3.6 Assay principle: plasma glucose

2.2.3.7 Assay principle: plasma apo A-I

2.2.3.8 Assay principle: plasma apo B

2.2.3.9 Assay principle: plasma NEFA

2.2.3.10 Assay principle: plasma fructosamine

2.2.4 Lipoprotein subclasses measurement

2.2.4.1 Gradient gel electrophoresis for the identification of HDL subclasses.

2.2.4.2 LDL subclasses measurement

2.2.5 Insulin measurement

2.2.6 Calculation of the homeostasis assessment model (HOMA)

2.2.7 Statistical analyses

CHAPTER 3. DETERMINATION OF THE GLYCAEMIC AND INSULINAEMIC INDICES OF EIGHT STAPLES IN THE KUWAITI DIET

3.1 Introduction

3.1.1 Glycaemic index overview

3.1.2 Identification of consumption patterns of bread and rice in Kuwait

3.1.3 Factors affecting the glycaemic responses to bread

3.1.4 Factors affecting the glycaemic response to rice

3.2 Statement of the problem

3.3 Objectives of the study

3.4 Study hypotheses
3.5 Methods
3.5.1 Test foods
3.5.2 Preparation of test foods
3.5.3 Recruitment
3.5.4 Study participants
3.5.5 Study design
3.5.6 Determination of glycaemic/insulinaemic responses and GI/II
3.5.7 Laboratory analyses
3.5.8 Statistical analyses

3.6 Results
3.6.1 Characteristics of subjects
3.6.2 Glycaemic and insulinaemic indices of test foods
3.6.3 Glycaemic and insulinaemic response data
3.6.4 Comparisons of GI with published values

3.7 Discussion
3.7.1 Glycaemic and insulinaemic indices of tested foods
3.7.2 Glucose and insulin responses of foods
3.7.3 Comparison of GI values with published values
3.7.4 Potential practical implications of the study

3.8 Conclusions

CHAPTER 4. INVESTIGATION INTO THE EFFECTS OF LOW GLYCAEMIC INDEX DIET IN ENERGY BALANCE AND AS COMPARED TO ENERGY RESTRICTION ON MODIFYING ALP IN OVERWEIGHT/OBESE T2 DM PATIENTS IN KUWAIT

4.1 Introduction
4.2 Statement of the problem
4.3 Objectives of the study
4.4 Study hypotheses
4.5 Significance of the problem to the Kuwaiti population

4.6 Ethical approval

4.7 Methods

4.7.1 Estimation of the GI of T2 DM diet in Kuwait

4.7.2 Sample size justification

4.7.3 Recruitment

4.7.4 Study participants

4.7.5 Study design

4.7.6 Intervention diets

4.7.6.1 LGI diet

4.7.6.2 LGI combined with hypocaloric diet

4.7.7 Questionnaires

4.7.7.1 24-hour dietary recall

4.7.7.2 VAS

4.7.7.3 GI knowledge questionnaire

4.7.7.4 Validity and reliability of the questionnaires

4.7.8 Data collection procedures

4.7.8.1 Demographics and anthropometric measurements

4.7.8.2 Dietary measurements

4.7.8.3 Fasting blood values

4.7.9 HOMA calculation

4.7.10 Calculation of daily GI and GL

4.7.11 Statistical analyses

4.8 Results

4.8.1 Patient recruitment

4.8.2 Baseline characteristics

4.8.3 Changes in anthropometric indices and blood pressure

4.8.4 Composition of the diet

4.8.4.1 Energy content of the diet

4.8.4.2 Macronutrient composition of the diet

4.8.4.3 GI and GL of the diet
CHAPTER 5. A STUDY OF THE EFFECTS OF LOW CARBOHYDRATE ATKINS DIET, WITH AND WITHOUT MODERATE EXERCISE, ON THE EXPRESSION OF THE Atherogenic Lipoprotein PHENOTYPE.

5.1 Overview 149
5.2 Aims of the study 150
5.3 Study hypotheses 150
5.4 Methods 150
   5.4.1 Participant selection 150
5.4.2 Sample size justification 151
5.4.3 Study design 151
5.4.4 Intervention groups 152
  5.4.4.1 LC diet group 152
  5.4.4.2 LC diet and exercise group 153
5.4.5 Measures 153
  5.4.5.1 Anthropometric measurements 153
  5.4.5.2 Dietary and fitness measurements 153
  5.4.5.3 Blood samples 154
  5.4.5.4 Laboratory measurements 154
5.4.6 Statistical analyses 154
5.5 Results 155
  5.5.1 Study progression 155
  5.5.2 Baseline characteristics of participants 156
  5.5.3 Prevalence of metabolic syndrome 156
  5.5.4 Changes in nutrient intakes 157
  5.5.5 Changes in anthropometric measures 160
    5.5.5.1 Changes in weight 160
    5.5.5.2 Changes in BMI 162
    5.5.5.3 Changes in the waist circumference 162
  5.5.6 Changes in fitness 165
  5.5.7 Changes in glycaemia 166
  5.5.8 Changes in plasma insulin and HOMA-IR 166
  5.5.9 Changes in features of an ALP 169
    5.5.9.1 Changes in plasma TAGs 169
    5.5.9.2 Changes in T-Cholesterol and LDL-C 169
    5.5.9.3 Plasma HDL-C 170
    5.5.9.4 Changes in NEFA levels 170
    5.5.9.5 Changes in LDL and HDL subclasses and LDL particle number 173
  5.5.10 Correlation analyses 175
5.6 Discussion

5.6.1 The effects on weight, BMI, and waist circumference

5.6.2 Effect on markers of insulin resistance

5.6.3 The effect on markers of ALP

5.6.3.1 Effects on plasma TAG

5.6.3.2 Effects on T. Cholesterol and LDL-C

5.6.3.3 Effect on HDL-C

5.6.3.4 Effects on LDL and HDL size and particle number

5.7 Conclusions

CHAPTER 6. GENERAL DISCUSSION

6.1 Measurements of glycaemic index

6.2 The effects of low GI diet in T2 DM patients in Kuwait

6.3 The effects of low-carbohydrate Atkins diet

6.4 Optimising a dietary approach for CVD risk reduction

6.5 Concluding remarks

6.6 Future work

REFERENCES

APPENDICES
CHAPTER 1
Chapter 1

INTRODUCTION

1.1. General introduction

Despite recent advances in the identification and modification of coronary risk factors, cardiovascular disease (CVD) remains a leading cause of premature death worldwide especially in developing countries such as Kuwait. CVD is associated with multiple risk factors, the most notable of which being overweight, obesity, and type 2 diabetes mellitus (T2 DM). In addition to contributing independently to CVD risk, these conditions are often associated with abnormalities of plasma lipids and lipoprotein concentrations that have been collectively called an atherogenic lipoprotein phenotype (ALP). Typically an ALP includes moderately elevated plasma triacylglycerol (TAG), low concentrations of high density lipoprotein cholesterol (HDL-C), and a predominance of atherogenic smaller denser LDL particles. A reduced incidence of this collection of lipid abnormalities has a significant beneficial quantitative impact on CVD morbidity and mortality.

A considerable range of studies increasingly suggest that an ALP can be favorably influenced by dietary modifications. Although initially designated neutral in their action on lipids, carbohydrates are now recognized as nutrients that play a major role in lipid abnormalities and CVD risk that are associated with obesity and T2 DM. There has been growing evidence of the role of carbohydrate quality (glycaemic index; GI), in the management of the dyslipidaemia of these conditions. Interest in the measurement of the GI of carbohydrate-rich foods has also grown considerably on the strength of evidence that carbohydrates classified as low GI carbohydrate are digested and absorbed slowly and lead to a lower glycaemic response and better metabolic risk profile than rapidly absorbed carbohydrates.

There is universal agreement that weight reduction is an essential part of dietary therapy for dyslipidaemia in obesity and T2 DM. It has consistently been shown that when weight reduction is achieved (and maintained) in these individuals a sustained decrease in TAG (Howard, 1993) and increase in HDL-C (Gardner et al., 2000) is achieved especially when combined with other lifestyle factors such as exercise.
A weight reduction of only 5-10% significantly reduces the risk associated with obesity and T2 DM (Wing et al., 1987a).

The following review of the literature will summarise the prevalence, incidence, and pathophysiology of obesity and T2 DM. The review will also focus on the current literature concerning the effects of GI, weight loss, Atkins diet, and exercise on lipid and lipoprotein abnormalities. Normal lipid and lipoprotein physiology will be reviewed briefly as a base from which these different effects will be examined.

1.2. Cardiovascular disease (CVD)

1.2.1. Prevalence

CVD is the disorders of the heart and/or blood vessels. It includes coronary heart disease (CHD), cerebrovascular disease, and peripheral vascular disease. CVD is the most common cause of death around the world. In the US alone, the prevalence of CVD among the adult population of 20 years and over is 37.1% (Rosamond et al., 2007). In the UK, CHD is the most common cause of premature death causing 22% of premature deaths in men and 12% premature deaths in women (British Heart Foundation, 2005a). In Kuwait, CHD is now the most common cause of adult death, with mortality from the disease increasing by 50% in the decade from 1972 to 1981 (Radovanovic, 1994). CHD represents a major cause of disability and is associated with enormous health care expenses with both direct and indirect costs. The cost of CHD for the UK economy is estimated to be a total of 7,900 million pounds annually (British Heart Foundation, 2005a).

1.2.2. Risk factors

CHD is a multi-factorial disease with a combination of modifiable and non-modifiable risk factors (Table 1.1). CHD is caused by atherosclerosis, a process associated with elevated serum total cholesterol (T.Cholesterol) and low density lipoprotein cholesterol (LDL-C) and low concentrations of high density lipoprotein cholesterol (HDL-C) (Anderson et al., 1991; Wilson et al., 1998; NCEP ATP-III, 2001). Chronically elevated glucose concentrations are also known to contribute to the development of atherosclerosis in people with diabetes, independent of other risk factors (Selvin et al., 2005). The risk factors for CHD are defined by guidelines and
classifications from different organizations in Europe (Wood et al., 1998; Balkau et al., 1999) and the United States (NCEP ATP-III, 2001). The early identification and modification of these risk factors is essential to reduce morbidity and mortality rates from CHD (Abate, 2000).

**Table 1.1.** Modifiable and non-modifiable risk factors of CVD (WHO, 2005).

<table>
<thead>
<tr>
<th>Modifiable</th>
<th>Non modifiable</th>
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<tr>
<td>Raised systolic BP</td>
<td>Age</td>
</tr>
<tr>
<td>Raised diastolic BP</td>
<td>Gender</td>
</tr>
<tr>
<td>Smoking</td>
<td>Family history of premature CVD</td>
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<tr>
<td>Hyperlipidaemia</td>
<td>Previous cardiovascular events</td>
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<td>Left ventricular hypertrophy</td>
<td>Premature cerebrovascular events</td>
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<td>Hyperglycaemia</td>
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<td>Renal disease</td>
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<td>Obesity</td>
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<td>Physical inactivity &amp; sedentary lifestyle</td>
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**1.2.3. Metabolic syndrome**

A prominent and important risk factor for CVD is the metabolic syndrome of insulin resistance. In 1988, Reaven (1988) introduced the term ‘syndrome X’, with insulin resistance as a common denominator for the syndrome. The metabolic syndrome is characterized by a constellation of metabolic risk factors, including abdominal obesity and an atherogenic dyslipidaemia consisting of elevated triacylglycerol (TAG), a predominance of small, dense LDL (also known as small dense LDL-III or sdLDL-C), low concentrations of HDL-C, elevated blood pressure, insulin resistance (with or without glucose intolerance), and prothrombotic and proinflammatory states. The first definition for the identification of the metabolic syndrome by the WHO (WHO, 1999) was quickly followed by other definitions from the European Group for the Study of Insulin Resistance (EGIR; 1999), the National Cholesterol Education Program adult treatment panel III (2001) and the International Diabetes Federation (IDF; 2005). These definitions are presented in Table 1.2.
<table>
<thead>
<tr>
<th>WHR: waist to hip ratio</th>
<th>WC: waist circumference</th>
<th>M: male</th>
<th>F: female</th>
<th>P: blood pressure</th>
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<td>WHR &gt; 0.85 (F)</td>
<td>WC &lt; 102 cm</td>
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<td>&gt; 1.8 mmol/L (M)</td>
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<td>2. Low HDL-C &lt; 1.0 mmol/L (M)</td>
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In the US about 22% of men and 24% of women aged ≥20 years have the metabolic syndrome, including diabetes (Ford et al., 2002). In Kuwait, the prevalence of the metabolic syndrome was 32.8% among 609 males and females according to the NCEP ATP-III guidelines (Al-Shaibani et al., 2004). Prevalence is also significantly affected by age, with 26% being between the ages 30–40y and 34.4% between the ages 40–60y.

Individuals with the metabolic syndrome have a 3-fold elevated risk for coronary artery disease (CAD) and stroke (Isomaa et al., 2001). In addition to having an effect on CAD, the components of the metabolic syndrome have also been closely associated with the development of T2 DM. In a study of 1,918 Pima Indians, insulin resistance was strongly associated with diabetes in a four year follow-up (Hanson et al., 2002). Also, body size and the lipid factors significantly predicted diabetes. The most common combinations of components of the metabolic syndrome seen in diabetics are obesity in combination with hypertension or dyslipidaemia, which is seen in about 50% of patients with T2 DM and in 10-20% of subjects with normal glucose tolerance (Isomaa et al., 2001).

1.3. Lipoproteins; structure and metabolism in relation to metabolic syndrome

Lipids are by definition insoluble in aqueous media such as plasma and are thus transported in blood as multi-molecular complexes called lipoproteins. There are four major classes of lipoproteins mainly synthesised in the liver and in the intestine (Jain et al., 2007). These classes are chylomicrons, very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) that differ in their hydrated density as a result of a decreasing proportion of hydrophobic lipids in their central core (cholesterol esters and triacylglycerol (TAG)) relative to protein in their surface coat (apoproteins; apo). Apoproteins and phospholipids are amphipathic molecules that act as an interface between plasma and the hydrophobic core lipids, and in effect, render these lipids soluble in plasma (Figure 1.1). In addition, apoproteins play an important role in stabilizing the structure of the lipoprotein and in acting as ligands for specific receptors, and thus determining the physiological behavior of lipoproteins (Jain et al., 2007).
Figure 1.1. Structure of a lipoprotein illustrating the hydrophobic core of TAG and cholesterol esters surrounded by amphipathic coat of apolipoprotein, phospholipids, and cholesterol.

Following a meal, the small intestine produces apo B-48 containing chylomicrons (Tso et al., 1984; Jain et al., 2007) which have the primary function of transporting dietary TAG and cholesterol from the intestinal lumen to sites of storage and metabolism (Schaefer & Levy, 1985). On entry to the blood stream, chylomicrons rapidly undergo lipolysis via the action of endothelial bound, lipoprotein lipase (LPL; Figure 1.2) and lose much of the TAG and acquire cholesteryl esters from other lipoproteins via the action of cholesteryl ester transfer proteins (CETP). These remnants are taken up by the liver via receptors that bind apo E. VLDL containing apo E, C-II, and B-100, are relatively large particles produced in the liver and have the function of transporting endogenous TAG and cholesterol to the peripheral tissues. VLDL particles are much smaller than chylomicron particles and therefore carry significantly less TAG than do chylomicrons. The catabolism of VLDL is similar to that of chylomicrons in that VLDL-TAG is lipolysed by LPL leaving cholesterol-rich remnants. Chylomicron remnant particles are considered to be particularly atherogenic (Karpe et al., 1994). In fact, both chylomicrons and VLDL remnants possess the potential to cause cholesterol deposition in the arterial wall (Williams, 1998). While all chylomicron remnants are removed by the liver, VLDL remnants
can have two fates; about 50% are normally removed directly by the liver, whereas
the other 50% is converted to LDL via the action of a close relative of LPL in the liver
hepatic lipase (HL).

**Figure 1.2.** Exogenous and endogenous lipid synthesis. Dietary (exogenous) fat is
absorbed into chylomicrons. In endogenous lipid synthesis the liver synthesizes TAG
and cholesterol esters and packages them to VLDL. LPL hydrolyze the TAG in
chylomicrons and VLDL which are further hydrolyzed by LPL to form IDL and then
LDL. Fatty acids generated from TAG hydrolyses are a source of energy or fat
storage.

LDL, an end product of VLDL metabolism, is composed of about 50% cholesterol
and is the major carrier of cholesterol to peripheral tissues. LDL shows structural
heterogeneity and exists in the plasma of all humans as a small number of discrete
subclasses (Griffin, 1997). These subclasses may be classified according to their
particle size, density, and flotation properties; the latter provides the most useful
description of LDL structural heterogeneity (Griffin, 1995). Three major classes of
LDL cholesterol exist; LDL-I, LDL-II, & LDL-III (Griffin, 1995). LDL-III represents a common marker of increased risk of CHD (Rizzo & Berneis, 2006a).

In contrast to LDL particles that deliver cholesterol to peripheral tissues, it is the apo-A-I containing HDL particles that help transport cholesterol to the liver from peripheral tissues (Jain et al., 2007; Link et al., 2007). The concentration of plasma HDL is often low (<1mmol/l) in people with T2 DM and the metabolic syndrome, patients with hypertriglyceridaemia, patients with sedentary lifestyle and smokers (NCEP ATP-III, 2001). As with LDL, HDL can be further subdivided into two principal subclasses; HDL2, which has a density range of 1.063-1.125g/ml and HDL3, which represents the range 1.125-1.210g/ml (Anderson et al., 1978).

The enzymes LPL and HL and lipid transfer protein CETP are key players in the metabolism of the major plasma lipoproteins. CETP mediates the transfer of hydrophobic lipids between VLDL, LDL and HDL, shuttling cholesterol esters from LDL and HDL to TAG-rich lipoproteins (chylomicrons and VLDL) in an equimolar exchange. As VLDL becomes rich in cholesterol esters both LDL and HDL can become transiently enriched in TAG, that on passage through the liver is hydrolyzed by HL, resulting in a smaller denser, LDL and HDL particles (Grundy, 1997).

1.4. Type 2 diabetes mellitus

Diabetes mellitus is associated with an elevated risk of CVD; the risk of CHD is 2.5-3 times higher in patients with diabetes and more likely to lead to mortality or prolonged morbidity (Gaede et al., 2003; Li et al., 2007b; Stamler et al., 1993). An important supporting line of evidence for the association of CVD risk factors with T2 DM came from the United Kingdom Prospective Diabetes Study (UKPDS) (Turner et al., 1998). This study analysed the strength of the relationship between various CVD risk factors and risk of myocardial infarction in patients with T2 DM and established LDL-C as the strongest predictor of CHD followed by diastolic blood pressure, smoking, HDL-C and finally, glycaemic control as assessed by HbA1c concentrations. The same study showed that a 1 mmol/l increase in LDL-C corresponded to a 57% increase in risk of a cardiovascular event as compared with an increase in risk of 15% for every 10mmHg increase in systolic blood pressure and an
increase in risk of 11% for every 1% increase in HbA1c (Turner et al., 1998). In addition, the risk of morbidity and mortality from stroke is also particularly high in individuals with diabetes (Idris et al., 2006).

1.4.1. Prevalence and incidence

The prevalence of T2 DM has been increasing dramatically worldwide (King et al., 1998; Mokdad et al., 2001; Mokdad et al., 2003). It is rapidly becoming a serious emerging public health concern not only in adults, but now more frequently in children and adolescents (Sinha et al., 2002; Pinhas-Hamiel et al., 1996). Over the next 20 years, the prevalence of diabetes is forecast to increase by 42% among adults living in the developed world and by 170% among adults in developing countries. Around 300 million people age 20 and older will have diabetes by the year 2025 (King et al., 1998). In 2001, 7.9% of US adults were diagnosed with DM which represents an increase of 61% since 1990 (Mokdad et al., 2003). The disease was shown to affect approximately 9.6% of adults aged 20y and older in the US in 2005 (U.S. Department of Health and Human Services, 2005b). In the UK, it is estimated that newly diagnosed T2 DM cases will rise to 19% above current levels by the year 2023 (Bagust et al., 2000).

As with CHD, diabetes is associated with substantial cost and economic consequences. In the US alone, the total annual economic cost of diabetes in 2002 was estimated to be $132 billion, or one out of every 10 health care dollars spent (American Diabetes Association, 2003). In the UK, large increases in healthcare demand and economic burden due to diabetes are expected in the next 40-50 years (Bagust et al., 2000).

1.4.2. Kuwait: background and T2 DM

Kuwait is typical of a traditional society that has undergone a very rapid modernization. The country occupies the north western corner of the Arabian Gulf and stretches over 17,818 km². The population in 2006 is approximately 2,418,393 among which 1,127,039 are Kuwaiti citizens (Ministry of Planning, 2003). A few decades after the discovery of oil (1940s), a nation of fishermen and traders was transformed into one of the richest and developed nations in the world.
This sudden transformation has led to significant improvements in lifestyle and standards of living and associated with this, a considerable transition in dietary intakes. With these dramatic changes food became subsidised. The country now depends heavily on imported produce which has created a greater abundance of food and dietary diversity (Al-Hooti et al., 2002). As such, Kuwait is an interesting example of a developing country with rapid rises in diet-related diseases (Abdella et al., 1996; Al-Adsani et al., 2004; Al-Asi, 2003; Al-Kandari, 2006; al-Muhtaseb et al., 1991; Al-Shaibani et al., 2004; Jackson et al., 2002; Akanji, 2002; Abdella et al., 1998; Radovanovic, 1994) such as T2 DM which is emerging as a leading chronic disease. The prevalence of diagnosed T2 DM in Kuwait in 1995 was 7.6% (Abdella et al., 1996). By 1998, the overall prevalence had escalated to 14.8% (Abdella et al., 1998). The disease presents at a relatively young age with a prevalence of 5.7% in the age group 20-39 and 18.3% in the age group 40-59 (Abdella et al., 1998). Despite the increased prevalence of T2 DM in Kuwait, little attention has been paid to public health nutrition and the therapeutic modification of diet.

1.4.3. Pathophysiology of T2 DM

The majority of patients with T2 DM are insulin resistant as compared to appropriately matched individuals with normal glucose tolerance (Reaven, 1995). Insulin resistance is an extremely common pathophysiological determinant for the development of T2 DM and dyslipidaemia (Reaven, 1995). A hypothesis that explains insulin resistance of T2 DM was postulated by Reaven (1988). This hypothesis states that as a result of chronic insulin resistance, compensatory hyperinsulinaemia develops to maintain glucose homeostasis. As a result, pancreatic hypersecretion of insulin fails and this is why T2 DM eventually develops. It has also been suggested that the most fundamental defects in insulin resistant individuals are hyperinsulinaemia, enhanced VLDL secretion and hypertriglyceridaemia (Lewis & Steiner, 1996). The Paris Prospective Study (Fontbonne & Eschwege, 1991), an 11 year study in 7028 diabetic and non diabetic men, found that CHD mortality was strongly associated with concentrations of fasting insulinaemia, increasing stepwise from the lowest to highest quintile of insulin concentration. Besides insulin resistance, abdominal obesity represents a major contributor to the pathogenesis of T2 DM (Ritchie & Connell, 2007). Several theories, which are not mutually exclusive.
have been put forward to explain the role of insulin resistance in abdominal obesity. Among these theories is the portal theory (Frayn et al., 2005). This theory states that since abdominal fat drains into the hepatic portal circulation, increased abdominal fat deposition is often associated with increased lipolysis and elevated plasma concentrations of non-estrified fatty acids (NEFA) which in turn can cause hepatic insulin resistance. Alteration in adipokine secretion by increased abdominal fat has also been proposed as a mechanism that links abdominal obesity with insulin resistance (Ritchie & Connell, 2007) since the latter may be considered as an inflammatory condition (Pittas et al., 2004).

1.5. Obesity

1.5.1. Prevalence and health consequences

Obesity is a major modifiable risk factor for CVD and increases the risk of developing conditions, such as T2 DM, dyslipidaemia and hypertension that, in turn, further increase CVD risk (Abate, 2000; Bjorntorp, 1992; Eckel & Krauss, 1998; Ferrannini et al., 1997; Li et al., 2007a). Individuals who are overweight or obese (specifically central obesity) are at greater risk for insulin resistance (Austin et al., 1990; Formiguera & Canton, 2004; Sowers, 2003). In an examination of the inter-relationships between body mass index (BMI), energy intake, and physical activity to CVD mortality, obesity and lower levels of physical activity were independently associated with decreased CVD survival (Fang et al., 2003).

Using a BMI of 25-30 kg.m$^2$ as overweight and BMI>30 kg/m$^2$ as obese, most of the states in the US have obesity prevalence rates equal to or greater than 25%, with 3 of those having a prevalence equal to or greater than 30% (U.S.Department of Health and Human Services, 2005a). In the UK, 41% of men and 33% of women are overweight. An additional 25% of men and 20% of women are obese (Foods Standard Agency & Department of Health, 2004). Obesity is associated with increased risk of T2 DM. In the Nurses’ Cohort Study, for an increase of 20-35 kg, the relative risk of developing T2 DM diabetes was 11.3, and for an increase of more than 35 kg, the relative risk was 17.3 (Colditz et al., 1990).
In Kuwait, the prevalence of obesity (Figure 1.3) may be increasing in both men (32.8%) and women (47.9%) of 20 years and older (WHO Regional Office for the Eastern Mediterranean, 2005). A comparison of two cross sectional samples studied in the year 1980-1981 and 1993-1994 for the prevalence of obesity showed that the frequency of overweight and obesity increased significantly among males and females between these two periods (Al-Isa, 2003). The prevalence of obesity increased by 15.4% and by 8.4% among men and women respectively. A recent study of the prevalence of obesity in Kuwait (Al-Kandari, 2006) indicated that most of the obese individuals, in a sample of 212 men and 212 women, were grade 2 obesity (30<BM<40). In addition, 71.2% of this sample was obese with obesity increasing with age and being more prevalent in women. In the same study, the high prevalence of obesity was found to correlate with the level of education, eating regularly at restaurants, and with the availability of a family cook and the socioeconomic status of the family. Obesity, in a T2 DM Kuwaiti population, was also found to be a significant risk factor for CHD (Abdella et al., 1998). In general, more than one third of patients with T2 DM are obese (Franz, 2004). Beside CHD, there are numerous other diseases that are either caused or made worse by the presence of obesity (O'Brien & Dixon, 2002); T2 DM parallels the epidemic of obesity and is at the top of the list of these diseases (Table 1.3).

**Figure 1.3.** Overall trends in obesity in the world from 1980-1998 (British Heart Foundation, 2005b). The diagram shows Kuwait with one of the highest prevalence rates for obesity with more than 1% increase in obesity each year.
Table 1.3. Obesity-associated health risks (O’Brien & Dixon, 2002)

<table>
<thead>
<tr>
<th>T2 DM</th>
<th>Osteoarthritis: knees, hips, feet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>Lower back pain</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>Infertility</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>Polycystic ovary syndrome</td>
</tr>
<tr>
<td>Stroke</td>
<td>Obstetric complications</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>Fetal abnormalities</td>
</tr>
<tr>
<td>Obesity hyperventilation syndrome</td>
<td>Depression</td>
</tr>
<tr>
<td>Asthma</td>
<td>Cancer: breast, bowel, prostate</td>
</tr>
<tr>
<td>Obstructive sleep apnea</td>
<td>Venous ulcers</td>
</tr>
<tr>
<td>Gallstones</td>
<td>Accident prone</td>
</tr>
<tr>
<td>Nonalcoholic steatohepatitis</td>
<td>Gout</td>
</tr>
<tr>
<td>Urinary incontinence</td>
<td>Gastroesophageal reflux</td>
</tr>
<tr>
<td></td>
<td>Skin disorders</td>
</tr>
</tbody>
</table>

1.5.2. Abdominal obesity

BMI provides an indication of total body fat content but does not take account of the distribution of body fat (Sowers, 2003). It therefore provides a useful marker of being overweight or obese but not central obesity. The pattern of fat distribution, as assessed by waist circumference, can be used as an alternative definition of obesity and is particularly important for cardiovascular risk assessment (Bjorntorp, 1992; Frayn, 2001; Li et al., 2007a; Ritchie & Connell, 2007). It is notable that in the last 15 years, the prevalence of abdominal obesity has been increasing continuously with increased obesity (Li et al., 2007a). In 1956, Vague introduced the idea of systematic evaluation of the role of fat distribution on risk for obesity-related morbidity (Vague, 1956). Vague coined the term android and gynoid obesity. In this study, android obesity was found to be more frequently associated with diabetes mellitus, coronary artery disease than gynoid obesity (Vague, 1956). Beside the finding that obesity is strongly predictive of CAD, the Framingham Heart Study (Diabetes Prevention Program Research Group, 2002) also demonstrated that the risk for CVD is significantly increased when abdominal obesity (waist circumference >102 cm in men and >88 cm in women) is present.

Central obesity is characterized by greater deposition of fat in the central part of the body, and has been shown to correlate with insulin resistance (Frayn, 2001; Ritchie &
Connell, 2007; Frayn et al., 2005; McFarlane et al., 2001). This is, as previously mentioned, because increased body fat, particularly abdominal fat, is associated with increased lipolysis and elevated plasma concentrations of NEFA (Frayn et al., 2005; Jensen, 1989). NEFA can interfere with the cellular mechanisms of insulin signaling and down regulate cellular sensitivity to insulin (Shulman, 2000). Since the risk for developing T2 DM is high in overweight and obesity, assessing weight and waist circumference in these individuals is particularly useful for identifying those at risk (Sharp et al., 2003). In Sharp’s study, all the anthropometric indices that were measured were positively correlated with total cholesterol (T. Cholesterol), insulin, glucose, TAG, and systolic and diastolic blood pressure and negatively correlated with HDL-C. Reduction in central abdominal fat has also been linked to a less atherogenic lipid profile (Klein et al., 2004; Norris et al., 2005).

1.6. Dyslipidaemia in T2 DM and obesity

The increased progression of atherosclerosis in diabetes is most likely to be the result of the impact of the major CVD risk factors that are more prevalent in diabetes, namely obesity and dyslipidaemia (Resnick & Howard, 2002). The Framingham Heart Study (Garge, 1994) found that dyslipidaemia was common in adults with diabetes and considered it to be an independent risk factor for CVD in this population. The key characteristics of T2 DM dyslipidaemia are moderate hypertriacylglycerolaemia, increased postprandial lipaemia a predominance of small dense LDL (sdLDL) and decreased HDL (especially HDL2) concentrations (Table 1.4; syvanne & Taskinen, 2005; Rizzo & Berneis, 2006a; Rizzo et al., 2007; Austin et al., 1994; Griffin & Zampelas, 1995). This collection of lipoprotein abnormalities frequently presents as a subclinical dyslipidaemia that is referred to as an atherogenic lipoprotein phenotype (ALP). This ALP is not restricted to persons with T2 DM; it is also common in obese individuals with insulin resistance (Grundy, 1997; Rizzo & Berneis, 2005). An ALP is found in 50-75% of patients with T2 DM (Beckman et al., 2002). Although an increased production of plasma apo B has not been universally accepted as being part of the definition of ALP, increased LDL particle number has emerged in recent years as a major source of increased CVD risk (Ahmed et al., 2007; Griffin et al., 1999; Griffin et al., 2000; Wagner et al., 2002).
Table 1.4. Characteristics of ALP (Griffin & Zampelas, 1995).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fasting plasma concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAG</td>
<td>1.5-2.3 mmol/l</td>
</tr>
<tr>
<td>sdLDL-III (density 1.044-1.060 g/ml)</td>
<td>&gt;40% of total LDL plasma</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>&lt;1 mmol/l</td>
</tr>
<tr>
<td>HDL2 (large HDL particle diameter 9.7-12.9 nm)</td>
<td>&lt;10% total HDL</td>
</tr>
<tr>
<td>Enhanced postprandial lipaemia</td>
<td></td>
</tr>
<tr>
<td>Cholesterol ester enriched VLDL and CM remnants</td>
<td></td>
</tr>
<tr>
<td>Insulin resistance</td>
<td></td>
</tr>
</tbody>
</table>

1.6.1. Pathophysiology of an ALP

1.6.1.1. Increased TAG rich lipoproteins

TAG-rich lipoproteins include several species of lipoproteins that are either newly secreted or modified in the circulation (which are called remnant lipoproteins). As stated before, remnant lipoproteins are formed by LPL in the circulation. During the catabolism of TAG-rich lipoproteins the cores of these lipoproteins are reduced in TAG content but are enriched in cholesterol esters via the action of CETP (Morton & Zilversmit, 1982). An elevated concentration of TAG-rich lipoproteins in the postprandial phase is a common feature of insulin resistant states, such as obesity and T2 DM, that may directly predispose to CHD (Grundy, 1999). A moderate rise in plasma TAG (>1.5 mmol/l) influences LDL size and density through a cycle of lipid exchange (Taskinen et al., 1996; Griffin et al., 1994). This concentration of plasma TAG allows enhanced CETP mediated exchange of cholesteryl ester from LDL and HDL for TAGs from VLDL and chylomicrons, leading to enrichment of LDL and HDL with TAG. Hydrolysis of core TAGs by HL, which is often increased in T2 DM (Baynes et al., 1991), results in the formation of sdLDL and small dense HDL (sdHDL; Figure 1.4).
Figure 1.4. Mechanism of generation of sdLDL and sdHDL in obesity and T2 DM (Ginsberg, 2000). Insulin resistant adipocytes fail to suppress the release of NEFA into the circulation in the postprandial phase leading to increased NEFA uptake by the liver for TAG synthesis. This in turn may stimulate the assembly and secretion of apo B and VLDL. The result is an increased number of VLDL particles and increased concentration of TAG in the plasma. This can accelerate neutral lipid exchange and promote a net transfer of TAG to LDL and HDL, leading to two outcomes: a cholesterol rich VLDL remnant particle that is atherogenic, and a TAG-rich cholesterol depleted HDL particle. The triglyceride-rich HDL particle can undergo further modification including hydrolysis of its TAG by HL thereby leading to the formation of sdHDL particles. Increased concentrations of VLDL TAG also enhance the transfer of TAG into LDL in exchange for LDL cholesteryl ester. The TAG-rich LDL can be hydrolysed by HL to the form sdLDL particles.

The increase in plasma TAG for VLDL assembly in the liver in insulin resistant states may be derived from three main sources; an increased flux of NEFA from adipose tissue to the liver, hepatic uptake of VLDL and chylomicron-derived remnants, and increased hepatic lipogenesis (Ginsberg et al., 2005). After the original exclusion of plasma TAG as a risk factor for CHD as a result of its association with other risk factors such as HDL, raised plasma TAG has re-emerged as an independent risk factor for CHD. This was largely due to the results of a meta-analyses of 17 prospective population studies, which established raised plasma TAG as an independent risk factor for CHD in a total of 46,413 men and 10,864 women (Hokanson & Austin, 1996).
1.6.1.2. Role of sdLDL in CVD

Evidence for the role of sdLDL in CVD has been derived from cross sectional, prospective and clinical intervention studies (Gardner et al., 1996; Griffin et al., 1990; Griffin et al., 1994; Grundy, 1997; Rosenson, 2004; Wood et al., 2006; Austin et al., 1988). Indeed, elevated concentrations of these particles has been associated with a 3 to 4-fold increase in the risk of acute myocardial infarction (Rizzo & Berneis, 2005; Austin et al., 1988). The NCEP ATP-III (2002) has therefore recognized a predominance of small dense sdLDL (LDL-III; Figure 1.5) as an emerging cardiovascular risk factor. Austin and colleagues (1990) were the first to investigate the clinical relevance of sdLDL. They analysed the LDL of 301 subjects by using non-denaturing gradient gel electrophoresis. In this study, two distinct phenotypes were identified; phenotype ‘A’ which was characterized by predominance of large, buoyant LDL particles, and phenotype ‘B’ which consisted of a major peak of sdLDL particles. Phenotype ‘B’ was specifically associated with increased plasma TAG and apo B and a decreased HDL-C and plasma apo A-I. While the majority of healthy subjects have LDL particles that are large and buoyant, about 30-35% of Europeans have a predominance of sdLDL particles. Phenotype ‘B’ has been associated with a number of other metabolic changes including raised intermediate density lipoproteins (IDL) (Austin et al., 1990) and reduced insulin sensitivity (Reaven, 1995).

![Figure 1.5. Plasma LDL subclasses of normal and ALP subjects (Griffin & Zampelas, 1995).](image)
The activity of CETP and endothelial lipases LPL and HL have been identified as major metabolic determinants of LDL particle size (Griffin & Zampelas, 1995). In order to evaluate the roles of these proteins in determining LDL subfraction distribution in T2 DM, a study of 137 patients with T2 DM (75 male & 62 female) and 140 matched controls (80 male & 60 female) was conducted (Krauss, 1998). The findings of this study supported the hypothesis that plasma TAG influences the formation of sdLDL particles through a cycle of lipid exchange via the action of CETP as described earlier (Section 1.6.1.1). Kinetic studies also indicated that the amount and type of VLDL secreted from the liver may determine the size of LDL formed in the circulation (Tan et al., 1999). More specifically, LDL derived from large VLDL was shown to be similar to sdLDL with respect to its lower fractional catabolic rate and longer residence time in plasma, suggesting that sdLDL may be the product of larger, TAG-rich VLDL particles (Packard et al., 2000).

Small, dense LDL has been associated with increased risk of atherosclerosis through several inter-related mechanisms. sdLDL binds to the arterial wall with greater affinity than native LDL (Demant & Packard, 1998). sdLDL is also more susceptible to glycation and oxidative modification (Yu et al., 2007) which contributes to their increased atherogenicity in diabetic patients (Wagner et al., 1989). Along with IDL, oxidized LDL can readily be taken up by arterial wall macrophages that thereby become foam cells (Wagner et al., 2003). Postprandial lipaemia has also been implicated in generating increased atherogenicity through sdLDL (Griffin & Fielding, 2001).

Recent findings from several primary and secondary prevention trials have indicated that raised plasma apo B is a better index of atherosclerotic risk than LDL-C, essentially because raised apo B provides a marker for an increased number of circulating LDL particles (Gotto et al., 2000; Ahmad et al., 2007). In addition, LDL-C:apo B ratio is an important tool to detect phenotype ‘B’ in T2 DM patients (Wagner et al., 2002). The ratio of apo B:non HDL-C (a measure of total cholesterol minus HDL-C) is also essential for the identification of dyslipidaemia that confers high CVD risk in patients with T2 DM (Wagner et al., 2002). While raised apo B (>1.3 g/l) is characterized by an abundance of sdLDL, a predominance of sdLDL (as >50%,
phenotype ‘B’) may not always be found in association with raised number of LDL particle (LDL apo B) (Griffin et al., 1999).

1.6.1.3. Low HDL cholesterol concentration

The concentration of plasma HDL-C is a major predictor of CVD risk (Rosenson, 2004; Gardner et al., 2000; Link et al., 2007). Large prospective studies have shown that low HDL-C independently increases the risk for coronary artery disease (Gordon et al., 1977; Link et al., 2007). In a follow-up study of risk factors in 229 T2 DM patients who had no history of CHD and stroke, low HDL-C was an independent risk factor for the development of CHD (Gordon et al., 1977). In addition, HDL-C has been shown to be a better discriminator of CHD than LDL-C and for every 0.026 mmol/l increase in HDL-C, CHD is reduced by 3% (Castelli, 1988).

Among other factors, it has been suggested that any benefit underlying elevated HDL cholesterol concentrations could be mainly attributed to the larger, more cholesterol-rich HDL₂ subfraction (Gardner et al., 2000; Gofman et al., 1966). The Kuopio Ischemic Heart Disease Risk Factor Study (Gofman et al., 1966) also indicated that the cardioprotective effect of elevated HDL cholesterol concentrations may be attributed to the HDL₂ subclass. HDL₂ levels have also been inversely correlated with abdominal fat, plasma insulin concentrations, and glucose intolerance (Ostlund et al., 1990). The weaker association between smaller HDL₃ and CVD risk is thought to arise from the shorter half-life, lower TG:CE ratio and lower anti-oxidative activity of these smaller particles (Hansel et al., 2004).

HDL particles in subjects with T2 DM are small and have a low free cholesterol content (Syvanne et al., 1995; Link et al., 2007; Syvanne & Taskinen, 2005). In insulin resistant individuals, HDL-C and apo A-I concentrations are reduced as a result of the action of CETP mediated transfer of cholesteryl ester from HDL to TAG rich lipoproteins (Link et al., 2007). Non-HDL-C has also been shown to represent a useful CVD risk marker for predicting the risk for CVD in T2 DM (Lu et al., 2003).
1.7. Dietary management of CVD risk in T2 DM and obesity

The clinical management of diabetes, insulin resistance, hypertension, obesity, and other risk factors aims to substantially reduce the risk of CVD. Similarly, weight loss and glycaemic control are key goals for reducing CVD risk in diabetes. Clinically significant weight loss of 5-10% of body weight has beneficial effects not only on blood lipids (Wing et al., 1987a; Wing et al., 1994; Blackburn et al., 2001; Wood, 2006) and risks associated with obesity (Blackburn et al., 2001; Krauss et al., 2006) but also on insulin sensitivity and glycaemic control (Krauss et al., 2006; Wing et al., 1987a; Wing et al., 1994; Heilbronn et al., 2002). It has been suggested that glycaemic control alone is not sufficient to reduce CVD risk in many patients (Schernthaner et al., 1983). The UKPDS data indicated that HbA1c ranked below LDL-C and HDL-C as risk factors for CAD and below LDL-C and diastolic blood pressure as a determinant of risk for fatal or non fatal myocardial infarction (Turner et al., 1998). Therefore, the treatment of dyslipidaemia in patients with T2 DM is essential. This is also indicated through the guidelines of NCEP ATP-III (2001) and the American Diabetes Association (2004b).

Dietary modification in T2 DM represents a simple tool for treatment and prevention of CHD. Epidemiological and experimental studies have identified many nutrients that either protect against or stimulate one or more of the risk factors for CHD (Wood et al., 1998; Frost et al., 1999; Wood, 2006). Epidemiological studies have clearly shown that diets high in fat and especially saturated fat are associated with insulin resistance (Mayer-Davis et al., 1997) and hyperlipidaemia (Tanasescu et al., 2004). Substitution of dietary saturated fat with a diet enriched in polyunsaturated fatty acid (PUFA) in an isoenergetic diet resulted in increased insulin sensitivity and reduced LDL-C (Summers et al., 2002) but these effects were not necessarily related to increased PUFA or decreased saturated fat intake in the PUFA group. In addition, diets enriched with PUFA have not always been associated with insulin sensitivity (Mayer et al., 1993). High monounsaturated fatty acid (MUFA) diets have also been advocated as dietary alternatives to improve insulin sensitivity (Vessby et al., 2001). However, within individuals with a high fat intake (37% of energy), beneficial effects on insulin sensitivity were not seen. The replacement of energy from saturated fat with carbohydrate stimulates hepatic synthesis and secretion of VLDL and TAG.
leading to an increase in TAG-rich lipoproteins and lower HDL-C concentrations
(Abbasi et al., 2000; Liese et al., 2007; Liu et al., 1983). It has therefore been
suggested that the quality of dietary carbohydrate should be considered as a
potentially modifiable factor that together with weight control and exercise may play
a role in CVD prevention and treatment in diabetes (Jenkins et al., 2002).

1.7.1. Glycaemic index of carbohydrates

In (1936) Conn and Newburgh noted that different carbohydrate containing foods
with the same macronutrient composition elicited different glycaemic responses. In
1981 Jenkins introduced the term glycaemic index as a means of quantifying the
glycaemic response to different dietary carbohydrates for the dietary management of
diabetes. Therefore, in determining the potential metabolic effects of a diet, the
physiological assessment of foods, represented by the glycaemic index (GI), is a
useful supplement to chemical analysis of their dietary fibre content and composition.
High GI carbohydrate-rich foods have been proposed to increase hunger and elevate
NEFA concentrations, leading to an increased risk of CVD (Wolever, 1990a).
Consequently, the WHO (1998) recommends the classification of dietary
carbohydrate according to the GI and endorses the usefulness of GI in dietary
planning.

1.7.1.1. Definition of glycaemic index (GI) and load (GL)

GI is a measure of the postprandial glucose response as a result of the consumption of
carbohydrate-rich food and provides a standardized comparison of the 2 hour
postprandial glucose response of a carbohydrate with that of white bread or glucose
(FAO/WHO, 1998). It is defined as the incremental area under the blood glucose
response curve elicited by a 50 g of available carbohydrate in portion of a
carbohydrate-rich food, expressed as a percentage of the glucose response to 50 g of a
reference material (usually 50 g of glucose in solution or white bread) in the same
subject. The GI can therefore be calculated by using the following formula

\[
GI = \frac{\text{Incremental area under 2-h plasma glucose curve for test food containing 50 g available CHO}}{\text{Incremental area under 2-h plasma glucose curve for 50 g glucose}} \times 100
\]
As such, low GI (LGI) carbohydrates (GI\(\leq55\%\)) are those that are digested and absorbed slowly and lead to a low glycaemic response, whereas HIGH GI foods (GI=70\% or more) are rapidly digested and absorbed and exhibit a high glycaemic response. A medium GI food will have a GI of 56-69\% (Figure 1.6). Much of the carbohydrates present in the Western diet contain refined polysaccharides, such as in bread and baked products, and sugars, found in juices and sodas. These have a HIGH GI, causing glucose and insulin to increase substantially (Ludwig, 2007). Other types of carbohydrates found in certain whole grains, beans, nuts, and vegetables have a lower GI and cause less of a postprandial increase in serum glucose and insulin.

The International Table of Glycaemic Index Values (Foster-Powell et al., 2002) was first published in 1995, and has been since updated to include the GI values for over 750 different types of foods (Ludwig, 2007). These tables provide the GI for each food with either glucose or white bread used as the reference food. Along with the GI values, the tables include values for the glycaemic load (GL) of certain carbohydrate-rich foods. The GL concept was first introduced in 1997 (Salmeron et al., 1997a; Salmeron et al., 1997b) to quantify the overall glycaemic effect of a portion of food. It is the product of dietary GI and total dietary carbohydrate, therefore it is representative of the quantity as well as the quality of carbohydrate consumed and provides a useful measure of the total glycaemic effect (Salmeron et al., 1997a; Salmeron et al., 1997b). The higher the GL, the greater the elevation in glycaemic and insulinaemic response (Foster-Powell et al., 2002).

Data from the Framingham offspring cohort study demonstrated that the intake of whole grain was inversely associated with insulin resistance, as measured by the homeostasis model assessment of relative insulin resistance (HOMA-IR), and a lower prevalence of the metabolic syndrome (McKeown et al., 2004). In this study, dietary GI, but not the GL, was positively associated with HOMA-IR and the prevalence of the metabolic syndrome. Consistent with this, Brand-Miller (2004) showed that the GI of carbohydrate rich foods was significantly associated with disease risk more often than the GL of the diet. Accordingly, there is accumulating evidence that a diet with a predominance of foods that elicit low glycaemic responses, induces modest clinically beneficial effects in diabetes (Salmeron et al., 1997a; Salmeron et al., 1997b; Opperman et al., 2004). CVD risk (Opperman et al., 2004; Liu et al., 2000;
Frost et al., 1999), and certain forms of cancer (Augustin et al., 2003), while the role of this diet for the management of obesity has recently been debated (Pawlak et al., 2002; Raben, 2002).

![Graph showing glycaemic response]

**Figure 1.6.** Glycaemic response after ingestion of pasta, wholemeal bread, and white bread (Frost & Dornhorst, 2000). Pasta has a relatively lower 2h postprandial response compared with white and wholemeal breads.

1.7.1.2. **Factors affecting GI**

The increase in plasma glucose after a meal is determined by the rate of absorption of a carbohydrate which, in turn, is controlled by the rate of gastric emptying, and rate of hydrolysis in the gastrointestinal tract (Augustin et al., 2002). The latter two processes are affected by the composition of the food, the tertiary structure, and the susceptibility of the starch molecules to enzymatic digestion (Jenkins et al., 1988a). The GI of carbohydrate-rich food provides an indication of the rate at which carbohydrate-rich foods are digested (Jenkins et al., 1981). One of the other factors that affect the rate of glucose absorption from a carbohydrate-rich food and hence the GI value is the proportion of amylose to amylopectin (Avenell et al., 2004b). Amylose is a linear molecule with D-glucose units linked in by α1–4 linkages. Amylopectin has both α1–4 and α1–6 linkages (Behall et al., 1988), and is thereby a branched structure (Figure 1.7). The rate of absorption is affected by the ratio of the
two types of starch (Behall et al., 1988). The higher the proportion of amylopectin, the higher the GI, because amylopectin is more easily hydrolyzed in the gut than is the single stranded amylase (Annison & Topping, 1994; Miller et al., 1992). For example, much of the variation in the GI of different rice brands is due to the variation in this ratio (Miller et al., 1992). Rice varieties that contain a higher proportion of amylose have been shown to have a slower rate of digestion and produce lower GI (Juliano & Goddard, 1986; Miller et al., 1992).

Figure 1.7. Structure of A; amylose and B; amylopectin. The ratio of amylose to amylopectin affects the rate of carbohydrate absorption and the GI.

In addition, studies have shown that diets rich in soluble non-starch polysaccharides (NSP), such as guar gum and β-glucan, produce lower postprandial blood glucose and insulin concentrations (Miller et al., 1992). When Wolever (1990a) studied the dietary fibre content and composition of 25 foods and related them to their GI, total dietary fibre was significantly related to GI ($r=-0.461$, $p<0.05$). Soluble NSPs are found in pulses, vegetables, whole fruits, oats, and barley. They form gelatinous gels in the stomach and delay gastric emptying and enzyme digestion. These, plus other factors that might affect the GI of the carbohydrate-rich foods are listed in Table 1.5.

1.7.1.3. Aspects of the practical measurement of GI

A frequent criticism of the GI is that it is subject to significant inter and intra individual variation (Brouns et al., 2005). However, Wolever (2006) emphasized that most of the variation appears in the plasma glucose response as assessed by incremental area under the curve elicited by a carbohydrate meal. This is reduced
when expressed as a ratio compared with the response to 50g glucose, as in the measurement of GI. Feeding experiments have shown that variation in glycaemic response to a single food between different individuals is small. For example, in determining the GI of 5 foods in 47 subjects (Wolever, 2006), about 50% of the variance of the AUC of glucose was due to variation between subjects, 25% was due to day to day variation within subjects, and 25% to variation between foods. When expressed as GI, the total variance was reduced by 45%, by a reduction in the between subjects variation (Wolever, 2006).

**Table 1.5. Factors affecting the GI of a carbohydrate rich food (Augustin et al., 2002).**

<table>
<thead>
<tr>
<th>Factors that affect the GI</th>
<th>Factors that decrease GI</th>
<th>Factors that increase GI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nature of the starch</td>
<td>↑ Amylose/Amylopectin</td>
<td>↓ Amylose/Amylopectin</td>
</tr>
<tr>
<td>Nature of monosaccharide components</td>
<td>Fructose</td>
<td>Glucose</td>
</tr>
<tr>
<td>Viscous fibre</td>
<td>↑ Guar</td>
<td>↓ Guar</td>
</tr>
<tr>
<td>Cooking/ Food processing</td>
<td>↑β-Glucan</td>
<td>↓β-Glucan</td>
</tr>
<tr>
<td>Parboiling</td>
<td>Extruding</td>
<td></td>
</tr>
<tr>
<td>Cold extrusion</td>
<td>Flaking</td>
<td></td>
</tr>
<tr>
<td>Cold extrusion</td>
<td>Popping</td>
<td></td>
</tr>
<tr>
<td>Particle size</td>
<td>Large particles</td>
<td>Grinding (small particles)</td>
</tr>
<tr>
<td>Ripeness and food storage</td>
<td>Unripeness</td>
<td>Ripeness</td>
</tr>
<tr>
<td>Cooling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Amylase inhibitors</td>
<td>↑ Lectins</td>
<td>↓ Lectins</td>
</tr>
<tr>
<td>Nutrient starch interaction</td>
<td>↑ Phytates</td>
<td>↓ Phytates</td>
</tr>
<tr>
<td></td>
<td>↑ Protein</td>
<td>↓ Protein</td>
</tr>
<tr>
<td></td>
<td>↑ Fat</td>
<td>↓ Fat</td>
</tr>
</tbody>
</table>

↑ High levels  
↓ Low levels

The measurement of the GI of foods has also been criticised in terms of its wide variation and precision between different labs. For instance, the GI values reported by different laboratories vary widely for some foods such as potato (Wolever et al., 1994a; Fernandes et al., 2005; Henry et al., 2005) and rice (Miller et al., 1992; Foster-Powell et al., 2002). In addition, the results of the most recent GI testing of 33 commercially available foods in the UK (breakfast cereals, breads, pasta, rice and potatoes) were different from previously published values of similar foods (Aston et al., 2007). However, this variation can usually be explained by real differences between the foods in terms of their processing and cooking methods, as well as other
factors that were described in Table 1.5. It is noteworthy that the between laboratory standard deviation (SD) for GI value was 9 in a recent inter-laboratory (28 laboratories around the world) study of GI (Wolever et al., 2007). An expert panel from the WHO (FAO/WHO, 1998) brought consensus to GI measurement as part of a global discussion on the role of dietary CHO in nutrition. This discussion provided guidelines and a reference methodology for the future testing of GI. More relevant GI methodological considerations regarding the number of subjects, sex, subject status, and pre-test conditions, carbohydrate test dose, blood sampling procedures, sampling times, test randomisation and calculation of glycaemic response (IAUC), have been reviewed more recently in light of the standardised GI testing methodology of the WHO (Brouns et al., 2005). The GI testing methodology of this review provided detailed and evidence-based guidelines for the measurement of GI. The method was standardised to improve quality control within laboratories and thus increase confidence in the measurement of GI (Brouns et al., 2005).

Although the GI and the carbohydrate content of foods are the strongest determinants of the variation in the mean glycaemic responses to different carbohydrate rich foods (Wolever et al., 2006), the presence of other constituents in the food, such as fat and protein, influences the total glycaemic response (Lovejoy et al., 1998; Nuttall et al., 1984). The term insulinaemic index (II) of foods was introduced to further elucidate this, ranking the impact of carbohydrates on postprandial plasma insulin instead of plasma glucose (Holter et al., 1997). The II of food provides additional information in the dietary management of diabetes (Bornet et al., 1997; Miller et al., 1992). However, although many studies have reported 'II' values beside the GI when assessing the physiological effects of food (Indar-Brown et al., 1992; Larsen et al., 2000; Ostman et al., 2001; Abdelgadir et al., 2005; Flint et al., 2006), and despite the high correlation between the two measures (Holter et al., 1997), the clinical usefulness of this food parameter is questionable as it varies in different groups of people (Wolever et al., 1998; Wolever, 2006). In mixed meals, particularly in milk-based meals, large inconsistencies between glycaemic and insulinaemic responses have been noted both in normal and diabetic subjects (Gannon et al., 1986).
1.7.1.4. Effect of GI on glycaemic control

There is compelling evidence to show that controlling postprandial glycaemia is important for macro and microvascular risk reduction in both type 1 (Diabetes Prevention Program Research Group, 2002) and T2 DM patients (UKPDS, 1998). For example, a 1% reduction in mean HbA1c (a measure of glycaemic control) is associated with a 21% reduction in severe end points related to diabetes such as mortality, heart failure, stroke, retinopathy, amputation and myocardial infarction (Stratton et al., 2000). Therefore in 2004 the American Diabetes Association recommended keeping glycaemic control, as assessed by HbA1c concentrations, to <7% (AHA, 2004).

The body of evidence to support a beneficial effect of low glycaemic index (LGI) diets on glycaemic control is very strong. For example, a meta-analysis of the randomized controlled trials that have examined the efficacy of the GI on overall blood glucose control indicated an additional benefit of using a LGI diet over that observed when total carbohydrate was considered alone (American Diabetes Association, 2004b). Glycated proteins, including both HbA1c and fructosamine, were reduced by 7.4% on the LGI diet. Specifically, there was a 0.4% reduction in HbA1c in diabetics who followed a LGI diet compared to high GI diets, whilst fructosamine concentrations were also lowered by 0.2 mmol/l. Measurement of plasma fructosamine is favoured as a short term marker of glycaemic control in studies that last for 6 weeks as the effect is reported to change maximally in 4-6 weeks (Jones et al., 1983). The longer the consumption of LGI foods, the larger the decreases in fructosamine concentrations (Brand-Miller et al., 2003a). Even when the improvement in glycaemic control was not significant (Heilbronn et al., 2002), the consumption of a LGI diet in T2 DM patients was associated with a 2-fold reduction in HbA1c compared with subjects consuming a HIGH GI diet. Another recent meta analysis (Opperman et al., 2004) of 16 studies that investigated the effect of LGI diets compared to HIGH GI diets on CVD risk, indicated an overall significant reduction in fructosamine concentrations in subjects receiving the LGI diet (-0.1 mmol/l; p=0.05). Mean HbA1c was also decreased significantly by 0.27% (p=0.03). A selection of studies on the effects of LGI diets on glycaemic control are summarized in Table 1.6.
<table>
<thead>
<tr>
<th>Study</th>
<th>Duration</th>
<th>Outcome</th>
<th>High GI Efﬁcacy</th>
<th>Low GI Efﬁcacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1.6: Efﬁcacy of GI foods on efﬁcacy proteins in healthy, overweight, impaired glucose tolerance, 1.1 DM and 1.2 DM subjects.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study</th>
<th>Duration</th>
<th>Outcome</th>
<th>High GI Efﬁcacy</th>
<th>Low GI Efﬁcacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1.6: Efﬁcacy of GI foods on efﬁcacy proteins in healthy, overweight, impaired glucose tolerance, 1.1 DM and 1.2 DM subjects.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1.7.1.5. Effect of LGI diet on obesity

The role of GI on appetite and body weight regulation has been a recent topic of debate (Raben, 2002; Pawlak et al., 2002). Some researchers have suggested that LGI foods are potentially beneficial in terms of weight management through their satiating qualities. Much of the evidence for this came from the WHO (1998) which reported that the consumption of a high fibre diet was beneficial with respect to weight management. For example, a prospective cohort study of 74,091 US female nurses, aged 38-63 years (Liu et al., 2003), reported that women who consumed more whole grains consistently weighed less than women who consumed less whole grains. Over a 12 years period, those with the greatest increase in intake of dietary fibre gained an average of 1.52 kg less than those with the smallest increase in intake of dietary fibre, independent of body weight at baseline. In this study, weight gain was inversely associated with the intake of high fibre, whole grain foods and positively related to the intake of refined grain foods. In addition, intervention studies such as the study by Slabber et al. (1994), indicated that women (n=30) who followed a LGI diet lost 9.4 kg as compared to 7.4 kg for those who followed a HIGH GI diets. These studies have therefore led to the conclusion that LGI foods typically induce higher satiety than HIGH GI foods, and are followed by a lower intake of energy intake at subsequent meals and therefore promote weight loss (Ludwig, 2000; Holt & Barnd Miller, 1994). The proposed explanation for this GI-induced satiety is based on the slower rate of digestion and absorption of LGI carbohydrates which results in a prolonged stimulation of nutrient receptors in the GI tract. This leads to prolonged feedback to the satiety centre in the brain through signals such as choleystokinin and glucagon like peptide-I (Lavin et al., 1996). These gut hormones are known to decrease appetite and regulate energy metabolism (Neary et al., 2004).

On the other hand, many studies have not shown beneficial effects of LGI diets on weight management. These studies are summarized in Table 1.7. Based on the presented studies, there is no evidence at present that low GI foods are beneficial compared to high GI foods with regard to long term body weight control. It is reasonable to conclude that while LGI diets improve metabolic cardiovascular factors, they do not reduce weight unless they are part of an energy-restricted diet.
Table 1.7. Studies against the role of GI in weight loss.

<table>
<thead>
<tr>
<th>Study</th>
<th>Duration (weeks)</th>
<th>n</th>
<th>Body Type</th>
<th>Diets</th>
<th>Body weight changes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bouche et al. (2002)</td>
<td>5</td>
<td>11 M</td>
<td>Overweight</td>
<td>Ad Libitum</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.3</td>
</tr>
<tr>
<td>Frost et al. (1994)</td>
<td>12</td>
<td>51 F + M</td>
<td>Overweight</td>
<td>LGI vs. HIGH GI foods</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-2.1</td>
</tr>
<tr>
<td>Jimenez-Cruz et al. (2003)</td>
<td>2</td>
<td>8 F + M</td>
<td>T2 DM patients</td>
<td>Ad Libitum</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>Kabir et al. (2002)</td>
<td>4</td>
<td>13 M</td>
<td>T2 DM patients</td>
<td>LGI vs. HIGH GI breakfast</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Not shown</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Not shown</td>
</tr>
<tr>
<td>Luscombe et al. (1999)</td>
<td>4</td>
<td>21 F + M</td>
<td>Overweight</td>
<td>LGI vs. HIGH GI foods</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.6</td>
</tr>
<tr>
<td>Raatz et al. (2005)</td>
<td>24</td>
<td>29 F + M</td>
<td>Obese</td>
<td>Energy controlled HIGH GI, LGI, LF</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-1.6</td>
</tr>
<tr>
<td>Raben et al. (1997)</td>
<td>2</td>
<td>20 F</td>
<td>Normal weight</td>
<td>Ad Libitum</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.7</td>
</tr>
<tr>
<td>Sloth et al. (2004)</td>
<td>10</td>
<td>45 F + M</td>
<td>Overweight</td>
<td>Ad Libitum</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-1.3</td>
</tr>
<tr>
<td>Tsihlias et al. (2000)</td>
<td>24</td>
<td>72 F + M</td>
<td>Normal weight &amp; overweight</td>
<td>LGI vs. HIGH GI breakfasts</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-1.7</td>
</tr>
<tr>
<td>Wolever et al. (1992)</td>
<td>6</td>
<td>6 F + M</td>
<td>Overweight &amp; obese</td>
<td>Hypocaloric</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-2.5</td>
</tr>
</tbody>
</table>

F female, M male, NS not significant, LF low fat
1.8.1.6. Effect of GI on insulin sensitivity

The GI of the diet has been shown to influence both glucose and insulin secretion (Ludwig, 2007). High GI diets have been linked with hyperinsulinaemia (McKeown et al., 2004; van Dam et al., 2000) which, in turn, may downregulate insulin receptors, reducing insulin efficiency, therefore resulting in insulin resistance. This condition acts in a vicious circle of increased glucose and insulin secretion (Figure 1.8) (Augustin et al., 2002).

![Diagram](image)

**Figure 1.8.** Potential mechanism for the relationship between high GI foods and insulin resistance (Augustin et al., 2002).

Several studies have examined the effects of LGI diets on hyperinsulinaemia and insulin resistance. In a 3 week study in women with increased risk of CHD, a diet with LGI was associated with increased insulin sensitivity (Frost et al., 1998). In another study (Jarvi et al., 1999) with a randomized cross over design, the effect of a LGI diet on insulin sensitivity was evaluated in 20 T2 DM patients. Insulin sensitivity was measured by the euglycemic hyperinsulinaemic clamp technique. Peripheral insulin sensitivity increased significantly after the consumption of the LGI diet. The incremental area under the curve for both blood glucose and plasma insulin was also lower by 30% compared to the high GI diet. These, and other studies (Dumesnil et al., 2001) have reported improved insulin sensitivity with low compared with high GI diets.
The influence of LGI diets on insulin sensitivity is thought to be regulated by NEFA concentrations (Wolever et al., 1995). High GI carbohydrates stimulate high insulin concentrations as a result of their rapid absorption. This is followed by a rapid glucose fall, often to below baseline values. This situation results in a counter-regulatory response with the release of NEFA in the late postprandial phase to a level well above that observed after a low GI meal, creating an insulin resistant environment. The improvement in insulin sensitivity seen in patients with CHD (Frost et al., 1998) or T2 DM (Jarvi et al., 1999; Wolever et al., 1992) on diets with LGI has been attributed to a greater suppression of release of NEFA.

However, some investigators have suggested that the fibre content of the diet is more closely related to insulin sensitivity than the GI. In the Insulin Resistance Atherosclerosis Study (Liese et al., 2005), 979 adults with normal or impaired glucose tolerance were studied for associations between carbohydrates, fibre intake, GI and GL, insulin sensitivity and fasting insulin. In this study, no association was observed between GI and insulin sensitivity or fasting insulin after adjustment for demographic characteristics, family history of diabetes, energy expenditure, and smoking. In contrast, fibre was positively associated with insulin sensitivity and inversely with fasting insulin. Therefore the extent to which fibre in a food is responsible for effects attributable to GI remains to be determined.

1.7.1.7. Effect of GI on an ALP

The results from studies on the effect of dietary GI on lipid markers of CVD with regard to plasma TAG and HDL-C, have, to date, been equivocal. This is probably due to a combination of factors including limited sample size, different designs, poor compliance, or due to the presence of confounding factors such as the fibre content of the diet. Although one of the largest cross sectional analysis of metabolic risk factors in 394 men in the Dutch town Zutphen (van Dam et al., 2000) found no association between GI and blood concentrations of T. Cholesterol, HDL-C, TAG, insulin or glucose, many other prospective and intervention studies have highlighted the potential of a LGI diet in improving the ALP.

Frost et al. (1999) found a negative relationship between the GI of the diet and HDL-C for both men and women in a cross sectional nutritional survey of 1420 British
adults, using a multiple regression model. This relationship was also found to be
more pronounced in women. The same study indicated that the GI of the diet was a
stronger predictor of HDL-C than dietary fat intake. The concentration of HDL-C is
expected to improve with a LGI diet since this diet is associated with reduced hepatic
gluconeogenesis and suppression of NEFA release (Rizkalla et al., 2002). In accord
with the original findings of Frost et al (1999) were the results of the Third National
Health and Nutrition Examination Survey of 13,907 adults (Ford & Liu, 2001). This
study indicated that a difference in dietary GI of 15 units corresponded to a difference
in HDL-C of 0.06 mmol/l.

The effect of LGI diets on plasma TAG has also been inconsistent. While many
studies have shown no association between the GI of the diet and plasma TAG (Frost
et al., 2004; Jimenez-Cruz et al., 2003; Sloth et al., 2004), others have reported a
significant fall in plasma TAG with a reduction in dietary GI (Dumesnil et al., 2001;
Fontvieille et al., 1992; Jarvi et al., 1999; Holter et al., 1997; Ebbeling et al., 2005;
Wolever et al., 1992; Jenkins et al., 1985). Even when the consumption of a LGI diet
was for a short period of time (only 6 days), a decreased concentration of plasma
TAG was observed in centrally obese men with raised baseline TAG concentrations
(Dumesnil et al., 2001). A recent-meta analysis (Opperman et al., 2004) of
randomized controlled trials (published in 1981 and 2003), with both crossover and
parallel designs, showed decreases in plasma TAG in response to a LGI diet in six
studies, though this finding was not significant due to the short duration of the studies.
In the same meta-analysis, mean LDL-C showed a non-significant decrease after a
LGI diet (0.15%, p=0.06). This decrease was greater in individuals with T2 DM as
compared with healthy subjects and subjects with CHD. T-Cholesterol was also
significantly lower in the LGI diet groups (-0.33 mmol/l; p<0.0001), with greater
reductions being evident in those with raised baseline levels.

A putative mechanism to explain the TAG-lowering effects of a LGI diet was first
described by Ludwig (2002). The explanation holds that between 2-4 hours after a
HIGH GI meal, blood insulin concentrations are higher than after a LGI meal with
similar nutrients leading to postprandial hypoglycaemia. After 4-6 hours, counter-
regulatory hormones are released due to the hypoglycaemia which stimulates lipolysis
and elevated circulating NEFA. The increased circulating NEFA increases TAG re-
estrification and VLDL syntheses and production in the liver. The raised plasma TAG induced by high GI carbohydrate, might also cause the remodeling of lipoproteins, shifting LDL towards smaller, denser particles and reducing both HDL concentration and size (Ginsberg et al., 2005; Ludwig, 2007).

Studies on the effects of LGI diets on LDL and HDL subclasses are limited (Dumesnil et al., 2001; Jarvi et al., 1999; Wolever et al., 2003; Rizkalla et al., 2004; Luscombe et al., 1999) and show inconsistent findings. Whereas the study of Luscombe et al (1999) in 21 obese T2 DM patients indicated no changes in sdLDL or sdHDL, Dumesnil et al (2001) observed an increase in LDL size. In contrast, Wolever (2003) found that a modest, long term reduced carbohydrate diet with increased fibre intakes (LGI), decreased LDL size and increased plasma TAG. The same study also showed increased HDL-TAG with the intake of a LGI diet which was concomitant with the changes in TAG concentrations. These effects were, as the author explained, attributed to the increased acetate availability in the colon as a result of high fibre consumption. With regards to the number of LDL particles, a reduction in plasma apo B was associated with the consumption of a LGI diet in one study (Rizkalla et al., 2004). While LDL particle size significantly increased in the study of Dumesnil et al. (2001) with the intake of LGI foods, no changes were apparent regarding LDL particle number. Studies on the effect of the LGI diet on lipid biomarkers are summarized in Table 1.8.
### Table 1.8. Effect of LGI diet on markers of ALP. Values represent mean concentrations of blood lipids (SD) by the end of the LGI diet.

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Duration</th>
<th>Subjects</th>
<th>GI reduced (units)</th>
<th>TAG</th>
<th>TC.cholesterol</th>
<th>HDL-C</th>
<th>LDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ebbeling et al. (2005)</td>
<td>Parallel</td>
<td>12 months</td>
<td>23 healthy obese</td>
<td>10</td>
<td>↓*↑+</td>
<td>↓</td>
<td>↑*</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-37</td>
<td>-8.5</td>
<td>1.5</td>
<td>(0.4)</td>
</tr>
<tr>
<td>Giacco et al. (2000)</td>
<td>Parallel</td>
<td>24 weeks</td>
<td>63 T1 DM</td>
<td>20</td>
<td>↓</td>
<td>↓</td>
<td>↑*</td>
<td>NM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.90</td>
<td>4.7 (0.8)</td>
<td>1.5 (0.4)</td>
<td>(0.21)</td>
</tr>
<tr>
<td>Luscombe et al. (1999)</td>
<td>Crossover</td>
<td>2x4 weeks</td>
<td>21 obese T2 DM</td>
<td>20</td>
<td>↓</td>
<td>↓</td>
<td>↑*</td>
<td>3.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.47</td>
<td>5.38 (0.23)</td>
<td>0.93 (0.04)</td>
<td>(0.21)</td>
</tr>
<tr>
<td>Jenkins et al. (1987)</td>
<td>Crossover</td>
<td>2x2 weeks</td>
<td>6 healthy T2 DM</td>
<td>29</td>
<td>↓</td>
<td>↓</td>
<td>↑*</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.98</td>
<td>3.97 (0.40)</td>
<td>1.04 (0.14)</td>
<td>(0.98)</td>
</tr>
<tr>
<td>Heilbronn et al. (2002)</td>
<td>Parallel</td>
<td>8 weeks</td>
<td>45 OW T2 DM</td>
<td>32</td>
<td>↓*</td>
<td>↑*</td>
<td>↓*</td>
<td>2.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.84</td>
<td>5.01 (0.17)</td>
<td>1.26 (0.08)</td>
<td>(0.15)</td>
</tr>
<tr>
<td>Tsihlias et al. (2000)</td>
<td>Parallel</td>
<td>3x6 months</td>
<td>72 T2 DM Hyperlipidemic</td>
<td>11</td>
<td>↓*</td>
<td>↓*</td>
<td>↑*</td>
<td>2.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.70</td>
<td>(0.29)</td>
<td>0.97 (0.05)</td>
<td>(0.14)</td>
</tr>
<tr>
<td>Dumesnil et al. (2001)</td>
<td>Crossover</td>
<td>3x7 days</td>
<td>12 OW men</td>
<td>35</td>
<td>↓*</td>
<td>↓*</td>
<td>↑*</td>
<td>3.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.31</td>
<td>(0.38)</td>
<td>0.92 (0.11)</td>
<td>(0.97)</td>
</tr>
<tr>
<td>Frost et al. (1994)</td>
<td>Parallel</td>
<td>12 weeks</td>
<td>51 T2 DM</td>
<td>5</td>
<td>↓*</td>
<td>↑*</td>
<td>↓*</td>
<td>4.4±</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.9 (0.2)</td>
<td>6.2 (0.3)</td>
<td>1.2±0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Jarvi et al. (1999)</td>
<td>Crossover</td>
<td>2x42 days</td>
<td>20 T2 DM</td>
<td>19</td>
<td>↓*</td>
<td>↓*</td>
<td>↓*</td>
<td>2.87</td>
</tr>
<tr>
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<td></td>
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<td>0.88 (0.28)</td>
<td>(0.70)</td>
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<td>Rizkalla et al. (2004)</td>
<td>Crossover</td>
<td>2x4 weeks</td>
<td>12 T2 DM men</td>
<td>30</td>
<td>↓</td>
<td>↓*</td>
<td>↑*</td>
<td>2.63</td>
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<td>1.29 (0.29)</td>
<td>(0.11)</td>
</tr>
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<td>Frost et al. (1998)</td>
<td>Parallel</td>
<td>3 weeks</td>
<td>61 women</td>
<td>14</td>
<td>↑*</td>
<td>↓*</td>
<td>↑*</td>
<td>NM</td>
</tr>
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<td></td>
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<td>5.1 (0.29)</td>
<td>1.4 (0.11)</td>
<td>(0.26)</td>
</tr>
<tr>
<td>Philippou et al. (2007)</td>
<td>Parallel</td>
<td>12 weeks</td>
<td>13 MS</td>
<td>7</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>3.7</td>
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<td>5.5 (0.08)</td>
<td>1.4 (0.06)</td>
<td>(0.15)</td>
</tr>
<tr>
<td>Bouche et al. (2002)</td>
<td>Crossover</td>
<td>2x5 weeks</td>
<td>11 Healthy</td>
<td>35</td>
<td>↓*</td>
<td>↑*</td>
<td>↑*</td>
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<td>4.9 (0.38)</td>
<td>1.01 (0.08)</td>
<td>(0.32)</td>
</tr>
<tr>
<td>Bellisle et al. (2007)</td>
<td>Parallel</td>
<td>12 weeks</td>
<td>96 women</td>
<td>NM</td>
<td>↑</td>
<td>↑*</td>
<td>↑*</td>
<td>3.30</td>
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<td>1.01</td>
<td>5.25 (0.08)</td>
<td>1.75 (0.06)</td>
<td>(0.15)</td>
</tr>
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<td>Wolever et al. (1992)</td>
<td>Crossover</td>
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<td>6 obese T2 DM</td>
<td>28</td>
<td>↓*</td>
<td>↓*</td>
<td>NM</td>
<td>NM</td>
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<td>0.54</td>
<td>(0.19)</td>
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</table>

† Decrease, ↑ increase, ↔ no change, NM not mentioned, OW overweight* Significant change from baseline, + Significant difference than high GI period or group, NM not mentioned, § Standard error of mean was used, ¥ No change in sdLDL, † percentage changes from baseline is reported.
1.7.2. Effects of weight loss on CVD risk

1.7.2.1. Energy-restricted diets

A large proportion of obese individuals with T2 DM and dyslipidaemia experience positive health benefits with modest weight loss (Bosello et al., 1997; Wing et al., 1987a; Truby et al., 2006; Dansinger et al., 2005). Modest weight loss, defined as a weight loss of 5-10% of baseline weight, is also associated with lower blood pressure (Mertens & Van Gaal, 2000). This amount of weight loss has been recommended for the reduction of CVD risk, and to assist patients who are unable to attain and maintain substantial weight reduction (Mertens & Van Gaal, 2000). With respect to T2 DM patients, there is evidence to suggest that weight reduction in the early stages of the disease might slow the progression of diabetes by improving insulin release in response to glucose (Goldstein, 1992). A weight loss of about 15% in normotensive, insulin resistant, moderately obese (BMI=32.6±1.1kg/m²) subjects has been shown to substantially improve insulin sensitivity in relation to the amount of weight lost (Yoshida et al., 2004). Moreover, in overweight, insulin resistant individuals, hyperinsulinaemia and insulin resistance were improved with weight loss, with the effect being more profound in insulin resistant individuals compared with insulin sensitive ones (McLaughlin et al., 2001). Studies have shown that weight loss, induced by energy restriction, also improves glucose tolerance and insulin action in overweight men with either normal or impaired glucose tolerance (McLaughlin et al., 2001). This improvement has been related to a reduction in central body fat, as estimated by a reduction in waist circumference.

With respect to the dyslipidaemia associated with metabolic syndrome, weight loss via energy restriction has been consistently shown to lower plasma TAG (Brinkworth et al., 2006; Yancy et al., 2004). Recent studies of the effect of weight loss on dyslipidaemia (Fernandez et al., 2004) showed that weight loss not only resulted in an improved lipid profile but also reduced the concentrations of plasma apo B which is associated with atherosclerosis and increased risk for CHD. In this investigation, a significant decrease in T.Cholesterol, TAG, and LDL-C was clearly demonstrated. Participants experienced reductions in total and LDL-C of 12% and 8% respectively, and a 15% reduction in TAG, while plasma HDL-C concentrations were 9% higher after 6 months. Plasma apo B concentrations were reduced by 8.7% and 6.5% over 3
and 6 months respectively. The changes in plasma apolipoprotein concentrations were significantly associated with changes in body weight, body composition, and total and abdominal fat. Modest weight loss in obese subjects via an energy restricted diet and the loss of abdominal fat, has been shown to reduce CVD risk independently of T2 DM (Markovic et al., 1998).

Weight loss can be achieved by a broad spectrum of dieting strategies. Some of these strategies include restrictions of portion size and energy and meal substitution. Some plans also minimise carbohydrate without fat restriction, or modulate macronutrient composition or the glycaemic load of the diet. The effect of four popular diets (Atkins, Zone, Weight watchers and Ornish) on body weight and CVD risk was assessed in a one year randomised trial in the US (Dansinger et al., 2005). All diets resulted in a modest but statistically significant weight loss at one year, with no significant difference between diets. In addition, each diet significantly improved several CVD risk factors. These included a reduction of LDL-C (although not significant for the Atkins group), and increased HDL-C as well as a non-significant decrease in plasma TAG over one year. In a more recent trial that used the same approach for testing the efficacy of four commercial weight loss diets (Atkins, Weight Watchers, Slim-fast, and Rosemary Conley), but this time in the UK (Truby et al., 2006), all diets produced significant weight loss with, again, no significant difference in the amount of weight lost between the four diets. Certain CVD risk factors were also improved with the consumption of certain diets. Since all the diet plans used in these studies produced weight loss and improved some markers of CVD risk related to obesity, this provides evidence to suggest that specific dietary plans may be more effective if targeted to individuals.

1.7.2.2. Low-carbohydrate diets

The reduction of dietary carbohydrate represents one possible strategy for weight management. Low carbohydrate (LC) diets provide one dietary strategy for weight loss. Among the numerous LC diets that have been adopted in search for an effective diet for weight loss is the Dr. Atkins New Diet Revolution (Atkins, 2003). This diet recommends two weeks of extreme carbohydrate restriction followed by a gradual increase in carbohydrate intake to 35g/day. It has been suggested that the reduced
energy intake resulting from a LC intake is not fully compensated for by an increase of other macronutrients (Brehm et al., 2003). Therefore the primary mechanism of weight loss with the Atkins diet is related to decreased energy intake. The decreased energy consumption with the LC diet might be due to the limited food choices within this diet plan, or alternatively the greater satiating potential of protein (Brehm et al., 2003). Furthermore, the production of ketones with the Atkins diet has also been proposed as a possible mechanism for the observed weight loss, since ketones are known to decrease appetite (Atkins, 2003). For these reasons, LC diets have been shown to produce greater initial weight loss than conventional weight-losing diets (Samaha et al., 2003; Stern et al., 2004; Foster et al., 2003). In addition, glycaemic control in T2 DM patients has been shown to be better with LC than with a low fat diet (Samaha et al., 2003). Many studies have also reported a decrease in hyperinsulinaemia with the intake of LC diets (Brehm et al., 2003; Samaha et al., 2003).

Regarding the effect on plasma lipids, LC diets generally result in a small increase in T. Cholesterol (Westman et al., 2002; Larosa et al., 1980; Volek et al., 2000). For example, T. Cholesterol and LDL-C, were shown to increase by 6% and 18% respectively in normolipidaemic subjects after 8 weeks on a LC diet (<10g/day) (Larosa et al., 1980). The increase in LDL-C, which is an established risk factor for CHD (Turner et al., 1998) together with the high consumption of saturated fat, represents one of the main concerns of consuming the Atkins diet. However there are some studies that have shown no significant changes in LDL-C concentrations with the intake of LC diets (Samaha et al., 2003; Foster et al., 2003).

Despite the potential health risks of a LC diet as a result of its potential to increase saturated fat intake and LDL-C, beneficial effects on plasma TAG have been shown with this diet. LC diets have been shown to be highly effective in decreasing plasma TAG. Most of the reduction in plasma TAG is shown in individuals with baseline hypertriglyceridaemia, with the effect, arguably, being independent of weight loss (Volek et al., 2000; Sharman et al., 2002; Meckling et al., 2002; Krauss et al., 2006). A mechanism to explain how a LC diets exerts favorable effect on plasma TAG was proposed by Volek (2005). This explanation centres around the effects of insulin on the dyslipidaemia of the metabolic syndrome, which was recently reviewed by
Ginsberg and colleagues (2005) and included decreased hyperinsulinaemia and VLDL synthesis and production.

In contrast to the effects of LC diets on lipids, there are only a limited number of studies on the effects of LC diet on LDL and HDL size. Some studies have shown that a LC diet shifts LDL particle distribution to a larger size which results in a significant increases in peak and mean LDL diameter, and decreases the proportion of sdLDL particles (Sharman et al., 2002; Wood et al., 2006; Volek et al., 2003; Sharman et al., 2004), with the effect being more profound in subjects with a predominance of sdLDL at baseline. Some of the short and longer term studies on the effects of LC diets on body weight and lipid biomarkers are presented in Table 1.9.

1.7.3. Effect of exercise on CVD risk

Long term success in weight management depends on a combination of energy expenditure and dietary modification (Avenell et al., 2004b). The NCEP ATP-III guidelines recommend lifestyle therapies to replace drug therapies for patients with an intermediate range of CHD risk.

In a longitudinal study of 9,777 men, who were followed for 19 years, men who maintained or improved adequate physical fitness were less likely to die from CVD during follow-up than men with relatively lower concentrations of activity (Avenell et al., 2004b). Reduced physical activity is often associated with obesity and has independent effect on CVD risk. Weight loss combined with an exercise program can delay, and possibly prevent, the development of diabetes in insulin resistant individuals (Laaksonen et al., 2005). The Diabetes Prevention Program (2002) examined a large cohort of individuals with insulin resistance. A subset of participants began a lifestyle modification program with a goal of ≥7% weight loss and 150 minutes of physical activity/week. After an average follow-up of 2.8 years, the lifestyle intervention group achieved nearly a 60% reduction in the incidence of diabetes compared with the control group. Exercise has also been shown to improve insulin sensitivity and glycaemic control through weight loss (Diabetes Prevention Program Research Group, 2002).
### Table 1.9: Effect of low-carbohydrate diets on weight loss, lipid, and lipoprotein concentrations.

<table>
<thead>
<tr>
<th>Study</th>
<th>Duration</th>
<th>Intervention</th>
<th>Weight loss (kg)</th>
<th>Lipid/HDL size</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>TAG</th>
<th>TAC</th>
<th>size</th>
<th>HDL size</th>
</tr>
</thead>
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<td>0.5</td>
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<td>+</td>
<td></td>
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<td>0.3</td>
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</tbody>
</table>
Exercise is well known to exert a substantial positive effect on CVD risk, especially with respect to plasma lipids and increasing HDL-C concentrations (Halverstadt et al., 2007). Regular exercise is shown to be particularly helpful in men with low HDL-C, elevated plasma TAG, and abdominal obesity (Halverstadt et al., 2007). The effect of aerobic exercise, after 10 weeks of intervention, on lipid concentrations was examined in 45 hypercholesterolemic, elderly women (Fahlman et al., 2002). In this study, subjects assigned to the aerobic exercise intervention experienced a 20% increase in HDL-C concentrations, and a 14% decrease in plasma TAG concentrations. These changes in HDL-C and TAG concentrations were independent of any change in body weight or dietary intake. Likewise, in hypercholesterolemic men, training was associated with modest reductions in T.Cholesterol, HDL_{3}-C, and apo A-I and apo B concentrations, along with a rise in HDL_{2}-C (Crouse et al., 1997). Although exercise training failed to influence the production of plasma oxidized LDL, it significantly decreased plasma T.Cholesterol and LDL-C concentration (Wang & Chow, 2004). In addition, 24 weeks of endurance exercise training resulted in an improvement in most of the lipid and lipoprotein measures (Halverstadt et al., 2007). This included a significant reduction in T.Cholesterol (2.1 ± 1.8 mg/dL, p<0.001), TAG (17±3.5 mg/dL, p<0.0001), and LDL-C (0.7±1.7 mg/dL, p<0.0001), with a significant increase in HDL-C subfractions (1.9±0.5 mg/dL, p=0.01 for HDL-C_{3} and 1.2±0.3 mg/dL, p=0.02 for HDL_{2}-C). The concentrations of sdLDL particles (103±27 nmol/L, p=0.02), and small HDL particles (−0.03±0.4 μmol/L, p=0.007) were also shown to be decreased, along with a significant increase in mean HDL particle size (0.1 ± 0.0 nm, p=0.04).

A possible mechanism for exercise-induced reduction in plasma TAG is through increased LPL mediated TAG clearance and reduced hepatic VLDL secretion (Gill & Hardman, 2003). Since exercise attenuates carbohydrate-induced postprandial hypertriglyceridemia, levels of physical activity should be taken into account when considering nutritional strategies for reducing the risk of CVD (Gill & Hardman, 2003). Accordingly, the Department of Health (2005) in the UK recommends that young people should achieve a total of at least 60 minutes of at least moderate intensity activity each day while adults should aim to accumulate 30 minutes of at least moderate intensity physical activity on five, and preferably, all days of the week.
1.7.4. Effect of exercise combined with LC diet on CVD risk

Data on the combined effects of exercise and a LC diet is very limited. Most studies on the effects of LC diets on markers of CVD did not consider exercise (Foster et al., 2003; Samaha et al., 2003; Yancy et al., 2004; Truby et al., 2006). In some cases the level of exercise was kept constant during the intervention by maintaining subjects’ baseline level (Volek et al., 2004a; Wood et al., 2007; Krauss et al., 2006), whilst other studies encouraged increased exercise, but no results were reported regarding this outcome (Brehm et al., 2003; Westman et al., 2002). In all cases, the detailed effect of exercise (or physical activity) with the consumption of low carbohydrate diets was not described.

Recently, an interesting study (Layman et al., 2005) examined the effect of LC high protein diets combined with exercise on body weight and blood lipids in 48 women with a BMI of 33 kg/m² during weight loss. In this 4-way study, two isoenergetic (1700 kcal-7140 KJ/d) dietary treatments (high protein, reduced carbohydrates and low protein, high carbohydrates) containing an equal percentage total energy/d as fat (30%) were examined with and without exercise. After 16 weeks, subjects in the LC and exercise groups lost more total weight (9.3 ± 0.8 kg vs. 7.3 ± 0.5 kg for the other groups, p<0.05). The LC plus exercise group had the largest relative weight loss (11.2%) from baseline weight and the high carbohydrate group had the smallest relative weight loss (8.4%). T. Cholesterol, LDL-C, HDL-C, and TAG improved in all groups but changes varied between treatments. For example, in the high carbohydrate and the high carbohydrate plus exercise groups, T. Cholesterol decreased by 9.2% while LDL-C decreased by 10.4%. These concentrations decreased by 3.7% and 1.7% respectively in the LC and the LC plus exercise groups. Changes in TAG were largest in the groups consuming the LC diet. Plasma TAG, in the LC and the LC plus exercise groups decreased by 20.2%. In the high carbohydrate and high carbohydrate plus exercise groups TAG concentration decreased by 5.2%. The study concluded that a diet with reduced carbohydrates combined with exercise additively improved body composition during weight loss; the effects on blood lipids however, differed between dietary treatments.
1.8. Statement of the problem

Obesity and an ALP are major risk factors for CVD, especially in individuals with T2 DM that can be modified through lifestyle changes. The purpose of the current research was therefore to investigate the effect of two lifestyle approaches for the reduction of obesity and the expression of an ALP in overweight/obese individuals with or without T2 DM. These approaches were:

- To examine the effects of a LGI diet alone versus a LGI combined with an energy restricted diet in T2 DM.
- To examine the effects of a LC Atkins diet as a weight loss diet alone versus an Atkins diet combined with a moderate exercise programme in otherwise healthy overweight or obese individuals.

1.9. Overall aim

The aim of these research studies was to examine different lifestyle approaches for the reduction of overweight/obesity and an ALP that confers high CVD risk in T2 DM and/or overweight and obesity. The effects of LGI diet were examined using patients with T2 DM in Kuwait. Therefore, it was essential during the first stages of the project to determine the GI of some staples in the Kuwaiti diet to be used during the intervention as data on this aspect of food was lacking in Kuwait. Once the GI of some staples foods in Kuwait had been determined, those foods with a LGI were selected to test the effects of LGI diets in patients with T2 DM in Kuwait.

The general objectives of the studies in this thesis were therefore as follows:

1. To determine the GI of the most abundant carbohydrate-rich foods staple to the Kuwaiti diet.
2. To examine the effects of a LGI diet as compared to a LGI diet combined with an energy restricted diet on modifying obesity and an ALP in T2 DM patients in Kuwait.
3. To examine the effect of a LC, Atkins diet alone compared to a LC combined with moderate exercise in reducing obesity and an ALP in overweight/obese individuals in a UK population.

The specific aims and objectives for each study are described in detail at the beginning of each chapter.
CHAPTER 2
Chapter 2

MATERIALS AND GENERAL METHODS

The following chapter outlines the materials and general methods used for the analyses of blood analytes in all studies presented in subsequent chapters. Individual protocols for each study are fully described within each chapter.

2.1. Materials

2.1.1. Equipment

**Accu-Check, UK**
- Soft Clix lancet device

**Alferwassermann, AL Woerden, Netherlands**
- SPACE autoanalyser

**Beckman Coulter, Buckinghamshire, UK**
- Optima XL100K Ultracentrifuge
- NVT 65 rotor
- NVT 65.2 rotor
- Ultra-Clear centrifuge tube (12ml)
- 70.1 Ti rotor
- 4.9 Optiseal centrifuge tubes

**Becton, Dickson and company, Oxford, UK**
- Plastic syringes 1 ml, 5 ml, 10ml
- Vacutainer tubes

**Berthold Technologies, Hertfordshire, UK**
- Luminescent plate reader Centro LB 960

**C.B.S. Scientific Company, Del Mar, California, USA**
- PGGE Pore Gradient Lipoprotein Electrophoresis System.
- 4-30% acrylamide gel
2.1.2. Chemicals and reagents

All chemicals and reagents were of analytical grade.

**Axis shield diagnostic, UK**
Optiprep

**Bio-Rad Laboratories, Germany**
Insulin quality control serum levels 1, 2 and 3

**Fisher Scientific, UK**
Glacial acetic acid, Methanol, Potassium bromide, Sodium chloride

**Molecular Light Technology Research Ltd, Cardiff, UK**
Immunochromiluminometric insulin assay

**Oxoid Ltd, UK**
Phosphate buffered saline (PBS)

**Randox Laboratories Ltd., County Antrim, UK**
Apolipoprotein A1, Apolipoprotein B, Apolipoprotein calibrator, Chemistry control level 1 and 2, Direct HDL, Direct LDL, Direct LDL/HDL calibrator, Gemcal calibrator, Glucose GOD/PAP test kit, Lipid control level 1, 2, and 3, Multisera level 2 and Multisera level 3, NEFA, System diluents, TG, Total cholesterol.
Sigma Chemical Company Ltd, UK
Coomassie blue R 250
Bromophenol blue
Sulphosalicylic acid

2.1.3. Electrophoresis buffer

38.14 g Tris (trishydroxymethylaminomethane)
17.31 g Boric acid
3.26 g EDTA
pH = 8.4

This was dissolved in 3 liters of water and stored at 4°C. HCl and Sodium hydroxide were used to bring the solution towards the desired pH.

2.2. Methods

2.2.1. Anthropometric and blood pressure

Height was measured using a wall-mounted stadiometer ("Seca" Somatometre 200 cm x 0.1 cm). Weight was measured using a calibrated dial scales (Seca 770 high capacity floor scale, 148 kg x 100 g). BMI was calculated using the standard formula (weight [kg]/height^2 [m]). Waist circumference was measured to the nearest 0.1 cm using a Holtain tape measure (Holtain Ltd., Dyfed, Wales). Blood pressure was measured three times using an electronic sphygmometer (Omron M4, Omron Healthcare, Sussex, UK) with the subject sitting at rest. An average of the three readings was then taken.

2.2.2. Collection of blood

Blood was taken by venepuncture of an antecubital vein both pre and postprandially from male and female volunteers. The blood was collected either into vacutainers or centrifuge tubes containing the following anticoagulants: K₂EDTA for the determination of total cholesterol (T.Cholesterol), TAG, LDL-C, HDL-C, apo A-I, apo B. NEFA, LDL and HDL subclasses, fluoride oxalate for the determination of plasma glucose, and lithium heparin for plasma insulin. The blood was immediately centrifuged at 2000g for 20 min at 4° C in a low speed centrifuge for the separation of plasma. Aliquots of plasma
were dispensed into appropriately labelled cryovials (1.8 ml) and stored at -80°C until analysis.

2.2.3. Biochemical analyses

2.2.3.1. Analyses using the SPACE autoanalyser

Fasting blood samples were analysed on the SPACE auto-analyser for glucose. T. Cholesterol, LDL-C, HDL-C, TAG, and NEFA using colourimetric enzymatic procedures. Apo A-I, and apo B were analysed by immunoturbidimetric assays. Specific quality controls (QCs) were measured at the beginning and end of each run. Assays were performed only if the QCs were within the accepted limit as determined by the QC manufacturer. Interassay CVs were calculated using 6-12 replicates while intraassay CVs were calculated using 6-18 replicates.

2.2.3.2. Assay principle: plasma T. Cholesterol

Cholesterol esters were hydrolysed to free cholesterol and free fatty acids by pancreatic cholesterol esterase. The liberated cholesterol and any free cholesterol present in the plasma were both oxidized by cholesterol oxidase, reaction:

\[
\text{cholesterol esters} + H_2O \xrightarrow{\text{cholesterol esterase}} \text{cholesterol} + \text{fatty acids}
\]

\[
\text{cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholestone} - 3\text{one} + H_2O_2
\]

\[
2H_2O_2 + 4\text{-aminantipyrine} + \text{phenol} \xrightarrow{\text{peroxidase}} \text{quinoneimine} + 4H_2O
\]

The absorbance of the quinoneimine pigment was measured bichromatically at 505/692 nm. The within assay precision gave CVs for level 1 (low) and level 2 (high) QCs of 2.9% and 1.6% respectively, while the between assay precision gave CVs of 3.2% and 3.0% for the low and high quality control respectively.
2.2.3.3. Assay principle: plasma TAG

The principle of the assay is similar to that for cholesterol; however, glycerol-3-phosphate is the substrate to be oxidized to form peroxide.

\[
\text{Triacylglycerol} + 3H_2O \xrightarrow{\text{lipase}} \text{glycerol} + \text{fatty acids}
\]

\[
\text{glycerol} + ATP \xrightarrow{\text{GK}} \text{glycerol-3-phosphate} + ADP
\]

\[
\text{glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{dihydroxystarone-phosphate} + H_2O_2
\]

\[
H_2O_2 + 4 \text{aminantipyrine} + 4 \text{chlorophenol} \xrightarrow{\text{POD}} \text{quinoneimine} + 4H_2O + HCl
\]

Where POD = peroxidase, GPO = glycerol-3-phosphate oxidase, GK = glycerol kinase, ATP = Adenosine-5'-triphosphate, ADP = Adenosine-5'-diphosphate. The absorbance was measured bichromatically at 505/692 nm and was proportional to the amount of the TAG present. Intra-assay precision gave CVs for level 1 (low) and level 2 (high) QCs of 2.3% and 4.0% respectively while the inter-assay precision gave CVs of 3.0% and 3.5% for the low and high control respectively.

2.2.3.4. Assay principle: HDL-C

The assay for HDL-C consists of two steps; the first step eliminates cholesterol present in chylomicrons, VLDL and LDL-C with the following enzyme sequence:

Step 1

\[
\text{cholesterol ester} \xrightarrow{\text{cholesterol esterase}} \text{cholesterol} + \text{fatty acids}
\]

\[
\text{cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholestone} + H_2O_2
\]

\[
2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2
\]
Step 2
Step 2 involves the release of HDL-C by detergents and measured specifically.

\[ \text{cholesterol ester} \xrightarrow{\text{cholesterol esterase}} \text{cholesterol} + \text{fatty acids} \]

\[ \text{cholesterol} + \text{O}_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholestone} + \text{H}_2\text{O}_2 \]

\[ 2\text{H}_2\text{O}_2 + 4 - \text{AA} + \text{HDAOS} \xrightarrow{\text{Peroxidase}} \text{Quinone pigment} + 4\text{H}_2\text{O} \]

Where 4-AA = 4-Aminoantipyrine
HDAOS = N-(2-hydroxy-3-sulphopropyl)-3,5-dimethoxyaniline.

When measured at 600 nm, the intensity of the quinone imine dye product was directly proportional to the cholesterol concentration. Within assay precision gave CVs for level 1 (low), level 2 (medium), and level 3 (high) QCs of 4.2%, 4.9%, and 3.9% respectively. The between assay precision gave CVs of 6.8%, 5%, and 5.3% respectively for the low, medium, and high QCs respectively.

2.2.3.5. Assay principle: LDL-C
The principle for the measurement of LDL-C was identical to that for HDL-C; with the exception of the detergent in the LDL reaction being TOOS (N-Ethyl-N-(2-hydroxy-3-sulphopropyl)-3-methylaniline) and HDAOS for HDL. Intra and inter-assay precision gave CVs for level 1 (low), level 2 (medium) and level 3 (high) quality controls of 4.7%, 3.8%, and 0.9% respectively. The inter-assay precision gave CVs of 5.7%, 4.7%, 1.8% for the low, medium and high QCs respectively.

2.2.3.6. Assay principle: plasma glucose
Glucose was determined by enzymatic oxidation in the presence of glucose oxidase (GOD). The resultant hydrogen peroxide reacts with phenol and 4-aminophenzone under the catalysis of peroxidase (POD) to produce a red – violet quinoneimine dye. The absorbance of the dye is measured bichromatically at 505/692 nm.
\[
glucose + O_2 + H_2O \xrightarrow{GOD} \text{gluconic acid} + H_2O_2
\]

\[
2H_2O_2 + 4 - \text{aminophenzone} + \text{phenol} \xrightarrow{POD} \text{quinineimine} + 4H_2O
\]

Intra-assay precision gave CVs of 1.6% for level 1 (low) QC, 1.3% for level 2 (high) QC. Inter-assay precision gave CVs of 0.4% for both level 1 and level 2 QCs.

**2.2.3.7. Assay principle: plasma apo A-I**

Apo A-I was measured turbidimetrically at 340 nm. The assay is based on the reaction of a sample containing human apo A-I and an antiserum to form an insoluble complex. The concentration of apo A-I in the sample can be determined by comparing the concentration with a standard curve from the absorbance of standards. Precision tests for QC and intra-assay CVs were 3.8%, 1.1%, and 7.3% for level 1 (low), level 2 (medium), and level 3 (high) QCs respectively. Inter-assay precision for level 1, level 2, and level 3 QCs were 0.7%, 1.5%, 5.3% respectively.

**2.2.3.8. Assay principle: plasma apo B**

Human total apo B was measured turbidimetrically. Apo B forms a precipitate with a specific anti apo B serum, which is measured turbidimetrically at 340 nm. The antigen-antibody complex produces a turbid precipitate, the level of which is directly proportional to the concentration of apo B.

A linear standard curve was constructed from an apo B standard (Randox) using saline (0.9% w/v), ranging from 0.0 – 2.18 g/L. Three quality controls were employed; level 1 (low), level 2 (medium) and level 3 (high). Precision tests were carried out on each QC and intra-assay CVs were 8.3%, 4.6%, and 6.8% for the low, medium and high QCs respectively. While inter-assay precision for the low, medium, and high quality controls were 8.7%, 6.0%, and 8.1% respectively.
2.2.3.9. Assay principle: plasma NEFA

NEFA was determined by the enzymatic reaction of acyl CoA synthetase and acyl CoA oxidase to produce peroxide; the peroxide reacts with 4-aminoantipyrine which results in the formation of a purple dye that absorbs at a wavelength of 550 nm. The intensity of the colour is directly proportional to the NEFA concentration.

\[ \text{NEFA} + \text{ATP} + \text{CoA} \xrightarrow{\text{Acyl CoA Synthetase}} \text{Acyl CoA} + \text{AMP} + \text{PPi} \]

\[ \text{Acyl CoA} + \text{O}_2 \xrightarrow{\text{Acyl CoA Oxidase}} 2,3-\text{trans-Enoyl-CoA} + \text{H}_2\text{O}_2 \]

\[ 2\text{H}_2\text{O}_2 + \text{TOOS} + 4-\text{AAP} \xrightarrow{\text{POD}} \text{purple adduct} + 4\text{H}_2\text{O} \]

Where 4-AAP = 4-aminoantipyrine, TOOS = N-ethyl-N-(2-hydroxy-3-sulphopropyl) m-toluidine. Multisera level 1 (low) and level 2 (high) quality controls were employed with intra-assay CVs of 5.4% and 5.6% respectively and inter-assay CVs of 4.9% and 5.4% respectively.

2.2.3.10. Assay principle: plasma fructosamine

Blood for fructosamine analyses was collected in EDTA tubes and fructosamine concentrations were measured quantitatively using a Randox enzymatic assay for human serum or plasma. The kit constitutes two reagents. Reagent 1 contains Proteinase K that digests the glycated protein to smaller fragments according to the following equation:

\[ \text{Glycated protein} \xrightarrow{\text{proteinase K}} \text{Glycated protein fragments} \]

Reagent 2 consists of Ketoamine oxidase (KAO) which oxidises the ketoamine bond of the fragments yielding the followings:

\[ \text{Glycated protein fragments} \xrightarrow{\text{KAO}} \text{Amino acids} + \text{H}_2\text{O}_2 \]
The reaction is colorimetric at 550nm and the amount of colour developed is directly proportional to the concentration of the glycated protein in the sample.

\[ H_2O_2 + \text{Chromogens} \xrightarrow{\text{Peroxidase}} \text{Color} + H_2O \]

The intra-assay %CV for level 1 (low) and level 2 (high) QC was 1.8 and 1.0 µmol/l respectively. The reference range for fructosamine analyses in healthy subjects was between 122-236 µmol/l.

2.2.4. Lipoprotein subclasses measurement

2.2.4.1. Gradient gel electrophoresis for the identification of HDL subclasses.

HDL subclasses were separated using commercially available, pre-cast, non-denaturing polyacrylamide gradient gels, using a PGGE Pore Gradient Lipoprotein Electrophoresis System. The 4/30 acrylamide gels were used for resolving HDL subclasses over an approximate molecular weight range of 50,000-2,000,000.

Whole plasma was adjusted to density 1.21 Kg/l by the addition of KBr. The adjusted plasma (1.0 ml) was transferred to a Beckman Ultra-Clear centrifuge tube (12 ml) and mixed with a KBr 1.21 Kg/l, 1% EDTA density solution. Tubes were housed in a Beckman 70.1 Ti rotor and centrifuged for 20 h at 147 000g, at 15°C. After centrifugation, 200 µl of the upper yellow supernatant band, containing total plasma lipoproteins, was aspirated and used for subsequent analysis. All pre and post-diet samples for each volunteer were run together, at the same time and in the same rotor.

In the next step, a 100 µl aliquot of the band was pre-stained with bromophenol blue (final concentration 5% w/v bromophenol blue). After the gels had been pre-equilibrated at 70 V, 65 mA for 30 min, 10 µl of each sample was introduced into a separate gel well. Following the addition of the samples, gels were run at 20 V, 50 mA for 20 min, followed by 70 V, 65 mA for 30 min, and finally at 120 V, 100 mA for 24 h. Gels were then removed from their casing and placed in fixing solution (sulphosalicylic acid, 10% w/v) for 30 min, followed by protein staining for 1 h (0.1% w/v coomassie blue R250.
methanol: glacial acetic acid: RO (reverse osmosis) water in a 5:1:4 ratio). Gels were destained with methanol: glacial acetic acid: RO water 50: 75: 874 (ml) for 2-3 days with regular changing of the de-stain. A Nikon D1X digital camera was used to photograph the gels. Photographic images were down-loaded directly by fire-wire to a PC and then analysed using Total-Lab 1D gel-scan software version 2.01. The software generated HDL subclass profiles, from which it was possible to measure electrophoretic migration distances of the HDL bands (Relative front, Rf). Two primary peaks were identified by the software that corresponded to HDL2 and smaller, less dense HDL3 subclasses (Figure 2.1). The intra-inter assay %CV for the percentages of HDL2 were 2.9% and 0.3% respectively.

![Figure 2.1. Example of a computer generated densitometric scan of electrophoresed plasma from one participant in the LGI diet study (Chapter 4) showing a peak magnitude and Rf for HDL subclasses analyses. The peak shown to the left correspond to LDL. The area under curve labeled 1 represents %HDL2 while the area under curve 2 represents %HDL3.](image-url)
2.2.4.2. LDL subclasses measurement

The separation of plasma LDL subclasses was carried out by iodixanol density gradient ultracentrifugation (DGUC) as previously described (Layman et al., 2005). The iodixanol DGUC is used specifically for the detection of the proportion of small, dense LDL-III and determination of LDL subclass phenotype. In the first step, 1.52 ml plasma was pre-stained with 200 μl Coomassie Blue R 250 (50 g/l in PBS) and mixed with 0.4ml Optiprep™ which is commercially available as 60% (w/v) iodixanol. This provided a working sample of 2 ml with a final concentration of 12%. For the upper layer, ‘Optiprep’ was mixed with PBS to provide 90g/l iodixanol solution. An aliquot of 3.4 ml of this solution was dispensed into a 4.9 Beckman ‘Optiseal’ centrifuge tube, followed by the careful under-layering of 1.5 ml of the denser plasma sample with a syringe and canunula. These tubes where then housed in a 16 pocket Beckman NVT65.2 near vertical rotor and centrifuged at 341 000g and 16 °C for 2.5 h in a Beckman Optima XL-100 Ultracentrifuge, with acceleration and deceleration program 5. All pre and post-diet samples for each volunteer were run together at the same time in the same rotor.

After centrifugation, the ‘Optiseal’ tubes were placed in a rack directly opposite to a vertical light box and photographed using a high resolution Nikon D1X digital camera, at a fixed distance from the Optiseal tubes. Photographic images of the Optiseal tubes containing the stained LDL bands were down-loaded via a fire-wire to a PC and analysed by using Total-lab 1D gel-scan software. The resultant LDL subclass profiles were assigned Rf values to all the peaks, including the major LDL peak (Figure 2.2). Rf values where then converted to density by cross referencing the value to a previously determined densities of a blank tube as previously described (Davies et al, 2003). LDL subclasses were described semi-quantitatively by this method in terms of LDL peak density and the percentage area under the LDL curve corresponding to small, dense LDL-III (%AUCB). The within and between rotor CVs for LDL peak density were 0.14% and 0.02% respectively (n=13). While the within and between rotor CV for the %AUCB was 2.26% and 0.30% respectively (n=13).
2.2.5. Insulin measurement

Blood was collected into a lithium heparin coated tubes. Insulin concentrations were measured quantitatively using the MLT immunochemiluminometric assay. The assay is a two-site immunoassay, utilizing an insulin-specific solid phase antibody immobilized on microtitre wells, and a soluble antibody labeled with a chemiluminescent acridinium ester. Both of these antibodies were mouse monoclonals. After the sample was incubated with the labeled antibody solution, unbound labelled antibodies were removed by washing. The microtitre plate luminometer measured the insulin by quantifying the bound luminescence.

Wash buffer was prepared by diluting 1 part buffer concentrate in 9 parts RO water. All kit components and samples were brought to room temperature before starting the assay. Standards (0-200 mU/L) were reconstituted by the addition of 1.0 ml RO water. 900 µl of the labeled antibody concentrate was dispensed into the labeled antibody diluent and mixed.
Aliquots of 100 µl of labeled antibody solution were dispensed into appropriate 96 microtitre wells. All samples (25 µl plasma) were run in duplicate. The plates were sealed and incubated for 2 h at 37°C. After incubation, the plates were washed three times with wash buffer, using an automatic plate washer. Finally, plates were read in a luminometer which calculated the relative luminescence (RLU) values and converted them into mU/L using MicroWin 2000 software. The intra-assay precision (CVs) of three controls were: 4.9% for QC level 1, 8.07% for level 2, and 7.6 % for QC level 3. The inter-assay precision for level 1, 2, and 3 were 6.6%, 6.0%, and 11.7% respectively. The conversion factor from mU/L to pmol/L: 1mU = 7.5 pmol

2.2.6. Calculation of the homeostasis assessment model (HOMA)

The homeostasis assessment model of insulin resistance (HOMA-IR) was calculated as described by Matthews et al (1985) using the following formula:

\[ \text{[Plasma insulin] (pmol/L)} \times \text{[Plasma glucose] (mmol/L)} / 22.5 \]

2.2.7. Statistical analyses

The details of statistical analyses are specific to each study and are therefore described in each chapter. In general, data was coded and entered into SPSS statistical analyses software (SPSS, version 14 for windows, Chicago, Illinois). All outcomes were assessed for normality using the Kolmogorov-Smirnov test and by checking normal and detrended normal probability plots. Homogeneity of the variance was assessed by Levene’s statistic. Skewed parameters were transformed and retested for normality. Geometric means were reported for log transformed parameters. The characteristics of the study subjects were expressed using descriptive statistics. Data was analysed for both between and within group effects. The effect of the intervention diet on all measured variables was assessed by one way repeated measure ANOVA. A within / between group 2-way ANOVA, with time as within subject factor and group as between subject factor was used to examine the effect of the interaction between time and diet. Pearson correlations coefficients \((r)\) were determined to examine the inter-relationships between variables. A \(p<0.05\) was considered statistically significant.
CHAPTER 3
DETERMINATION OF THE GLYCAEMIC AND INSULINAEMIC INDICES OF EIGHT STAPLE FOODS IN THE KUWAITI DIET

3.1. Introduction

T2 DM is a major clinical problem in Kuwait with a prevalence rate of 15% (Abdella et al., 1998) and a link to dyslipidaemia as a common CVD risk factor (Akanji, 2002). Appropriate dietary advice is recommended for the management of T2 DM and its related risk factors. In this respect, identification of food composition is essential for T2 DM patient management. However, food composition alone would not predict the potential effect of the diet, as carbohydrate-rich foods with similar composition are known to cause different glycaemic responses (Rasmussen et al., 1992).

The GI is an important parameter of food quality which classifies carbohydrate-rich foods on the basis of their physiological effect on blood glucose. It represents the plasma glucose response to a food, expressed as a percentage of the glucose response to a reference food, generally glucose (Jenkins et al., 1981). Since the presence of other constituents besides carbohydrates in the food, such as fat and protein, can influence blood concentrations of insulin independently of the glucose response (Lovejoy et al., 1998), the insulinaemic index (II) of foods is another important measure that provides additional information on the GI in the dietary management of diabetes (Bornet et al., 1997; Miller et al., 1992). An understanding of the physiological basis of carbohydrates as classified by these two food parameters, GI and II, may assist in optimizing healthy food choices for Kuwaiti T2 DM patients, as there is accumulating evidence on the therapeutic potential of LGI diets in these patients (Heilbronn et al., 2002) and in subjects with dyslipidaemia (Jenkins et al., 1985). The GI may provide a rationale for the selection of carbohydrate-rich foods for the dietary management of T2 DM patients. The largest published GI database (Foster-Powell et al., 2002) contains values for over 750 different types of food, including branded products and foods from around the world, but not from Kuwait. Therefore, integrating the GI concept into the Kuwaiti diet is limited by the lack of data. This study was the first to be undertaken for the determination of the GI of eight staple foods commonly eaten in Kuwait. Since both GI and II should be
considered when attempting to optimize the quality of dietary carbohydrates for individuals with diabetes (Miller et al., 1992), both GI and II were measured in the present study.

3.1.1. Glycaemic index overview

GI provides a mean for assessing the metabolic responses to carbohydrate-rich foods by ranking them according to how much they raise postprandial plasma glucose concentration. It is defined as the incremental area under the blood glucose curve of a test food compared to a reference food: glucose or white bread (Foster-Powell et al., 2002). Based on the GI, CHO may be divided into 3 groups. LGI carbohydrates (GI<55%) are those that are digested and absorbed slowly and lead to a low glycaemic response, whereas HIGH GI foods (GI=70% or more) are rapidly digested and absorbed and exhibit a high glycaemic response. A medium-GI food will have a GI of 56-69%.

Interest in the measurement of GI for research and commercial applications increased significantly when the FAO/WHO (1998) recommended that the bulk of the CHO should be from LGI food. More recently, the American Diabetes Association (2004b) highlighted the importance of the GI as an additional benefit over that observed when total carbohydrates are considered alone. While in several countries (Australia, Canada, Sweden) the GI concept has been integrated into the dietary guidelines and some food companies have marketed specifically-labeled LGI products, the GI of foods in Arabic countries has not received a great deal of attention, and only a few studies have investigated the GI of some staple Arabic foods (Miller et al., 2002; Miller et al., 2003; Mehio et al., 1997). In the International Tables of GI (Foster-Powell et al., 2002) of the GI of 750 food items, only 7 are of Arabic origin.

3.1.2. Identification of consumption patterns of bread and rice in Kuwait

It is recognized that the Middle East region has a special need for food consumption data (Harrison, 1998) and Kuwait is not exempt from this. A limited number of studies, however, have provided some insights into food consumption patterns in Kuwait (Kuwait Institute for Scientific Research, 1980; Kamel & Martinez, 1984b; Dehghan et al., 2005;
Dashti & Al-Awadi, 2001; Sawaya et al., 1998; Al-Hooti et al., 2002). Although food plays a very important social and ceremonial role in Kuwait, the country does not have a large food manufacturing industry. Instead, it relies heavily upon imported foods from all over the world, which makes food abundant and diverse. Grains and cereal grains including rice are some of the major imported foods in Kuwait.

Bread is an important staple in the diet of Kuwaitis and is eaten once or more per day by almost 81% of this population (Kamel & Martinez, 1984). Breads, usually pita and Iranian bread, are eaten mainly at breakfast and dinner (Kamel & Martinez, 1984). 45.6% of the Kuwaitis consume white pita bread at least once per day, and about 15.5% consume Iranian bread once/day, while only 9.4% consume a serving of brown pita bread/day (AlAjmi, 2004). Most of the breads consumed in Kuwait are manufactured locally by the Kuwait Flour Mills and Bakeries Company. These manufacturers supply food distributors that serve consumers in Kuwait with high quality products such as breads, biscuits, pasta, and flour. The flour that is produced by the company from wheat is used to manufacture breads that are consumed by most Kuwaitis. These breads include white and brown pita breads, both normal and light brands (Lebanese khubus), and white and brown bread (toast). On the other hand, Iranian breads are made within small bakeries which are distributed all around Kuwait.

Rice, especially white rice, represents the other main source of carbohydrate in the diet of Kuwaitis who consume it as a main course at lunch (Kamel & Martinez, 1984). Peshawar white rice is a polished long-grained variety of rice, famous for its delicate flavor. The rice, usually cultivated in Pakistan, is imported to Kuwait and distributed by the Kuwait Supply Company using ration cards issued to Kuwaiti families based on their family size. Rice in Kuwait is cooked by different methods. The preferred method of preparing rice is to boil it in an excess amount of water, then drain it and leave it to simmer for a period of time (mashkhol). According to Kamel & Martinez (1984), 74% of Kuwaitis consume rice daily and a substantial proportion consume it twice a day. Recently, the percentage of people that consumed rice on a daily basis has been declining. Dehghan et al. (2005) recently observed that in a sample of 201 Kuwaitis, only 48% reported consuming rice once per day. This study was undertaken in an
attempt to develop a food-frequency questionnaire specifically for use in nutritional surveys in Kuwait. In the same study all the participants reported consuming cereals at least once per day. More precisely, the mean number of servings of cereals and cereal products consumed by participants was 5.3/day.

3.1.3. Factors affecting the glycaemic responses to bread

The ingredients of the bread as well as baking processes influence the glycaemic response to breads (Englyst et al., 1982). Consequently, the GI of the same breads varies widely according to its country of manufacture. In the International Tables of GI (Foster-Powell et al., 2002), of 750 different food items, the GI value for 95 types of bread are included. These GI range from LGI (barley bread 75% whole grains; 27%) to HIGH GI (porous French baguette; 95%).

The baking process of bread usually involves high temperature and pressure. These conditions increase the availability of starch to amylase and hence increase the digestibility of starch in the mouth and stomach as a result of starch gelatinization and changes in the chemical nature of the granule structure (Fardet et al., 2006). Baking breads using different temperatures can therefore affect the glycaemic responses. For example, baking breads at a low temperature for a long period of time (similar to the conditions used for baking pumpernickel bread: 2 hr at 120° C) decreased the GI of a high-amylose barley bread by 30%, as a result of the formation of crystalline starch (Akeberg et al., 1998).

Resistant starch, defined as the fraction of starch that is not digested in the small intestine and is fermented in the large intestine (Englyst et al., 1992), is likely to be negatively correlated with the GI (Annison & Topping, 1994) as it is slowly absorbed in the small intestine resulting in decreased postprandial glucose and insulin responses. Moreover, the addition of viscous soluble fibres to bread affects the GI through thickening of the unstirred layer at the level of the intestinal mucosa which limits diffusion and hence the rate of glucose absorption into the epithelial-cell, leading to decreased GI (Lund et al., 1989). Organic acids in breads, as additives or as natural constituents in the bread, also affect the GI by slowing down gastric emptying (Darwiche et al., 2001). Furthermore.
the addition of monoacylglycerols to prevent staling of bread slows the rate of starch degradation by forming a complex with amylose (Fardet et al., 2006), thereby decrease the corresponding GI.

Studies have shown that the glycaemic response of wholemeal bread is similar to that of white bread (Foster-Powell et al., 2002). Wholemeal bread is made from white flour with added bran. Therefore, breads with a high proportion of whole cereal grains should be called wholegrain instead of wholemeal breads (Jenkins et al., 1988b). The former could be useful in reducing the postprandial blood-glucose profile in individuals with T2 DM (Jenkins et al., 1988b).

3.1.4. Factors affecting the glycaemic response to rice

Rice is the most important staple food and the primary dietary source of CHO for over half of the world's population. It plays an important role in meeting energy requirements and nutrient intake. As with bread, rice is difficult to classify as a LGI or HIGH GI food because of the wide range of its GI. The GI of white rice ranges from as low as 45% to as high as 112% when glucose (GI = 100%) was used as the reference food (Foster-Powell et al., 2002). A variety of factors, both intrinsic and extrinsic, have been reported to influence the rate and extent of starch digestibility in rice among other starches.

Much of the variation in the GI of rice is due to differences in the ratio of amylose to amyllopectin. Rice varieties that contain a higher proportion of amylose have been shown to have a slower rate of digestion and produce lower GI and II (Juliano & Goddard, 1986). Miller et al. (1992) clearly showed that rice with a higher amylose content of 28% had lower GI and II than normal amylose rice varieties with 20% amylose. In the same study, the GI of the rice ranged from 64 ± 9 for Doongara white high amylose rice to 93 ± 11 for Pelde white rice. Rice with a high amylose content in the raw state forms more resistant starch on processing compared with the low and intermediate amylose rice due to the presence of higher amounts of the linear component of starch (Sagum & Arcot, 2000).
Beside the amylose content, cooking rice affects its glycaemic response and a greater rise in blood glucose and insulin has been reported after the consumption of cooked as opposed to raw starch (Colling et al., 1981). The amount of water and the processing temperature used during cooking rice influence the formation of resistant starch (Sagum & Arcot, 2000). When rice is heated in excess water, the structure of the starch granules is altered by the loss of crystallization of amylopectin, followed by swelling, hydration and gelatinization. When starch is gelatinized and allowed to cool, the amylose molecules align themselves or associate with each other and form retrograded starch, (Sagum & Arcot, 2000) thereby increasing the level of enzyme-resistant starch through recrystallization (Englyst et al., 1992).

3.2. Statement of the problem

Integration of GI concept into the dietary management of T2 DM patients in Kuwait is not presently possible, as the GI of the Kuwaiti staple foods has not been tested. This situation prevails in the face of a high prevalence of T2 DM in Kuwait with its associated risk factors such as dyslipidaemia, and a considerable body of evidence on the therapeutic potential of LGI diets in these patients. Thus in this study the GI and II of eight staple foods in the Kuwaiti diet were determined.

3.3. Objectives of the study

1. To determine the GI and II of eight types of carbohydrate foods staple to the Kuwaiti diet. These foods are: white pita (WP), light white pita (LWP), brown pita (BP) and light brown pita (LBP), white bread (WB), brown bread (BB), Iranian bread (IB), and white rice (WR).

2. To evaluate the glycaemic and insulinaemic responses in healthy volunteers after the ingestion of eight staple Kuwaiti foods (mentioned above).

3. To compare the GI of some staples in the current study with those of published values in the International Tables of GI.
3.4. Study hypotheses

1. The GI of the staples tested in the current study will have a wide range.
2. Significantly different blood glucose and insulin responses will be elicited by equivalent amounts of carbohydrate from different types of food with different carbohydrate content.
3. The postprandial glucose responses of the test foods will be closely related to their insulin responses.
4. GI values for the different type of breads and rice determined in this study will substantially differ from their published values in the International Tables of GI (Foster-Powell et al., 2002).

3.5. Methods

3.5.1. Test foods

The eight test foods included seven types of bread, staple to the Kuwaiti diet, and a type of white rice. The following breads were examined for their glycaemic and insulinaemic responses; white pita (WP), light white pita (LWP), brown pita (BP), light brown pita (LBP), white bread (WB), brown bread (BB) and Iranian bread (IB). With the exception of IB, all breads were produced locally by the Kuwait Flour Mills & Bakeries Company. IB is usually made in small Iranian bakeries. The white rice examined in this study was the same as that provided by the Kuwait Supply Company to all Kuwaiti families. The rationale for choosing the specified test breads and rice was to study eight carbohydrate-rich foods that are extensively consumed by the general Kuwaiti population, including T2 DM patients.

Portion sizes of WP, BP, WB, BB, IB and WR were determined by the commercial analysis of moisture, ash, protein, fat and total dietary fibre (Leatherhead International Limited, UK). Available carbohydrate was calculated by difference. The portion size of LWP and LBP breads was determined from the nutritional information on package labels as they were the only types of bread with such labels. Table 3.1 summarizes the nutrient composition of the test foods.
Table 3.1. Weight (g) and composition of bread portions containing 25g available carbohydrate (Available carbohydrate=total carbohydrate by difference - total dietary fibre).

<table>
<thead>
<tr>
<th></th>
<th>WP†</th>
<th>LWP†</th>
<th>BP+</th>
<th>LBP†</th>
<th>WB+</th>
<th>BB+</th>
<th>IB+</th>
<th>WR†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>44.80</td>
<td>43.86</td>
<td>53.41</td>
<td>48.83</td>
<td>54.35</td>
<td>62.97</td>
<td>46.12</td>
<td>86.20</td>
</tr>
<tr>
<td>Protein</td>
<td>4.8</td>
<td>5.4</td>
<td>5.8</td>
<td>6.7</td>
<td>5.8</td>
<td>6.7</td>
<td>4.7</td>
<td>2.8</td>
</tr>
<tr>
<td>Moisture</td>
<td>12.5</td>
<td>N/A</td>
<td>15.1</td>
<td>N/A</td>
<td>19.1</td>
<td>17.8</td>
<td>13.5</td>
<td>48.7</td>
</tr>
<tr>
<td>T. Fat</td>
<td>0.63</td>
<td>0.22</td>
<td>1.12</td>
<td>0.34</td>
<td>2.1</td>
<td>2.4</td>
<td>0.92</td>
<td>0.4</td>
</tr>
<tr>
<td>Ash</td>
<td>0.89</td>
<td>N/A</td>
<td>1.4</td>
<td>N/A</td>
<td>1.0</td>
<td>1.1</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Dietary fibre</td>
<td>1.12</td>
<td>0.22</td>
<td>5.0</td>
<td>N/A</td>
<td>1.12</td>
<td>1.5</td>
<td>3.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Total Carbohydrate</td>
<td>26.1</td>
<td>25.3</td>
<td>30.0</td>
<td>26.2</td>
<td>26.5</td>
<td>28.7</td>
<td>26.2</td>
<td>25.8</td>
</tr>
</tbody>
</table>

†Nutrient composition based on analyses (Leatherhead International Limited).
†Nutrient composition based on food label.
N/A Not available

3.5.2. Preparation of test foods

All breads were purchased the night before the test, weighed to the desired amount, packed into plastic bags and freshly consumed on the day of the study. Because of the low carbohydrate density of the breads, the available carbohydrate content of the test portion was lowered to 25g to prevent delivery of an over sized meal. Breads were cut into portions, each containing 25g carbohydrate. Crust ends of the WB and the BB were sliced off and discarded.

Rice was boiled in excess salted water in a covered pot for 10 minutes and strained. The rice was then simmered for a short period of time (5-8 min). The cooked rice was prepared the night before the test, kept in the fridge and microwaved before consumption. All test foods were taken with a standardized amount of water (250 ml).
3.5.3. Recruitment

The study was approved by the University of Surrey Ethics Committee (EC 204/37 SBMS). Volunteers were recruited for the study by invitation posters placed at a local supermarket in Kuwait (Dasma Co-operation). Subjects responded sporadically over the recruitment period which lasted for the month of October 2005. A minimum of 10 subjects were required for testing each type of bread to provide sufficient power and precision for the GI measurement (Brouns et al., 2005). The study cohort consisted of 7 females and 7 male subjects. Each subject needed between 6-9 weeks to complete the study.

3.5.4. Study participants

Volunteers were interviewed before the study commenced using a screening questionnaire to assess suitability for participation. Inclusion criteria included being male or a non-pregnant healthy female aged 20-70 years and being willing and able to comply with the study protocol and give informed consent. Exclusion criteria included a gastrointestinal condition affecting digestion or absorption of nutrients, the use of drugs affecting gastrointestinal motility or nutrient digestion or absorption, hepatitis, surgery or infection within the last three months.

3.5.5. Study design

In a randomized crossover design, the control and test foods were fed in random order on separate occasions. The study protocol was standardized using the WHO criteria (1998) in line with recent European recommendations (Brouns et al., 2005). To determine the GI, each of the 14 volunteers consumed 25g of the test food or the standard (25g of a glucose anhydrous solution) after a 12-14 hours overnight fast (Figure 3.1). Finger-prick capillary blood samples were taken at 15-30 minute intervals over the next 2 hours after the meal (at 0, 15, 30, 45, 60, 90, 120 min). Time 0 was taken as the beginning of the first bite or sip. Prior to blood sampling, subjects were encouraged to warm their hand to increase blood flow. Fingers were punctured with an Accu-Check Soft Clix lancet device (UK). Blood was collected in 300µl with fluoride oxalate tubes (microvette, Sarsted, Germany).
Volunteers consumed eight different types of food in random order, with a minimum of two days between test breads. Subjects were randomly assigned to test different foods, therefore not all the subjects tested the same food (Appendix 1). To control for the variation in fasting blood glucose concentrations on the day of the test, subjects were asked to eat the same meal the evening prior to each test meal. Participants were also asked to abstain from exercise and heavy activities 24 hours prior to testing. Volunteers tested each type of food on two separate occasions while they tested the reference food (glucose) on three separate occasions. They were asked to ingest the breads within 10-15 min and the 250 ml glucose solution within 5-10 min.

Repeated twice for each test food and three times for the standard (glucose load)

**Figure 3.1:** Schematic presentation of the study day protocol. Each volunteer (n=14) ingested the test food twice and the glucose load three times. A fasting capillary blood sample was obtained by finger prick and at intervals of 15, 30, 45, 60, 90, and 120 min after the ingestion of the test food or the standard food (glucose load).
3.5.6. Determination of glycaemic/insulinaemic responses and GI/II

Plasma glucose and insulin response curves for the two-hour test period were produced by taking the mean of duplicate measures of blood glucose and insulin concentration at each time point for each volunteer, for each bread tested. A mean glucose / insulin for the 10 volunteers for each time point was then used to construct the response curve.

The GI and II were determined from plasma glucose and insulin response curves produced over the two-hour test period. The GI was calculated from the incremental area under the curve (IAUC) for glucose after the consumption of a test food divided by the IAUC after the consumption of a control food containing the same amount of carbohydrate. The insulin response (II) was calculated from the IAUC of the insulin response after consumption of a test food divided by the IAUC after consumption of a control food containing the same amount of carbohydrate. In both cases the 'control food' was 25 g of glucose. Hence, the GI and II of each diet were calculated for each person using the following equations:

\[ GI = \frac{\text{Incremental area under 2-h plasma glucose curve for test food}}{\text{Incremental area under 2-h plasma glucose curve for glucose}} \times 100 \]

\[ II = \frac{\text{Incremental area under 2-h plasma insulin curve for test food}}{\text{Incremental area under 2-h plasma insulin curve for glucose}} \times 100 \]

In this calculation, blood glucose/insulin values that were lower than the first value (at 0 min) were ignored. The IAUC of glucose and the IAUC of insulin responses for the standard (25 g glucose) were derived from the mean of the three IAUC for each volunteer. IAUC calculations were taken from Brouns et al. (2005) and incorporated into an Excel macro.
For the calculation of individual GI and II values, the IAUC was calculated separately for each food in every volunteer to indicate the total increase in blood glucose and insulin after the intake of test food. This was calculated as the sum of the trapezoids between the blood glucose curve and the horizontal line. Since each bread was tested twice in each volunteer, an average GI value was calculated from these two determinations. Then the GI for each test food was calculated as a mean of the respective average GI for each individual. Glycaemic indices are classified as high (70 to 100), intermediate (55 to 69), or low (55 and lower).

3.5.7. Laboratory analyses

Blood samples were immediately centrifuged (Eppendorf centrifuge 5810 R) and plasma glucose concentrations were measured in the Amiri hospital in Kuwait using an automated glucose oxidase method (Beckman Synchron CX®3 system). The rest of the plasma was stored in -80°C in the Amiri hospital and shipped later on dry ice to the University of Surrey for the determination of plasma insulin.

The synchron CX3 system determines glucose concentration by an oxygen-rate method employing a Beckman oxygen electrode. 10μL of the sample is injected into a reaction cup containing a glucose oxidase solution. The rate of oxygen consumption which is directly proportional to the concentration of glucose in the sample, is determined through electronic circuits

\[ \beta - D - \text{glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{gluconic acid} + H_2O_2 \]

Hydrogen peroxide is then destroyed in the presence of catalase without yielding oxygen by the addition of ethanol to the reagent according to the following reaction

\[ H_2O_2 + \text{Ethanol} \xrightarrow{\text{Catalase}} \text{Acetaldehyde} + H_2O \]

In order to ensure the complete destruction of peroxide, iodide and molybdate are added to the enzyme reagent causing the following reaction

\[ H_2O_2 + 2H^+ + 2I^- \xrightarrow{\text{Molybdate}} I_2 + H_2O \]
Three levels of controls were used at the beginning and end of each assay. The intra-assay coefficients of variation for the low, medium, and high quality controls were calculated to be 2.8% (low QC), 2.2% (medium QC), and 2.4% (high QC). The inter-assay coefficient of variation were 4.4% (low QC), 2.7% (medium QC), and 2.1% (high QC).

Insulin was measured using immunochemiluminometric assay as described in section 2.2.4

3.5.8. Statistical analyses

Statistical analyses were performed using SPSS version 14 (SPSS for Windows®, Chicago, Illinois). Results were plotted as glucose and insulin curves and expressed as means ± SEM unless otherwise specified. Mean IAUC values for glucose and insulin, were calculated, according to Brouns et al. (2005) ignoring any areas below the fasting level. Normality was assessed using the Kolmogorov-Smirnov test. Leven’s test was used to investigate the equality of variance in different treatment groups. As homogeneity of variance was verified, data were tested by ANOVA. To compare the responses to different foods, a 2-way ANOVA with factors for food type (between subjects) and time (within subject) was used. A one sample t-test was used to compare the GI and II of the different breads with the standard glucose load. Pearson’s correlation coefficient was used to examine relationships between variables of interest. Differences were considered statistically significant if $p<0.05$. 

73
3.6. Results

3.6.1. Characteristics of subjects

The demographic and clinical characteristics of the subjects are shown in Table 3.2. All subjects consumed each of the test foods on two separate occasions and the standard glucose load three occasions (19 occasions). The subjects completed a total of 202 tests. An additional nine tests were repeated when the concentration of fasting blood glucose exceeded the normal reference range (>7 mmol/l), and it was assumed that this represented a non-fasted value.

Table 3.2. Characteristics of subjects values are means (SD)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>14</td>
</tr>
<tr>
<td>Male/female</td>
<td>7/7</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>35 (10.8)</td>
</tr>
<tr>
<td>Mean weight (kg)</td>
<td>65.14 (9.4)</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>5.2 (0.7)</td>
</tr>
<tr>
<td>Fasting blood insulin (pmol/l)</td>
<td>11.8 (8.5)</td>
</tr>
</tbody>
</table>

Mean blood glucose concentration before ingestion of the test food on the 19 occasions was 5.2±0.07 mmol/l (n=94). There was no statistically significant difference between fasting plasma glucose (p=0.99) or fasting plasma insulin (p=0.60) for any of the tests at baseline.

3.6.2. Glycaemic and insulinaemic indices (GI & II) of test foods

The GI and II values for the foods are presented in Figure 3.2 (see Appendix 2). The GI of the BP was significantly higher than the GI of LWP bread (p=0.026), with no significant difference between the rest of the foods, although the GI of these foods varied from 58.6% to 76.4%. All tested breads and rice had a significantly lower GI than the glucose standard (GI=100). The II of BP bread, on the other hand, was significantly different from the II of WP (p=0.042) and LWP (p=0.022). Except for IB which had a
higher II than that of glucose, none of the food tested in the current study had a significantly different II from that of the glucose standard. There was a trend in the differences in II between BP and the glucose standard. Correlation analyses indicated that there was a significant association between the GI and the II of foods tested ($r=0.238$, $p=0.034$).

![Bar chart](image)

**Figure 3.2**: Glycaemic and insulinaemic index for the 8 foods (n=10). Values represent means ± SEM. *Significantly different from the control $p<0.05$, **$p<0.005$, and ***$p<0.0005$. † Represent a trend in the difference between the food and the control. Differences between a & b and c & d are significant, $p<0.05$.

### 3.6.3. Glycaemic and insulinaemic response data

Mean blood glucose concentrations following ingestion of the eight foods in normal subjects at each time point are shown in Figure 3.3 and Figure 3.4 (see Appendix 3). The mean blood glucose concentration was similar before ingestion of each test food, varying from $5.1 \pm 0.2$ to $5.3 \pm 0.3$ mmol/l. The consumption of glucose standard, BP, WP, LBP, and WR resulted in a mean peak glucose concentration at 30 min after ingestion while plasma glucose concentration peaked at 45 min postprandially for LWP, WB, BB and the
IB. The highest peak was obtained with the 25 g glucose load at 45.7% above basal level, followed by white bread (36.2% increase), and the lowest was with the light white pita bread (25.7%).

Patterns of mean postprandial blood glucose increments are shown in Figure 3.5 and 3.6. The control (25 g glucose) and the white rice had relatively early negative blood glucose increments compared with the breads. The glucose showed a high early blood glucose increment peak and was followed by a rapid decline. The brown pita and the white bread, on the other hand, had relatively higher incremental levels which were maintained above the baseline level before it decreased at a later stage. The other breads showed the same pattern of postprandial glucose increments. There was a significant effect of time and significant interaction between time and food type ($p<0.0005$) with no significant effect of bread on mean plasma glucose increments ($p=0.122$).

Mean plasma insulin concentrations following ingestion of the eight foods in normal subjects are shown in Figure 3.3 and Figure 3.4. Mean insulin concentration was similar before the ingestion of test foods (11.86±0.87 pmol/l (SEM), $p=0.603$). The consumption of the glucose load, LWP, and WR resulted in a mean peak insulin concentration at 30 min after ingestion, while plasma glucose concentration peaked at 45 min postprandially for WP, BP, LBP, WB, BB and the IB. A two-way ANOVA, with time and food type on plasma insulin increments, indicated that there was a statistically significant effect for time ($p<0.0005$) and a trend for an interaction between time and food type on insulin levels ($p=0.079$) with no significant effect of food type ($p=0.414$).
Figure 3.3: Mean plasma A: glucose, B: Insulin responses in 14 healthy volunteers after the ingestion of 25g glucose load and the ingestion of pita breads (white, brown, light white, and light brown; n=10). Values are means ± SEM. Error bars have not been shown if they are smaller than the symbol or overlap other bars.
Figure 3.4: Mean plasma A: glucose B: insulin responses in 14 healthy volunteers after the ingestion of 25g glucose load and the ingestion of white, brown, and Iranian breads and white rice (n=10). Values are Means ± SEM. Error bars have not been shown if they are smaller than the symbol or overlap other bars.
Figure 3.5: Mean plasma A: glucose increments B: insulin increments for food types vs. time for pita breads (white, brown, light white, and light brown). Values are Means ± SEM. Error bars have not been shown if they are smaller than the symbol or overlap other bars.
Figure 3.6: Mean plasma A: glucose B: insulin increments for food types vs. time for: white, brown, and Iranian breads and white rice. Values are Means ± SEM. Error bars have not been shown if they are smaller than the symbol or overlap other bars.
3.6.4. Comparisons of GI with published values

A comparison was made between the GI values calculated from the current study and those that were available for similar foods from the International Tables of GI. Four out of the eight foods tested in this study were compared with published GI values for similar foods from a different country origin (Table 3.3). The GI of these foods was comparable to their previously published values.

Table 3.3 Comparison between GI values derived from the current study and published values in the International Tables of GI.

<table>
<thead>
<tr>
<th>Food Type</th>
<th>GI %</th>
<th>Current study</th>
<th>International Tables of GI</th>
</tr>
</thead>
<tbody>
<tr>
<td>White pita bread</td>
<td>59</td>
<td></td>
<td>57</td>
</tr>
<tr>
<td>White bread</td>
<td>68</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Brown bread</td>
<td>64</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>White Rice</td>
<td>62</td>
<td>69</td>
<td></td>
</tr>
</tbody>
</table>
3.7. Discussion

3.7.1 Glycaemic and insulinaemic indices of tested foods

The GI values of the carbohydrate rich staples were determined in the present study using a standard protocol (Brouns et al., 2005). The primary objective of this study was to establish the GI of some staple foods in the Kuwaiti diet, that had been previously reported by others (Kamel & Martinez, 1984; Al-Hooti et al., 2002) that could then be used to investigate the effects of lowering the GI in T2 DM patients (Chapter 4). The results of the analyses of the 24-hour recalls collected previously from individuals with T2 DM (Chapter 4; pilot study) were also used to identify the staples in the diet of these patients. The glycaemic index of foods is similar in normal and diabetic subjects regardless of the individuals’ glucose tolerance status (Jenkins et al., 1981; Wolever et al., 1998). Therefore results of the GI testing of the food in this study can be applied to other individuals, including T2 DM patients. Studies have shown an association between dietary GI and the risk of CVD in T2 DM patients (Jenkins et al., 1988a).

Using published criteria (Foster-Powell et al., 2002), it is possible to classify brown pita as a high GI bread. The rest of the breads in this study (WP, LWP, LBP, WB, BB, and IB) and the white rice can be designated as having an intermediate GI value despite the observation that WP bread and LWP had relatively lower GI values (59% and 56% respectively). These GIs were comparable or lower than many other types of breads consumed worldwide (Foster-Powell et al., 2002). For example, the GI of white Turkish bread, bagel, and French baguette are 87%, 72% and 95% respectively.

The II of the breads on the other hand could not be distinguished from the glucose reference, with Iranian bread having an even higher II than the glucose reference ($p=0.032$), and the brown pita bread having a higher II that was approaching significance compared to the glucose reference ($p=0.053$). The II was usually higher on the relative scale than the GI of the breads. This was difficult to explain as the amount of fat and protein in these breads were negligible. Both fat and protein in the diet increases insulin responses to a carbohydrate load (Lovejoy et al., 1998; Nuttall et al., 1984). These breads might therefore possibly induce a higher glycaemic response in T2 DM patients as
insulin secretion in these individuals is subnormal (Weyer et al., 1999). However, the overall association between the GI and the II was positive. In general, the insulinaemic index is less clinically useful than the GI as it varies in different groups of people (Wolever et al., 1998).

When the breads were compared statistically for their measured GI, brown pita bread, interestingly, had a higher GI value than the light white pita bread. This finding was unexpected as many studies have shown that the GI of wholemeal bread is similar to that of white bread (Foster-Powell et al., 2002). A possible explanation might lie in the presence of fibre in the brown pita rather than the type of fibre per se. In manufacturing these pita breads, the bran which is added to the wheat flour consists of insoluble fibre inclusions. It is possible that these constituents disturb the gluten network of the bread and favor digestion, thereby increasing the bread’s GI. The other possible explanation is that the addition of bran to the wheat flour in manufacturing brown pita necessitates intense kneading to incorporate the fibre satisfactorily. Intense kneading is known to have a positive effect on the rate of digestion, therefore increasing the GI value (Fardet et al., 2006). While the relatively higher GI value (68%) of the light brown pita as compared to the other white pitas (GI of white pita=59% and light white pita=56%) is consistent with this idea, further GI testing of wholemeal breads will be necessary to substantiate these explanations. Moreover, the physical structure of the starch (porous texture with highly gelatinized starch) rather than the nature of the raw material influences the bread’s glycaemic response (Fardet et al., 2006). Therefore, the variation observed among some breads in this study in terms of their GI could be due to the baking process itself which plays an important role in determining the texture of the food. In manufacturing breads, it would be preferable to use wholegrain flour with the entire wheat fraction (bran, germ, and endosperm) which usually slows down the digestive process of starchy foods. The effect of fat and protein on the GI of the staples in the current study cannot be determined as the amount of fat and protein present in the test foods may not be sufficient to affect their overall glycaemic response. For example, a number of studies (Gannon et al., 1988; Nuttall et al., 1984) have indicated that about 20-30g dietary protein is required to stimulate insulin and decrease glycaemic responses of the carbohydrate food.
The results of this study could lead to beneficial health effects if the relatively low GI breads are substituted for the high GI brands. This involves replacing brown pita bread with light white pita or white pita breads. The importance of this arises from the finding that even with small changes in the GI of the diet, a significant reduction of CVD risk will follow (Liu et al., 2000). With the exception of the white and light white pitas, the GI of white rice in this study was relatively lower than the breads (GI=62%). Therefore, rice can be substituted for the other breads that are commonly consumed in Kuwait, especially at lunch, as the majority of people prefer rice dishes at lunch. The rice however should not be replaced with Iranian breads in the diet of T2 DM patients as these breads have a significantly higher insulinaemic index than the rest of the breads tested in the present study. The least-eaten bread among individuals with T2 DM in Kuwait, as determined by a 24-h dietary recall (Chapter 4), was reported for light white pita (3.8%; GI=56%), while the highest consumption was reported for the light brown pita (GI=68%). This was in line with the general perception among diabetic patients that wholemeal bread contains higher dietary fibres than the white bread and would be expected to have a lower glycaemic response. This study did not support the patient perception that the brown pita had a lower glycaemic response than the white light pita. However, studies on whole cereal grains showed a reduction of the postprandial glucose profile with the consumption of wholegrain bread (Jenkins et al., 1988b).

Individuals with diabetes have a limited range of low GI foods available, especially in terms of cereal products (Bjorck & Elmstahl, 2003; Jarvi et al., 1999). Of the hundreds of cereal products listed in the GI tables, only 16 have a GI <70. Results of this study clearly showed that most of the breads consumed generally in Kuwait by both the normal and the diabetic population are in an intermediate GI range. This finding prompts the speculation that the high prevalence of diabetes among Kuwaitis might be related to the quantity (glycaemic load; GL) rather than the quality of carbohydrate they consume. However, such a sweeping conclusion cannot be made. Indeed, examining the effect of GL on glycaemic response is important, as both the GI and GL determine the individual’s glycaemic response to a food or meal (Sheard et al., 2004). The GL is the product of the GI of a food and its total available carbohydrate content. With respect to GL, a stepwise elevation in postprandial plasma glucose and/or insulin concentrations was associated
with stepwise increases in the GI in healthy individuals (Brand-Miller et al., 2003b). Also, it should be remembered that these foods are seldom eaten alone but rather as part of a mixed meal or consumed as components of a complex diet. The glycaemic and insulinaemic responses produced by these staples could therefore be affected by the addition of fat and protein to the diet, the presence of which affects gastric emptying and insulin secretion (Nuttall et al., 1984; Welch et al., 1987). However, many studies support the predictability of the GI in mixed meals (Wolever & Jenkins, 1986; Wolever & Bolognesi, 1996).

3.7.2. Glucose and insulin responses to test foods

The second objective of this study was to compare the pattern of plasma glucose and insulin responses to the different test foods. This was examined through plotting the measured plasma glucose/insulin increments vs. time for each food to obtain the corresponding curves. The glycaemic and insulinaemic responses to the reference food (glucose) were assessed three times in each individual to reduce the effect of the within-individual variation in glucose tolerance.

When depicted graphically, the plasma glucose and insulin increments show that the glycaemic responses were different from the control. However, this difference did not reach statistical significance. In hindsight, this was not unexpected once the GI of the staples was determined in the first part of the study. In view of the narrow range of GI values for the staples, it was predictable that the glycaemic responses of the test foods would not be significantly different. The source of carbohydrate would affect postprandial glucose responses only if the GI values of different foods differed by a sufficiently large amount. Furthermore, other factors such as the variation between and within subjects might affect the ability to detect differences in plasma glucose or insulin responses to different foods.
3.7.3. Comparison of GI values with published values

Since the breads examined in the current study have never been tested previously, it was important to calibrate the study with breads tested in other countries. Consequently, three of the breads tested in this study (white pita bread, white bread, and brown bread) in addition to the white rice were compared to values that have been previously published by other countries in the International Tables of GI (Foster-Powell et al., 2002). The GI of the test foods were closely related to previously published values. This gives confidence that the International Tables of the GI are a reliable resource for researchers as well as for clinicians and dietitians in Kuwait for the dietary management of T2 DM. However, the preamble to these tables reinforces the need for testing local foods in some countries, as the GI varies considerably for different varieties of breads. Therefore, these tables should be used for planning diets for people with T2 DM only if the measurement of the GI of local products is not feasible.

The GI value of brown pita has not been published by any country in the International Tables of GI. However, one recent study of the GI and GL of commercial foods (Henry et al., 2005) was the first to determine the GI of brown pita (wholemeal pita) in the UK. According to this study, the GI calculated for brown pita was 56%, which placed it among medium GI foods, whereas in our study, brown pita was classified as high GI bread (76%). This difference was larger than the 10-15% which others (Wolever et al., 1991) have ascribed to errors in the measurement of GI. This large difference in GI is difficult to explain given the host of factors that could contribute to this variation, including different baking conditions and fibre content of the wholemeal bread tested in these studies.

White rice is the other major staple in the Kuwaiti diet. The GI of the rice tested in this study compared well with the published values (Henry et al., 2005; Foster-Powell et al., 2002). However, in the present study, only one cooking method of rice was examined (boiling in excess water and straining). The other most common way in Kuwait of cooking rice is by boiling it in a lesser amount of water (machbos). The GI of cooked rice using this method should also be tested as the process of boiling consistently decreased the amount of amylose in all rice varieties (Sagum & Arcot, 2000), thereby
affecting their GI. Overall, Kuwaiti people commonly boil foods in too much water for too long a period of time (Kamel & Martinez, 1984). Since the classification of rice into a high or low GI rice depends mainly on the amylose content of commercial varieties (Miller et al., 1992), determination of the GI of different brands that are commonly consumed in Kuwait is necessary, as consumers will have no way of knowing the amylose content of any rice from the food label (if present). Indeed, rice needs to be tested locally, brand by brand (Foster-Powell et al., 2002). Beside the amylose content, starch digestibility in rice is generally influenced by several other factors, such as the degree of gelatinization during cooking, the granule particle size, starch-protein interactions, amylose/lipid complexes and the level of resistant starch (Sagum & Arcot, 2000). Moreover, rice in Kuwait is almost always eaten with a protein source, so examining the glycaemic response of rice in the context of a mixed meal rather than as an individual food (as in this study) will allow the examination of the possible confounding effects of fat and protein.

3.7.4. Potential practical implications of the study

A major practical implication from the findings of this study is to encourage the use of GI for better planning the diet of T2 DM patients in the context of disease management in Kuwait. As the study is the first to test the physiological effect of staples in Kuwait, these findings should provide valuable evidence to support changes in dietary recommendations for these staples in T2 DM. In planning diets in these individuals, the International Tables can be used as a representative tool for selection of carbohydrate foods of low GI values. A low GI diet plan can be applied by following the 2005 Dietary Guidelines for Americans (Department of Health and Human Servises & U.S. Department of Agriculture, 2006) and the recommendations of the WHO/FAO (1998); that is, the GI should be used to compare foods of similar composition within food groups. However, further studies are needed to identify low GI staples in Kuwait and promote their usage. The production of palatable low GI breads that combine several factors, including the intact cereal grain, using baking conditions that favor a lower glycaemic response (long time/low temperature) and the generation of organic acids through sourdough fermentation should be encouraged by bread manufacturers in Kuwait. Lowering of the GI was seen when including a β-glucan rich barley genotype in
flat-baked bread products (Liljeberg et al., 1996). It should be remembered though, that the nutritional quality of breads should not be based on the GI parameter solely. Adding fats to bread, for example, to lower its GI should not be encouraged as high consumption of fat is related to CHD risk (Wijendran & Hayes, 2004). The use of minimal processing conditions might also be useful to maintain starch crystallinity and reduce the amylase availability and GI of cereal flakes (Fardet et al., 2006). Since the majority of the people in Kuwait (65.9%) use food within at least one day (ALAjmi, 2004); a greater understanding of the influence of storage conditions and times on the formation of retrograde starch is required.

It is undoubtedly easy for people in Kuwait to switch from the brown pita bread to breads made with wholegrain flour or intact cereal grain such as the new multigrain bread which has recently been marketed by the Kuwait Flour Mills and Bakeries Company. Besides having the lowest GI of these breads, they have the beneficial effect of lowering LDL-C (Sloth et al., 2004), the strongest predictor of CHD in T2 DM patients (Turner et al., 1998).

Since the glycaemic response to mixed meals can be reasonably predicted from the GI of constituent foods (Wolever & Bolognesi, 1996), and provided that the GI of the individual foods is comparable with those of the International Tables of GI, then integration of information on GI into the Kuwaiti diet, including the mixed meal concept, should be achievable.
3.8. Conclusions

To conclude, most of the breads, with the exception of brown pita bread consumed in Kuwait, were shown to have a medium GI value. The concept of GI as a mechanism of exchange of one source of carbohydrate for another can therefore be simply applied by replacing brown pita with white or light white pita breads. This simple change could have major implications in the dietary management of T2 DM and its associated CVD risk factors.
Chapter 4

INVESTIGATION INTO THE EFFECTS OF A LOW GLYCAEMIC INDEX DIET IN ENERGY BALANCE AND AS COMPARED WITH ENERGY RESTRICTION ON MODIFYING AN ALP IN OVERWEIGHT/OBESE T2 DM PATIENTS IN KUWAIT

4.1. Introduction

T2 DM is a major clinical and public health problem among the Kuwaiti population of 20 years of age and older with an overall prevalence rate of 14.8 % (Abdella et al., 1998). T2 DM is associated with elevated risk of CVD and the risk of coronary heart disease (CHD) is 3-fold higher in patients with diabetes and more likely to lead to mortality and prolonged morbidity (Stamler et al., 1993). The National Cholesterol Education Program Adult Treatment Panel III (2001) defined diabetes as CHD ‘risk equivalents’ and recommend equally aggressive treatment as those used for the secondary prevention of CHD. Typically, T2 DM is associated with a dyslipidaemia that increases the risk of CHD. T2 DM dyslipidaemia is characterized by high TAG concentrations, predominance of sdLDL-C and decreased concentrations of HDL-C (Goldberg, 2001). This triad of abnormalities in plasma TAG, LDL and HDL is collectively known as the atherogenic lipoprotein phenotype (ALP). Glycaemic control alone in T2 DM patients is not sufficient to reduce CVD risk (Schernthaner, 2005). The UKPDS showed that HbA1c ranked below LDL-C and HDL-C as risk factors for CAD and below LDL-C and diastolic blood pressure in determining risk for fatal or non fatal myocardial infarction (Turner et al., 1998). Therefore, the NCEP ATP-III (2001) guidelines placed emphasis on lipids and lipoprotein abnormalities as they can be modified though diet and lifestyle changes. To date, the most effective dietary approach for the treatment of this ALP remains controversial. In a low fat diet, replacement of saturated fat, which is considered atherogenic, with carbohydrates adversely increases plasma TAG concentrations especially in individuals with an ALP (Abbasi et al., 2000). An alternative approach is therefore to replace high glycaemic index (GI) foods with less refined lower GI foods. The GI is a degree to which a food will raise the plasma glucose concentration. It compares a test food, gram for gram of available carbohydrates with a standard test meal
(Jenkins et al., 2002). Carbohydrate-rich diets with a high GI cause a high postprandial glucose and insulin response (Jenkins et al., 2002; Wolever, 1990b) and are associated with decreased insulin sensitivity (Frost et al., 1998). Moreover, high GI diets have been shown to raise plasma TAG and lower HDL, whereas diets containing low GI (LGI) foods tend to lower plasma TAG (Dumesnil et al., 2001). While randomized, controlled trials have shown that glycated haemoglobin was reduced by 0.43% points on a low-GI diet (Brand-Miller et al., 2003a), the detailed effects of a LGI diet on the ALP; a phenotype with a high prevalence in T2 DM patients in Kuwait, have not been previously tested.

There is universal agreement that weight reduction is an essential part of dietary therapy for dyslipidaemia in individuals with T2 DM. It has been consistently shown that when weight reduction is achieved (and maintained) in T2 DM patients, a sustained decrease in TAG concentrations is observed (Howard, 1993). Many studies have also shown an increase in HDL-C as well as an improvement in the ratio of total to HDL-C in T2 DM patients with weight reduction (Ginsberg et al., 2005b). In overweight or obese individuals with T2 DM, a weight reduction of 5-10% is associated with metabolic improvement in glycaemic control, insulin sensitivity and CVD risk factors (Blackburn, 1995; Ditschuneit et al., 2002; Wing et al., 1987a). Results from the Women Nurses Health Study (Liu et al., 2000) suggest that the effect of dietary GI is more important in individuals with a greater degree of obesity, as there was no association of dietary GI with CHD in persons with a body mass index of <23 kg/m². Therefore, any dietary advice for T2 DM patients should take their degree of obesity into account. Despite the fact that weight reduction is an important therapeutic goal for the reduction of CHD risk in T2 DM in Kuwait (Al-Adsani et al., 2004), available data is insufficient to construct dietary guidelines for risk reduction in this population.

4.2. Statement of the problem

In spite of the high prevalence of CVD risk factors in T2 DM patients in Kuwait, and despite the evidence that these risk factors are modifiable through the manipulation of dietary GI, this has never been investigated previously in Kuwait. Therefore, the primary
aim of the current study was to investigate the efficacy of a LGI diet in improving an ALP as a CVD risk profile in T2 DM patients in Kuwait. In addition, it is unclear whether energy-restricting the diet in addition to lowering its GI will confer additional benefits to those observed during energy balance especially in individuals with increased CVD risk such as T2 DM patients. Therefore, a secondary aim of the present study was to compare the efficacy of the LGI diet with energy restriction as compared to energy balance.

4.3. Objectives of the study

To examine, in overweight/obese individuals with T2 DM in Kuwait, two dietary approaches for the reduction of an ALP as a major CVD risk factor within this population. These approaches are a LGI diet and a LGI combined with an energy-restricted, weight-losing diet.

Specifically:

1. To investigate the effect of a LGI diet compared to high GI diet on glycaemic control and ALP as markers of CVD risk in overweight/obese patients with T2 DM in Kuwait primarily TAG concentrations.
2. To compare the efficacy of a LGI diet to that of LGI diet combined with an energy-restriction with respect to the expression of an ALP in order to predict the most effective dietary approach for CVD risk reduction in Kuwaiti overweight/obese T2 DM patients.
3. To investigate the effect of a LGI diet on self-reported appetite and food intake in overweight/obese individuals with T2 DM using a subjective appetite rating system (VAS) and 24-hour dietary recall.
4. To demonstrate the applicability of LGI dietary advice and its potential use as a management tool for CVD risk reduction in Kuwaiti overweight/obese T2 DM patients.
4.4. Study hypotheses

1. A LGI diet will produce significant improvement in glycaemic control and in the expression of an ALP in overweight/obese patients with T2 DM in Kuwait.

2. An energy-restricted LGI diet will further enhance the improvement in glycaemic control and the expression of an ALP in T2 DM Kuwaiti patients compared to LGI diet alone.

3. A LGI diet will significantly decrease appetite as measured by VAS scores.

4.5. Significance of the problem to the Kuwaiti population

Any clinical or dietary findings from this study will be of great significance to the health professionals and nutrition care providers in Kuwait as the relevant dietary data on this type of dietary intervention is lacking. In the attempt to manage the risk factors for CVD in T2 DM patients, dietary treatment is of substantial importance. Prevention and risk reduction are primary themes for current research in Kuwait (Al-Adsani et al., 2004; al-Muhtaseb et al., 1991; Mojiminiyi et al., 2006; Jackson et al., 2002; Akanji, 2002; Abdella et al., 1998). Thus, findings from this study will translate into future programs for diabetes management and in constructing procedures for nutrition education appropriate for the Kuwaiti population.

The study will focus primarily on determining the best approach for the dietary management of CVD risk in overweight/obese individuals with T2 DM in Kuwait. To the best of our knowledge, this study is the first to examine dietary manipulation, both in terms of GI and/or energy restriction, and to examine its applicability and relevance in the diabetic Kuwaiti population. Outcomes from this study, therefore, enable nutrition professionals in the country to gain insights into the dietary intake of this population compared to international recommendations in order to promote more positive behaviors and build-up a comprehensive plan to meet the nutritional requirements of this population. In addition, the study will help nutrition professionals develop an awareness of risk management and evaluate possible strategies for CVD risk reductions in T2 DM.
This might require a comprehensive care approach and a shift in the way many patients with diabetes in Kuwait are currently being treated.

4.6. Ethical approval

Ethical approval was sought from the University of Surrey Advisory Committee on Ethics (Appendix 4). A letter of approval for conducting the study in the Ameri hospital, Kuwait, was also obtained (Appendix 5). The study was supervised by a panel of professionals at both study sites; the University of Surrey and the Ameri hospital in Kuwait. The panel of experts including nutrition specialists, diabetologists, research assistants, and dieticians were involved for guidance and research quality assurance especially throughout the data collection period.

4.7. Methods

4.7.1. Estimation of the GI of T2 DM diet in Kuwait

Before the commencement of the study, a detailed literature review was conducted to search for any dietary study that might provide insights into the GI of the diet of Kuwaitis. This search indicated that there had been no work that defined the GI of the diet of Kuwaitis previously. Therefore, it was decided to undertake a pilot study in order to estimate the GI of the diet of T2 DM patients in Kuwait. Twenty-six subjects with T2 DM; 11 males and 15 females, age 26 ±12.4 years (SD) and BMI= 31.7±6.7 (kg/m2) (SD), who attended the dietician outpatient clinic at the Ameri hospital, were interviewed and a 24-hour dietary recalls were collected. This was done through the corresponding outpatient dietician at a routine outpatient visit and the recalls were analyzed using WinDiet software (WinDiet, research version, Aberdeen, UK). These recalls were collected in both weekdays (n=17) and weekend days (n=9). The majority of subjects were obese with a mean BMI of 31.7±6.7 (SD). Analyses of these records indicated that the mean GI of the diet of subjects with T2 DM was 68% which falls in the high GI range for the diet. Table 4.1 summarizes the basic outcomes from this pilot study including anthropometric, blood pressure, and dietary information.
Table 4.1. Characteristics and dietary composition (per day) of patients with T2 DM in Kuwait (pilot study).

<table>
<thead>
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<th>Mean</th>
<th>SD</th>
</tr>
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<td>12.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
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<td>17.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
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<td>31.7</td>
<td>6.7</td>
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<tr>
<td>Waist circumference (cm)</td>
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</tr>
<tr>
<td>Systolic B.P (mmHg)</td>
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<td>129.5</td>
<td>13.5</td>
</tr>
<tr>
<td>Diastolic B.P (mmHg)</td>
<td>22</td>
<td>82.9</td>
<td>15.0</td>
</tr>
<tr>
<td>Energy (kcal/d-KJ/d))</td>
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<td>2565-1113</td>
<td>428-1797.6</td>
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<td>16.6</td>
<td>11.6</td>
</tr>
<tr>
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<td>16.1</td>
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<tr>
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<tr>
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<td>26</td>
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</tr>
</tbody>
</table>

4.7.2. Sample size justification

Primary Outcome Variable: Plasma triglyceride (TAG).

Luscombe et al (1999) observed a 0.33 mmol/l fall in TAG concentrations on a LGI diet. Based on these data, a common standard deviation of 0.28 was assumed. Using these data a sample size of 15 participants / group would be predicted to have 80% power to detect a difference in means of 0.33 mmol/l in the TAG concentration between groups. Allowing for a 25% dropout rate, 20 participants/group were recruited.
4.7.3. Recruitment

Recruitment was based on existing health records held in the Ameri hospital for each patient. Volunteers were recruited through clinical databases by the assistance of diabetologist. The recruitment period lasted for three months (February-April 2006). Overweight and/or obese volunteers were recruited only from the Ameri health region, which included the primary health care centres in the area. The Ameri hospital was chosen for the target population because it was the most active in terms of diabetes management in Kuwait.

4.7.4. Study participants

Inclusion criteria were

- Patient with established T2 DM according to WHO criteria (1999)
- Aged 30-70 years
- Overweight or obese (BMI ≥ 27 kg/m²)
- On stable medication for at least 6 months
- HDL cholesterol < 1 mmol/l for men, < 1.2 mmol/l for women
- TAG > 1.3 mmol/l
- Average GI of the diet (determined from 24 hour dietary recall) ≥ 65

Participants were excluded if they had a BMI of ≥ 45 kg/m² or if they had serious microvascular complications. Patients were seen by a diabetologist on a regular basis for at least 6 months prior to participation in the study. All patients were in good health except for diabetes. Medications, including, oral hypoglycaemic agents and lipid-lowering, were maintained at the same doses throughout the study period.

4.7.5. Study design

The study was a controlled, randomized parallel design with two dietary treatments and a control group. The duration of the intervention was 6 weeks. The dietary treatments were the LGI diet and the LGI combined with an energy-restricted weight reducing diet with a 6 week dietary intervention period preceded by a 2 week run in period and a 6 week follow up (Figure 4.1). The study consisted of 3 cohorts. During the run-in period,
participants were asked to maintain their normal diet, while in the follow up period participants had free choice of either continuing with the intervention diet or going back to their habitual diet. The dietary intervention was of an open, free living design in which participants purchased their own food and prepared their own meals.

Participants were randomly assigned to one of the following groups; a control group, a LGI diet group or an energy-restricted LGI group. In all three groups, subjects were requested not to alter their level of physical activity throughout.

![Study design diagram]

Figure 4.1. Study design.
4.7.6. Intervention diets

4.7.6.1. LGI diet

The principal components of the LGI diet were based on the GI testing of some local breads and rice staples for the participants under study. This investigation was carried out before running the study (Chapter 3). In addition, the International Tables of the GI values (Foster-Powell et al., 2002) were utilized for modifying individuals habitual high GI diet to a lower GI diet, designed to be isocaloric with respect to their previous dietary intake. The intervention aimed to achieve large differences in the GI of the high carbohydrate foods with no changes in the macronutrient or fibre intakes. It was important throughout the study that subjects in the control and LGI diet groups maintained their weight as this leg of the intervention was designed to test the effect of LGI diet alone on plasma lipids and lipoproteins. The habitual intakes of each individual were identified through the first 24-hour dietary recall collected personally before the run-in period (Appendix 6). A diet sheet was developed to provide participants with detailed and practical advice on the principles of the LGI diet (Appendix 7). It included tips on how to reduce the GI of the diet, meal and snack ideas, as well as a substitution list of high GI with LGI foods. The instructions were designed by the researcher to aid participants on understanding the principles of a LGI diet, and in implementing them. Individualized dietary advice was provided through the investigator and a trained dietitian who was already working in the hospital and who was currently dealing with patients with T2 DM in the hospital outpatient clinic. Dietary advice was based on the habitual intake and provided through visit 2 during the second week of the intervention period (Figure 4.1).

4.7.6.2 LGI combined with energy-restricted diet

The degree of energy restriction was estimated and calculated individually for each participant using the Harris Benedict equation calculator available online (Cornell University, 2001). The calculator simply incorporates the age, gender, and weight of the participant into the equation and calculates the basal energy requirement. This is then multiplied by the activity factor to account for the physical activity of the individual as described previously (Ebbeling et al., 2005). A weight loss of 5-10% was targeted for
this group. The distribution of energy in grams of macronutrients was drawn from the recommendations of the American Diabetes Association (Franz et al., 2004) for people with diabetes. According to these guidelines, approximately 10-20% of the daily energy intake should be derived from protein. Both animal and vegetable sources of protein should be provided in the diet. The remaining 80-90% of energy is to be distributed between dietary fat and carbohydrate. Less than 10% of this energy should be from saturated fats and up to 10% energy from polyunsaturated fats, leaving 60-70% of the total energy from monounsaturated fats and carbohydrates. The distribution of calories from fat and carbohydrate was varied and individualized based on the habitual intakes and nutrition goals. The carbohydrate content of the diet comprised of LGI foods. The diabetic food exchange list (American Diabetes Association & The American Dietetic Association, 2003) was then used to plan menus for each participant with emphasis on LGI foods. Participants were provided with menus based on preferences. Monitoring Glycaemic status and body weight, on any diet, is essential to assess the effectiveness of the nutrition plan. Therefore, participants in the energy restricted diet group were asked to monitor their glucose concentrations and weight regularly in order to make dietary modifications if necessary.

4.7.7. Questionnaires

Three questionnaires were utilized in this study. Dietary intakes were assessed using the 24-hour dietary recalls method. Participants’ feelings of hunger/satiety was assessed before and one hour after each meal during the day using 100 mm self rated visual analogue scales (VAS) (Rogers & Green, 1993) and the knowledge of individuals with T2 DM was assessed using the GI knowledge questionnaire.

4.7.7.1. 24-hour dietary recall

The method of 24-hour recall was used to assess the dietary intake of participants and to measure compliance with the intervention diet as this method has been shown to be the most feasible to identify the quantity of food items consumed by Kuwaitis (Kamel & Martinez, 1984). These recalls were collected on one occasion before the study commenced and three times during the run-in and the intervention phase of the study and
once more at the end of the follow-up period (Appendix 6). This method of dietary assessment was used as being the most appropriate for dietary researches in Kuwait, as participants are generally reluctant to fill in written questionnaires. The researcher interviewed participants and provided dietary advice for them to follow. All foods and drinks consumed by the respondent were reported on a standard form used in the hospital with the use of measurement aids and visuals, including charts and drawings to quantify the foods and beverages when the interview was conducted face to face. Three dietary recalls were conducted in person and three were collected over the telephone. The dietary interview length ranged from 15-30 minutes depending on the number of foods reported in the dietary recall.

4.7.7.2. VAS

A pre-post meal VAS (Appendix 8) was collected from participants on three occasions within the run-in and intervention period and a last one in the follow-up period of the study. These standardized scales are known to be easy and quick to use and do not involve the use of descriptive terms (Stubbs et al., 2000). Four variables, hunger, fullness, desire to eat, and prospective consumption were tested through the questionnaire. Participants were familiarized with the scale in advance in the first visit during the run-in period. They were requested to make a vertical mark on each line that best matched how they felt at the time. The dates for VAS collection were pre-scheduled for each patient and the dietician in the hospital reminded each participant to do so the night before the collection.

4.7.7.3. GI knowledge questionnaire

The GI knowledge questionnaire was developed by the researcher for the purpose of testing how much participants knew about the GI of the diet and also as a tool for assessing the success of the nutrition education and dietary advice directed to participants in both intervention groups (Appendix 9). The knowledge of participants regarding the GI of the diet was assessed immediately after the initial dietary advice by the dietician and at the end of the intervention period. The questionnaire attempted to test GI knowledge regarding foods that are low and high in GI. The questionnaire was
developed using GI values from the International Tables of GI and findings from the GI testing of breads and rice (Chapter 3) in order to make it more appropriate for the study population.

4.7.7.4. Validity and reliability of the questionnaires

The method of 24-hour dietary recall was used for assessing dietary intakes of the study subjects. The 24-hour dietary recall is a valid method for estimating the dietary intake of energy, macro and micronutrients in the diet (Greger & Etnyre, 1978). In order to increase the reliability of the 24-hour recall interview in this study, a standardized form was used to assure consistency for the collection of the information from each patient (Appendix 10). Collecting a 24-hour dietary recall by means of telephone interview is also considered a valid method and provides an adequate description of food intake (Bogle et al., 2001). In fact, the telephone interview produces acceptable estimates of the means and distributions of nutrient intakes and has the advantages of markedly reducing the cost, time, logistical, and personnel constraints associated with nutrition surveys (Posner et al., 1982).

VAS rating of appetite was used to measure appetite and satiety in relation to the test diets. This instrument is also considered a valid method for subjectively rating appetite. VAS are recommended to be used within subjects, using a repeated measure designs so as to compare the effect of treatment under similar circumstances (Stubbs et al., 2000).

The GI knowledge questionnaire was assessed for both face and content validity. A panel of four University of Surrey faculty members evaluated the validity of the instrument. These faculty members were chosen to be experts in the area of nutrition and GI of food. A description of the objectives of the study was provided to the experts along with the questionnaire to determine whether the instrument was capable of measuring the objectives specified in the study. In order to ensure the validity of the Arabic version of the questionnaire, two diabetologists and two dietitians who worked in the Ameri hospital answered the questions independently. Items that were answered the same by the four respondents were retained. Reliability, on the other hand, was assessed by
comparing the scores of a segment of experts to those of intermediate and limited knowledge in the area of GI.

4.7.8. Data collection procedures

Data was collected starting from the month of February, until the end of August, 2006. Permission was sought from the Public Authority for Applied Education and Training, the Investigator's sponsors, to conduct the study in Kuwait (Appendix 11).

Recruitment was based on existing health records held in the Ameri hospital for each patient. Suitability for inclusion was assessed during patient routine visits to the clinics, when the GI of the diet was calculated also from the 24-hour dietary recall collected during this visit, along with other information, within the patient's health records. At this point, suitability was checked using a checklist especially designed for the study (Appendix 12).

Selected participants were informed about the study design through individual interviews during routine clinical visits. The health care providers in the hospital always encouraged patients to participate in the study. Upon agreement of participation, participants were provided with a patient information sheet (Appendix 13) and consent forms were obtained from each participant (Appendix 14). The consent form included an explanation that participation was voluntary and that participants were guaranteed confidentiality and freedom to withdraw from the study at any point. Time of selection for the starting date for each participant was done prior to the first visit and was based on the time which was the least disruptive to the schedule of the patient.

4.7.8.1. Demographics and anthropometric measurements

A demographic as well as anthropometric questionnaire (Appendix 15) were used at baseline and subsequent visits. Height was measured using a wall mounted stadiometer ("Seca" Somatometre 200 cm x 0.1 cm). Weight were measured using a calibrated dial scales (Healthometer Professional Scales, 148 kg x 500 g). BMI was calculated using the standard formula (weight [kg]/height² [m]). Waist circumference was measured to the
nearest 0.1 cm using a standard tape measure at the narrowest point between the iliac crest and the lowest rib. Blood pressure was measured using an electronic sphygmometer (Omron M4, Omron Healthcare Netherlands) with the participant sitting at rest.

4.7.8.2. Dietary measurements

A 24-hour dietary recall was used to calculate the food intake of participants. In order to assist with proper portion size recording, standard measuring utensils and common serving dishes were displayed when collecting the first dietary recall in each phase of the study. The researcher asked participants for reasonable accuracy in recalling the dietary intake. The dietary intakes were analyzed using WinDiets software (WinDiets, research version, Aberdeen, UK) to give a full nutrient analysis. Considering the cultural variation in the diet, specificity of the amount and combination of dishes was required to make the report appropriate for analysis. The number of exchanges in selected Kuwaiti composite dishes (Sawaya et al., 1998) was also used to calculate the macronutrient content of the diet.

4.7.8.3. Fasting blood values

Fasting blood samples were taken at baseline (week 0) and 6 weeks. They were analyzed for glucose, T. Cholesterol, LDL-C, HDL-C, TAG, NEFA and insulin, LDL and HDL subfractions. 30 ml of fasting blood was drawn by venepuncture using commercially available vacutainer tubes (Becton Dickinson, Oxford, UK). All blood samples were taken by venepuncture by trained phlebotomists who worked in the laboratory within the hospital. Blood was collected into flouride oxalate for glucose analyses, lithium heparin for insulin assay and K₂EDTA for all lipid assays. Blood was immediately centrifuged at 2000g for 20 min at 4°C in an Eppendorf centrifuge 5810R low speed centrifuge for the separation of the plasma. Aliquots of plasma were dispensed into appropriately labeled Sarsted tubes (2 ml). They were stored at -80°C in the Ameri hospital and then shipped to the UK on dry ice through a courier company for subsequent analyses at the University of Surrey. Glucose, insulin, lipids and lipoprotein measurements were dcarried out as described previously (Section 2.2.3).
4.7.9. HOMA calculation

HOMA-IR was calculated using online calculator (Diabetes Trials Unit., 2004). The calculation of HOMA by this method involves measurements of both glucose and insulin concentrations. The calculator also provides estimates of steady state beta cell function (%B) and insulin sensitivity (%S) which has been shown to correlate with stimulatory models such as the hyperinsulinaemic clamp (Diabetes Trials Unit., 2004).

4.7.10. Calculation of daily GI and GL

The GI figures of individual foods were derived from the world literature with main reference to the international table of GI (Foster-Powell et al., 2002), and the results of the GI testing of local Kuwaiti breads and rice (Chapter 3). Similar foods were determined by ingredients breakdown (Olendzki et al., 2006) and food preparation methods that were described as part of the collection of the 24-hour dietary recall. Mixed dishes involved breaking down the ingredient of the mixed dish to consider direct or similar matches of individual ingredients which were then matched to foods from the GI recourses mentioned above. Mean GI/GL values of the day were calculated using the method recommended by Wolever et al (1994b). Briefly, an average GI was computed by summing the grams of carbohydrate per serving multiplied by its GI. All divided by the grams of carbohydrate per day. Average dietary GL was calculated in the same way but without dividing by the total carbohydrate per day. The calculation of GI used glucose as the reference food. The following equations summarises the calculation:

\[
\text{Dietary GI} = GI \times \frac{CHO}{CHO(g/d)}
\]

\[
\text{Dietary GL} = GI \times \frac{CHO}{100}
\]
4.7.11. Statistical analyses

Statistical analyses were performed using SPSS statistical package version 14 (SPSS for windows, Chicago, USA). Data were checked for normality using Kolmogorov-Smirnov analyses and checking both the normal and detrended normal probability plots. Homogeneity of the variance was assessed by Leven’s statistic. Skewed parameters were transformed as appropriate for analyses. WC, plasma TAG, LDL-C, NEFA and insulin concentrations were not normally distributed therefore they were log transformed and the geometric means were reported. Data for plasma glucose could not be transformed to a normal distribution therefore they were analyzed using the non parametric Kruskal-Wallis test for the between groups effect. Mean daily intakes of energy and nutrients were calculated from an average of the three 24-hour recalls collected in each phase of the study. VAS were also averaged for each period of the study and repeated measure ANOVA was used to study the changes in VAS scores, over time. Pre versus post dietary effects of all the different variables within each group were assessed by a paired Student t-test. The effect of the GI variables across groups was assessed using ANOVA. Post-hoc pairwise analyses with Tukey’s HSD was used when statistical differences were evident between groups. For categorical outcomes; HOMA-IR, groups were compared by using the chi-square test. Values are means±SEM unless otherwise indicated. All box plots were plotted using the program default option of medians and interquartiles. Correlations coefficients were measured to examine relationships between changes in different variables. Statistical significance was defined as $p<0.05$. 
4.8 Results

4.8.1 Patient recruitment

It should be noted that due to time constraints and recruitment problems, the investigator was unable to conduct the third part of the study (the follow-up phase). Of the 56 participants who started the trial, four withdrew during the run-in phase of the study. Reasons for these withdrawals included pregnancy of one female participant while three participants could not come to the clinic; one because of knee injury and the other two because of individual work pressures. Of the 52 participants who were allocated to treatment groups, 2 females withdrew; one because of long period of traveling and the other one no longer wished to diet. The percentage of withdrawals from the study was still less than expected (12% versus 25%). The flow of participants through the study is shown in Figure 4.2

![Flowchart of Subject Enrollment and Analysis](image)

Figure 4.2. A schematic presentation of the flow of subjects through the study.
4.8.2. Baseline characteristics

The anthropometric and clinical characteristics of participants at baseline according to allocated groups are shown in Table 4.2. The mean age of participants did not differ significantly between the three groups neither did the weight, BMI, and WC. Average diabetes duration was 7 years±3.1 (SD) years and this did not differ between the allocated groups, or between men or women. HDL-C and TAG concentrations presented in Table 4.2 were taken from the patient’s health record.

Table 4.2. Characteristics of participants in each dietary group at baseline. Values are means (SD).

<table>
<thead>
<tr>
<th></th>
<th>Control (n=16)</th>
<th>LGI (n=16)</th>
<th>Energy-restricted LGI (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/F</td>
<td>7/9</td>
<td>9/7</td>
<td>7/11</td>
</tr>
<tr>
<td>Age (y)</td>
<td>51.9 (12.8)</td>
<td>55.3 (8.9)</td>
<td>48.2 (8.0)</td>
</tr>
<tr>
<td>Diabetes duration (y)</td>
<td>7 (3.1)</td>
<td>8.1 (2.9)</td>
<td>6.6 (2.6)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>91.8 (15.1)</td>
<td>88.1 (12.2)</td>
<td>88.3 (17.3)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>34.6 (4.8)</td>
<td>33.0 (3.0)</td>
<td>34.4 (5.4)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>104.3 (23.5)</td>
<td>103.8 (7.2)</td>
<td>110.4 (12.9)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>127 (7.2)</td>
<td>129.9 (8.7)</td>
<td>131.1 (11.4)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>81.4 (3.7)</td>
<td>84.7 (4.7)</td>
<td>82.3 (5.7)</td>
</tr>
<tr>
<td>TAG (mmol/l) †</td>
<td>2.1 (0.9)</td>
<td>2 (0.7)</td>
<td>2.1 (1.0)</td>
</tr>
<tr>
<td>HDL (mmol/l) †</td>
<td>0.8 (0.2)</td>
<td>0.9 (0.2)</td>
<td>0.9 (0.2)</td>
</tr>
<tr>
<td>GI (%)*</td>
<td>74.0 (7.9)</td>
<td>73.4 (5.6)</td>
<td>70.7 (6.7)</td>
</tr>
<tr>
<td>GL*</td>
<td>208.1 (63.9)</td>
<td>200 (36.1)</td>
<td>199.4 (49.3)</td>
</tr>
</tbody>
</table>

†Values were based on last biochemical profile within 3 months before the start of the trial.
*Values represent habitual intakes
4.8.3. Changes in anthropometric indices and blood pressure

There was no significant difference in the systolic or diastolic blood pressure between the groups at baseline. After 6 weeks intervention, the systolic blood pressure was significantly different across groups \((p<0.0005)\) with the changes in the energy-restricted LGI diet group being significantly lower than the control \((p<0.0005)\) and the LGI diet group \((p=0.001; \text{ Table 4.3})\). The changes in the systolic blood pressure were strongly associated with weight loss \((r=0.766, p<0.0005)\).

Weight, BMI, and WC measurements were assessed at week 0 and 6. At baseline, weight was not statistically different between groups \((p=0.67)\). Only participants in the energy-restricted LGI diet group reduced their weight significantly \((p=0.001)\) over the 6 week period. Within this group two participants gained weight \((1.05 \text{ kg})\) and they were included in subsequent analyses as the trial was designed originally to represent a clinical situation. Mean absolute weight loss \((\text{kg})\) from baseline to 6 weeks was \(2.3\pm1.6\) (SD) as shown in Figure 4.3. The percentage of weight loss over the 6 week period within the weight loss group was \(2.6\pm1.6\%\) (SD). In this group, there was a relationship between baseline weight and weight loss \((r=0.57, p=0.013)\). Also, changes in body weight were associated with changes in GL of the diet \((r=0.312, p=0.028)\) but not the GI \((r=-0.308, p=0.213)\). Weight loss in the energy-restricted LGI group was significantly different than the control group \((p=0.001)\) and the LGI diet group \((p=0.017)\). These results are shown in Table 4.3 and Figure 4.3.

BMI was not significantly different between groups at baseline \((p=0.511)\). The average BMI for the allocated groups placed them in the obese range \((\text{BMI}\geq30)\). BMI for the energy-restricted group decreased significantly from baseline \((p<0.0005)\). This reduction was significant when compared to the control \((p<0.0005)\) and the LGI diet group \((p=0.009)\) with no difference between these latter two groups (Figure 4.4). Changes in the BMI were strongly related to changes in total energy intake /day \((r=0.987, p<0.0005)\).
Table 4.3. Anthropometric measurements at baseline and at week 6. Values are means (SEM)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>LGI</th>
<th>energy-restricted LGI</th>
<th>p (ANOVA)</th>
<th>Pairwise testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>92.8 (3.8)</td>
<td>88.3 (3.0)</td>
<td>88.3 (4.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 weeks</td>
<td>92.9 (3.9)</td>
<td>87.9 (2.9)</td>
<td>85.9 (3.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Changes (6 weeks -baseline)</td>
<td>0.5 (0.5)</td>
<td>-0.3 (0.7)</td>
<td>-2.4 (0.4)**</td>
<td>0.001</td>
<td>b, c</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>34.8 (1.2)</td>
<td>33.0 (0.8)</td>
<td>34.4 (1.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 weeks</td>
<td>35.1 (1.2)</td>
<td>33.0 (0.8)</td>
<td>33.5 (1.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Changes (6 weeks -baseline)</td>
<td>0.24 (0.6)</td>
<td>0.07 (0.3)</td>
<td>-0.9 (0.6)**</td>
<td>&lt;0.0005</td>
<td>b, c</td>
</tr>
<tr>
<td>WC (cm)†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>109.8 (1.0)</td>
<td>104.4 (1.0)</td>
<td>110.2 (1.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 weeks</td>
<td>110.1 (1.0)</td>
<td>101.2 (1.0)</td>
<td>103.6 (1.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Changes (6 weeks -baseline)</td>
<td>0.09 (0.9)</td>
<td>-3.1 (1.1)*</td>
<td>-6.8 (6.5)**</td>
<td>0.001</td>
<td>b</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>127 (1.8)</td>
<td>129.9 (2.2)</td>
<td>131.1 (2.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 weeks</td>
<td>127 (1.9)</td>
<td>128.4 (2.1)</td>
<td>123 (2.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Changes (6 weeks -baseline)</td>
<td>0.4 (0.5)</td>
<td>-1.5 (1.4)</td>
<td>-7.5 (1.1)***</td>
<td>&lt;0.0005</td>
<td>b, c</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>81.4 (1.9)</td>
<td>84.7 (1.2)</td>
<td>82.3 (1.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 weeks</td>
<td>81.6 (1.2)</td>
<td>83.4 (1.2)</td>
<td>80.7 (1.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Changes (6 weeks -baseline)</td>
<td>0.2 (0.4)</td>
<td>-1.1 (0.8)</td>
<td>-1.6 (0.6)*</td>
<td>0.117</td>
<td>NS</td>
</tr>
</tbody>
</table>

†Values are geometric means (SEM).
*Represents within-group difference of p<0.05, **p <0.005, and ***p<0.0005.
a: significant changes between control and LGI. b: significant changes between control and energy-restricted LGI group. c: significant changes between LGI and energy-restricted LGI group
NS, not significant
Figure 4.3 Weight changes during the trial. A: changes from baseline with different diet, $p<0.0005$. B: absolute weight loss in the LGI plus weight loss diet group. Each bar represent an individual participant. ***significantly different from baseline, $p<0.0005$. a: changes from baseline significantly different from the control and LGI group.
WC at baseline was not different between the groups. The Average WC (cm) for the whole study participants at baseline was 108.9±13.1 (SD) which placed participants in a high risk category according to NCEP ATP-III criteria. At week 6 there was a significant reduction in WC in both intervention groups. Participants in the LGI diet group decreased their WC by 3% ($p=0.01$), whereas participants in the energy-restricted LGI group had a 6% reduction from baseline ($p<0.0005$) with no changes in the control group ($p=0.926$). The energy-restricted LGI group showed a decrease in their WC relative to the control (Figure 4.4, $p=0.001$). Changes in the WC for the participants in the intervention groups was associated with changes in the GI of the diet ($r=0.404$, $p=0.004$).

4.8.4. Composition of the diet

Dietary information was obtained from all participants by 24-hour recall before the trial (habitual diet) and then on the 1st, 7th, and on day 15 during the run-in period. The recalls were also collected three times within the intervention period; in week 2, 4, and 6. An average of the three days for each phase of the study was taken for analysis. It should be clarified here that the habitual dietary intakes were included in the figures only for comparisons with the run-in diet in order to show that the latter was representative of the habitual intake and not confounded by mis-reporting as a result of inclusion in a trial. There were no changes in the habitual diet relative to the run-in diet. Changes between diets were subsequently assessed using only the difference between the run-in and the intervention diets.

4.8.4.1. Energy content of the diet

The overall average habitual energy intakes (kcal/d-KJ/d) for participants was 2430.7±70.6-10209±296.5 (mean±SEM). There was no differences in energy intake between groups or between males and females. Over the dietary intervention period, mean energy intake was reduced within all dietary groups (2%, 1%, and 19% for the control, LGI and energy-restricted LGI groups respectively), but with a significant reduction only in the energy-restricted LGI diet group when compared to the control and the LGI diet group ($p<0.0005$; Figure 4.5).
A decrease of 3500 kcal/week (14700 KJ/week) can be estimated to achieve a weight loss of approximately 0.5 kg/week. Therefore a 3290 kcal (13818 KJ) decrease (470*7) of calories in the energy-restricted LGI diet group was estimated to achieve a weight loss of 0.47 kg/week. In the present study, subjects in the energy-restricted group had a mean weight loss of 2.4 kg (SD=1.4) over a 6 week period. This is equivalent to a weight loss of 0.4 kg/week.

**Figure 4.4.** Changes in A: BMI (kg/m²) and B: WC (cm) between groups over 6 weeks. *significantly different from baseline, p<0.05 and **p<0.005. Different letters indicate that changes from baseline on the different diets differed significantly, p<0.005.
4.8.4.2 Macronutrient composition of the diet

Macronutrient intakes of participants in the three groups are shown in Table 4.4. At baseline, there was no significant difference in the total energy intake or the percentage energy from CHO, protein, T.Fat, S.fat, MUFA or PUFA between the groups. The energy-restricted LGI group decreased their intake of CHO significantly ($p=0.004$) relative to the control but not the LGI group. Total fat intakes also decreased significantly for the energy-restricted LGI diet group compared to both the control and the LGI diet group. Therefore the decrease in total energy intake in the energy-restricted LGI group was brought about by a decrease in both the CHO and the total fat of the diet but with no changes in the overall contribution of these macronutrient to the total energy/day (Figure 4.6 and 4.7). Consumption of dietary fibre was not different between groups at baseline. The mean intakes of fibre for the whole sample was 24.0g±5.6 (SD) at baseline. This did not change for any of the groups over the intervention period (Table 4.4).

![Figure 4.5](image)

**Figure 4.5.** Changes in total energy (kcal/d) between groups over 6 weeks. ***significantly different from baseline, $p<0.0005$. a: changes from baseline significantly different than control and LGI group, $p<0.0005$. 

114
<table>
<thead>
<tr>
<th></th>
<th>ANOVA (d)</th>
<th>Powerwise Test</th>
<th>Energy-Restricted LCT</th>
<th>LCT (d=19)</th>
<th>Control (d=0)</th>
<th>Nutrient/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>50000, &gt;</td>
<td>11.7 (0.7)</td>
<td>128.3 (0.7)</td>
<td>21.9 (0.7)</td>
<td>9.7 (0.5)</td>
<td>7.9 (1.3)</td>
<td>Baseline</td>
</tr>
<tr>
<td>5000, &gt;</td>
<td>9 (0.5)</td>
<td>194.1 (1.3)</td>
<td>22.7 (0.7)</td>
<td>9.3 (1.3)</td>
<td>7.9 (1.6)</td>
<td>Baseline</td>
</tr>
<tr>
<td>2000, &gt;</td>
<td>9 (0.5)</td>
<td>194.1 (1.3)</td>
<td>22.7 (0.7)</td>
<td>9.3 (1.3)</td>
<td>7.9 (1.6)</td>
<td>Baseline</td>
</tr>
<tr>
<td>SN</td>
<td>0.96</td>
<td>9.3 (1.3)</td>
<td>7.9 (1.6)</td>
<td>Baseline</td>
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<td></td>
</tr>
<tr>
<td>q</td>
<td>9.3 (1.3)</td>
<td>7.9 (1.6)</td>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SN</td>
<td>0.96</td>
<td>9.3 (1.3)</td>
<td>7.9 (1.6)</td>
<td>Baseline</td>
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<td></td>
</tr>
<tr>
<td>q</td>
<td>9.3 (1.3)</td>
<td>7.9 (1.6)</td>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table: A4.1** (Kcal/LK) and macronutrient intakes (g) per day in each group at week 0 (baseline) and week 6. Values are means (SEM).
Figure 4.6. Changes in daily energy from A: CHO and B: protein as total energy/day over 6 weeks for the different groups. Values represent medians with interquartiles.
Figure 4.7. Changes from baseline in % of total daily energy from A; T.Fat, B: S.Fat, C: PUFA, and D: MUFA in each group. Values represent medians with interquartile.

4.8.4.3. GI and GL of the diet

The average GI of the habitual diet for the whole sample was 73%±6.8 (SD). There was no significant difference in the mean dietary GI between groups at baseline. After six weeks, both the LGI and the energy-restricted LGI group decreased their dietary GI significantly compared to the control group (p<0.0005) with no statistical differences between these two groups. The percentage reduction in the GI of the LGI diet group was
12.5% (1.5), while the percentage reduction in the GI of the diet of the energy-restricted LGI group was 10% (1.5). The average GI intakes of participants is presented in Table 4.4, whilst changes between baseline and the end of the study are shown in Figure 4.8.

Figure 4.8. Changes in A: GI and B: GL of the diet. **significantly different from baseline, $p<0.005$, ***$p<0.0005$. Different letters indicates that changes from baseline on different diets differed significantly, $P<0.0005$.

Baseline GL for the participants in the three groups was similar (Table 4.4). Over the intervention period, the energy-restricted LGI group decreased their GL significantly compared with the control ($p<0.0005$), and the LGI diet group ($p<0.005$). Changes in the
GL were positively associated with changes in total carbohydrate intakes ($r=0.412$, $p=0.003$), but not changes in the GI of the diet ($r=0.233$, $p=0.103$).

Participants’ knowledge within the intervention groups improved significantly over time ($p<0.05$) as measured by the correct number of responses in the GI knowledge questionnaire with no significant difference between the two intervention groups ($p=0.487$). Changes in the GI of the diet were not associated with improvement in GI knowledge ($r=-0.08$, $p=0.647$).

4.8.5. Changes in glycaemic control

4.8.5.1. Changes in fasting glucose

Plasma glucose was not normally distributed even after log transformation. Therefore data was analyzed using Kruskal-Wallis test. Analysis revealed no significant change in glucose concentrations between the groups ($p=0.366$; Figure 4.9.), despite the observation that plasma glucose concentrations were lower by 10% in the LGI group compared to 3% in both the control and the energy-restricted LGI groups.

4.8.5.2. Changes in fructosamine concentrations

Baseline concentrations for fructosamine were higher in the LGI group compared with the control and the energy-restricted LGI groups ($p<0.008$). ANOVA for the differences in the concentration of fructosamine within each group was used to compare between groups and a comparison within each group was made to study the changes using a paired sample t-test. Analyses revealed that there was no significant change in the concentration of plasma fructosamine in the control group but a significant decrease in both the LGI ($p<0.005$) and the energy-restricted LGI groups over time ($p<0.0005$). Comparison between the groups showed that the LGI group had a significantly lower fructosamine concentration relative to the control ($p=0.011$) but not the energy-restricted LGI group ($p=0.752$). There was also a tendency for plasma fructosamine to decrease in the energy-restricted LGI group compared with the control group ($p=0.055$). These results are shown in Table 4.5 and the changes within each group are presented graphically (Figure 4.9).
Figure 4.9. Changes in A: fasting plasma glucose concentrations (mmol/l) and B: fructosamine concentrations (µmol/l) between groups over the intervention period. ** Significantly differently from baseline, \( p<0.005 \), ***\( p<0.0005 \). Different letters indicates that changes from baseline on the different diets differed significantly, \( p<0.05 \).
4.8.5.3. Changes in insulin concentrations and HOMA-IR

At baseline, there was no statistical difference between the three groups. There was a 23% reduction in plasma insulin in the LGI diet group ($p=0.005$) and a 13% reduction in the energy-restricted LGI group ($p=0.036$) but no change in fasting concentrations in the control group ($p=0.652$). After 6 weeks, there was no statistical difference in plasma insulin between the intervention groups (Table 4.5) but the difference between the control and LGI group was of borderline significance ($p=0.066$).

Baseline HOMA-IR between groups was significantly different for the three groups ($p=0.029$). A between group comparisons of the changes in HOMA-IR in each group indicated no significant difference between groups (Table 4.5). There was also no association between changes in HOMA and weight loss ($r=-0.163$, $p=0.259$).

A HOMA-IR index of $>2$ was taken to indicate IR (Balkau et al., 1999). A chi-squared test of independence (Table 4.6) indicated that there was no significant changes in the number of participants with insulin resistance in the control group ($p=1.00$), LGI group ($p=0.149$), or the energy-restricted LGI group ($p=0.70$), although decreases in the number of participants with HOMA-IR were seen in the latter two intervention groups.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline (mmol/L)</th>
<th>Changes (6 weeks)</th>
<th>Insulin (nmol/L)</th>
<th>Furosemide (mmol/L)</th>
<th>Glucose (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>0.029 (0.03)</td>
<td>-1.0 (0.021)</td>
<td>2.6 (0.047)</td>
<td>9.8 (0.069)</td>
<td>2.8 (0.063)</td>
</tr>
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<td>Trend in a</td>
<td>0.13 (1.13)</td>
<td>-1.93 (1.11)</td>
<td>4.49 (0.3)</td>
<td>1.17 (1.12)</td>
<td>0.69 (1.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>110.0</td>
<td>-3.6 (0.3)</td>
<td>4.4 (0.3)</td>
<td>3.1 (0.3)</td>
<td>8.3 (0.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>99.6 (0.3)</td>
<td>-2.38 (0.05)</td>
<td>4.38 (0.002)</td>
<td>1.82 (0.06)</td>
<td>7.35 (0.013)</td>
</tr>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

TABLE 4.5: Changes in glycemia and insulinemia over the intervention period.
Table 4.6. Chi square test of independence for HOMA-IR.

<table>
<thead>
<tr>
<th>Group</th>
<th>HOMA-IR&gt;2</th>
<th>HOMA-IR&lt;2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Control (n=16)</td>
<td></td>
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<tr>
<td>Baseline</td>
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<td>6 weeks</td>
<td>9</td>
<td>56.3</td>
</tr>
<tr>
<td>LGI (n=16)</td>
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<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>9</td>
<td>56.3</td>
</tr>
<tr>
<td>6 weeks</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>Energy-restricted LGI (n=18)</td>
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<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>8</td>
<td>44.4</td>
</tr>
<tr>
<td>6 weeks</td>
<td>6</td>
<td>33.4</td>
</tr>
</tbody>
</table>

4.8.6. Changes in the features of an ALP

4.8.6.1. Changes in plasma TAGs

At baseline there was no significant difference in plasma TAG between the three groups. After 6 weeks of intervention there was a 12% increase in plasma TAG within the control group (p=0.037), whilst plasma TAG in the LGI and the energy-restricted LGI group decreased by 12% (p=0.006) and 16% respectively (p<0.0005). When the groups were compared, both the LGI and the energy-restricted LGI group showed decreased plasma TAG (p<0.0005) relative to the control. These results are shown in Figure 4.10 and Table 4.7. Changes in plasma TAG were associated with changes in WC (r=0.261, p=0.068). Other correlations are shown in Figure 4.11

4.8.6.2. Changes in T. Cholesterol and LDL-C

At baseline, participants in each group were similar in terms of their T.Cholesterol and LDL-C concentrations. Over time, there were no significant changes in the concentration of T.Cholesterol within or between groups, but there was a reduction in plasma LDL-C concentration in all three groups (p=0.001). Decreases of 5%, 9%, and 10% were observed for the control, LGI, and the energy-restricted LGI groups respectively. However, the percentage reduction was significant for both the LGI (p=0.006) and the
energy-restricted LGI group \((p=0.003)\) when compared with the control group with no significant difference between the two intervention groups. The ratio of LDL:HDL after 6 weeks dietary intervention was also lower than at baseline \((p<0.0005)\).

### 4.8.6.3 Changes in HDL-C

The three groups had similar concentrations of plasma HDL-C at baseline. After 6 weeks, the concentration of HDL-C increased significantly from baseline in the LGI diet group \((p<0.001)\) and the energy-restricted LGI group \((p<0.005)\). ANOVA indicated a significant difference between groups in HDL-C concentration \((p<0.0005)\). Post-hoc analyses indicated that there was a significant increase in HDL-C concentrations in the LGI and the energy-restricted LGI diet groups relative to the control group \((p=0.006\) and \(p=0.003\) respectively), but no significant difference between the two intervention groups (Table 4.7 and Figure 4.10).

### 4.8.6.4. Changes in plasma LDL and HDL subclasses

The majority (92%) of the participants expressed a predominance of sdLDL (%AUCB≥50%; LDL subclass pattern B). Of the remaining participants, 4% had a predominance of larger, buoyant LDL (Pattern A; sdLDL<40, a predominance of LDL-II) and 4% were of an intermediate pattern I (Pattern I; 50>sdLDL>40). By the end of the intervention period, one participant had switched from pattern B to pattern I. The proportion of sdLDL was significantly associated with plasma TAG at the beginning \((r=0.35, P=0.013)\) and the end of the trial \((r=0.34, p=0.018)\) in the control, LGI, and LGI plus energy restricted diet groups. Pattern B subjects had a mean plasma TAG and HDL-C concentration of 2.1±0.9 (SD) and 0.83±0.2 respectively at the beginning of the trial and 1.9±0.9 (SD) 0.92±0.2 respectively at the end of the trial. Participants with pattern A had a TAG and HDL-C concentration of 1.5±0.4 and 1.02±0.2 at the beginning of the trial and 1.4±0.2 and 1.0±0.2 respectively after 6 weeks intervention.
Weeks. Changes from baseline for the different diets were significantly different from the control group, p<0.05.

Figure 4.10. Changes in A: Plasma TAG (mmol/l), B: HDL-C (mmol/l) and C: LDL-C (mmol/l) concentration between groups over 6 weeks.
<table>
<thead>
<tr>
<th>Variable</th>
<th>p</th>
<th>F(1, n) = 16.6</th>
<th>95% CI</th>
<th>EC5 (n=10)</th>
<th>EC5 (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides (TG)</td>
<td>&lt; 0.0005</td>
<td>-0.03 to -0.07</td>
<td>-0.03 to -0.07</td>
<td>0.01 to 0.03</td>
<td>0.01 to 0.03</td>
</tr>
<tr>
<td>Cholesterol (CH)</td>
<td>&lt; 0.0005</td>
<td>-0.03 to -0.07</td>
<td>-0.03 to -0.07</td>
<td>0.01 to 0.03</td>
<td>0.01 to 0.03</td>
</tr>
<tr>
<td>HDL-C (HDL)</td>
<td>&lt; 0.0005</td>
<td>-0.03 to -0.07</td>
<td>-0.03 to -0.07</td>
<td>0.01 to 0.03</td>
<td>0.01 to 0.03</td>
</tr>
<tr>
<td>LDL-C (LDL)</td>
<td>&lt; 0.0005</td>
<td>-0.03 to -0.07</td>
<td>-0.03 to -0.07</td>
<td>0.01 to 0.03</td>
<td>0.01 to 0.03</td>
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<tr>
<td>NEFA (NEFA)</td>
<td>&lt; 0.0005</td>
<td>-0.03 to -0.07</td>
<td>-0.03 to -0.07</td>
<td>0.01 to 0.03</td>
<td>0.01 to 0.03</td>
</tr>
</tbody>
</table>

N=10. EC5 = Effective Concentration 5. CI = Confidence Interval. Table F.7. Changes in plasma lipids and lipoproteins in each group. Values are means (SEM).
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<th>LCT (n=16) (u=16)</th>
<th>LCT (n=16) (u=18)</th>
<th>Energy-Restricted (n=16)</th>
<th>Energy-Restricted (n=18)</th>
<th>Baseline Peaks Density</th>
<th>Baseline Peaks Density</th>
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</thead>
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<td>SN</td>
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<td>103.0</td>
<td>103.0</td>
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<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

TABLE 48: Changes in lipoprotein constitution and particle number in each group. Values are means ± SEM.
The percentage of the larger HDL$_2$ subclass increased significantly in the two intervention groups after 6 weeks of intervention compared to the control ($p=0.047$ and $p=0.013$ for the LGI and the energy-restricted LGI diet group respectively) with no significant differences between the two intervention groups. An example of the increase in HDL$_2$ subclass in one subject as shown by a computer generated densitometric scan is presented in Figure 4.14. Mean values for the LDL peak density, %AUCB and %HDL$_2$ are shown in Table 4.8, whilst changes in these values across time are presented graphically in Figure 4.12. A strong association was observed between weight loss and the change in the %AUCB ($r=-0.51$, $p=0.032$) in the energy-restricted LGI diet group. Changes in sdLDL were also associated with changes in the WC ($r=0.387$, $p=0.006$).

At baseline apo B and apo A-I were not significantly different between groups. Plasma apoproteins did not change significantly either within or between the dietary intervention groups after 6 weeks. HDL-C and apo A-I were shown to be positively related ($p<0.0005$) at baseline ($r=0.64$) and at 6 weeks ($r=0.67$).

4.8.7. Changes in NEFA

There was no significant difference in the concentration of plasma NEFA between groups at baseline. After 6 weeks intervention, there were significant changes in plasma NEFA in all groups; NEFA concentrations fell in both the LGI ($p=0.033$) and the energy-restricted LGI group ($p<0.0005$) but there was a significant increase in the control group ($p=0.023$). Both the LGI and the energy-restricted LGI diet groups had decreased NEFA concentrations significantly relative to the control group.

4.8.8. Changes in subjective appetite rating

VAS data were collected in 33 participants; 11 in the control group, 10 in the LGI diet group, and 12 in the energy-restricted LGI diet group. At baseline there was no significant difference between groups in scores for hunger ($p=0.161$), desire to eat ($p=0.288$), fullness ($p=0.223$), or prospective food consumption ($p=0.119$). A 2-way ANOVA for the scores after 6 weeks indicated a significant effect of time ($p<0.0005$), and a significant interaction between time and treatment ($p=0.006$, $p=0.019$, $p=0.065$).
$p=0.018$ for the hunger, desire to eat, fullness, and prospective consumption scores respectively), with no difference between groups. These results are shown in Figure 4.15.

**Figure 4.12** Changes in A: % HDL$_2$ and B: sdLDL. Values are means±SEM. *Significantly different from baseline, $p<0.05$, **$p<0.005$, ***$p<0.0005$. Different letters indicate changes from baseline on the different diets. $p<0.05$. 
Figure 4.13 Changes in sLDL (%AUCB) in one participant A: before and B: after 6 weeks of dietary intervention with LGI diet (a reduction from 29.6% to 21.8%).

Figure 4.14 Changes in %HDL₂ in one participant A: before and B: after 6 weeks of dietary intervention with LGI diet (an increase from 28.2% to 36.9%).
Figure 4.15. VAS scores for hunger, desire to eat, fullness, and prospective consumption at baseline and after 6 weeks. Significant interaction between time and treatment for all the scores after 6 weeks, $p<0.05$. 
4.9. Discussion

The present study was undertaken to investigate the effect of a LGI diet on CVD risk in a sample of overweight/obese T2 DM patients in Kuwait, with special attention given to outcomes related to ALP. A secondary objective was to investigate the efficacy of a LGI diet compared to a LGI, energy-restricted, weight-losing diet in improving diabetic dyslipidaemia known as an ALP in overweight/obese T2 DM patients in Kuwait. Due to time constraints and recruitment problems, the investigator was unable to conduct the third part of the study (the follow-up phase).

4.9.1. Dietary intakes and GI

The GI of the diet was calculated as a mean of three GI values obtained from 24-hour recalls collected in each phase of the study. The calculation of GI from food records by others (Wolever et al., 1994) has resulted in normally distributed values, and thus was considered to be appropriate for parametric statistical analyses. Estimation of the GI/GL from 24-hour dietary recalls was also possible and has been used previously to assess the association between these dietary parameters and body weight and outcomes from chronic diseases (Olendzki et al., 2006). The average GI of the three groups at baseline was 73%; this corresponds to a GI that has been previously reported for a diabetic population (Wolever et al., 1994b). The average GI of the diet of T2 DM patients in Kuwait was successfully reduced from 73% at baseline to 61% in 6 weeks; a reduction of 12 units. A similar reduction was reported by others in diabetics and in patients with CHD (Brand et al., 1991; Collier et al., 1988; Frost et al., 2004; Tsilhias et al., 2000; Frost et al., 1994). On average, studies reported a GI reduction of 16 units (Brand-Miller et al., 2003a). Overall, a reduction of 12-14 units (Brand-Miller et al., 2003a; Collier et al., 1988; Fontvieille et al., 1992) has been associated with improved glucose control, serum cholesterol, and TAG concentrations in diabetics. In the present study no significant differences in the changes in GI was observed between the LGI group (12±1.5 units reduction) and the energy-restricted LGI diet group (10±1.7 units reduction).

Both the International Tables of GI (Foster-Powell et al., 2002) and the results of the GI testing of commonly consumed breads and rice in Kuwait (Chapter 3) were used as tools
to construct the LGI diet. The main approach used to lower the GI of the diet for the two intervention groups was the substitution of LGI foods for those with a high GI content. In order to decrease the potential effects of the variations in dietary fibre, dietary GI was changed mainly through altering the physical structure of the starchy foods (whole vs. ground beans or seeds), bread types, and substituting pasta for rice and potatoes in the intervention groups. Both intervention diets were constructed in accordance with the dietary recommendations for patients with diabetes (Franz et al., 2004). The dietary manipulation was carried out in a free living situation where participants had free access to food with no supply of specific LGI foods. Therefore the results should be more reflective of outcomes from a clinical setting rather than a metabolic ward. The finding that the GI of the diet was significantly decreased in the two intervention groups, suggests both palatability of the diet and applicability of the GI concept to the dietary management of T2 DM in Kuwait.

Patient’s GI knowledge of foods improved significantly over time in both intervention groups. This was measured through the GI questionnaire which was also used as an educational tool where any deviation from the correct responses was used to reinforce the nutritional message. Interestingly, changes in GI knowledge were not related to changes in the GI of the diet. This suggests that dietary programs for T2 DM patients in Kuwait should also aim at behavioural changes and not only be directed towards improving dietary knowledge.

In comparison to the ADA recommendations for people with diabetes (Franz et al., 2004), participants in the current study had a baseline percentages of energy from protein (16%), CHO (51%), and T.Fat (33%) that were comparable with these recommendations. The percentages of S.Fat, PUFA, and MUFA, as a percentage of total energy, were also in-line with the recommendations of the ADA (10%, 6%, and 10% respectively) and did not change significantly over the intervention period. These intakes were also close to those reported in other countries including 9960 diabetics in the US (Banini et al., 2003), 132 diabetics in the UK (Eeley et al., 1996), 164 diabetics in Australia (Barclay et al., 2006) and 342 diabetics in Canada (Wolever et al., 1994b). The consumption of CHO
and total fat (g) in the energy-restricted LGI diet decreased significantly compared with the control and the LGI diet groups, whilst the overall percentage contribution of these macronutrients to total energy remained constant.

### 4.9.2. Changes in blood pressure and anthropometry

The reduction in systolic blood pressure that accompanied a reduction in the GI in the current study is of great importance since the UKPDS recognize hypertension (and hyperglycaemia) as an independent and additive risk factor for diabetic complications (Stratton et al., 2006). This reduction was achieved only in the energy-restricted LGI diet group compared with the control and the LGI diet group. Changes in the systolic, but not diastolic, blood pressure were significantly related to weight loss ($r=0.766$, $p<0.0005$). Blood pressure control and lower blood pressures in hypertensive T2 DM patients has been recommended by the ADA (American Diabetes Association, 2004a) as an important strategy to prevent the progression of CVD morbidity and mortality.

The intention was to keep the weight of the individuals in the control and the LGI diet groups constant during the 6 weeks study, while decreasing the weight of those in the energy-restricted LGI diet group. This was achieved in the study through a reduction in the total amount of energy/day in the energy-restricted LGI group while maintaining the GI of the diet similar to people in the LGI diet group. Participants in the energy-restricted LGI group lost a significant amount of weight compared with the control ($p=0.001$) and the LGI diet group ($p=0.017$). Mean weight loss for this group was $2.4\pm1.6$ kg (SD). However, this finding should not be overemphasized as the average weight loss in this group was only 3% and mainly achieved by four participants. Studies, in T2 DM patients, have reported metabolic improvement in glycaemic control, insulin sensitivity and CVD risk factors with a modest weight reduction of 5-10% (Blackburn, 1995; Ditschuneit et al., 2002; Wing et al., 1987a). In contrast, a minimum weight loss of 10%, from baseline body weight, was needed to demonstrate significant improvements in the risk profile (Bosello et al., 1997). Wing and colleagues (1987a) reported that a weight loss of less than 5% in diabetics was not associated with any significant changes in the risk profile. The level of weight loss achieved in the current study through energy
restriction was similar to that which has been shown to accompany the consumption of LGI foods in energy balance studies (Livesey, 2007). The amount of weight loss in this study therefore makes it possible to analyse the effects of LGI separately from the potentially confounding effect of weight loss, mediated through increased satiety (Dumesnil et al., 2001). In the energy-restricted group, no association between weight loss and changes in dietary GI was found. Instead, changes in the BMI were strongly associated with changes in total energy intakes (Kcal/d; $r=0.987$, $p<0.0005$). This finding, in addition to the observation that participants in the LGI diet group did not lose weight, suggests that weight loss in this study was produced by decreased energy intake/day and was not related to decreased appetite as a result of the consumption of the LGI diet. This finding was consistent with other studies that have tested dietary GI with respect to weight changes and reported negative outcomes (Kabir et al., 2002; Sloth et al., 2004). It should be emphasised that the changes in some scores of the VAS questionnaire were initially due to difference in the scores for the groups at the start of the study. Therefore it seems that the LGI diet in the current study did not produce significant changes in the hunger or satiety among groups who consumed the LGI diet.

A significant reduction in WC was observed within both the LGI and the energy-restricted LGI diet groups. The reduction of WC within the LGI diet group is of particular interest. The importance of this arises from the finding that the decrease in WC within this group was achieved without energy restriction or weight loss. This is certainly of relevance since weight loss in obese individuals represents a serious challenge, especially in T2 DM as a result of their poor dietary compliance (Wing et al., 1987b). This is also important as a reduction in central abdominal fat has been shown to be related to a less atherogenic lipid profile (Markovic et al., 1998; Sharp et al., 2003). Changes in WC in the current study were associated with changes in the GI of the diet ($r=0.404$, $p=0.004$). It seems, therefore, that the LGI diet in the current study, whilst not affecting weight loss, had the potential to decrease abdominal fat not through regulation of appetite but rather through other mechanisms that might involve the lowering of hyperinsulinaemia (Dumesnil et al., 2001). Increased abdominal fat tissue is often associated with increased lipolysis and elevated plasma concentrations of NEFA which
elevates hepatic TAG synthesis as a result of increased substrate availability (Frayn, 2001). Thus, the decreased WC in the LGI diet group is consistent with the lowering of circulating NEFA concentrations. In light of this finding, the conclusion drawn by the European Group for the Study of Insulin Resistance that WC and BMI are equally strong indicators of the risk of insulin resistance is important. In addition McLaughlin et al. (2004) found that insulin resistance might exist in a significant proportion of the normal weight population. This highlights the importance of the LGI approach in decreasing markers of insulin resistance, not only in obese people, but also in centrally obese insulin resistance individuals.

4.9.3. Effects of GI on glycaemia and insulinaemia

When obese T2 DM patients consumed a LGI meal, the overall glycaemic response was 40% lower and the peak glucose concentration was 25% lower in the LGI meal group compared to an isoenergetic high GI meal (Cohen et al., 1990). Additionally, studies have also suggested that a high concentrations of fasting plasma glucose may be reduced by decreased dietary GI (Heilbronn et al., 2002). The increased risk of CVD with the habitual consumption of high GI meals is believed to be partly related to hyperglycaemia induced oxidative stress (Ludwig, 2002). Although participants in the current study had a high concentration of fasting glucose at baseline (9.7 mmol/l ± 3.7 SD), no changes were observed in these concentrations on the intervention diets. However, the lack of significance, whilst not expected, was in agreement with many previous studies (Brand et al., 1991; Collier et al., 1988; Jenkins et al., 1985; Jenkins et al., 1987; Wolever et al., 1992). A meta-analysis of eleven studies by Brand-Miller (1994), eight of them in diabetics, reported an average reduction of only 5% in mean plasma glucose concentrations after a LGI diet. For the most part, the reduction was associated with weight loss (Wolever et al., 1992). In contrast, weight loss, in the present study, did not affect glucose concentrations in the energy-restricted LGI group, as changes in these values did not show any relationship with changes in weight, possibly because of the low amount of weight lost.
Glycaemic control, as measured by fructosamine concentrations, improved significantly within both intervention groups but only significantly in the LGI group when compared with the control group (p=0.011). Measurement of fructosamine concentrations are favoured as a short term marker in studies that last for 6 weeks as the effect is reported to change maximally in 4-6 weeks (Jones et al., 1983). There is compelling evidence that postprandial glycaemia is an important risk factor that needs to be controlled in both type 1 (Diabetes Prevention Program Research Group, 2002) and T2 DM patients (UKPDS, 1998). Studies are conclusive on the effect of a LGI diet on glycaemic control in T2 DM patients. In a recent meta-analyses by Opperman et al. (2004), seven randomized control trials that measured fructosamine concentrations were included. Mean fructosamine concentrations were reduced significantly on a LGI diet compared with high GI diets (-0.1 mmol/l, p=0.05) over a period of 4.6 (SD 3) weeks. Previous to this , another meta-analysis, of 10 studies that investigated the effect of LGI diet on glycaemic control in T2 DM patients (Brand-Miller et al., 2003a), showed that over a 4 week period, mean fructosamine concentrations were 0.2 mmol/l lower than baseline (range 0.04–0.35). Improvement in glycaemic control with the consumption of LGI diets has been achieved even in a very short term study that lasted for only two weeks (Jenkins et al., 1988a). Indeed, the longer the consumption of a LGI diet, the larger the decreases in fructosamine concentrations (Brand-Miller et al., 2003a). These studies, with others that were not included in the meta-analyses mentioned above (Salmeron et al., 1997a; Salmeron et al., 1997b), all reported an improvement in glycaemic control with a LGI diet. Even when the improvement in glycaemic control was not significant, such as in the study of Heilbronn et al.(2002), HbA1c was reduced by -2.8%; two-fold more in subjects consuming a LGI energy-restricted diet when compared to subjects consuming a high GI, energy restricted-diet. A LGI diet may improve glycaemic control by lowering early postprandial hyperglycaemia and decreasing the risk of postabsorptive hypoglycaemia (Ludwig, 2002). Only a few studies have reported negative results with the use of LGI diets in diabetics (Cohen et al., 1990; Tsihlias et al., 2000; Luscombe et al., 1999). most probably because the GI was not reduced sufficiently, or due to the small sample size. Therefore the use of LGI in the dietary management of T2 DM in Kuwait would seem to be advantageous.
It is important to note that even when fasting glucose concentrations were unchanged in the LGI group, a fall in the concentration of fructosamine, as an index of overall glycaemic control, was apparent in this group ($p<0.0005$). This suggests that changes in the fructosamine are not necessarily due to a decreased glucose response postprandially (Jenkins et al., 1988a). In addition, diet may influence protein glycation by other mechanisms such as the rate of protein turnover or antioxidant status (Davie et al., 1992). However, the uncoupling between the concentrations of fructosamine and fasting glucose with the use of LGI has been previously reported (Wolever et al., 1992; Fontvieille et al., 1992; Wolever & Mehling, 2003; Jenkins et al., 1987). The selection of participants in the present study was not based on well controlled diabetes, as participants were recruited mostly from the out-patient clinic in the Amiri hospital where most of the patients are referred from primary clinics as a result of poor diabetic control. Therefore it could be argued that changes in the glycaemic control due to dietary modifications are not referred to those associated with less well controlled diabetes. However, studies on diabetic subjects with obesity, high fasting plasma glucose, and dyslipidaemia showed similar and sometimes more favourable outcomes (Fontvieille et al., 1992; Dumesnil et al., 2001).

All participants in the current study had elevated fasting insulin concentrations at baseline (129.6±16.9 pmol/l (SEM)). When tested individually, there was a significant reduction in these concentrations both within the LGI diet group ($p=0.005$) and the energy-restricted LGI diet group ($p=0.036$). The percentage reduction in the insulin concentration observed in the LGI diet group was almost double the reduction of the concentration in the energy-restricted LGI group (23% vs. 13% reduction). However, whilst this did not reach significance, and the changes in the former group was of borderline statistical significance when compared to the control group ($p=0.066$). An association between the GI of the diet and insulin concentrations is well documented; decreased hyperinsulinaemia and increased insulin sensitivity is often associated with a LGI diet (Rizkalla et al., 2004; Miller, 1994; Jarvi et al., 1999; Liese et al., 2005; Frost et al., 1998). On the other hand, a high GI diet may stimulate more insulin secretion than a LGI diet because of the relative postprandial hyperglycaemia and increased incretin.
levels (Ludwig, 2002), such as glucose-mediated insulinotropic polypeptide (GIP). Although weight-reducing diets have a powerful insulin sensitizing effect (Wing et al., 1987a; Wing et al., 1994), in the present study this was not different from the LGI or the control group, as weight loss, whilst statistically significant, was not clinically significant (Blackburn, 1995; Ditschuneit et al., 2002; Wing et al., 1987a). Moreover, the lack of significant insulin reduction in the energy-restricted group might be due to the reduced intake of carbohydrate during energy restriction which could potentially reduce the insulin sensitizing ability of the LGI diet (Heilbronn et al., 2002). Wing and co-workers (1994) noted that the amount of energy restriction and weight loss has independent effects on glycaemic control and insulin sensitivity in T2 DM patients. Insulin resistance in the current study was defined by HOMA-IR >2 (Balkau et al., 1999). 31% of the insulin resistant subjects in the LGI diet group in the present study became more insulin sensitive as compared to 11% in the energy-restricted LGI group with the number of participants with insulin resistance in the control group remaining constant (56%). These findings suggest that the number of individuals with insulin resistance was decreasing in the intervention groups but the study was probably underpowered for this measure.

4.9.4. Effects of GI on an ALP

4.9.4.1. Effect on plasma TAGs

One of the main findings of the current study was the favourable effects produced on plasma TAG in overweight/obese T2 DM patients by using a dietary approach that placed emphasis on the use of LGI foods. This finding extends data from previous short term studies that showed a beneficial reduction of TAG concentrations with the use of LGI diets in subjects with elevated baseline plasma TAG and/or T2 DM (Dumesnil et al., 2001; Fontvieille et al., 1992; Frost et al., 1994; Jenkins et al., 1985; Wolever et al., 1992; Jenkins et al., 1987). Reductions in plasma TAG have reached 20% in one study (Wolever et al., 1992) compared to a control diet and 35% (from 2.0 to 1.3 mmol/l) in only 6 days in T2 DM patients in another study (Dumesnil et al., 2001). Studies have clearly shown that elevated plasma TAG is an independent risk factor for CVD (Austin et al., 1998). Nonetheless, some studies have shown negative outcomes with respect to
TAG concentrations (Sloth et al., 2004; Kabir et al., 2002; Frost et al., 2004; Jimenez-Cruz et al., 2003).

A meta-analysis of over 70 studies concluded that reduction of a kilogram in body weight is associated with a 0.015 mmol/l decrease in plasma TAG (Dattilo & Kris-Etherton, 1992). Although there was a 16% fall in TAG concentrations in the energy-restricted LGI group compared to a 12% reduction in the LGI diet, the difference did not reach statistical significance and there was no additive effect of weight loss on TAG concentrations. This finding might suggest that the amount of weight loss achieved by participants in this study was not enough to substantially affect TAG concentrations. As mentioned previously, the study was designed to decrease the weight of subjects in the energy-restricted LGI group by 5-10%. However, this was not achieved successfully and only a 3% reduction was obtained. This is further supported by a lack of association between weight loss and changes in TAG concentrations in the energy-restricted group (p=0.78).

Changes in TAG concentrations were associated with changes in the GI of the diet (r=0.35, p=0.015). One of the possible explanations for a LGI-induced lowering of plasma TAG is through a decreased production of VLDL by the liver as a result of increased insulin sensitivity, limiting the availability of NEFA for VLDL production (Martins & Redgrave, 2004). It is also possible that an increase in insulin sensitivity that is associated with LGI diet could increases the rate of clearance of TAG concentrations through stimulation of LPL. There is evidence for a decrease in the rate of TAG clearance as a result of decreased LPL activity in T2 DM patients (De Man et al., 1996). Decreased TAG clearance is also known to have a detrimental effect on LDL heterogeneity (Griffin & Fielding, 2001). Moreover, the LGI diet is expected to contain larger amount of resistant starch. Resistant starch is fermented by the colonic microflora producing short chain fatty acids which have been shown to exert metabolic effects on lipids through the reduction of VLDL production (Marcil et al., 2002). It appears that central adiposity is more relevant, within the context of GI, than body weight in inducing
favourable changes in plasma TAG as indicated by the trend in the relationship between changes in TAG concentrations and changes in WC ($r=0.261, p=0.068$).

Despite the fact that the participants in the control group received no intervention, an unexpected increase in plasma TAG was observed within this group. This finding was surprising given that the lipid profiles for these participants were stable as compared with the previous lipid profile (6 months). However, individual biological variation in plasma TAG concentrations has been previously reported and may explain this result (Nazir et al., 1999). This finding emphasises the importance of a RCT design that is able to accommodate such changes. It is consistent with the rapid progression of macrovascular complications within this population in Kuwait, suggesting the relevance of this study to the Kuwaiti population in the context of diabetes risk management. In Kuwait, about 75% of patients with T2 DM had either mixed hyperlipidaemia or predominant hypertriglyceridaemia (Akanji, 2002) a major CVD risk factor. Additionally, in 108 Arabic diabetic patients, the only significant difference in lipid concentrations between diabetic patients with and without CAD was for plasma TAG (Daghash et al., 2007). In the present study, two participants in the control group had hyperchylomicronaemia manifested by the presence of visibly lipaemic plasma both in baseline and post trial samples. Most of these patients have an underlying lipid disorder, such as LPL deficiency, that is usually exacerbated by diabetes (Julien et al., 1997). The plasma TAG of these two subjects made a significant contribution to the overall change in the control group.

4.9.4.2. Effect on T. Cholesterol and LDL-C

There were no changes within or between diets with respect to plasma total cholesterol concentrations. This finding was not surprising given that some of the largest epidemiological studies to date, such as the Survey of British Adults (Frost et al., 1999) and the Zutphen Elderly Study (van Dam et al., 2000) failed to show any relationship between LGI diet and total plasma cholesterol. Even in a long term study of 6 months (Tsihlias et al., 2000) there was no effect of LGI breakfast cereal consumed by T2 DM patients on total plasma cholesterol compared to high GI cereal. Studies which have
reported a reduction in the concentration of T.Cholesterol in T2 DM patients with a LGI diet achieved a reduction of 19 GI units (Opperman et al., 2004). with a larger decreases in cholesterol being reported in patients with elevated baseline concentrations (T.Cholesterol>5.2 mmol/l). Others have shown a significant reduction in total cholesterol with an even greater reduction of 26 GI units (Fontvieille et al., 1992). Therefore, the lack of significance might be explained by the smaller reduction in GI achieved in the current study. Moreover, mean baseline cholesterol concentrations in participants were below the 5.2 mmol/l cut-off for high cholesterol concentration. It should also be remembered that total cholesterol concentration is now recognised to be only a weak predictor of CHD risk within populations (Fruchart & Packard, 1997).

A pronounced reduction in LDL-C in subjects with T2 DM has been previously reported when the GI of an energy-restricted diet was reduced (Heilbronn et al., 2002). This study involved 45 male and female subjects who were followed over 12 weeks of energy restriction. A 13.2% reduction of LDL-C was found, with the LGI diet lowering LDL-C to a greater extent than the high GI diet (p = 0.02). In weight maintenance studies (Jarvi et al., 1999; Jenkins et al., 1985), LGI diets have been shown to reduce LDL-C compared to high GI diets in subjects with raised plasma TAG concentrations and in T2 DM patients with poor glycaemic control.

The majority of participants (92%) in the current study had a predominance of sdLDL (pattern B) at baseline. After 6 weeks intervention, while only one participant switched from pattern B to I, a significant reduction in the concentration of sdLDL, in the intervention groups compared to the control group (p=0.001) was evident with no difference between the intervention groups. A raised plasma TAG concentration is a critical determinant of LDL particle size (Griffin, 1997) therefore, a reduction in sdLDL can only be observed when TAG decreases concomitantly (Halle et al., 1999). Baseline plasma TAG concentrations were elevated; TAG>1.5 mmol/l. This concentration of TAG promotes the transfer of plasma TAG from TAG rich lipoproteins to LDL and HDL in exchange for cholesteryl esters leading to the formation of sdLDL (Griffin, 1997) and increased HDL clearance (Lamarche et al., 1999). A reduction in plasma TAG reduces
CVD risk by redistributing LDL toward larger, less dense, and thus less atherogenic LDL particles (Griffin, 1997). In the current study, no changes in the number of LDL particles, as indicated by plasma apo B, was found between or within diets. Previous studies have reported a reduction in apo B concentrations in response to LGI diets (Rizkalla et al., 2004; Jarvi et al., 1999). A predominance of sdLDL and raised LDL particle number (raised plasma apo B concentration) frequently co-exist in the dyslipidaemia found in T2 DM (Tan et al., 1995). In the present study, none of the study population presented with hyper-apo B (apo B>1.3 g/l) at the beginning or at the end of the intervention period. This was an unexpected finding since most of the subjects were pattern B with a predominance of sdLDL. However, this uncoupling between the quantity and quality of LDL particles was previously reported in free living groups with an ALP when TAG concentrations increased over 2.5 mmol/l (Griffin et al., 1999). This observation led the author to suggest that there might be a distinct mechanisms for the regulation of LDL particle number and the distribution of LDL particle size. It should, therefore, be emphasised that the effect of the diet on CHD risk in at least 16% of the study population be interpreted with caution as CVD risk may be overestimated in these individuals (Griffin et al., 1999). In the current study, despite the reduction in WC within the intervention groups, no changes were detected in the concentration of apoB in these groups. Apolipoprotein B was previously shown to be very sensitive to the accumulation of abdominal fat (Despres & Lamarche, 1993). This could be due to the fact that baseline apo B was not originally elevated and that a longer period of time was needed for these changes to take place.

4.9.4.3. GI and the effect on HDL-C

The plasma concentration of HDL-C is well known to be inversely associated with CHD in T2 DM (Leelawattana et al., 2003). A low plasma HDL-C concentration is associated with an increased rate of HDL clearance (Brinton et al., 1991), which in turn is increased when HDL is enriched in TAG (Lamarche et al., 1999). HDL-C is claimed to be a better discriminator of CHD than LDL-C and for every 0.026 mmol/l increase in HDL-C, CHD is reduced by 3% (Castelli, 1988). In the current study, the mean HDL-C concentration increased significantly in both the LGI and the energy-restricted LGI groups compared to
the control group, with no difference between these two groups. This increase was consistent with results found in the 1987 survey of British adults (Frost et al., 1999) and the study of US adults (Ford & Liu, 2001). The latter study clearly showed a significant reduction of HDL-C of -0.06 mmol/l per 15 unit increase in GI, after adjusting for gender, BMI, smoking, alcohol intake and physical activity, after the consumption of high GI foods. In the former study, a 29% reduction in CHD morbidity in women and 7% in men was associated with a difference between the lowest and highest quintiles of GI. An increase in HDL-C was expected in these studies because LGI foods are associated with decreased hepatic gluconeogenesis and a prolonged suppression of NEFA release for VLDL synthesis (Rizkalla et al., 2002). Studies that did not show an increase in the concentration of HDL-C compared to high GI diets (Heilbronn et al., 2002; Jarvi et al., 1999; Jenkins et al., 1988a) were usually of short duration (Opperman et al., 2004). More importantly, the proportion of HDL₂ in the present study increased significantly in both of the intervention groups compared to the control group. This is important as studies indicated that any benefit underlying elevated HDL cholesterol concentrations could be mainly attributed to the HDL₂ subfraction (Salonen et al., 1991). The increase in % HDL₂ observed within the intervention groups compared to the control group with the consumption of LGI diet might be a consequence of decreased VLDL production and CETP-mediated exchange of lipid. It could also be attributed to enhanced HL activity in these individuals with LGI diet, as a result of insulin sensitivity (Martins & Redgrave, 2004). While overweight/obese diabetic men and women showed changes in both HDL-C and HDL particle profile (%HDL₂), there were no significant changes in plasma apo A-I in the intervention groups, despite associations between the HDL-C and apo A-I particles both at baseline and after intervention. With many key metabolic factors participating in the reverse cholesterol transport, it is difficult to conclude which mechanism is specifically involved with respect to apo A-I. Overall, the correlation between changes in TAG concentrations with changes in LDL size (r=-0.280, p=0.049) and HDL size (trend in the correlation; r=-0.239, p=0.094) were consistent with the concept that lipoprotein changes are driven by changes in plasma TAG concentrations.
4.9.5. Implications of the findings for the Kuwaiti population

One important outcome that might be of particular interest to the health professionals and nutrition care providers in Kuwait is that lowering the GI of the diet produced a favourable improvement in the diabetic risk profile that might be easier to implement in terms of compliance than dietary manipulations designed to cause weight loss. To the best of our knowledge this study was the first to involve any dietary manipulation in Kuwait both in terms of GI and/or energy restriction. A dietary factor is considered beneficial, only if the changes produced by such a diet do not adversely affect other risk factors. The fact that the LGI diet, in the present study, favourably altered many of the risk factors associated with the atherogenic profile of overweight/obese T2 DM patients, without adversely affecting other risk factors appears promising. The reduction of risk amongst participants with two dietary manipulation; LGI diet and LGI with energy restricted diet was equivalent for most of the risk parameters. Lowered systolic blood pressure, however was only significant in the energy-restricted diet group. Therefore, energy restricted diets should be considered for hypertensive T2 DM patients. It should be emphasised that many factors in the design of the study were successful. Among these factors was the maintenance of dietary fat and fibre consumption throughout the study thereby controlling some confounders that could affect the validity of the GI. In addition, measurements of sdLDL added strength to the study as this measure is considered to be one of the hallmarks of diabetic dyslipidaemia (Krauss, 1994). The monitoring and additional attention given to subjects who were clearly suffering from a lack of dietary advice and counselling, was shown to successfully increase compliance. The targeting of the nutritional messages should get further attention. Beside dietary modifications, nutritional plans should be directed toward behavioural changes as the improved GI knowledge in the current study did not reflect lower GI intake. The possibility that the LGI diet approach might not produce favourable changes in risk profile in non-obese individuals should be tested, as hypertriglyceridaemic subjects are known to be more responsive to the type of diet.
4.10. Conclusions

The present study showed that modest changes of dietary GI in overweight/obese T2 DM patients in Kuwait produced a favourable reduction in plasma TAG that was associated with other potentially beneficial changes in lipid and lipoprotein markers of an ALP such as increased HDL-C, and decreased LDL-C. The LGI diet was also associated with favourable changes in the composition of LDL and HDL particles. In addition, diabetic control was improved by lowering dietary GI. These changes in response to a LGI diet were of an equal magnitude to that achieved when weight reduction was less than 5% compared to baseline. In conclusion, these two dietary manipulations resulted in equivalent improvements in CVD risk profile in overweight/obese T2 DM patients.
CHAPTER 5
The two intervention groups and the control group all had similar mean LDL peak densities, %AUCB, and HDL₂ at baseline. There was no significant changes in the levels of LDL peak density in any of the intervention groups. However, sdLDL in both the LGI and the energy-restricted LGI group decreased significantly relative to the control group ($p=0.005$ and $p=0.003$ respectively). There was no significant difference between the intervention groups. These results are presented in Table 4.8 and Figure 4.12. An example of the decrease in sdLDL in one subject as shown by computer generated densitometric scan is presented in Figure 4.13.

**Figure 4.11** Correlation between changes in A: sdLDL, B: %HDL₂, C: NEFA and D: %GI and changes in plasma TAG. The strength and direction of correlation is individually presented in the Figure as correlation coefficient ($r$).
Chapter 5

A STUDY OF THE EFFECTS OF LOW CARBOHYDRATE ATKINS DIET, WITH AND WITHOUT MODERATE EXERCISE, ON THE EXPRESSION OF AN ATHEROGENIC LIPOPROTEIN PHENOTYPE.

5.1. Overview

Obesity is a major modifiable risk factor for CVD. It increases the risk of developing conditions, such as T2 DM, dyslipidaemia and hypertension that, in turn, increase CVD risk (Abate, 2000). More importantly, evidence is accumulating that the distribution of fat in the body, and especially abdominal fat, is more highly associated with the risk of CVD (Diabetes Prevention Program Research Group, 2002). Although obesity itself does not form part of an ALP, visceral obesity has been linked with increased plasma TAG concentrations (Sharp et al., 2003) and a predominance of smaller, dense LDL particles (Blackburn et al., 2003), both of which are major components of an ALP. Therefore, since the accumulation of abdominal fat may underlie the development of an ALP, weight loss may be an important factor for the management of this high risk dyslipidaemia. Interest in low carbohydrate (LC) diets has grown, largely on the strength of evidence that they can effectively reduce weight (Stern et al., 2004; Seshadri et al., 2004; Samaha et al., 2003; Foster et al., 2003). However, the role of LC diets in reversing the lipid and lipoprotein abnormalities associated with an ALP is not well understood, and is controversial, as these diets derive a large proportion of their energy from fat; a macronutrient with a potentially detrimental impact on CVD risk (Lovejoy et al., 1998). In addition, the combined effects of a LC diet with other lifestyle modifications, such as moderate exercise, is an area where evidence is lacking. Therefore, the present study aimed to investigate the effect of a LC diet on an ALP in overweight/obese individuals, and to examine how moderate exercise may influence any dietary effects.
5.2. Aims of the study

The aims of this study were two-fold:

1. To investigate the effect of a 6-month, low-carbohydrate, Atkins diet on the expression of an ALP and fasting insulin concentrations, as markers of CVD risk in overweight/obese men.

2. To compare the efficacy of a low-carbohydrate Atkins diet, with and without the inclusion of moderate exercise, on the expression of an ALP, to establish the most effective approach for reducing CVD risk in overweight/obese men.

5.3. Study hypotheses

1. A low carbohydrate diet will produce significant improvements in fasting insulin and the expression of an ALP in overweight/obese men.

2. A low carbohydrate diet combined with exercise will further enhance improvements in fasting insulin and the expression of an ALP in overweight/obese men as compared to low carbohydrate diet alone.

5.4. Methods

5.4.1. Participant selection

Participants were selected for the study based on the following inclusion criteria:

- Males 21-65 years of age.
- BMI 27-40kg/m².
- Not on any weight reduction regimen or currently losing weight.
- Living in close proximity to Guildford.
- Ability to exercise to a moderate intensity.
Exclusion criteria were:

- History of heart disease, renal disease, liver disease, gout, clinical depression, anorexia, and/or drug or alcohol abuse.
- T1 DM or T2 DM.
- Currently using weight loss drugs.
- Uncontrolled thyroid disease.
- Treated with lipid-lowering or anti-hypertensive medication.

Methods of subject recruitment were based on media advertising, and posters and flyers within the Guildford area, utilizing the university webpage and adverts placed inside the ‘Dr Atkins New Diet Revolution’ book in bookstores. Selected participants were randomized to their dietary intervention groups using computer generated random numbers (GraphPad Software Inc, USA).

5.4.2. Sample size justification

The sample size was originally estimated by the collaborators (Dr. Helen Truby, Dr. Kath Heart, and Mrs Rebecca Hiscutt) and was calculated on the primary outcome measure of plasma insulin (pmol/l). The calculation was based on that used in a previous study at the University of Surrey; The BBC Diet Trials; (Morgan et al., 2004). In order to detect a 40pmol/l difference in pre to post change in fasting insulin concentrations between groups with an 80% power and significant at $p=0.05$, 20 subjects per group were required. A standard deviation of 45.2 was assumed. Therefore, the study included a total of 60 participants distributed between the two intervention groups to withstand an estimated drop-out rate of 50%.

5.4.3. Study design

The study was a randomised trial of parallel design, with two intervention groups; a LC diet group and a LC diet plus exercise group (Figure 5.1). The study was a collaborative project that involved other investigators who were responsible for the recruitment and
management of the volunteers, and collection and analyses of dietary and fitness data. My principal role was to undertake all the biochemical analyses for the study. The trial was part of a larger, 12 months randomised trial on the effect of LC diets on CVD risk factors in overweight and obese men.

![Study design diagram](image)

**Figure 5.1.** Study design.

### 5.4.4. Intervention groups

#### 5.4.4.1. LC diet group

The dietary principals of the LC Atkins diet were based on the Dr Atkins Diet Revolution book (Atkins, 2003), with modifications to make the diet as nutritionally balanced as possible. Dietary advice and diet sheets were provided to ensure compliance. Diet sheets were constructed for each phase of the study; an induction phase, ongoing weight loss, pre-maintenance, and maintenance phases. Participants in the LC group were instructed to maintain their baseline levels of activity.
5.4.4.2. LC diet and exercise group

All efforts were made to make the dietary intervention in this group identical to that of the first group. In addition, participants were asked to perform three moderate exercise sessions of 30 minutes duration per week. The exercise session included brisk walking, use of an exercise bike, or gym achieving 50-70% maximum heart rate as assessed by heart rate monitors which were provided for the participants. Exercise bikes and gym passes were provided for the duration of the study. Participants who were already exercising at baseline, were asked to increase their exercise level.

5.4.5. Measures

5.4.5.1. Anthropometric measurements

Anthropometric measures were taken as described in section 2.2.5.1.

5.4.5.2 Dietary and fitness measurements

A 7-day quantified diet diary was used to calculate the food intake of participants at baseline and 6 months intervention. In order to assist with proper portion size recording, the diaries contained pictures of portion sizes. The dietary intakes were analyzed using WinDiets software research version (The Robert Gordon University, Aberdeen, Scotland).

Fitness was assessed using the Aerobic Adaptation Test (AAT) developed by Church and co-workers (2001). This test utilises an exercise bike to detect changes in submaximal work capacity with minimal participant discomfort. Participants were asked to maintain a constant speed (60 rpm), and then work load (wattage) was gradually increased each minute until 70% of maximum heart rate was achieved. Improved fitness, or otherwise, was then assessed by changes in the final wattage at baseline, 2 months, 4 months and at the end of the study.
5.4.5.3. Blood samples

Blood samples were taken and treated as described in section 2.2.1 at baseline, 2, 4, and 6 months.

5.4.5.4. Laboratory measurements

Total plasma TAG, T. Cholesterol, HDL-C, LDL-C, apo A-I, apo B, NEFA, and glucose were measured as described in Section 2.2.2.1. Plasma insulin concentrations were measured as described in Section 2.2.4. Small, dense LDL (sdLDL) and HDL subclasses were measured by techniques described in Sections 2.2.3.2 and 2.2.3.1 respectively.

5.4.6. Statistical analyses

Data was coded and entered into SPSS statistical software, version 14 (SPSS for windows, Chicago, Illinois). Data was analysed for normality using the Kolmogorov-Smirnov test. The following variables were not normally distributed and were therefore log transformed before analyses; weight, plasma insulin, and plasma TAG. The characteristics of the study participants were expressed using descriptive statistics. Data were analysed for both between (treatment) and within (time) group effects. As participants in the LC and exercise group did not increase their activity level significantly over time compared to the LC diet alone group, it was decided to combine the analyses in order to examine the effect of LC diet alone on CVD risk. The effect of LC diet in each group on all measured variables was assessed by one-way repeated measure ANOVA for those variables with interim measurements. A Tukey post-hoc test was then applied to locate individual differences. Variables that were assessed only at baseline and at the end of the study were examined for within group differences by paired sample t-test. The between group differences were tested using the unpaired Student t-test for the changes from baseline. A two-way ANOVA, with treatment and time as factors was conducted to test the differences between different groups with time. Only complete sets of data were used for analyses. Pearson correlations coefficients (r) were determined to examine the relationship between variables. A \( p < 0.05 \) was considered statistically significant.
5.5. Results

5.5.1. Study progression

The flow of participants through the study is outlined in Figure 5.2. Out of 56 participants recruited, 12 withdrew at different stages of the study for various reasons (21% attrition rate). The reasons for withdrawal included inability to tolerate the intervention diet, loss of interest in the diet, inability to attend appointments or medical reasons unrelated to the diet.

![Diagram of participant flow](image)

* Represents reasons for withdrawal.

**Figure 5.2.** Flow of participants through the trial.
5.5.2. Baseline characteristics of participants

All participants who started the trial were overweight or obese as defined by increased weight and elevated BMI (>27 kg/m²). Baseline anthropometric measurements are shown in Table 5.1. There was no significant difference in age, weight, BMI, or waist circumference between the two groups at baseline. Out of 42 participants with baseline lipid and lipoprotein data, 7 (17%) initially presented with a predominance of sdLDL and were therefore classified as Pattern ‘B’. Twenty one (50%) of the participants had raised plasma TAG (TAG>1.5 mmol/l), and 12 (29%) had low HDL-C (HDL-C<1mmol/l).

Table 5.1. Baseline characteristics of study participants.

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>p (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC</td>
<td>LC + exercise</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.7 (7.6)</td>
<td>48.1 (9.7)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>102.1 (13.1)</td>
<td>105.5 (13.2)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.4 (3.1)</td>
<td>33.1 (3.4)</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>108.9 (6.8)</td>
<td>112.4 (10.6)</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>6.2 (0.5)</td>
<td>6.2 (0.6)</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)§</td>
<td>58.4 (1.8)</td>
<td>63.3 (2.0)</td>
</tr>
</tbody>
</table>

Values represent means (SD), § values represent geometric means.

5.5.3. Prevalence of metabolic syndrome

The NCEP ATP-III criteria (2001) for the identification of the metabolic syndrome (MS) were applied to detect the presence of MS in the study participants at baseline and upon completion of the trial. The presence of three features of MS shown in Table 5.2 was taken as being indicative of the MS. The most common combination for the syndrome in the study participants was increased waist circumference combined with low HDL-C and raised plasma TAG concentrations.
Table 5.2. Prevalence of metabolic syndrome in study participants at baseline (b) and at 6 months (6 m) based on the NCEP ATP-III criteria (2001).

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Waist circumference &gt; 102 cm</th>
<th>Fasting plasma glucose &gt; 6.1 mmol/l</th>
<th>HDL-C &lt; 1 mmol/l</th>
<th>Fasting Plasma TAG &gt; 1.5 mmol/l</th>
<th>Metabolic Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>b 6 m</td>
<td>b 6 m</td>
<td>b 6 m</td>
<td>b 6 m</td>
<td>b 6 m</td>
</tr>
<tr>
<td>All</td>
<td>46 25</td>
<td>25 18</td>
<td>12 7</td>
<td>20 11</td>
<td>14 4</td>
</tr>
<tr>
<td>Diet</td>
<td>22 11</td>
<td>9 6</td>
<td>6 4</td>
<td>7 4</td>
<td>4 2</td>
</tr>
<tr>
<td>Diet + exercise</td>
<td>24 14</td>
<td>16 12</td>
<td>6 3</td>
<td>13 7</td>
<td>10 2</td>
</tr>
</tbody>
</table>

5.5.4. Changes in nutrient intakes

Nutrient intakes were analysed at baseline and after 6 months intervention by averaging the intakes recorded in the 7-day food diaries. For all participants, the average energy intake/day was reduced significantly \((p<0.0005)\) from \(2628\pm156\) kcal/d-\(11038\pm655\) KJ/d (SEM) at baseline to \(1760\pm111\) kcal/d-\(7392\pm466\) KJ/d at 6 months; an energy deficit of \(868\) kcal/day (Figure 5.3). There was a significant difference in mean energy intake/day between groups, as a result of a greater decrease in energy intake in the exercising group versus the diet alone group (Table 5.3).

Changes in the macronutrient composition of the diet in grams are presented in Table 5.3. At baseline, the percentage of total energy from macronutrients was not significantly different between groups (Figure 5.3 and 5.4). After 6 months, there was a significant increase from baseline in the percentage of daily energy from total fat \((p<0.0005)\), saturated fat \((p<0.0005)\), PUFA \((p=0.002)\), MUFA \((p<0.0005)\), and protein \((p<0.0005)\), while there was a significant reduction in the percentage of energy from carbohydrate per day \((p<0.0005)\) for the whole sample. Comparisons between groups indicated that the only significant difference between the intervention groups with respect to macronutrient composition of the diet was in protein as a fraction of total daily energy intake, with this percentage being significantly increased in the exercise group compared with the LC diet alone group \((p=0.044;\ Figure 5.3)\).
\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|}
\hline
\textbf{Period} & \textbf{Baseline} & \textbf{6 months} & \textbf{Change} & \textbf{Baseline} & \textbf{6 months} & \textbf{Change} & \textbf{Baseline} & \textbf{6 months} & \textbf{Change} \\
\hline
0-36 & 0.16 & 0.22 & 0.06 & 0.17 & 0.23 & 0.06 & 0.18 & 0.24 & 0.06 \\
0-12 & 0.15 & 0.20 & 0.05 & 0.16 & 0.22 & 0.06 & 0.17 & 0.23 & 0.06 \\
0-36 & 0.14 & 0.19 & 0.05 & 0.15 & 0.20 & 0.05 & 0.16 & 0.21 & 0.05 \\
0-12 & 0.13 & 0.18 & 0.05 & 0.14 & 0.19 & 0.05 & 0.15 & 0.20 & 0.05 \\
\hline
\end{tabular}
\caption{Changes in macronutrients in grams over the intervention period.}
\end{table}
Significant difference between a and b.

Figure 5. Average ± SEM of A: total energy; B: percentage CHO from total daily energy; and C: percentage protein from total daily energy over 6 months.

- **A**: Energy (kcal/d)
  - LC + exercise vs. LC: p < 0.005, *p < 0.05
  - LC vs. Baseline: p < 0.005

- **B**: Daily energy from CHO
  - LC + exercise vs. LC: ***p < 0.005

- **C**: Daily energy from protein
  - LC + exercise vs. LC: ***p < 0.005
  - LC vs. Baseline: p < 0.005

**Note:** Energy (kcal/day)
Figure 5.4 Average ± SEM of A: T. Fat, B: S. Fat, C: PUFA, and D: MUFA at baseline and after 6 months of intervention. *Within group difference of $p<0.05$, **$p<0.005$, and ***$p<0.0005$ from baseline.

5.5.5. Changes in anthropometric measures

5.5.5.1. Changes in weight

Weight was measured at baseline and thereafter once every 2 months until the end of the trial. At baseline there was no significant difference in the weight between the intervention groups ($p=0.332$). After 6 months, there was a significant reduction in the weight of participants in both groups ($p=0.001$; Figure 5.6). The percentage of weight
loss in the LC diet group was 6.4% which was not significantly different from the percentage of weight reduction in the LC plus exercise group (6.3% reduction). Also, the percentage of weight change was not statistically different between the two groups at any point during the intervention. Participants achieved rapid weight loss during the first two months of the trial (4.8% ±3.3 (SD) and 5.7%±3.8 for the LC diet group and the LC plus exercise group respectively). Weight loss then slowed down in both groups. Mean absolute weight loss from baseline to 6 months for the whole sample was 6.6±6.1kg (SD). By the end of the trial, there was a mean reduction of 6.4%±5.6 (SD) from the initial body weight in all subjects. Two participants, one in the LC diet group and one in the LC plus exercise group, regained weight at 6 months (1.20 kg and 0.40 kg respectively). The absolute weight loss for all subjects from baseline to 6 months is presented in Figure 5.5, while the average weight changes from baseline for the intervention groups are presented in Figure 5.7.

Figure 5.5. Absolute changes in weight after 6 months of intervention with A: low-carbohydrate diet and B: low-carbohydrate with moderate exercise. Each bar represents one subject.
5.5.5.2. Changes in BMI

The BMI for all the participants following a LC diet decreased significantly from 32.3±3.3 kg/m² (SD) to 30.2±3.6 kg/m² (p<0.001; Figure 5.6). Participants in both groups showed a decrease in their BMI compared to baseline (p<0.001). While the LC diet group had a significantly lower BMI than the LC plus exercise group at baseline (p<0.05), 2-way ANOVA showed no significant effect of diet on BMI (p=0.078), or any time and group interaction (p=0.309).

5.5.5.3. Changes in the waist circumference (WC)

WC was significantly reduced in all participants as a whole (p=0.001; Figure 5.6) from 110.6±9.1 cm (SD) at baseline to 104.1±10.1 at 6 months, with a significant decrease in this measure for the whole cohort over time. At baseline, 43 participants (97.7%) presented with WC in excess of 102cm. After 6 months intervention, only 25 participants (56.8%) had a WC >102 cm. At the end of the trial, there was no significant difference between the two groups (p=0.259) or any significant interaction between time and group (p=0.548) in changes in the WC. Figure 5.7 shows a comparison between groups with respect to WC.
Figure 5.6. Average ± SEM of A: weight, B: BMI, and C: waist circumference for study participants as a whole at 0 (baseline), 2, 4, and 6 months of intervention. ***Significantly different from baseline, $p<0.001$. 
Figure 5.7. Average ± SEM of A: weight, B: BMI, and C: waist circumference at 0 (baseline), 2, 4, and 6 months of intervention in the LC diet and the LC plus exercise group. *Within group difference of $p<0.05$, and ***$p<0.001$ from baseline.
5.5.6. Changes in fitness

Changes in fitness were defined by differences in the final exercise work load (wattage) based on the aerobic adaptation test (Church et al., 2001). The level of fitness from baseline to 6 months was only increased significantly within the LC plus exercise group ($p<0.05$; Figure 5.8). There was a significant effect of time ($p=0.006$) and a trend towards greater fitness level in the LC plus exercise group compared with LC diet group ($p=0.05$) but no interaction between time and treatment ($p=0.383$). The only time that a significant increase in the fitness level was observed within the exercise group was between the second and fourth month of the intervention ($p<0.011$). At this time point, there was a trend towards increased fitness in LC group alone ($p=0.060$) and therefore no significant difference between groups in fitness at this time point was evident.

![Figure 5.8](image)

**Figure 5.8.** Average ± SEM of fitness at 0 (baseline), 2, 4, and 6 months of intervention in the LC diet and the LC diet plus exercise group. **Within group difference of $p<0.01$ from baseline.**
5.5.7. Changes in glycaemia

Mean fasting glucose concentrations for the whole cohort throughout the study can be seen in Figure 5.9. No significant effects of time were seen on fasting plasma glucose in the whole cohort \((p=0.097)\). Although there was a 6.4% reduction in plasma glucose concentration in the LC plus exercise group from baseline (from 6.2±0.1 mmol/l (SEM) at baseline to 5.7±0.4 mmol/l at 6 months), comparison between groups indicated no significant effect of time \((p=0.10\), Figure 5.10\), group \((p=0.524)\) or significant interaction between time and group \((p=0.090)\).

5.5.8. Changes in plasma insulin and HOMA-IR

Mean fasting plasma insulin for the two groups at baseline was not different \((p=0.797)\). After two months, there was a tendency towards lower plasma insulin in the LC plus exercise group relative to baseline \((p=0.058)\), but there were no significant changes within any of the groups \((p=0.473\) and \(p=0.501\) for the LC diet and the LC diet plus exercise group respectively; Figure 5.10). At the end of the trial there was a 5% reduction of insulin concentrations in the LC diet group and 12% reduction in the LC diet plus exercise group. However, these changes did not reach significance \((p=0.501)\). The changes in a surrogate marker of insulin sensitivity, HOMA-IR, were not significant either within or between groups after 6 months of intervention (Figure 5.9 and 5.10).
Figure 5.9. Average ± SEM of A: fasting glucose, B: fasting insulin, and C: HOMA-IR for the study participants as a whole at 0 (baseline), 2, 4, and 6 months of intervention.
Figure 5.10 Average ± SEM of A: glucose, B: insulin, and C: HOMA-IR at 0 (baseline), 2, 4, and 6 months of intervention in the diet and the LC diet plus exercise group. †Within group tendency towards lower insulin levels. $p=0.058$. 
5.5.9. Changes in features of an ALP

5.5.9.1. Changes in plasma TAGs

The mean concentration of plasma TAG for the whole cohort decreased significantly (Figure 5.11) from 1.5±1.1 (SEM) mmol/l at baseline to 1.1±1.1 mmol/l at 6 months ($p<0.005$). The LC diet without exercise group showed reductions in plasma TAG at 4 ($p<0.01$) and at 6 months ($p<0.05$) compared with baseline values. The exercise group showed significant reductions in plasma TAG within the first two months ($p<0.05$) of intervention which was maintained at 6 months. Although there were reductions in plasma TAG of 15% and 19% in the LC and LC plus exercise groups respectively, there was no significant effect of treatment ($p=0.131$; Figure 5.12) or interaction between time and treatment ($p=0.358$) but a significant effect of time ($p=0.003$). The ratio of TAG:HDL-C decreased significantly in the overall cohort ($p=0.001$) but there was no significant difference between groups ($p=0.649$).

5.5.9.2. Changes in T. Cholesterol and LDL-C

There was no significant difference in total plasma cholesterol or LDL-C during any period of the trial in the LC group (Figure 5.11) but a significant decrease in total plasma cholesterol after two months in the LC plus exercise group ($p<0.05$). The concentration of LDL-C within the latter group also decreased significantly after two months relative to baseline ($p=0.016$) but then increased between two and four months ($p=0.022$). By the end of the interventions, there was no significant difference in these lipids in either group compared to baseline. Between group comparison indicated there was no significant effect of time ($p=0.079$ and $p=0.449$) and no significant interaction between time and treatment ($p=0.291$ and $p=0.385$) for total cholesterol or LDL-C (Figure 5.12). Full details on the concentrations of plasma lipids and lipoproteins are provided in Appendix 16.
5.5.9.3. Plasma HDL-C

The overall cohort had a significantly ($p=0.047$) higher plasma concentration of HDL-C at 6 months (1.4±0.1 mmol/l (SEM)) compared with baseline (1.3±0.1 mmol/l); Figure 5.11). There was no significant change in the concentration of HDL-C from baseline to 6 months in the LC diet group ($p=0.197$) or the LC plus exercise group ($p=0.136$). However, an increase in HDL-C was evident between the second and sixth months in the LC plus exercise group ($p<0.01$). There was a significant effect of time ($p=0.011$) but no significant effect of group ($p=0.972$) or interaction between time and treatment ($p=0.834$). These changes are shown in Figure 5.12. The ratio of total cholesterol: HDL-C decreased significantly for the whole cohort at 6 months compared with baseline ($p=0.004$).

5.5.9.4. Changes in NEFA concentrations

The whole cohort showed a tendency ($p=0.060$) towards lower plasma NEFA concentrations at 6 months (0.68±0.04 mmol/l (SEM)) compared with baseline (0.76±0.05 mmol/l). There was no difference in plasma NEFA between groups at baseline. A within group analysis of the concentration of fasting NEFA indicated a tendency ($p=0.059$) towards decrease in this concentration in the LC diet plus exercise group but not the LC alone diet group ($p=0.442$). There was no significant changes in plasma NEFA between groups after 6 months of intervention ($p=0.477$). Changes in NEFA within and between groups can be seen in Table 5.4.
Figure 5.11. Average ± SEM of A: TAG, B: HDL-C, and C: LDL-C for study participants as a whole at 0 (baseline), 2, 4, and 6 months of intervention with low-carbohydrate diet. *Significantly different than baseline at $p<0.05$ and **$p<0.01$. and ***$p<0.001$. 
Figure 5.12. Average ± SEM of A: plasma TAG, B: HDL-C, and C: LDL-C at 0 (baseline), 2, 4, and 6 months of intervention in the diet and the diet plus exercise group. *Within group differences of $p<0.05$ and ** $p<0.01$ from baseline.
5.5.9.5. Changes in LDL and HDL subclasses and LDL particle number

The overall cohort had a significantly ($p<0.0005$) higher percentage of the larger, more lipid-rich HDL$_2$ subclass and higher concentration of plasma apoA-I ($p=0.026$). The proportion of small, dense LDL (sdLDL), as well as peak LDL density, was significantly reduced within the whole cohort at 6 months compared with baseline ($p<0.0005$ for each group), but this was not accompanied by any significant change in total plasma apo B (a surrogate for the number of LDL particles in fasting plasma) ($p=0.440$). Within group analyses revealed that HDL$_2$ increased in both the LC diet alone ($p=0.056$) and LC diet plus exercise groups ($p=0.002$) after 6 months relative to baseline (Figure 5.13). The proportion of sdLDL, as measured by the percentage AUC representative of subclass pattern ‘B’ was decreased in both the LC diet ($p=0.002$) and LC plus exercise group ($p=0.055$) after 6 months (Figure 5.13). A reduction in LDL peak density was also evident in both intervention groups ($p=0.014$ and $p=0.001$; LC diet and the LC diet plus exercise groups respectively). There was also evidence of an increase in plasma apoA-I in the LC diet group ($p=0.066$), but no changes in plasma apo B in any of the groups. When the groups were compared, there was no significant difference in any of the above lipoprotein subclass profiles. Changes in lipoprotein subclasses and plasma apoproteins can be seen in Table 5.4.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Month 1</th>
<th>Month 2</th>
<th>Month 3</th>
<th>Month 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL (mg/dL)</td>
<td>45</td>
<td>43</td>
<td>46</td>
<td>47</td>
<td>48</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>120</td>
<td>125</td>
<td>120</td>
<td>125</td>
<td>120</td>
</tr>
</tbody>
</table>

*Represents within group trend in the changes from baseline.*

**Represents within group difference of p < 0.05 and p < 0.005.*
Figure 5.13. Changes in A: sdLDL and B: HDL$_2$ in each group. * represent within group trend in the changes from baseline. ** Significantly different from baseline, $p<0.005$.

5.5.10. Correlation analyses

Changes in plasma TAG correlated significantly with changes in body weight ($r=0.459$, $p=0.003$) within the whole cohort. Plasma insulin ($r=0.388$, $p=0.010$), sdLDL ($r=0.430$, $p=0.008$) and LDL peak density ($r=0.385$, $p=0.019$) all correlated significantly with weight loss. Improvements in fitness for the whole sample were significantly and negatively associated with changes in sdLDL ($r=-0.507$, $p=0.002$) and LDL peak density ($r=-0.465$, $p=0.006$) but not with changes in weight ($r=0.032$, $p=0.822$). Changes in fitness was not associated with changes in plasma TAG ($r=0.142$, $p=0.563$). Changes in HOMA-IR were significantly associated with changes in plasma TAG ($r=-0.311$, $p=0.048$) for the whole cohort. Alterations in the principal macronutrient content of the diet did not appear to account for the relationship between changes in lipoproteins and BMI (Appendix 17).
5.6. Discussion

The aims of the present study were to investigate the effects of two lifestyle approaches on surrogate markers of CVD risk. These approaches were a low carbohydrate diet alone and a low carbohydrate diet with moderate exercise. These aims were based on the hypothesis that a low carbohydrate diet will increase insulin sensitivity and decrease the expression of the ALP, and that moderate exercise, when combined with a low carbohydrate diet, will further enhance improvement in these CVD risk markers as compared to a low carbohydrate diet alone.

5.6.1. The effects on weight, BMI, and waist circumference

The amount of weight loss in this trial (6.6 kg) was expected given that this magnitude of weight loss has been frequently demonstrated in low carbohydrate diet trials (Volek et al., 2004b; Samaha et al., 2003; Truby et al., 2006; Foster et al., 2003). The percentage of weight loss achieved in the present study (6.4%) would be expected to confer health benefits in obese individuals in terms of CVD risk (Wing et al., 1987a). A favorable improvement in markers of CVD risk was evident in the cohort as a whole and within both intervention groups. This improvement was also evident with respect to the number of participants meeting the ATP-III criteria for metabolic syndrome. By the end of the trial, the number of participants expressing 3 or more features of the metabolic syndrome had decreased from 14 to 4. Generally, the metabolic syndrome increases CHD risk by 3.7 fold in men (Sattar et al., 2003).

Weight loss has profound effects on lipid metabolism and this is perhaps the most likely mechanism to explain how a low carbohydrate diet exerts favorable effects on plasma TAG. Weight loss in both groups was evident from the reduction in total energy achieved in the sample as a whole (from 2471 kcal/d-10378 KJ/d to 1760 kcal/d-7392 KJ/d) in addition to the macronutrient composition of the diet, which suggested that participants were compliant with the expected macronutrient goals of the diet (27±5.4% (SD) %, 55±9.4%, and 14±9.4% energy from protein, fat, and carbohydrates respectively). Although the dietary advice for participants in both groups was to restrict
intake of carbohydrate, but not energy, and despite the speculation that a low carbohydrate diet facilitates weight loss by promoting adipose tissue metabolism (Atkins, 2003), weight loss in both groups, in the present study, appeared to result from decreased energy intake. This reduction in energy might be attributable to several factors including limited food choices in a low carbohydrate diet, the simplicity of the diet or as a result of a greater effect on satiety with the higher consumption of protein.

When the intervention groups were compared with respect to weight loss and BMI, the low carbohydrate diet and the low carbohydrate diet plus exercise groups showed no significant differences after the intervention. This finding was quite unexpected as the role of exercise in assisting and maintaining weight loss has been well established by others (Wing, 1999; Avenell et al., 2004a; Blair, 1993). Studies have found that the combined effects of a low carbohydrate diet with exercise is additive on improving body composition (Layman et al., 2005). The lack of difference between groups could therefore be related to a difference in the total energy intakes. Although men tend not to upregulate their energy intake in response to exercise (Stubbs et al., 2002), men in the exercise group in the present study may have increased their consumption of food after exercise to compensate for their increased energy expenditure. After 6 months intervention the exercise group had a lower estimated average energy intake compared with the diet alone group (p=0.019). However, under-reporting of food intake during exercise programs have been previously reported, and evidence from previous studies indicates that self-reported food intake during transitions from one level of energy expenditure to another is a major source of error in recording dietary intake (Ambler et al., 1998). In the current study, changes in fitness were not associated with weight loss. The absence of this association is consistent with other reports (Jakicic et al., 1999; Ross et al., 2000) in which weight loss produced by exercise required more than 30 minutes of daily activity. Therefore, it is probable that while participants in the low carbohydrate diet plus exercise group had a small but significant increase in their fitness compared to baseline, the intensity, duration, and frequency of exercise utilised by these individuals was insufficient to decrease weight in relation to the low carbohydrate diet alone. Indeed,
these three exercise components are important determinants of the clinical outcomes of any exercise program (LaFontaine & Roitman, 2002).

In hindsight, when fitness was analysed, the lack of significance between groups in terms of weight loss at 6 months was not surprising given the fact that there was no significant difference between groups in fitness at that point in time (6 months). The increase in fitness observed within the exercise group was only significant at four months of intervention when compared with baseline but was not significantly different from the low carbohydrate diet alone group. It seems that the latter group was possibly highly motivated and may have sought out other healthy lifestyle options such as increased physical activity, although 86% of participants in the low carbohydrate diet group complied with instructions not to alter their exercise levels over the course of the study. This is a common confounding feature in studies that examine the effect of lifestyle changes on CVD risk (Petrella et al., 2005). The proposed assumption is further supported by the tendency towards an increased level of fitness level in the low carbohydrate diet alone group between the second and fourth month of intervention \((p=0.06)\); when the exercise group had maximum changes in fitness. After this period, fitness waned and eventually the groups re-attained their baseline fitness levels. The reason as to why participants in the exercise group have ceased exercise is not clear. It is probable that participants within this group exercised intensely during the first months of the trial which led them to exhaustion and therefore decreased compliance. A low carbohydrate diet has been associated with a higher heart rate and increased exertion in one study (Haub et al., 2006) compared with a control diet. The practical implications of this finding therefore deserve some address. Participants in the exercise group were able to comply with a low carbohydrate diet, but not an exercise program, this finding in itself, is an interesting outcome since dietary compliance is one of the main factors that might compromise the success of any nutritional therapy. It also highlights the difficulty obese individuals face in following a regular exercise program with minimal supervision.

Centrally obese individuals are known to have a greater risk of developing diabetes and CVD (Diabetes Prevention Program Research Group, 2002). The reduction in waist
circumference for the overweight/obese participants in this study (from 110.7 cm at baseline to 104.1 cm after 6 months) therefore represents an important outcome in terms of CVD risk reduction. A major risk associated with abdominal obesity is the insulin resistance of metabolic syndrome. Among the factors that promote insulin resistance in central obese individuals is increased lipolysis of abdominal fat and the exposure of the liver to elevated concentrations of NEFA (Björntorp, 1992). Elevated plasma NEFA can, in theory, lead to the increased production of VLDL by the liver. Increased NEFA concentrations have been identified as a major metabolic defect in many diseases including T2 DM (Frayn et al., 2005). Consequently, the trend towards reduction of NEFA concentrations in the overall cohort after 6-months represents another important outcome from the study in terms of CVD risk reduction.

5.6.2. Effect on markers of insulin resistance

Impaired fasting glucose (fasting plasma glucose > 6.1 mmol/l) is one of the NCEP ATP-III (2001) criteria for the clinical diagnosis of insulin resistant patients with the metabolic syndrome. Of the 25 participants who had a baseline glucose concentration that exceeded 6.1 mmol/l in the current study, seven had lowered their plasma glucose concentration below this value by the end of the trial. However, no changes in mean fasting plasma glucose over 6 months was evident for the cohort as a whole or between the intervention groups. Consistent with this finding, a weight loss of a similar magnitude as in the present study was not associated with significant changes in glucose concentrations in another trial of a low carbohydrate diet that also lasted for 6 months (Samaha et al., 2003). In general, the reduction in glucose concentrations that is associated with the consumption of low carbohydrate diets is limited to individuals with diabetes (Samaha et al., 2003), although it is also related to the magnitude of weight loss.

Measurements of fasting insulin represent a more useful way to identify individuals with insulin resistance since hyperinsulinaemia is highly correlated with insulin resistance (Reaven, 1988). In the present study, subjects showed characteristics of insulin resistance with raised insulin concentrations together with evidence of increased visceral fat (waist circumference > 102 cm). Although it is expected that fasting concentrations
of insulin would decrease after 6 months after a significant reduction in weight, and more relevantly, reduction in waist circumference, no significant changes in fasting concentrations of insulin were evident in the study sample after 6 months. However, a trend towards lower insulin concentrations between baseline and 2-months of intervention in the low carbohydrate plus exercise group \( p=0.058 \) was evident when there was maximum weight loss, though this effect was transient and had disappeared by the end of the trial. The lack of significance with respect to insulin concentrations might be attributable to the nutrient composition of the diet. Participants at the end of the trial had a higher percentage of saturated fat and protein intakes compared with baseline, therefore it is possible that this higher intake had an adverse effect on insulin concentrations, as high fat and high protein diets are known to increase plasma concentrations of insulin (Lovejoy et al., 1998). In addition, participants in the exercise group had a greater increase in the percentage of protein as total energy when compared with the LC diet alone \( p=0.044 \) which might have counteracted the effect of exercise within this group. Overall, studies on the effect of low carbohydrate diets on markers of insulin resistance are controversial. While some studies (Samaha et al., 2003; Brehm et al., 2003; Meckling et al., 2004) showed an increase in insulin sensitivity with the consumption of low carbohydrate diet, others showed no significant difference between low carbohydrate and low fat, energy restricted diets (Meckling et al., 2002; Foster et al., 2003; Stern et al., 2004) on insulin concentrations.

5.6.3. The effect on markers of ALP

5.6.3.1. Effects on plasma TAG

The findings in the present study are in agreement with that of many other studies that have investigated the effects of low carbohydrate diets on lipids and lipoproteins, and showed that these diets generally reduce plasma TAG (Volek et al., 2004b; Sharman et al., 2002; Volek et al., 2000; Foster et al., 2003). Interestingly, the reduction in plasma TAG in the present study was apparent in both intervention groups, with there being no significant difference between them. A kilogram reduction in body weight is associated with a 0.015 mmol/l decrease in plasma TAG (Dattilo & Kris-Etherton, 1992). Reductions in plasma TAG ranging from 11.4% to 60.4% of baseline concentrations have
been reported to result from weight loss in response to a low carbohydrate diet (Brehm et al., 2003; Sondike et al., 2003; Volek et al., 2003; Westman et al., 2002). Elevated plasma TAG is known to predispose to CHD (Grundy, 1999), and studies clearly indicate that elevated plasma TAG is an independent risk factor for CVD (Austin et al., 1998). Although weight loss beneficially affects TAG concentrations, changes in TAG on low carbohydrate diets are usually greater than those associated with weight loss alone (Wood, 2006). A possible mechanism for the reduction in plasma TAG induced by a low carbohydrate diet is through increased TAG clearance by LPL or decreased VLDL/TAG production by the liver in response to decreased carbohydrate substrate delivery. Consequently, VLDL particle quantity, but not size, is reduced (Volek & Feinman, 2005). In addition, a low carbohydrate diet will also be lower in GL as a result of the limited carbohydrate consumption per day. The findings in Chapter 4 and from others (Bellisle et al., 2007; Colombani, 2004) indicate that this type of diet decreases TAG concentrations in hyperlipidaemic subjects as it stabilises plasma glucose concentrations and reduces the availability of NEFA for VLDL production.

Exercise has been consistently shown to decrease plasma TAG. In the largest controlled exercise trial to date, the Health Risk Factors, Exercise Training, and Genetics (HERITAGE) study, plasma TAG was decreased by 2.7% in 299 normolipidaemic men who participated in 5 months of exercise training (Leon et al., 2000). In the present study, TAG concentrations after the intervention were shown to be similar between the diet alone and diet plus exercise groups. The lack of a significant difference in fitness between these groups no doubt contributed to this finding, so that plasma TAG concentrations may only have been affected by weight loss. The changes in plasma TAG were associated with changes in weight loss therefore since the latter was also not different between groups, this may explain why plasma TAG was unaffected by the interventions. This is further supported by the lack of association between changes in plasma TAG with changes in fitness in the exercise group.

A surrogate marker of insulin resistance is the ratio of TAG:HDL-C (Bloomgarden, 2003). Many studies have shown a significant reduction in this ratio in response to a low
carbohydrate diet (Sharman et al., 2002; Volek et al., 2004b; Westman et al., 2002; Meckling et al., 2004). The present study was in agreement with these studies in that this ratio was decreased with the consumption of the low carbohydrate diet \( (p=0.001) \) for 6 months but with no significant difference between groups.

5.6.3.2. Effects on T. Cholesterol and LDL-C

There was no overall increase in total plasma cholesterol by the end of the 6 months intervention compared with baseline values. While studies that have examined blood lipid responses to low carbohydrate diets have generally reported small increases in total cholesterol (Westman et al., 2002; Larosa et al., 1980; Volek et al., 2000), one earlier study reported increases in total cholesterol and LDL-C of 6% and 18% respectively in normolipidaemic subjects after 8 weeks (Larosa et al., 1980).

One of the main concerns of consuming low carbohydrate diets is that they are relatively high in total and saturated fat. Diets high in saturated fat, in particular, are well known to increase plasma concentrations of LDL-C which is an established risk factor for CHD (Turner et al., 1998). An increase in LDL-C has been shown when dietary saturated fat was increased at the expense of other macronutrients in hyperlipidaemic subjects (Sondike et al., 2003). In the present study, plasma LDL-C was similar at 6 months to baseline values within groups, and the changes in LDL-C did not differ significantly between the groups. The lack of any significant effects on LDL-C is important given the popular concern over low carbohydrate diets raising this lipoprotein. Other studies have also reported no significant changes in LDL-C with the consumption of a low carbohydrate diet (Howell et al., 1997; Samaha et al., 2003). It has been suggested that a low carbohydrate diet might be more appropriate for the treatment of individuals with obesity and dyslipidaemia related to raised TAG and low HDL and inappropriate for individuals with high baseline LDL-C concentrations (Sondike et al., 2003). The Atkins diet has been associated with favorable effects on lipids and LDL-C (Dashti & Al-Awadi, 2001; Volek et al., 2004b; Wood, 2006; Foster et al., 2003). This finding would suggest that any adverse effect of increased total and saturated fat on LDL-C is offset by weight loss.
5.6.3.3. Effect on HDL-C

The majority of studies have shown a beneficial increase in HDL-C in response to a low carbohydrate diet (Volek et al., 2000; Yancy et al., 2004; Volek & Feinman, 2005; Krauss et al., 2006; Foster et al., 2003). This increase in HDL-C is believed to be as a consequence of a decrease in plasma TAG and CETP-mediated neutral lipid exchange and resultant remodelling of HDL or through the down regulation of hepatic scavenger receptor B1 concentrations (Hatahet et al., 2003). This receptor binds HDL-C and facilitates reverse cholesterol transport of HDL cholesterol to the liver. Recently, the activity of CETP was found to be unchanged with the intake of a low carbohydrate diet (Wood et al., 2006) suggesting that the mechanism behind the elevation of HDL-C might result from peripheral cholesterol removal and estrification and reduced incorporation of TAG into HDL particles (Wood, 2006). Moreover, a greater increase in HDL-C after training program may occur in individuals with elevated plasma TAG at baseline (Couillard et al., 2001). The present findings were in agreement with the above studies in that HDL-C concentrations significantly increased with the consumption of the low-carbohydrate diet in both groups but with no significant difference between them. Usually manoeuvres which reduce serum TAG tend to increase HDL-C (Garg, 1998), it is therefore reasonable to find that as weight-loss in this study did not affect plasma TAG differentially, changes in HDL-C concentrations were also equivalent between groups.

Among overweight, insulin resistant individuals, hyperinsulinemia, hypertriglyceridemia, low HDL-C concentrations, and a net increase in the daily energy expenditure produced by regular endurance exercise may eventually induce mobilization of body fat and weight loss. In turn, this may ultimately reduce the amount of abdominal fat, improve insulin action, lower plasma TAG, and increase plasma HDL-C concentrations (Despres & Lamarche, 1994). HDL-C in the current study increased significantly within the exercise group between the second and the forth month of the trial which was concomitant with the significant increase in fitness during this period. In the present study, although there was no change in total cholesterol concentrations over time for the whole sample, a significant reduction in the ratio of total:HDL-C from baseline was evident.
5.6.3.4 Effects on LDL and HDL size and particle number

The majority of men in the current study did not express a predominance of small, dense LDL i.e. they expressed LDL subclass pattern ‘A’, which was unexpected since they had BMIs of 27 and over. Only seven participants were classified as LDL pattern ‘B’ at the start of the study. Of those, two were in the diet group and five were in the diet plus exercise group. Individuals exhibiting higher concentrations of sdLDL have a greater than three-fold increased risk of CVD (Austin et al., 1988). Interestingly, all subjects with pattern ‘B’ switched to pattern ‘A’ after 6 months on a low carbohydrate diet. The reduction in sdLDL seen in the current study was in agreement with other studies that examined the effect of carbohydrate consumption on LDL particle size (Dreon et al., 1994; Sharman et al., 2002; Krauss et al., 2006; Wood et al., 2006). The reduction in sdLDL was seen even when participants were normolipidemic, normal weight men who consumed a low carbohydrate diet for 6 weeks (Sharman et al., 2002). Those with a predominance of sdLDL particles had significant increases in mean and peak LDL particle diameter and the percentage of larger and lighter LDL particles after the consumption of a low carbohydrate diet, while those with pattern ‘A’ remained pattern ‘A’ by the end of the intervention. Reduction in sdLDL can only be observed when plasma TAG decreases concomitantly (Halle et al., 1999). These dietary-induced changes in sdLDL have been attributed to reductions in VLDL and IDL particles. In the present study, participants on both diets experienced an increase in the proportion of large LDL and decrease in sdLDL. This change is associated with reduced plasma TAG and hence decreased neutral lipid exchange of TAG for cholesterol esters into the LDL particle by CETP. The resulting lower concentration of TAG, compared with cholesterol esters in LDL particles, would lead to the production of larger LDL (Griffin, 1997). In the present study, changes in fitness were significantly associated with changes in sdLDL ($r=-0.507$, $p=0.002$) and LDL peak density ($r=-0.465$, $p=0.006$) emphasizing once more the importance of increasing exercise among obese individuals for the reduction of CVD risk.

The concentration of plasma apo B is an alternative measurement for LDL particle number because one apo B is present per particle of LDL. Plasma apo B constitutes an
independent risk factor for CHD (Vega et al., 1991). Plasma concentrations of apo B have been shown to decrease by 4.1% after 10 weeks on a low carbohydrate diet (Lofgren et al., 2005). In the present study, despite the reduction in TAG concentrations and energy intake seen in both groups, there was no reduction in apo B over time or between groups. In theory, the lower energy intake might have been expected to decrease the availability of TAG in the liver for VLDL synthesis leading to an increase in apo B degradation and a decrease in VLDL secretion. Reductions in apo B usually result from a decrease in VLDL secretion or an increase in hepatic clearance of VLDL or a combination of these two processes (Sniderman et al., 2001). The present findings might suggest that a low-carbohydrate diet has differential effects on the regulation of LDL particle size (sdLDL) and particle number (apo B concentration).

There is a strong inverse relationship between the large HDL2 subclass and CAD (Johansson et al., 1991). In the present study, both intervention groups experienced a significant increase in the proportion of HDL2. However, since there was no significant difference between groups in the reduction in plasma TAG, the increase in HDL2 was also predictably similar between groups. It is possible that low carbohydrate-induced reductions in plasma TAG might not always be coupled with increases in HDL2 (Seshadri et al., 2004). The results in this study were in agreement with other reports (Seshadri et al., 2004), in that HDL2 increased in the low carbohydrate diet group. Others have reported a significant increase in large HDL particles with no change in the quantity of small HDL particles (Wood et al., 2006). Apo A-I concentrations, on the other hand, increased significantly at 6 months compared to baseline ($p=0.026$) which was concomitant with the increase in HDL-C observed in this study.
5.7. Conclusions

Taken together, the findings of the present study demonstrated that a 6-month low carbohydrate diet had some beneficial effects on markers of an ALP, in particular decreasing plasma TAG, increasing HDL-C and decreasing the proportion of sdLDL. The results also indicate that the high consumption of fat and protein during a low carbohydrate diet might offset or counteract any beneficial effects of a low carbohydrate diet on hyperinsulinaemia. Furthermore, most of the favourable changes seen with the low carbohydrate diet were associated with the weight loss as a result of decreased energy intake. This study was unfortunately unable to differentiate between the effect of exercise and the low carbohydrate diet on an ALP, probably because of the difficulty that obese individuals faced in undertaking a regular exercise program, under minimal supervision.
CHAPTER 6
This research project focused on four different lifestyle strategies for ameliorating an ALP in a population at increased risk for CVD either because of the presence of T2 DM and/or being overweight or obese. Three of the tested lifestyle modifications were based on dietary interventions, while the last approach combined both dietary and activity factors. A dual approach was used to examine the impact of modifying the quality of dietary carbohydrate on an ALP, using low GI and a low GI / hypo-energetic diets in overweight/obese T2 DM patients. The effects of the quantity of carbohydrate on an ALP was also examined in a dual approach, in a comparison of a low carbohydrate Atkins diet with or without a moderate exercise program in obese subjects. These dietary combinations were designed to provide informative, multiple comparisons in two extremely different populations of overweight and/or obese UK Caucasians, and patients with T2 DM from Kuwait.

6.1 Measurements of glycaemic index

In the scientific literature, there is a considerable body of evidence on the therapeutic potential of LGI diets in patients with T2 DM (Wolever et al., 1992; Brand-Miller et al., 2003a; Brand-Miller, 2004; Franz et al., 2004; Ludwig, 2002; Meckling et al., 2004; Salmeron et al., 1997b; Schulze et al., 2004; Tsilhias et al., 2000; Willett et al., 2002; Wolever et al., 2003). However, it was not possible to address any dietary intervention aimed at modifying the GI of the diet in Kuwait without first testing some local foods for their GI, as data regarding this was lacking. Moreover, many researchers (Aston et al., 2007; Miller et al., 1992; Foster-Powell et al., 2002) have indicated the importance of measuring the GI of specific foods rather than applying GI values to groups of similar foods. Before determining the GI of some carbohydrate-rich staples in Kuwait, it was necessary to establish the pattern of intake of carbohydrate-rich foods in this country by undertaking an initial pilot study. This provided a valuable insight into some of the nutritional habits and dietary intakes of T2 DM patients in Kuwait. 24-hour dietary recalls were collected, together with anthropometric and blood pressure measurements in
26 T2 DM patients. After careful examination of dietary recalls, it was decided to examine the GI of the most commonly consumed, carbohydrate-rich foods. The food diaries also provided an estimate of the average dietary GI of foods being consumed by these patients.

Seven types of bread plus a type of rice that is commonly consumed by T2 DM patients were selected as staples for GI testing, and examined for their physiological effects and nutrient composition (Chapter 3). Six of these breads were shown to have GI values that fell within an intermediate GI range (56-68 for the tested breads) which was comparable to the GI values of similar foods reported in the literature (Foster-Powell et al., 2002; Henry et al., 2005). The exception to this was a brown pita bread which gave a high GI value of 76. Only one UK study has reported the GI of brown pita bread as being lower than in the present study (Henry et al., 2005). The difference in GI in this case is likely to be due to differences in the processing or baking of these breads. This again emphasised the importance of testing GI locally, and brand by brand, as it was impossible to predict from the literature where local differences in GI may have occurred. On the strength of this finding, it was recommended that Kuwaiti T2DM patients replace the high GI brown pita bread with one of the other breads tested, preferably with light white pita or white pita breads. The importance of this finding lies in the evidence to suggest that even small changes in the GI of the diet can produce significant reductions of CVD risk (Liu et al., 2000). Although the insulinaemic response to the selected staples was relatively higher than expected in relation to the GI values, it correlated well with GI. It was concluded that additional testing of these staples in T2 DM patients is required to firmly establish their total glycaemic effect.

6.2 The effects of low GI diet in T2 DM patients in Kuwait

The effects of two low GI diets, with and without energy restriction, were examined for markers of CVD risk in T2 DM in Chapter 4. Kuwait has one of the highest prevalence rates of T2 DM and its associated risk factors (Abdella et al., 1996; Al-Adsani et al., 2004; al-Muhtaseb et al., 1991; Jackson et al., 2002; Akanji. 2002: Abdella et al., 1998). The intake of a low GI diet in this population was associated with multiple, favourable
effects on CVD risk markers without adversely affecting any of the other risk markers that were examined. This finding was generally consistent with a wealth of literature from similar studies (Augustin et al., 2002; Kabir et al., 2002; Leeds, 2002; Luscombe et al., 1999; Miller, 1994; Rizkalla et al., 2004; Wolever et al., 1992; Fontvieille et al., 1992; Frost et al., 2004; Opperman et al., 2004; Wolever, 2006), and included improved glycaemic control, decreased plasma TAG and LDL-C concentrations, and increased HDL-C. In addition, both LDL and HDL particle size were increased in response to a reduction in dietary GI. Despite the observations of others that plasma apo B, a marker of LDL particle number (Marcovina et al., 1988; Van Lennep et al., 2000), is decreased by lowering the GI of the diet (Rizkalla et al., 2004; Kabir et al., 2002), neither plasma apo B or apo A-I changed in the present study, perhaps as a result of the small sample size, as the study was powered primarily to detect a change in plasma TAG.

A number of mechanisms have been proposed to explain how GI may influence lipid metabolism (Augustin et al., 2002). These include: insulin-mediated effects on the rate-limiting enzyme in cholesterol synthesis (HMG-CoA reductase) because of slow carbohydrate absorption; decreased bile acid and cholesterol absorption from the ileum as a result of the fibre content of low GI foods; inhibition of cholesterol synthesis by the short chain fatty acid propionate due to colonic fermentation of fibre; and finally, a reduced inflammatory response mediated by HDL which blocks the interaction of T cells and thereby inhibits TNF-α and IL-1β.

Both intervention groups, the low GI and the hypo-energetic low GI, showed significant reductions in their waist circumference. This reduction was related to the decrease in dietary GI achieved in both group ($r=0.404$, $p=0.004$). This finding within the low GI diet group, that was not energy restricted, is of particular importance for two reasons. First, the reduction in waist circumference within this group was achieved without energy restriction or weight loss, and secondly reduction in central abdominal fat is correlated with a lower atherogenic lipid profile (Markovic et al., 1998; Sharp et al., 2003).
This study was surprisingly unable to show any difference in the effects of a LGI diet, with or without energy restriction, on modifying the expression of an ALP, possibly because of the limited amount of weight loss that was achieved by participants in the energy restricted group. Studies in T2 DM patients have reported metabolic improvement in glycaemic control, insulin sensitivity and CVD risk factors with a modest weight reduction of 5-10% (Blackburn, 1995; Ditschuneit et al., 2002; Wing et al., 1987a). However, below this level of weight loss, no significant changes in the risk profile were found by others (Wing et al., 1987a). It is reasonable then to draw two conclusions regarding these findings. Firstly, in view of the drop in the systolic blood pressure in the energy-restricted low GI group in the current study, energy-restricted diets appear to be appropriate for the management of CVD risk in hypertensive T2 DM patients. Secondly, dietary manipulations aimed at reducing the GI of the diet are more acceptable to the participants and therefore easier to achieve in terms of compliance than dietary manipulations aimed at weight loss. Although compliance with the energy restricted diet was poor, as indicated by the very limited amount of weight loss in this group, compliance with the LGI diet in both of the intervention groups was considerable. Indeed, the LGI foods were highly acceptable to the study participants as proven by the fact that they, like others (Miller, 1994), indicated a preference to continue the LGI diet after the completion of the trial. It can therefore be postulated that dietary manipulation to achieve a lower GI was, at least in practical terms, easier to achieve than an energy restricted diet and did not present any adverse effects on markers of CVD risk. Despite this, weight loss should continue to be recommended in overweight/obese T2 DM patients, since weight loss has been shown consistently to have a potent effect in enhancing insulin sensitivity in overweight individuals (Avenell et al., 2004a; Yoshida et al., 2004), increasing diabetes control in T2 DM patients (Wing et al., 1987a), and improving lipid and lipoprotein profiles in both of these populations (Dashti et al., 2003; Dumesnil et al., 2001; Fernandez et al., 2004). Therefore, different weight loss strategies should be considered in order to increase dietary compliance. Instead of using a single dietary regimen for weight loss, such as the low energy diets that have been used for the study of T2 DM, a broad spectrum of dietary options to accommodate individual food preferences and lifestyles should be considered (Dansinger et al., 2005). In other words.
a single type of diet is not the best for everybody. When the efficacy of four commercial weight loss programs (Atkins, Weight Watchers, Slim-Fast, and Rosemary Conley) in the UK were compared in the BBC Diet Trials (Truby et al., 2006), there was no significant difference in the amount of weight loss between the four diets, but certain diets produced more favourable effects on CVD risk factors than others. This indicates that while a variety of popular diets can be used to reduce body weight, dietary effects on CVD risk factors are more specific to the individual diet.

The duration of the LGI study in Kuwait was moderate (6 weeks) in relation to other LGI studies (Dumesnil et al., 2001; Frost et al., 2004; Heilbronn et al., 2002; Jarvi et al., 1999; Rizkalla et al., 2004), but the dietetic advice and support was regular and intensive. The practical implementation of this diet and attainment of its health benefits might therefore be difficult to achieve in a free-living population without this nutritional support.

Although knowledge can produce changes in behavior and decision making (Ford & Jones, 1991) and provides cues for action (Becker et al., 1977), the results of the present study indicate that knowledge alone was insufficient to produce behavioral changes. The results point clearly to a need to develop intensive educational programs for diabetics that aims to increase knowledge and thus promote behavioral modifications.

A major limitation, as with any dietary intervention, was the method of dietary assessment, which had a strong bias towards underestimating habitual energy intakes (Black et al., 1991). Accurate dietary analysis was further hindered in Kuwait by the lack of validated nutritional analysis software specific for the Kuwaiti population. Establishing a food analysis database specific for the Kuwaiti population is required. There was also no dietary follow-up of participants after 6 weeks of intervention, making it difficult to draw conclusions about the sustainability and acceptability of the diet in the longer term.
The study of the effects of low GI diets in T2 DM patients was the first to involve dietary manipulations in Kuwait, both in terms of GI and/or energy restriction. Although participants in this study did not achieve the desired amount of weight loss (5-10%) by energy restriction, dietary compliance among Kuwaiti T2 DM patients is well known to be generally very poor (Akanji, 2002) making a weight loss of as little as 3% in this population not only a unique achievement but also a highly successful outcome (Dr. Mojiminiyi & Dr. Akanji, personal communication, 2007). Individual variations in lipoprotein response to changes in dietary composition have been linked, at least in part, to genetic differences amongst individuals (Dreon & Krauss, 1997). Therefore, outcomes of the current study cannot be generalized beyond an overweight/obese T2 DM Kuwaiti population.

6.3 The effects of low-carbohydrate Atkins diet

Another approach that was used to study the impact of diet on an ALP was to examine the effects of a low carbohydrate, Atkins diet for the management of overweight/obese subjects (Chapter 5). The effect of a low carbohydrate diet either alone or in combination with an exercise program was examined to provide evidence for the influence of physical activity in modulating the effects of this type of diet. A low carbohydrate diet (Brehm et al., 2003; Dashti et al., 2003; Jarvi et al., 1999; Samaha et al., 2003; Wood et al., 2006) and exercise (Anderson & Hoie, 2005; Blair, 1993; Fahlman et al., 2002; Petrella et al., 2005; Ross et al., 2000) has been shown to be beneficial in reducing some of the risk factors associated with CVD in overweight/obese individuals.

Dietary compliance in this study was measured by dietary records and supported by other measures. For example, the nutrient intake in the low carbohydrate and the low carbohydrate plus exercise group was analysed at baseline and at 6 months by averaging the intake of 7-day food diaries. For all participants, the average energy intake/day was reduced significantly (p<0.0005) from 2628±156 kcal/d-11038±31 KJ/d (SEM) at baseline to 1760±111 kcal/d-7392±466 KJ/d at 6 months, an energy deficit of 868 kcal/d-3646 KJ/d. An energy deficit of this magnitude for one week (868 x 7 = 6076) would be expected to produce a weight loss of 0.87 kg per week. Mean weight loss for all
participants together was 6.6 kg over 6 months, indicating a weight loss of 0.26 kg/week. Although the food diaries indicated that the participants adhered to the low carbohydrate diet, these diaries might not accurately reflect real consumption, as under-reporting in obese individuals is common (Barkeling et al., 1990).

The outcome of the low carbohydrate study is consistent with others (Brehm et al., 2003; Dansinger et al., 2005; Dashti et al., 2003; Samaha et al., 2003) in showing beneficial reductions in plasma TAG and increases in plasma HDL-C in the whole sample. The low carbohydrate diet in both groups, however, was not associated with decreased insulinemia. This was an unexpected finding. Other studies that achieved a similar weight loss have shown significant reductions in plasma insulin (Brehm et al., 2003; Volek et al., 2004b; Foster et al., 2003), and confirmed the association between weight loss and decreased insulin concentrations and improved insulin sensitivity. It is possible that the relatively high proportion of fat and protein in the low-carbohydrate diet in the current study had an adverse effect on insulin concentrations. At the end of the study participants had a higher percentage of saturated fat and protein intakes compared to baseline. High fat and high protein diets are known to increase plasma concentrations of insulin (Lovejoy et al., 1998; Nuttall et al., 1984). However, it seems that the effects of these macronutrients were counter balanced by weight loss and therefore the overall effect of diet on insulin concentrations was neutral.

One of the main concerns in consuming low carbohydrate diets is that they are relatively high in total and saturated fat. Diets high in saturated fats with 12-16 carbons are well known to increase plasma concentrations of LDL-C (Summers et al., 2002), an established risk factor for CHD (Turner et al., 1998). Findings from the current study would suggest that any adverse effect of increased total and saturated fat on LDL-C is offset by weight loss. Nonetheless, blood lipid concentrations should be regularly monitored in individuals following the Atkins diet, since LDL, though not significantly changed in the present study, might increase with the continued consumption of such a diet (Sondike et al., 2003).
The recommendation to increase exercise levels was clearly not met by the participants. While 86% of participants on the low carbohydrate diet alone complied with the instructions not to alter their exercise levels over the course of the study, only 63% of subjects in the low carbohydrate and exercise group complied with instructions to increase their exercise levels from baseline (Rebecca Hiscutt, personal communication, 2007). As a consequence, it is not surprising that there was no difference in markers of CVD risk factors between the dietary interventions, with and without exercise in the present study. Despite this, the recommendation to increase exercise as a strategy for the secondary prevention of CVD should be maintained because the role of exercise in assisting and maintaining weight loss has been well established by others (Wing, 1999; Avenell et al., 2004a; Blair, 1993). Moreover, there is evidence to suggest that the combination of exercise and energy restriction is favorable for altering the distribution of weight loss, increasing the loss of fat mass more than the fat-free mass (Ross & Janssen, 1999; Ross et al., 2000). Lastly, assessment of CVD risk in the Atkins study was based on a limited number of biomarkers of CVD risk that were chiefly lipid in origin. There are other CVD risk factors that play an initiating event in atherosclerosis that could be potentially improved by exercise. One of these risk factors is endothelial dysfunction. Individuals with endothelial dysfunction have a 3-4 fold increased risk of coronary events that is independent of traditional risk factors (Al Suwaidi et al., 2002). Exercise may improve endothelial function and enhance coronary blood flow in individuals at risk of CVD, thus reducing the overall risk of atherosclerosis (LaFontaine & Roitman, 2002). In general, physical activity levels are reported to be low both in the UK (National Centre for Social Research, 2004) and in Kuwait (Al-Asi, 2003).

6.4 Optimising a dietary approach for CVD risk reduction

The study of the low carbohydrate Atkins diet in Chapter 5 was of relatively short duration (6 months). Whether subjects will comply with such a diet over the long term is questionable (Foster et al., 2003). Therefore, a low GI/GL diet with a large amount of whole grains, fruit and vegetables such as the one used in the GI study in Kuwait (Chapter 4) might represent a potentially more flexible dietary approach for CVD risk reduction. The low carbohydrate diet was associated with a 17% reduction in plasma
TAG concentrations (Chapter 5), whilst T2 DM patients showed a reduction in plasma TAG of between 12%-16% on a low GI diet. In light of the increased association between raised plasma TAG and increased CVD risk, these two approaches might appear equally efficacious. However, a generalization of the findings should not be made as both studies involved relatively small subject numbers.

In a comparison of methods for assessing dietary intake, the use of diet diaries in the Atkins study was associated with more under-reporting than the 24-hour dietary recalls from T2 DM patients in Kuwait, (estimated weight loss from diet recalls =0.47 kg/week, actual weight loss =0.40 kg/week versus estimated weight loss from food diaries =0.87 kg/week, actual weight loss =0.26 kg/week for the GI and the Atkins study respectively). Despite the fact that the food diary method is considered to be more accurate for assessing dietary intake on an individual basis, since it directly measures portion sizes and does not depend on memory of the subject (Wrieden et al., 2003), the 24-hour recall used in the GI study seemed to better reflect actual intakes of overweight/obese T2 DM patients in Kuwait. While this finding does not necessarily support the superiority of the latter method over diet diaries in overweight/obese populations in the UK, it might indicate a possible confounding effect of cultural differences between the two groups (Harrison et al., 2000).

Findings from the energy restricted low GI study in T2 DM patients in Kuwait indicated a need for effective methods to promote healthy weight loss in this population. The Atkins diet that was tested in the present study in a UK overweight / obese population may represent an option for increasing dietary compliance for weight loss in T2 DM patients in Kuwait. Beneficial effects of the Atkins diet have been shown in T2 DM (Samaha et al., 2003). The Atkins Diabetes Revolution: the ground breaking approach to preventing and controlling type 2 diabetes book (Vernon & Eberstein, 2004) might be a useful dietary alternative to the Atkins diet for diabetics. The plan presented in this book is similar to the Atkins weight loss diet except that the intake of carbohydrates is progressively increased in relation to glycaemic control.
In diabetics, the adoption of multiple healthy habits has been definitively associated with a better health-related quality of life, even after adjustment for demographic and socioeconomic characteristics, diabetic complications and severity, and the related co-morbidities (Li et al., 2007b). Therefore, if weight loss is successfully achieved in overweight or obese individuals, then a maintenance diet would be a low GI diet combined with an exercise program. This might offer the optimal choice between low fat and low carbohydrate diets, since low fat diets may lead to increased glycaemia and insulinaemia (Ludwig et al., 1999). The metabolic benefits of such an approach in decreasing CVD risk might be significantly greater than those obtained from either a low-fat or low-carbohydrate diet alone.

An ALP consists of a collection of high risk abnormalities in plasma lipoproteins that cannot be measured by routine laboratory analyses. This is mainly because of technical difficulties in the measurement of lipoprotein subclasses (Rizzo & Berneis, 2006b). However, because of their strong link with CVD risk (Griffin, 1997; Griffin et al., 1994; Grundy, 1997; Gardner et al., 2000; Salonen et al., 1991; Ahmad et al., 2007), and potential value as therapeutic targets (NCEP ATP III, 2002), it has been suggested that measurements of LDL and HDL subclasses in high risk individuals (such as participants in the current studies) should be encouraged (Rizzo & Berneis, 2006b). Indeed, measurement of both LDL and HDL subclasses in the studies herein has strengthened evidence for the beneficial effect of the different dietary treatments used.

6.5 Concluding remarks

This thesis has examined multi-factorial lifestyle approaches for the reduction of CVD risk in overweight/obese T2 DM patients, with a focus on the modification of an ALP in overweight/obese individuals. The effects of these approaches on an ALP in each study were similar, in that they all showed favourable effects, to varying degrees, on lipids and lipoproteins. Both of the intervention studies resulted in reductions in metabolic risk factors for CVD, including a decrease in plasma TAG and increase in the HDL-C concentrations across the two studies. Because of poor compliance to the energy-restricted diet in the low GI study in Kuwait and to exercise regimens in the Atkins study.
no additional benefits of these approaches were shown. Despite this, weight loss and a high level of exercise should continue to be recommended for CVD risk reductions in high risk individuals with T2 DM and obesity. A low GI diet for T2 DM and a low-carbohydrate diet for overweight/obese individuals with an ALP would seem to offer a simple and acceptable approach for reducing CVD risk.

6.6 Future work

The outcome from these studies has generated questions that could represent principal objectives for future investigation. Future work could encompass the following:

Essential work that needs to be done as a priority would be to complete the low GI diet trial in T2 DM patients in Kuwait by conducting a follow-up phase. This should be done in order to reach a definite conclusion about the acceptability of the tested diet and the long term effects in these individuals. In addition, the measurement of gut hormones (CCK, PYY3-36, leptin, and ghrelin), as originally intended, would provide evidence for the effects of the low GI diet on energy metabolism and appetite regulation.

The GI of other carbohydrate-rich foods that are commonly used in Kuwait must also be determined. Indeed, establishing a GI database that is specific for Arabian foods should be a theme of future national (Kuwaiti) research, as the International Tables of GI values provide only a limited amount of information.

The staples that were examined in this thesis are seldom eaten alone. Therefore it would be worth examining the effects on the overall glycaemic response of adding fat and /or protein to these staples.

A multi-factorial intervention study for the reduction of an ALP, not only in T2 DM patients but also in otherwise healthy overweight/obese individuals in Kuwait, should be undertaken. In addition to dietary changes, this should also include the examination of changes in behavior and physical activity as major lifestyle components that influence and frequently confound dietary effects.
REFERENCES
References


Rizkalla, S.W., Bellisle, F. & Slama, G. (2002). Health benefits of low glycaemic index foods, such as pulses, in diabetic patients and healthy individuals. British Journal of Nutrition, 88 (Suppl 3.), S255-S262.


APPENDICES
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### Appendix I. Subject Randomization Form

232
**Appendix 2** Mean (SEM) of glycaemic and insulinaemic indices for tested foods.

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<th>GI (%)</th>
<th>II (%)</th>
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<td>n=10</td>
</tr>
<tr>
<td></td>
<td>Mean (SEM)</td>
<td>Mean (SEM)</td>
</tr>
<tr>
<td>White pita</td>
<td>58.6 (3.5)***</td>
<td>82.4 (13.4)a</td>
</tr>
<tr>
<td>Light white pita</td>
<td>56.2 (2.7)***a</td>
<td>77.3 (11.1)a</td>
</tr>
<tr>
<td>Brown pita</td>
<td>76.4 (5.0)***b</td>
<td>152.5 (23.6)b</td>
</tr>
<tr>
<td>Light brown pita</td>
<td>67.7 (5.2)***</td>
<td>99.6 (19.1)</td>
</tr>
<tr>
<td>White bread</td>
<td>67.8 (3.1)***</td>
<td>125.7 (16.4)</td>
</tr>
<tr>
<td>Brown bread</td>
<td>64.3 (3.9)***</td>
<td>98.1 (14.4)</td>
</tr>
<tr>
<td>Iranian bread</td>
<td>66.9 (6.8)***</td>
<td>139.5 (15.6)</td>
</tr>
<tr>
<td>White rice</td>
<td>62.5 (4.7)***</td>
<td>96.5 (16.3)</td>
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<tr>
<td>Glucose</td>
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<td>100</td>
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</tbody>
</table>

*Significantly different from the glucose load, p<0.05, **p<0.005, ***p<0.0005
Different letters within each column represents significant difference, p<0.05.
### Appendix 3. Mean plasma glucose at 0, 15, 30, 45, 60, 90, 120 min elicited by eight Kuwaiti foods in 10 healthy subjects.

Mean blood glucose concentration (mmol/l). n=10

<table>
<thead>
<tr>
<th>Food</th>
<th>0 min</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>90 min</th>
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Appendix 4. Ethical approval for the LGI study from the University of Surrey.

06 July 2007

Professor Linda Morgan
S B M S

Dear Professor Morgan

Influence of glycaemic index diet and a low glycaemic combined with weight loss diet on atheogenic risk in type 2 diabetics (EC/2005/94/SBMS)

On behalf of the Ethics Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the submitted protocol and supporting documentation.

Date of confirmation of ethical opinion: 06 October 2005

The list of documents reviewed and approved by the Committee is as follows:-

Document Type: Application
Dated: 20/08/05
Received: 31/08/05

Document Type: Insurance Proforma
Received: 31/08/05

Document Type: Research Protocol
Received: 31/08/05

Document Type: Appendix 1 – 24 Hour Dietary Recall
Received: 31/08/05

Document Type: Appendix 2 – Suitability Checklist
Received: 31/08/05

Document Type: Appendix 3 – Patient Information Sheet
Received: 31/08/05

Document Type: Appendix 4 – Consent Form
Received: 31/08/05
Appendix 5. Approval letter to conduct the LGI study in the Amiri hospital, Kuwait.

Diabetes Unit
Department of Medicine
AMIRI HOSPITAL - KUWAIT

Date: 28 September 2005

Dear Sir/ Madam

This is to confirm that we, the diabetic team at AlAmiri hospital in Kuwait, had reviewed the protocol that Mrs Badriyah Al-jazaf presented to us regarding the study of the influence of the low glycemic index and a low glycemic index combined with weight loss diet on cardiovascular disease risk associated with Type 2 diabetes mellitus. We have no objection over conducting this study in the hospital and using our patients as study subjects.

Should you have any other questions regarding this please contact team members in AlAmeri hospital, telephone & fax +965 246 8874

Dr. A. Ben Nakhri MD, consultant diabetologist

Dr. M Al-Aroux MD, consultant diabetologist

Dr. A Alattar MD, consultant diabetologist
Appendix 7. LGI diet sheet

المؤشر السكري المنخفض

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<td>Plums</td>
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<td>grapes</td>
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<tr>
<td>Rice</td>
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<tr>
<td>Spaghetti</td>
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<tr>
<td>Yam</td>
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<tr>
<td>Table sugar</td>
<td>90</td>
</tr>
<tr>
<td>Baked potatoes</td>
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</tr>
<tr>
<td>Carrots</td>
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<tr>
<td>Cornflakes</td>
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<td>Bananas</td>
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<tr>
<td>Instant rice</td>
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<tr>
<td>Rice Cake</td>
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<tr>
<td>French Bread</td>
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دلـيلك لوجـبات الفهـرس السكـري المنخفض
البدأ في وجبة المؤشر السكري المنخفض

المؤشر السكري هو قياس مدى سرعة امتصاص السكر في الدم بعد تناول الوجبة. الأغذية ذات المؤشر السكري المرتفع تحتوي على كربوهيدرات سريعة الامتصاص في الدم، في حين أن الأغذية ذات المؤشر السكري المنخفض تأخذ فترة أطول لامتصاص الدم السكر.

المؤشر السكري هو قياس يبدأ من 0 إلى 100. كلما ارتفعت المقياس باتجاه المئة كانت سرعة امتصاص السكر في الدم أسرع.

أهمية المؤشر السكري
- المؤشر السكري المنخفض يعني انخفاض أكثر للسكر في الدم بعد تناول الوجبات.
- الوجبات ذات المؤشر السكري المنخفض تثبت حساسية الإنسولين للجسم.
- المؤشر السكري المنخفض تثبت مدى تحكم مرض السكري بكمية السكر في الدم.
- الأغذية ذات المؤشر السكري المنخفض تعطي شعور بالشبع لمدة أطول.

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</tr>
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<td>موز ناضج</td>
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</tr>
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</tr>
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<td>أرز البسمتي</td>
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</tr>
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</table>
كيفية خفض المؤشر السكري للوجبات

الخطوة الأولى - عمل قائمة للكربوهيدرات الرئيسية (الخضروات-الفواكه-الخبز-الحليب).
معكرونة-الرز-البقوليات-المكولات المشوية .. الخ في الوجبة و معرفة عدد الالوفات التي تؤكل فيها هذه الأغذية خلال الأسبوع.

الخطوة الثانية - النظر إلى المؤشر السكري لكل صنف من الأغذية على القائمة. إذا لم تتمكن من إيجاد أحد الاصناف في القائمة، حدد محتويات الوجبة إذا كانت محتويات الوجبة إذا كانت محتويات الوجبة مثال: كير (الطحين الأبيض-السكر-شراب النكهة).
صنف على أنها أغذية عالية المؤشر السكري، و العكس صحيح.

الخطوة الثالثة - معرفة الأغذية ذات المؤشر السكري المرتفع في الوجبة، الأغذية ذات المؤشر السكري الأعلى من 70، ومحاولة ترتيبها. اختيار القليل من الأغذية ذات المؤشر السكري المتوسط (المؤشر السكري بين 55 و 70) التي يمكن الاستغناء عنها.

الخطوة الرابعة - استبدل الأغذية المفضلة وابداً بتناولها في الوجبات اليومية. إذا تم اختيار أحد الاصناف في الوجبة، حاول تبديله بأحد الاصناف المفضلة.

الخطوة الخامسة - شبع عادة اختبار السكر في الدم لإثبات فعالية الوجبة بناءً على معرفة أثر الكربوهيدرات المختلفة على مستوى السكر في الدم.
اقتراحات الوجبات

الإفطار

الإفطار ذات المؤشر السكري المنخفض يحتوي على خبز أو توست حبوب كاملة، الخبز بالنخالة و
الكثير من الفواكة الطازجة. تجنب السكريات.

Scheme

- خبز أسمر مع جبن قليل الدسم.

- روبي خالي الدسم مع فاكهة طازجة و النخالة.

- نخالة كاملة خليج لبناني يقدم مع بيض مخلوط.

- توست بالبيض مع شرب الفواكة لمرضى السكري.

- كعك كامل النخالة مع مربي لمرضى السكري و فاكهة.

- توست أسمر مع جبن قليل الدسم قابل للدهن و فاكهة.

- توست الشعر و بيض - اومليت مع فاكهة.

الغداء

في وقت الغداء، ابتعد عن الخبز الأبيض و الصمون، اعمل سندويش مستخدما خبز حبوب كاملة،
و سلطة مستخدمة فيها الكثير من الخضروات الطازجة المتنوعة و صلصة الخل. السمك أيضا
 مصدر جيد للبروتين.

- شوربات منزلية: خضروات، عدس، أو هريس (تستطيع إضافة المزيد من الخضروات).

- سندويشات لحوم خفيفة تصنع من الخبز كامل الحبوب، الخبز الينابيعي الأسمر، مع سلطة
الفواكة أو الخضروات.

- سلطات المعكرونة مع الخل و الخضروات الطازجة و جبن قليل الدسم.

- سلطة حمص، مع دجاج مشوي و الخل (خلط معها بعض البقوليات).
العشاء

ركز وجبتك على الكربوهيدرات ذات المؤشر السكري المنخفض، مع كميات من الخضروات النشوية والكثير من السلطات الطازجة. كل لحم خالية من الدهون، الدواجن أو السمك. إنه وجبت المنتظمة بفاصهة طازجة.

مكرونة، حبوب، أرز، بفوليات، خبز كامل الحبوب،

خضروات طازجة وسلطات.

لحم خالية من الدهون، دجاج، و سمك.

استبدال البقوليات، البازلاء بمصدر بروتيني إذا أحببت.

وجبة خفيفة (اختار أثنتان من القائمة)

باختيار وجبتين خفيفتين مع حلويات ذات مؤشر سكري منخفض.

فواكة طازجة أو مجففة.

مكسرات (كمية قليلا).

ملطيات قليلة الدسم مع فواكة طازجة.

تفاح.

بسكويت نخالة

قطعة شوكولاتة خالية من السكر (كمية قليلة).

ناشوز
الاغذية المرتفعة والمنخفضة في المؤشر السكري

<table>
<thead>
<tr>
<th>إفطار ذات مؤشر سكري مرتفع</th>
<th>إفطار ذات مؤشر سكري منخفض</th>
</tr>
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<tr>
<td>عصير توت</td>
<td>عصير البرتقال</td>
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</tr>
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<tr>
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<tr>
<td>مربي فراولة</td>
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<td>سندوتش بالخبز الأبيض مع جلي</td>
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<td>سلطة البطاطس</td>
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<td>فشار</td>
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<td>شوكولاتة لمرضى السكري</td>
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<td>كيك بالكريمة</td>
<td>(سكر صناعي) روب بالفواكة</td>
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</table>

246
نظام إشارة المرور للمؤشر السكري المنخفض

تستطيع تحديد الغذاء ما إذا كان ذات مؤشر سكري مرتفع أو منخفض بتباع إشارة الوقوف لائحة الأغذية:  

أخضر يعني منخفض - يمكن أكل هذه الأغذية يوميا. 

أصفر يعني معتدل - يمكن أكل هذه الأغذية أحيانا حوالي ثلاث مرات في الأسبوع.

أحمر يعني مرتفع - نادرا ما تؤكل هذه الأغذية.

إذا لم يكن الغذاء موجودا في القائمة ابحث عن غذاء مماثل للغذاء الذي تبحث عنه في القائمة.

المؤشر السكري لهم سيكون تجريبيا متشابه.

<table>
<thead>
<tr>
<th>المجموعة الغذائية</th>
<th>الأخضر يوميا</th>
<th>أصفر مرتين أو ثلاث مرات في الأسبوع</th>
<th>أحمر مرة في الأسبوع أو أقل</th>
</tr>
</thead>
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| الخبز والحبوب     | منتجات الحبوب الكاملة بدون سكريات، عسل، سكر أسمر، أو شراب الذرة  
                      خبز كامل الحبوب  
                      مكرونة كاملة الحبوب  
                      أرز إسمر  
                      حبوب بدون سكر  
                      بسكويت بالحبوب  
                      كعك مهللي مع عصير فاكهة  
                      سكر صناعي  
                      بذخ الصال بالحبوب الكاملة  
                      غالبا يمكن أكله مكرونة  
                      حبوب قليلة النخالة  
                      بسكويت محلى  
                      خبز أبيض  
                      خبز مع سكر  
                      حبوب مع سكر  
                      أرز أبيض  
                      بسكويت محلى |
| الفواكة          | تفاح  
                      مشمش  
                      توت  
                      بطيخ أصفر  
                      كرز، عنب  
                      جريب فروت، كيوي  
                      مانجا، برتقال  
                      خوخ  
                      اجاص، يندي  
                      موز (قليلة النضح)  
                      أفضل  
                      فواكة مجمدة  
                      بطيخ  
                      اناناس  
                      تمر  
                      فواكة مملة مع شراب و سكر  
                      فواكة ملمحة مع سكر |
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<td>وجبات خفيفة</td>
<td>أغلب الفواكة (غير الموز أو البطيخ)</td>
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</table>

طرقية التحضير تؤدي إلى الاختلاف
- إضافة الدهن يؤدي لتحويل الاختيار الأخضر أو الأحمر إلى الاختيار الأصفر.
- العديد من السكر الصناعات المحلية يؤدي لتحويل اختيار الأغذية باللون الأخضر أو الأصفر إلى الاختيار الأحمر.
- أغلب الفواكة والخضروات لديها مؤشر سكري منخفض.
- أغلب الأغذية النشوي مثل البطاطس، الجزر، الخبز الأبيض، الحبوب، السكويت، لديها مؤشر سكري مرتفع.
- الأغذية قليلة الكربوهيدرات مثل اللحوم، البيض، الحليب ومنتجات الألبان لديها مؤشر سكري منخفض، لكن البروتينين يجب أن يكون معتدل، حيث أن تناول الكثير من الدهون و الكثير من السعرات الحرارية يمكن أن يقلل فوائد الوجبة المنخفضة المؤشر السكري.
Low Glycaemic Index Diet Sheet

Your Dietary Guide to a Low GI Diet
How to reduce the GI of the diet

- **Step 1** - Make a list of the main carbohydrates (vegetables, fruits, breads, grains, cereals, pasta, rice, juices, beans, soups, baked goods, etc.) in the diet and note the number of times a week that particular food is eaten.

- **Step 2** - Look up the Glycemic Index for each food on the list. If you can't find a particular food listed, consider the foods ingredients. If they are all highly processed (refined white flour, sugar, corn syrup) rank the food HIGH. If the food has some processed and some unprocessed ingredients, rank it a MEDIUM. If almost all the ingredients are in their natural state, rank the food as LOW. This is a good rule of thumb, but it's not exact. Also, overcooked foods will tend to have a higher GI than undercooked foods. This is especially true for pasta, vegetables, grains, and cereals.

- **Step 3** - Identify the high glycemic foods in the diet, foods with a GI over 70, and try to eliminate as many as you can. Pick out some of the medium GI foods (GI between 55 and 70) you can do without.

- **Step 4** – Substitute foods that are enjoyed and start working them into every day diet. If eating out and have to select a high GI food try to offset it by ordering something with a very low GI. Or, consider ordering a vinaigrette dressing on a salad to bring the average GI of your meal lower. Remember, this isn't an “all or nothing” diet. No one eats perfectly all the time. Just do the best you can and watch what happens.

- **Step 5** – Encourage the habit of testing blood glucose to prove it works. This will help people knowing the effect of various carbohydrates on their blood glucose level.
Meal Ideas

Breakfast
A low-GI breakfast includes whole-grain breads or toasts, oat based cereals, and lots of fresh fruits. Avoid syrups and stay away from instant hot cereals.

- Cup of tea or coffee
- Brown bread with Low fat cheese
- Light yogurt with fresh fruit and bran
- Low-GI cold cereal with skim milk and fruit
- Whole wheat pita bread stuffed with scrambled egg
- French toast with diabetic maple syrup and fruit
- All-bran muffin with diabetic jam and fruit
- Brown toast with melted low-fat cheese and fruit
- Rye toast and egg-white omelette and fruit

Lunch
At lunch time, stay away from processed white bread and rolls. Instead, make a sandwich using wholegrain rye bread. Try salads made with lots of varied fresh vegetables and vinaigrette dressing. Fish is also a good protein addition

- Homemade soups: vegetable, lentil, minestrone, or barley (you can add extra vegetables)
- Sandwiches made with lean meats on whole-grain wheat, rye, pita bread, along with vegetable and fruit salad
- Pasta salad with vinaigrette dressing and assorted fresh vegetables and reduced-fat cheese
- Mixed salad, hummus, with grilled chicken and vinaigrette dressing (toss in some beans)
Dinner

Base your meal on a low-GI carbohydrate, with generous amounts of non-starchy vegetables and plenty of fresh salads. Eat lean meats, poultry, and fish. End your balanced meal with a serving of fresh fruit.

- Pasta, grains, rice, beans, whole grain breads
- Fresh vegetables and salads
- Lean meats, chicken, and fish
- Substitute beans, peas, or lentils for protein sources if you prefer

Snack (choose 2 from the list)

By choosing snacks with a low GI or low-fat desserts, you can help prevent some of the symptoms and complications associated with gusher foods.

- Fresh or dried fruits
- Nuts (small serving)
- Low-fat ice cream with fresh fruit
- Apple
- Oatmeal biscuits
- Sugar free chocolate bar (small serving)
- Baked tortilla chips
Starting a low GI diet

The Glycemic Index (GI) is a measure of how fast after eating a food glucose gets absorbed into the bloodstream. Foods high on the GI contain carbohydrates that are rapidly absorbed into the bloodstream and, foods with low GI values take relatively longer to release sugars into the bloodstream.

The GI uses a scale from 0 to 100. As you move up the scale, towards 100, the quicker the food source will elevate your blood sugar levels. At the top of the scale (100) is normally pure glucose.

The significance of the glycaemic index

Low GI means a smaller rise in blood glucose levels after meals
Low GI diets can improve the body’s sensitivity to insulin
Low GI can improve diabetes control
Low GI foods keep you feeling fuller for longer

<table>
<thead>
<tr>
<th>High GI Foods</th>
<th>Moderate GI Foods</th>
<th>Low Glycemic Foods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Orange Juice</td>
<td>Apple</td>
</tr>
<tr>
<td>Baked Potato</td>
<td>White Rice</td>
<td>Pear</td>
</tr>
<tr>
<td>Corn Flakes</td>
<td>Popcorn</td>
<td>Skim Milk</td>
</tr>
<tr>
<td>Cheerios</td>
<td>Corn</td>
<td>Green Beans</td>
</tr>
<tr>
<td>Graham Crackers</td>
<td>Brown Rice</td>
<td>Lentils</td>
</tr>
<tr>
<td>Honey</td>
<td>Sweet Potato</td>
<td>Kidney Beans</td>
</tr>
<tr>
<td>Watermelon</td>
<td>(Ripe) Banana</td>
<td>Grapefruit</td>
</tr>
<tr>
<td>White Bread</td>
<td>Orange</td>
<td>Barley</td>
</tr>
<tr>
<td>Table Sugar</td>
<td>Apple Juice</td>
<td></td>
</tr>
<tr>
<td>Raisins</td>
<td>Basmati Rice</td>
<td></td>
</tr>
<tr>
<td>High GI Breakfast</td>
<td>Low GI Breakfast</td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>------------------</td>
<td></td>
</tr>
<tr>
<td>Cranberry Juice Cocktail</td>
<td>Orange Juice</td>
<td></td>
</tr>
<tr>
<td>Cantaloupe</td>
<td></td>
<td>68</td>
</tr>
<tr>
<td>Raisins</td>
<td></td>
<td>65</td>
</tr>
<tr>
<td>Corn flakes</td>
<td></td>
<td>92</td>
</tr>
<tr>
<td>Cherrios</td>
<td></td>
<td>74</td>
</tr>
<tr>
<td>Plain Bagel</td>
<td></td>
<td>72</td>
</tr>
<tr>
<td>English Muffin</td>
<td></td>
<td>77</td>
</tr>
<tr>
<td>White Toast</td>
<td></td>
<td>70</td>
</tr>
<tr>
<td>Waffles</td>
<td></td>
<td>76</td>
</tr>
<tr>
<td>Pancake Syrup</td>
<td></td>
<td>76</td>
</tr>
<tr>
<td>Strawberry Preserves</td>
<td></td>
<td>74</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>High GI Lunch Food</th>
<th>Low GI Lunch Food</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken Rice Soup</td>
<td>Minestrone Soup</td>
</tr>
<tr>
<td>Sandwich on white bread</td>
<td></td>
</tr>
<tr>
<td>Peanut Butter and Jelly Sandwich on White Bread</td>
<td>Peanut Butter and Diabetic fruit spread on Whole Wheat Bread</td>
</tr>
<tr>
<td>Potato Salad</td>
<td></td>
</tr>
<tr>
<td>Watermelon</td>
<td>Macaroni Salad</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>High GI Dinner Food</th>
<th>Low GI Dinner Food</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instant Rice</td>
<td>Basmati Rice</td>
</tr>
<tr>
<td>Baked Potato</td>
<td>Boiled, New Potato</td>
</tr>
<tr>
<td>Instant Mashed Potatoes</td>
<td>Sweet Potato</td>
</tr>
<tr>
<td>French Fries</td>
<td></td>
</tr>
<tr>
<td>White Rice</td>
<td></td>
</tr>
<tr>
<td>Bread Stuffing</td>
<td></td>
</tr>
<tr>
<td>Taco Shell</td>
<td></td>
</tr>
<tr>
<td>Hamburger Bun</td>
<td></td>
</tr>
<tr>
<td>Macaroni and Cheese</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>High GI Snack Food</th>
<th>Low GI snack Food</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretzels</td>
<td>Peanuts</td>
</tr>
<tr>
<td>Corn Chips</td>
<td>Popcorn</td>
</tr>
<tr>
<td>Graham Crackers</td>
<td>Diabetic Chocolate Chip Cookies</td>
</tr>
<tr>
<td>Rice Cakes</td>
<td>Cherries</td>
</tr>
<tr>
<td>Water Crackers</td>
<td>Low Fat Ice Cream</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The Low Glycemic Index Traffic Light System

You can determine if a food has a High or Low Glycemic Index by referring to the stoplight list of foods:

- Green means low – eat these foods every day
- Yellow means moderate – eat these foods sometimes—about three times a week
- Red means high – eat these foods rarely

If the food is not on the list, find a food that is similar to one that is on the list—the glycemic index will probably be similar.

<table>
<thead>
<tr>
<th>FOOD GROUP</th>
<th>Green often (every day)</th>
<th>Yellow 2 or 3 times a week</th>
<th>Red Once a week or less</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breads, Grains and Cereals</td>
<td>Whole grain products without dextrose, maltose, honey, molasses, brown sugar or corn syrup Whole Grain Breads Whole Grain Pasta Whole grain brown rice, Oatmeal Bran and Whole Grain cereals with no sugar added Whole grain crackers Stone Ground whole wheat bagels or Pitas Bran muffins sweetened with fruit juice, fructose or artificial sweeteners</td>
<td>Tortillas (whole wheat can be eaten more often) Regular pasta Instant oatmeal or other instant cereals</td>
<td>White bread Breads with added sugar Cereals with added sugar White rice Cookies or other baked goods with added sugar Crackers</td>
</tr>
<tr>
<td>Fruits</td>
<td>Bananas (less ripe are the best)</td>
<td>Watermelon, pineapple, Dates, Canned fruits with added sugar or in syrup, Frozen fruits with added sugar</td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>----------------------------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Apples, Apricots, Berries, Cantaloupe/honeydew melons, Cherries, Grapes, Grapefruit, Kiwis, Mangos, Oranges, Peaches, Pears, Plums, Tangerines</td>
<td>Raisins, Dried fruits</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vegetables</th>
<th>Peanut butter (no sugar added)</th>
<th>Mashed Potatoes, French Fries, Baked Potatoes, Potato Chips, Corn, Popcorn, Turnips, Parsnips, Sweet pickles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artichokes, Asparagus, Dried Beans (all kinds), Broccoli, Cabbage, Cauliflower, Celery, Cucumbers, Eggplant, Green Beans, Green Onions, Lentils, Mushrooms, Onions Peas, Spinach, Squash and Zucchini, Sweet potatoes and yams, Tomatoes, Peppers</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Meats, Eggs, Beans, Nuts</th>
<th>Peanut butter (no sugar added)</th>
<th>Sugar cured meat, Cold cuts processed with sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean Beef, Chicken, Turkey, Fish, Shellfish, crabmeat, shrimp, scallops, Lamb, Beans, Lentils, Nuts</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Milk and Dairy</th>
<th>Whole milk, Low fat ice cream, Yogurt with added sugar</th>
<th>Regular Ice Cream, Chocolate milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheese, Low fat milk, Yogurt (no added sugar), Eggs, Butter</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cottage Cheese</td>
<td>Sour Cream Frozen Yogurt</td>
</tr>
<tr>
<td>---------------</td>
<td>----------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td><strong>Fats &amp; Condiments</strong></td>
<td>Olive oil/canola oil, vinegar, Mustard, mayonnaise, Soy sauce, salsa, Spreadable fruit (no added sugar), Tomato sauce</td>
<td>ketchup</td>
</tr>
<tr>
<td><strong>Snack Foods</strong></td>
<td>Most fruits (not bananas or watermelon), Yogurt (no sugar added), cheese, Peanuts, Nuts, Celery</td>
<td>Low fat frozen yogurt, or ice cream, Peanut butter (no sugar added)</td>
</tr>
<tr>
<td><strong>Beverages</strong></td>
<td>Low fat milk, Unsweetened tea</td>
<td>Diet Soda, Whole fruit juices</td>
</tr>
</tbody>
</table>

- **Preparation Makes a Difference**
  - Adding fat will make a yellow choice a **green** or **red** choice a **yellow**.
  - Extra sugar or sweetened sauces will make a **green** or **yellow** food a **red** choice.

- Understand that most fruits and many vegetables have a low glycemic index.
- Most starchy foods like potatoes, carrots, breads, cereals, crackers, have a high glycemic index.
- Foods that are low in carbohydrate like meats, eggs, milk, cheese and other dairy products have a low glycemic index, but the portions of these need to be moderate, since too much fat and too many calories can overcome the benefits of the low glycemic index diet.
Appendix 8. VAS questionnaire

اسم:
الرقم:
الالج:
التاريخ:

يرجى وضع علامة على الخط الأمامي حتى تكون كل سؤال ضع العلامة طبقًا لما تشعر به في هذه اللحظة.

ما مده شعورك بالجوع؟
لا أشعر بالجوع أبداً في حياتي

ما مدى رغبتك في الأكل؟
قوي جداً

ما مده شعورك بالشبع؟
أبداً لم أشعر

ما هي كمية الأكل التي تستطيع تناولها الآن؟
كمية كبيرة

بعد ساعة من الوجبة

ما مده شعورك بالجوع؟
لا أشعر بالجوع أبداً في حياتي

ما مدى رغبتك في الأكل؟
قوي جداً

ما مده شعورك بالشبع؟
أبداً لم أشعر

ما هي كمية الأكل التي تستطيع تناولها الآن؟
كمية كبيرة
Please place a vertical mark through the line for each question. Mark the line according to how you feel at this moment. Regard both ends of the lines as indicating the most extreme sensations you have ever felt.

**Immediately before meal**

**How hungry do you feel?**

- Not hungry at all
- Very week
- Not at all full
- Nothing at all

**How strong is your desire to eat?**

- Very strong

**How full do you feel?**

- Extremely full

**How much food do you think you could eat?**

- A large amount

**1 hour after Meal**

**How hungry do you feel?**

- Not hungry at all
- Very week
- Not at all full
- Nothing at all

**How strong is your desire to eat?**

- Very strong

**How full do you feel?**

- Extremely full

**How much food do you think you could eat?**

- A large amount
استبيان المؤشر السكري لل診対

يعزز من مشاركتك في بحث لمؤشر السكري للأغنية على مدى خطورة الإصابة بأمراض القلب في النوع الثاني من السكر تم تقديم هذا الاستبيان. قد يختار الإجابة الأسباب من بين الإجابات الثلاث.

1. أي نوع من الخزير يصف على أنه ذو مؤشر سكري منخفض نسبياً
   • الخزير العربي الأبيض
   • الخزير الأيسر
   • لا أعلم

2. عندما أقوم بتناول وجبة خفيفة في النهار أتناول
   • سكويت دايجستيف
   • سكويت ماري
   • لا أعلم

3. من الأفضل تناول الثاني على وجه التطور
   • الكورن فليكس
   • رقيق الإفطار الكاملا
   • لا أعلم

4. أي من النباتي له مؤشر سكري منخفض نسبياً
   • المانجا
   • التفاح
   • لا أعلم

5. بعض الأغاني ترفع مستوى السكر في الدم بشكل سريع. هذه الأغانية تشمل
   • الأرز الأبيض
   • الفاصوليا
   • لا أعلم

6. إذا كان هناك نوع من السلطة فإلى ذلك اختار
   • سلطة البطاطس مع المانيايز
   • سلطة المعكرونة مع المانيايز
   • لا أعلم

7. الأرز المصري يحتوي على مؤشر سكري ............... من الأرز المصري.
   • أعلم
   • لا أعلم

8. أفضل تناول الثاني في وجهة العشاء
   • شوربة العدس
   • شوربة المعكرونة
   • لا أعلم

9. أي من النباتي له مؤشر منخفض نسبياً
   • البطاطس المهروسة
   • البذور
   • لا أعلم

10. الهدف من تناول الأغنية ذات المؤشر السكري المنخفض هو
    • تنظيم نسبة السكر في الدم
    • تخفيض الوزن
    • لا أعلم

11. من الأفضل استبدال البذور الأبية بالثاني
    • الخزير الأبيض
    • توست السكر ذو
    • لا أعلم

12. المؤشر السكري للصلع عندما يوضع على خال النخالة
    • يقل
    • يزيد
    • لا أعلم

وشكرنا لمشاركتك في تعابير الإستبيان...
Glycaemic index Knowledge Questionnaire

As part of your participation in the study of the influence of a low GI diet on the metabolic risk profile in type 2 diabetes, we have created this questionnaire for you. Tick one of the boxes opposite to the appropriate answer to each question. Your responses will be completely confidential but will help us to find out how much you know about the glycaemic index.

1. Which type of bread has relatively a lower GI
   - White pita bread
   - Wholemeal bread.
   - Don’t know.

2. If you think about eating a low GI snack it is better to have
   - Digestive biscuits.
   - Marie biscuits.
   - Don’t know.

3. This food is a better choice for breakfast since it has a lower GI
   - Cornflakes.
   - All-Bran.
   - Don’t know.

4. Which of these has a lower GI
   - Mango.
   - Apples.
   - Don’t know.

5. Some foods tend to raise glucose levels rapidly. Such foods include
   - White rice.
   - Beans.
   - Don’t know.

6. This type of salad has a lower GI
   - Potato salad with low fat mayonnaise.
   - Pasta salad with low fat mayonnaise.
   - Don’t know.

7. Basmati rice has a.............GI than short grain rice
   - Lower.
   - Higher.
   - Don’t know.

8. As a dinner item, it is better to choose
   - Lentil soup.
   - Pasta soup.
   - Don’t know.

9. Which of these foods have a relatively lower GI
   - Mashed potatoes.
   - Pasta.
   - Don’t know.
10. The main idea of eating a low GI diet is to
   • Control glucose level.
   • Lose weight.
   • Don’t know.

11. It is better to replace wholemeal bread with
   • White bread.
   • Sourdough bread.
   • Don’t know.

12. The GI of jam when combined with wholegrain bread become
   • Higher than that of the jam alone.
   • Lower than that of the jam alone.
   • Don’t know.

When you have completed filling out the above form please hand it over to the dietitian. Thank you for your contribution of time and effort.
Appendix 10 Standard interview form for collection of 24-hour dietary recall

24-hour dietary interview

24-hour dietary interview format to collect detailed information about all foods and beverages.

The interview will include the following:

1) Quick List: Respondent will be asked to recall all foods and beverages consumed in a 24-hour period the day before the interview without interrupting.

2) Time, Occasion and Place: Respondent will be asked to report the time and place each food was eaten and what they would call the eating occasion for the food. Afterwards, a list of frequently forgotten foods will be shown to probe the respondent for any forgotten foods or drinks.

3) Food Details: Specific food probes will be used to collect detailed information for each food reported. This includes a complete description of each food and the amount eaten.

4) Final Review: The reported foods will be reviewed with the respondent in chronological order. Any additional foods remembered during the process will be added to the record as well as modifications for reported foods.
Appendix 11. Approval letter from sponsors to conduct the study in Kuwait

Embassy of the State of Kuwait
Cultural Office
London

28 May 2006
GPA053

chers/guests,

It is with great pleasure that we inform you of the approval of the study conducted in Kuwait. This letter serves as a formal acknowledgment of your permission to conduct the study in the cultural context of Kuwait.

The study has been approved by the Ministry of Education, Culture and Higher Education. The approval was granted on the condition that the study should be conducted with the utmost care and respect for the cultural norms and values of Kuwait.

Please find attached the formal letter of approval from the Ministry of Education, Culture and Higher Education. This letter should be treated as an official document and should be kept on file.

The study will commence on 1st March 2006. We are looking forward to a fruitful collaboration with you and your team.

Yours sincerely,

[Signature]

[Name]

Correspondence should be addressed to The Cultural Counsellor & Head of the Office
60A Knightsbridge, London SW1X 7JX
Telephone: 020-7761 8500 Fax: 020-7761 8505
www.kuwaitculturaluk.com

266
Appendix 12. Suitability checklist

Suitability Checklist

Clinic Name: ____________________________
Patients Name: _________________________ Phone #: _________________________

Type 2 diabetes

Suitable

Age: ________ years

BMI: ________ kg/m²

Microvascular complication

HDL-C: ________ mmol/l

TAG: ________ mmol/l

Name: ____________________________ Signature: ____________________________ Date: ________

(Medical supervisor)
Appendix 13. Patient information sheet.

معلومات عن الدراسة

العنوان: هل تستطيع الأغذية المنخفضة في مؤشر السكري من تحسين مستوى الدهون الضارة لدى المرضى الذين يعانون من النوع الثاني من السكري

شكراً على مساعدتك في المشاركة في دراسة الموضوع أعلاً. قبل مساعدتك على المشاركة من المهم أن تفهم أهمية هذه الدراسة. لا ستخدم النتائج لقراءة المعلومات التالية بحذر وملاحظتها مع عيتك إذا أحببت. وإن كنت بحاجة للمعالجة والاستفسار عن أي شيء غير واضح أو احتجت المعلومات إضافية فلا تتردد في السؤال والرجاء أخذ الوقت الكافي لتحقيق ما إذا كنت تستطيع في الدراسة أم لا.

ما هو الهدف من الدراسة؟

تعتبر التغذية الصحية حجر الأساس في علاج السكري. لعدة سنوات وصفت منظمات التغذية العالمية بخيارات الوجبات الصحية في محاولة أن تؤدي إلى تقليل الدهون والكربوهيدرات التي يمكنها أن تؤدي إلى ارتفاع نسب السكر في الجسم.

لحل هذه المشكلة تم اقتراح تناول الأغذية ذات الأثر السكري المنخفض. يعتبر المؤشر السكري قياس مدى ارتفاع السكر في الدم بعد تناول الوجبات. إذا كان مؤشر السكري المنخفض فإن ارتفاع السكر بعد تناول الوجبة منخفض. أن الهدف من الدراسة هو مقارنة تأثير الأغذية ذات الأثر السكري المنخفض والأغذية ذات الأثر السكري الأكثر ارتفاعاً على الجهاز العصبي التلقائي عند الأشخاص الذين يعانون من النوع الثاني للسكري. ودراسة تأثير هذه الأغذية على خطورة الإصابة بأمراض القلب.

ما هي مدة الدراسة؟

سيكون هناك أسبوعان في بداية الدراسة حيث تعرف فيها على عاداتك الغذائية ثم يتبعها أربع أسابيع من البحث و brains.

في الختام، يعتبر ذلك مقابلات من قبل الباحث للمعايرة في الوقت المناسب بالنسبة للدراسة، كيف تكون متوازن وجذاب العمليات. كما يمكنك متابعة الدراسة حيث تقوم بأنتج وهبة البدائل لتحديث البيانات.

وتاريخ عيانك.

الجزء الأول من الدراسة:

سوف تقوم ببناء اختبار لتمد بعد 14 ساعة من الصيام بعد الوجبة. سوف يتم تدريبك بما يجب تناوله من الأغذية في هذه الفترة (ستة أسابيع) مع تقديم اقتراحات بما يجب تناوله أو الابتعاد عنه في هذه الفترة. في منتصف هذه الفترة الزمنية سيعود التاريخ الغذائي وفقاً لما وصفت البدائل المستخدمة في النهاية للسنا الإسابيع تتبع نقص متوسط هيئة دم آخر.

الجزء الثاني من الدراسة:

سوف يأخذ عينات الدم في بداية ونهاية هذه الفترة أيضاً وتاريخك الغذائي في منتصف الدراسة. وذلك بعد تزويديك بقائمة الأغذية التي يجب تناولها أو الابتعاد عنها. ونهاية هذه الفترة سوف تتبع الدراسة.
الجزء الثالث من الدراسة:
في هذه الفترة ستكون لديك حرية الاختيار بين تناولك للوجبة التي تتناولها في 6 أسابيع السابقة أو وجبتك الاعتادية التي كنت تتناولها قبل فترة الدراسة.

هل هناك التزامات أخرى يجب الأخذ بها خلال فترة الدراسة?
إلى جانب الوجبات لا يوجد أي التزامات أخرى. سوف تقوم بأخذ الأدوية المقررة لك من السابق وممارسة ما كنت تمارسه من أعمال.

هل توجد أثار سلبية لهذه الدراسة?
لا توجد هناك أي أثار سلبية لتناول الأغذية حيث أنها أغذية معتادة متوافرة في الأسواق والجمعيات التعاونية.

ما هي وسائل المشاركة في البحث?
نحن نأمل أن تزودنا الدراسة بمعلومات يتم على أساسها توصيات غذائية للكويتيين الذين يعانون من السكر. بالإضافة إلى ذلك نقوم بإجراء تحليل لا تجري عادة في مختبرات الدولة وهي حساسة وتحدد نسبة احتمالية إصابتك بأمراض القلب في المستقبل.

ملاحظات:
في حالة الاستفسار الرجاء الاتصال بالأرقام التالية:
أ. بدري الجزاف 9718936
أ. ليلى عبد الغفور 9715691

وتمتى لك الشفاء العاجل بإذن الله...
Introduction
Thank you for agreeing to consider taking part in this study. Before you decide whether to participate it is important for you to understand why research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask us if there is anything that is not clear or if you would like further information, and please take time to decide whether or not you wish to take part.

What is the study about?
Diet is the cornerstone in the treatment of diabetes. For many years we have recommended a high carbohydrate, low fat diet for people with type 2 diabetes, but there has been concerns recently that this may not be ideal, and that, in particular, it may have an adverse effect on some blood fat abnormalities that are seen in people with diabetes, and which may be among the factors which increase the risk of heart disease in diabetes. One solution for the problem has been the introduction of diets containing foods with a low glycemic index (GI). The GI is a measure of the rise in blood glucose after eating a particular food; foods with low GI produces less of a rise in blood glucose; carbohydrate in foods like pasta, fruits and vegetables is absorbed slowly as compared to carbohydrate in white rice potatoes that is absorbed rapidly, giving these foods a low and high GI respectively. Low GI foods are beneficial to diabetes and appear to have favorable effect on blood lipids, especially the TAG, a lipid that has been pertinently associated with heart disease risk. A low GI foods combined with a weight loss diet may have an even more beneficial effect on blood lipids. However studies comparing the relative effect of low GI diets alone and combined with weight loss are lacking.

Why have you been invited to participate?
We plan to carry out the study in overweight people with type 2 diabetes who have been on stable medication for at least 6 months and who are currently eating a high GI diet. As far as we understand you fit these criteria.

Do you have to take part?
Absolutely not. There is no pressure on you to participate in this study, and it is entirely up to you to decide whether to do so or not. If you decide to take part, you will be asked to sign a consent form. If you decide to take part you are still free to withdraw from the study at anytime. I you decide not to take part or if you withdraw from the study, this will in no way affect the standard of care which you will continue to receive.

What will the study involve?
This is a dietary intervention study which will involve a 2 week run-in phase followed by a 6 weeks period of dietary modification and another 6 weeks period of follow up. The study will not involve a change in your medication unless advised by your diabetologist.

Visit 1 (week 0)
This will be done during your routine visit to AlAmeri hospital. You will be interviewed by your diabetologist to make sure that you are qualified to participate in the study. She/he will outline the details of the study and the type of dietary modification which you would be asked to implement. If you agree to take part, you will be asked to sign a consent form and you will also be interviewed by the study dietitian (whom you may have seen before) and asked to recall all the foods/drinks consumed within the day before.

You will then be asked to follow your usual diet and activity level for two weeks. During this period you will be asked also to fill a questionnaire rating your appetite in 3 predetermined dates. Your dietitian will give you a phone call the night before to remind you to do so.

Visit 2 (week 2)
You will be asked to come in having fasted overnight. Prior to the meeting with the study dietitian, a blood sample for measuring blood lipids, glucose, and glucose control will be taken. Your weight, height, and waist circumference will be measured. After this you will be allowed tea or juice and will be able to take your regular medication as appropriate.

For the next 6 weeks you will be assigned to one of three groups. You will be asked to either 1. Follow your usual diet. 2. A low GI diet or 3. A low GI with a weight reducing diet. You will be given advice by the study dietitian on what foods to consume and suggestions for menus. During the six week period, you will be getting phone calls from the study dietitian to provide help and answer your questions regarding the study diet and also to collect 2 dietary recalls. You will also be asked to fill a questionnaire rating your appetite on 3 dates within this period.

Visit 3 (week 6)
You will be asked to come in having fasted overnight. Prior to the meeting with the study dietitian, a blood sample for measuring blood lipids, glucose, and glucose control will be taken. Your weight, height, and waist circumference will be measured. After this you will be allowed tea or juice and will be able to take your regular medication as appropriate.

You will be also interviewed by the study dietitian and asked to recall all the foods/drinks consumed within the day before. You can ask any question and talk about your concerns also during this visit.

Visit 4 (week 12)
As in visit 2. After that you will be free to continue following the intervention diet for 6 more weeks or get back to your habitual intake.

Are there any adverse effects of either dietary intervention?
The risks to you are very low since you will be asked to consume only foods which are readily available in supermarkets. Low GI diet usually contains high dietary fiber and
may occasionally cause minor abdominal discomfort when the diet is started, but these effects soon pass as you get used to the diet. We do not anticipate any other adverse effects.

**What are the possible benefits of taking part?**

We hope that the study will provide information on which to base future recommendations for diet in people with diabetes. If the dietary modification which we are studying is beneficial you will have had the opportunity to try the diet and to have done so under instruction with a considerable amount of useful information given to you. At the end of the study we will let you know how you responded to the diet.

**Confidentiality**

Any information which we obtain about you and the results of your blood tests will be held in confidence by members of the study team. During the study, you and your results will be identified by a code number. You will be informed of any results obtained which may affect your future treatment.

**Expenses**

We are unable to offer you any remuneration for participating in the study. However, transportation will be available in the event that a lift to/from study sites is needed during the study period.

**Contact for future information**

Should you require any further information or have any concerns now or at any time during the study please contact

Mrs Badriya Al-jazaf 9718936
Dr. Tahani Al-niama 9752209
Mrs. Layla Abdulghafoor 9715691

*Thank you for your time and for considering to participate in this study*
Appendix 14. Consent form

I agree to the following terms related to the study.

1. I understand and agree to the study procedures and any possible risks associated with the study.

2. I understand that the study involves [describe the study's purpose and procedures].

3. I agree to the confidentiality of my personal information and that it will be used only for the purpose of this study.

4. I understand that the study is a [mention relevant context such as medical research, psychology, etc.].

5. I agree to participate in the study and that my participation is voluntary.

6. I understand that I can withdraw from the study at any time and that this withdrawal will not affect my relationship with the study team.

7. I agree to follow all instructions given by the study team.

8. I understand that my participation in this study is not influenced by any financial or other incentives.

9. I understand that the study team will handle any data collected in a confidential manner.

10. I understand that this study is approved by the relevant ethical review board.

11. I agree to all the terms and conditions stated above.

Signed:

[Participant's Signature]

Date:

[Today's Date]

I / We hereby accept and agree to the above-stated terms and conditions.

Signature:

[Participant's Signature]

Date:

[Today's Date]

I / We hereby accept and agree to the above-stated terms and conditions.

Signature:

[Participant's Signature]

Date:

[Today's Date]
Consent Form

I, the undersigned voluntarily agree to take part in the study on the effect of low glycemic index food decreasing the risk of heart disease in patients with diabetes.

I have read and understood the Information Sheet provided. I have been given a full explanation by the investigators of the nature, purpose, location and likely duration of the study, and of what I will be expected to do. I have been advised about any discomfort and possible ill-effects on my health and well-being which may result. I have been given the opportunity to ask questions on all aspects of the study and have understood the advice and information given as a result.

I agree to comply with any instruction given to me during the study and to co-operate fully with the investigators. I shall inform them immediately if I suffer any deterioration of any kind in my health or well-being, or experience any unexpected or unusual symptoms.

I agree to the investigators contacting my hospital about my participation in the study, and I authorise AlAmeri hospital to disclose details of my relevant medical or drug history, in confidence.

I understand that all personal data relating to volunteers is held and processed in the strictest confidence, and in accordance with the UK Data Protection Act (1998). I agree that I will not seek to restrict the use of the results of the study on the understanding that my anonymity is preserved.

I understand that I am free to withdraw from the study at any time without needing to justify my decision and without prejudice.

I confirm that I have read and understood the above and freely consent to participating in this study. I have been given adequate time to consider my participation and agree to comply with the instructions and restrictions of the study.

Name of volunteer .................................................. ..................................................
(BLOCK CAPITALS)

Signed ...........................................................................

Date ...........................................................................

Name of researcher .................................................. ..................................................
(BLOCK CAPITALS)

Signed ...........................................................................

Date ...........................................................................
Appendix 15. Demographic and anthropometric questionnaire

Date QC! ýýJý/fin.
Participant Name

Gender
Male □ Female □

Age
years

Nationality
Kuwaiti □ others/specify □

Height
metres

Weight
kg

Waist
cm

BMI
kg/m²

Systolic (mmHg)  Diastolic (mmHg)  Heart Rate (b/min)
BP/HR

Medication

Cigarette smoking Yes □ How many/day □ No □

Investigator Signature ..................................................  Date .......................
Appendix 16: Changes in lipids and lipoprotein values over the intervention period.

<table>
<thead>
<tr>
<th></th>
<th>Mean (SEM)</th>
<th>P paired t-test</th>
<th>Mean (SEM)</th>
<th>P unpaired t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>Diet (n=20)</td>
<td>Diet + exercise (n=21)</td>
<td></td>
</tr>
<tr>
<td>TAG (mmol/l)§</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.5 (1.1)</td>
<td>1.3 (0.4)</td>
<td>1.6 (0.4)</td>
<td></td>
</tr>
<tr>
<td>2 months</td>
<td>1.1 (1.1)</td>
<td>1.0 (0.4)</td>
<td>1.1 (0.4)</td>
<td></td>
</tr>
<tr>
<td>4 months</td>
<td>1.1 (1.1)</td>
<td>0.9 (0.4)</td>
<td>1.2 (0.4)</td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>1.1 (1.1)</td>
<td>0.9 (0.4)</td>
<td>1.1 (0.4)</td>
<td></td>
</tr>
<tr>
<td>6 months-baseline (% change)</td>
<td>&lt;0.0005</td>
<td>0.3 (0.2)*</td>
<td>0.4 (0.8)**</td>
<td>0.873</td>
</tr>
<tr>
<td>T. Cholesterol (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5.9 (0.2)</td>
<td>5.9 (0.2)</td>
<td>6.1 (0.3)</td>
<td></td>
</tr>
<tr>
<td>2 months</td>
<td>5.7 (0.2)</td>
<td>5.9 (0.3)</td>
<td>5.6 (0.2)</td>
<td></td>
</tr>
<tr>
<td>4 months</td>
<td>5.8 (0.1)</td>
<td>5.9 (0.2)</td>
<td>5.8 (0.2)</td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>6.0 (0.2)</td>
<td>6.0 (0.3)</td>
<td>6.3 (0.3)</td>
<td></td>
</tr>
<tr>
<td>6 months-baseline (% change)</td>
<td>0.633</td>
<td>0.1 (1.0)</td>
<td>0.1 (1.4)</td>
<td>0.904</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.3 (0.1)</td>
<td>1.4 (0.1)</td>
<td>1.3 (0.1)</td>
<td></td>
</tr>
<tr>
<td>2 months</td>
<td>1.2 (0.0)</td>
<td>1.2 (0.1)</td>
<td>1.2 (0.1)</td>
<td></td>
</tr>
<tr>
<td>4 months</td>
<td>1.3 (0.1)</td>
<td>1.3 (0.1)</td>
<td>1.3 (0.1)</td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>1.4 (0.1)</td>
<td>1.5 (0.1)</td>
<td>1.5 (0.1)</td>
<td></td>
</tr>
<tr>
<td>6 months-baseline (% change)</td>
<td>0.047</td>
<td>0.1 (0.4)</td>
<td>0.1 (0.3)</td>
<td>0.954</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.5 (0.1)</td>
<td>3.5 (0.2)</td>
<td>3.6 (0.2)</td>
<td></td>
</tr>
<tr>
<td>2 months</td>
<td>3.3 (0.1)</td>
<td>3.6 (0.2)</td>
<td>3.1 (0.1)</td>
<td></td>
</tr>
<tr>
<td>4 months</td>
<td>3.5 (0.1)</td>
<td>3.6 (0.2)</td>
<td>3.5 (0.2)</td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>3.5 (0.1)</td>
<td>3.8 (0.2)</td>
<td>3.5 (0.2)</td>
<td></td>
</tr>
<tr>
<td>6 months-baseline (% change)</td>
<td>-0.02</td>
<td>0.849</td>
<td>0.1 (0.7)</td>
<td>0.297</td>
</tr>
</tbody>
</table>

§Values are geometric means.
*Represent within group difference of $p<0.05$ and **$p<0.005$.  

276
Appendix 17. Correlations between dietary changes and changes in BMI and lipids and lipoproteins.

<table>
<thead>
<tr>
<th></th>
<th>Changes in TAG (mmol/l)</th>
<th>Changes in HDL (mmol/l)</th>
<th>Changes in %LDL3</th>
<th>Changes in %HDL2</th>
<th>Changes in BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Changes in T.Fat (g)</td>
<td>$R = -0.164$</td>
<td>$0.320$</td>
<td>$-0.207$</td>
<td>$-0.157$</td>
<td>$-0.314$</td>
</tr>
<tr>
<td></td>
<td>$P = 0.514$</td>
<td>$0.195$</td>
<td>$0.426$</td>
<td>$0.562$</td>
<td>$0.155$</td>
</tr>
<tr>
<td></td>
<td>$N = 18$</td>
<td>$18$</td>
<td>$17$</td>
<td>$16$</td>
<td>$22$</td>
</tr>
<tr>
<td>Changes in S.Fat (g)</td>
<td>$R = -0.068$</td>
<td>$0.121$</td>
<td>$-0.024$</td>
<td>$-0.348$</td>
<td>$-0.199$</td>
</tr>
<tr>
<td></td>
<td>$P = 0.789$</td>
<td>$0.632$</td>
<td>$0.927$</td>
<td>$0.186$</td>
<td>$0.374$</td>
</tr>
<tr>
<td></td>
<td>$N = 18$</td>
<td>$18$</td>
<td>$17$</td>
<td>$16$</td>
<td>$22$</td>
</tr>
<tr>
<td>Changes in PUFA(g)</td>
<td>$R = -0.237$</td>
<td>$0.083$</td>
<td>$-0.361$</td>
<td>$0.052$</td>
<td>$-0.160$</td>
</tr>
<tr>
<td></td>
<td>$P = 0.343$</td>
<td>$0.744$</td>
<td>$0.155$</td>
<td>$0.849$</td>
<td>$0.476$</td>
</tr>
<tr>
<td></td>
<td>$N = 18$</td>
<td>$18$</td>
<td>$17$</td>
<td>$16$</td>
<td>$22$</td>
</tr>
<tr>
<td>Changes in MUFA (g)</td>
<td>$R = -0.164$</td>
<td>$0.323$</td>
<td>$-0.198$</td>
<td>$-0.038$</td>
<td>$-0.259$</td>
</tr>
<tr>
<td></td>
<td>$P = 0.517$</td>
<td>$0.191$</td>
<td>$0.447$</td>
<td>$0.888$</td>
<td>$0.245$</td>
</tr>
<tr>
<td></td>
<td>$N = 18$</td>
<td>$18$</td>
<td>$17$</td>
<td>$16$</td>
<td>$22$</td>
</tr>
<tr>
<td>Changes in protein (g)</td>
<td>$R = -0.001$</td>
<td>$0.243$</td>
<td>$-0.002$</td>
<td>$-0.228$</td>
<td>$-0.394$</td>
</tr>
<tr>
<td></td>
<td>$P = 0.998$</td>
<td>$0.332$</td>
<td>$0.995$</td>
<td>$0.395$</td>
<td>$0.069$</td>
</tr>
<tr>
<td></td>
<td>$N = 18$</td>
<td>$18$</td>
<td>$17$</td>
<td>$16$</td>
<td>$22$</td>
</tr>
<tr>
<td>Changes in CHO (g)</td>
<td>$R = -0.135$</td>
<td>$-0.350$</td>
<td>$-0.045$</td>
<td>$0.066$</td>
<td>$-0.009$</td>
</tr>
<tr>
<td></td>
<td>$P = 0.593$</td>
<td>$0.154$</td>
<td>$0.865$</td>
<td>$0.807$</td>
<td>$0.967$</td>
</tr>
<tr>
<td></td>
<td>$N = 18$</td>
<td>$18$</td>
<td>$17$</td>
<td>$16$</td>
<td>$22$</td>
</tr>
</tbody>
</table>

$R$; Correlation coefficient, $P$; p-value, $N$; sample size