A Dietary Interventional Study Moderating Fat Intake in Saudi Subjects with Metabolic Disease

By

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DECLARATION

I declare that this thesis is a accurate record of my results obtained by myself within the labs at University of Warwick, Clinical Science Research Laboratories and, the data that has arisen is detailed in this thesis. All sources of support and technical assistance have been stated in the text of the acknowledgments. None of the work has been previously submitted for a higher degree.

All sources have been specifically acknowledged by means of reference.

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Synopsis

The gut-derived bacteria, endotoxin (lipopolysaccharide), have been observed to be raised in patients with type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVD) which appears to represent a source of diet induced inflammation exacerbating metabolic disease. To further the current studies on endotoxin induced inflammation investigations examined a cohort of adult Saudi Arabian women with obesity/weight gain and or T2DM to ascertain (1) the impact of a post-prandial high SFA rich meal on systemic inflammation; (2) the direct effect of a 3 month diet intervention on cardiometabolic health (3) and assess how subtle changes in dietary interventions can impact on metabolic risk in different patient groups and what dietary components appear important.

A total of 92 Saudi adult women with varying metabolic states [18 non-diabetic (ND) control subjects (Age 24.4±7.9 years; BMI 22.2±2.2 Kg/m^2), 24 overweight-plus obese (overweight$^+$) subjects (Age 32.0±7.8 years; BMI 28.5±1.5 Kg/m^2) and 50 T2DM patients (Age 41.5±6.2 years; BMI 35.2±7.7 Kg/m^2)] were recruited for this 3-month intervention study. Anthropometric data and fasting blood samples were taken at pre- and 3 months post-intervention with glucose, insulin, HOMA-IR, lipid profile and endotoxin measured. To establish whether a high-fat meal alters circulating endotoxin in different metabolic disease states, all subjects were given a high fat standardized meal (75g fat, 5g carbohydrate, 6g protein) after an overnight fast of 12–14 h. Blood samples were drawn via cannula at baseline (0 hour) and post-prandially (1, 2, 3, and 4 hours). For the dietary intervention, participants were prescribed a 500Kcal deficit energy diet less than their daily recommended dietary allowances. Targeted macronutrient composition was 20%-30% fat, <10% of saturated fatty acids, 50%-60% carbohydrates, 15%-20% protein and at least 15g of fibre per 1000 kcal.

At baseline and with the exception of HDL-cholesterol, all anthropometric, glycemic parameters, lipid profile and endotoxin were significantly higher in the T2DM group. For the high fat challenge, the most notable changes were the post-prandial increases in the triglycerides, insulin, HOMA-IR and endotoxin levels, and subsequent significant decrease in HDL-cholesterol in all groups ($p<0.05$). These same patterns of changes were observed after 3 months in the overweight^{+} and T2DM group. Endotoxin was found to be significantly and positively associated with total and LDL-cholesterol ($p<0.05$), modestly with triglycerides and inversely with HDL-cholesterol (p=NS). For the dietary intervention, significant improvements were noted in all anthropometric measures in the T2DM group and BMI in the overweight^{+} group ($p<0.01$). The noted weight loss was secondary to the significant decrease in carbohydrates, fats and total caloric intakes ($p<0.05$) which translated to a better cardiometabolic health in both groups noted clearly through lipid profile changes. Endotoxin was found to be inversely associated with fiber intake ($p<0.05$). Fiber intake was found to be positively associated with HDL-cholesterol ($p<0.05$), which appeared to be an important dietary component to be associated with health improvements.

This current thesis expanded our knowledge and understanding on how a high fat oral challenge exacerbates cardiometabolic and inflammatory conditions (including endotoxemia) in Saudi Arabian women with different metabolic states, and how a 3-month caloric restriction may induce weight loss that leads to improved cardiometabolic health. Observations from the present thesis highlight strategies that may potentially be of clinical use in future dietary intervention studies in patients with T2DM and obesity in the Middle Eastern region.

Abbreviations

AbSc	Abdominal Subcutaneous
AbSc AT	Abdominal Subcutaneous Adipose Tissue
Ad	Adipocyte
Acyl-CoAs	Acyl-coenzyme A
ADIPOR	Adiponectin Receptor
ADSF	Adipocyte-specific Secretory Factor
AGT	Angiotensinogen
ALLN	N-Acetyl-Leu-Leu-Nle-CHO (Calpain Inhibitor I)
AMPK	AMP-activated Protein Kinase
ANCOVA	Analysis of Covariance
ANOVA	Analysis of variance
ANG-II	Angiotensin-II
AP-1	Activator Protein-1
Ap2	Adipocyte Fatty Acid Binding-protein
ASP	Acylation-stimulating Protein
AT	Adipose Tissue
ATMs	Adipose tissue macrophages
ATP	Adenosine triphosphate
BAT	Brown Adipose Tissue
BBB	Blood-brain Barrier
BMI	Body Mass Index
BP	Blood pressure
BSA	Bovine Serum Albumin
ºC	Celsius
CAC	Coronary Artery Calcification
$CaCl_2$	Calcium Chloride
CAD	Coronary artery disease
CART	Cocaine-and Amphetamine-Regulated Transcript
cDNA	Complementary (to mRNA) Deoxyribonucleic acid
CD14-KO	CD14-Knock out
CE	Cholesterol Esterase
CHD	Coronary Heart Disease
Cm	Centimetres
CNS	Central Nervous System
CO	Cholesterol Oxidase
CO_2	Carbon Dioxide

CONSORT	Consolidated Standards for Reporting of Trials
CRH	Corticotrophin-releasing Hormone
CRP	C-reactive Protein
CSF	Cerebrospinal Fluid
Ct	Cycle Threshold
CV	Coefficient of Variance
CVD	Cardiovascular Disease
Da	Daltons
DAG	Diacylglycerol
db/db	Leptin Receptor-deficient Mouse
DC	Detergent Compatible
ΔCt	Delta Cycle Threshold
DEXA	Dual Energy X-ray Absorption
dH_2O	Distilled Water
DM	Diabetes mellitus
DMEM	Dulbecco's Minimum Essential Medium
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic Acid
DNase	Deoxyribonuclease
dNTPs	Deoxynucleotides Triphosphates
DRI	Dietary reference intake
DTT	Dithiothreitol
ECL	Enhanced Chemiluminescence
ECSIT	Evolutionarily Conserved Signalling Intermediate in Toll Pathways
EDTA	Ethylene diamine tra acetic Acid
ECG	Electrocardiogram
EGF	Epidermal Growth Factor
ELISA	Enzyme-linked Immunosorbant Assay
EMBL-EBI	European Bioinformatics Institute
ER	Endoplasmic Reticulum
ERK	Extra cellular signal-regulated kinase
FAs	Fatty Acids
FABP-2	Fatty Acid Binding-protein-2
FABPs	Fatty Acid Binding-proteins
fa/fa	Obese Zucker Rat
FAM	RT-PCR Reporter Fluorochrome/Dye Label
FDP	Finnish Diabetes Prevention

FFAs	Free Fatty Acids
FFQ	Food Frequency Questionnaire
FGF	Fibroblast Growth Factor
FIZZ	Found in Inflammatory Zone
FPG	Fasting plasma glucose
FPLC	Fast Protein Liquid Chromatography
g	Gram
GI	Gastrointestinal
GK	Glycerokinase
GLP-1	Glucagon-like peptide 1
GLUT-4	Glucose- transporter-4
GLUTs	Glucose Transporters
G6Pase	Glucose-6-phosphatase
GSK-3	Glycogen Synthase Kinase-3
HBA	Hydroxybenzoic Acid
HBSS	Hank's Balanced Salt Solution
HDL	High density lipoprotein
HDL-C	High density lipoprotein cholesterol
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic Acid
HMW	Higher Molecular Weight
H_2O	Water
HOMA	Homeostasis Model Assessment
HOMA- IR	Homeostasis Model Assessment of Insulin Resistance
hr	Hour
HRP	Horseradish Peroxidase
HsCRP	High-sensitive C-Reactive Protein
HSL	Hormone sensitive lipase
HUVECs	Human umbilical vein endothelial cells
ICAM-1	Intercellular Adhesion Molecule-1
IDF	International Diabetes Federation
IFG	Impaired Fasting Glycaemia
IGF-1	Insulin-like Growth Factor-1
IGF-1BP	IGF-1 Binding protein
IGT	Impaired Glucose Tolerance
IκB	Inhibitor of NF-Kb
IKK	Inhibitor of NF-κB Kinase
IKKα	Inhibitor of NF-κB Kinase-α
IKKβ	Inhibitor of NF-κB Kinase-β

IKKγ/NEMO	Inhibitor of NF-κB Kinase-γ
IL	Interleukin
IR	Insulin Receptor
IRAK	IL-1 Receptor-associated Kinase
IRS	Insulin Receptor Substrate
JNK	c-Jun N-terminal Kinase
Kcal	Kilo-calorie
kDa	Kilodaltons
Kg	Kilogram
L	Litre
LAL	Limulus amebocyte lysate
LBP	Lipopolysaccharide-binding protein
LCN2	Lipocalin-2
LDL cholesterol	Low density lipoprotein cholesterol
Look Ahead	Action for Health in Diabetes
LMW	Low Molecular Weight
LPL	Lipoprotein lipase
LPS	Lipopolysaccharide
LXR	Liver X Receptor
M	Molar
M2	Macrophages
M_r	Molecular Weight
mal1	Adipocyte/macrophage Fatty Acid-binding Protein-5
MALP-2	Macrophage-activating Lipopeptide-2
MAPK	Mitogen-activated Protein Kinase
MCP-1	Monocyte Chemotactic Protein-1
mCD14	Membrane cluster of differentiation 14
MD2	Myeloid differentiation protein-2
MEKK1	Mitogen-activated Kinase Kinase-1
$MgCl_2$	Magnesium Chloride
MS	Metabolic Syndrome
µg	Microgram
mg	Milligram
min	Minute (time)
µl	Microlitre
ml	Millilitre
mM	Millimolar
MMPs	Matrix Metalloproteinases

MNT	Medical nutrition therapy
MONICA	Multinational Monitoring of Trends and Determinants in CVD
MPO	Myeloperoxidase
mQH_2O	Milli Q water (ultra-filtered water)
mRNA	Messenger Ribonucleic acid
MyD88	Myeloid Differentiation Primary Response Gene-88
NaCl	Sodium Chloride
NF-κB	Nuclear Factor-Kb
NEFA	Non-esterified Fatty Acid
NIK	NF-κB-inducing Kinase
ng	Nanogram
nm	Nanometre
NO	Nitric oxide
NOC	Non-obese control
NPY	Neuropeptide Y
N.S	Non-significant
ob/ob	Leptin-deficient Mouse
OB-Rb	Leptin Receptor
OD	Optical Density
Om	Omental
Om Ad	Omental Adipocytes
Om AT	Omental Adipose Tissue
Overweight$^+$	Overweight plus
P	Phosphorylated
PAI-1	Plasminogen Activator Inhibitor-1
PBMCs	Peripheral Blood Mononuclear Cells
PBS	Phosphate-buffered Saline
PBS-T	Phosphate-buffered Saline containing 0.1% Tween 20
PCOS	Polycystic ovary syndrome
PCR	Polymerase Chain Reaction
PDK-1	3-phosphoinositide-dependent Protein Kinase-1
PEG	Poly ethylene glycol
PET	Positron Emission Tomography
PEPCK	Phosphoenolpyruvate Carboxylase
PI3K	Phosphoinositide-3 Kinase
PIP2/PtdIns(4,5)P_2	Phosphatidylinositol-4,5-bisphosphate
PIP3/PtdIns(3,4,5)P_3	Phosphatidylinositol-3,4,5-trisphosphate

PKB/Akt	Protein Kinase B
PKC	Protein Kinase C
PKR	Protein Kinase R
pNA	P-nitroaniline
POMC	Proopiomelanocortin
POD	Peroxidase
PPAR-γ	Peroxisome Proliferator Activated Receptor-γ
PPRE2	PPAR-γ Response Element-2
PPREs	PPAR-γ Response Elements
psi	Pounds Per Square Inch
PVDF	Polyvinylidene-fluoride
PVN	Paraventricular Nucleus
RAGE	Receptor for Advanced Glycation End-products
REACH	The REduction of Atherothrombosis for Continued Health
RELM	Resistin-like Molecule
RELMs	Resistin-like Molecules
RETN	Human Resistin Gene
Retn	Mouse Resistin Gene
RIA	Radioimmunoassay
RIPA	Radio-Immunoprecipitation Assay
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
RSG	Rosiglitazone
RT	Room Temperature
RTn	Reverse Transcriptase
RT-PCR	Real-time PCR
s	Second (time)
S_A	Adiponectin Sensitivity Index
SAPE	Streptavidin-Phycoerythrin
Sc	Subcutaneous
Sc Ad	Subcutaneous Adipocytes
Sc AT	Subcutaneous Adipose Tissue
Ser	Serine Residue
SD	Standard Deviation
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	SDS-polyacrylamide Gel Electrophoresis
SEM	Standard Error of the Mean
SFA	Saturated Fatty Acids

SH2	Src Homology 2
sICAM-1	Soluble Intercellular Adhesion Molecule-1
S-Resistin	Short Resistin
SNPs	Single Nucleotide Polymorphisms
SOCS	Suppressor of Cytokine Signalling
SOCS-3	Suppressor of Cytokine Signalling-3
sTNF-R2	Soluble TNF-receptor 2
SVF	Stromo-Vascular Fraction
3T3-L1	Mouse Embryonic Fibroblast Cell Line
TAK1	TGF-β-activated Kinase-1
Taq	Thermus Aquaticus (DNA polymerase)
TBS	Tris-buffered Saline
TBS-T	Tris-buffered Saline containing 0.1% Tween 20
TCA	Trichloroacetic Acid
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TEME	NN, N', N'-Tetramethylethelenediamine
TG	Triglyceride
TGF-β	Transforming Growth Factor-β
TIR	Toll-interleukin 1 Receptor-resistance
TLR	Toll-like Receptor
TLRs	Toll-like Receptors
TLR2	Toll-like receptor- 2
TLR4	Toll-like receptor- 4
TLR4-KO	Toll-like receptor-4 knock out
TNF-α	Tumour Necrosis Factor-α
TNFR	TNF-α Receptor
TNF-R2	TNF-α Receptor-2
TRAF	TNF Receptor-associated Factor
TRAF-6	TNF Receptor-associated Factor-6
Tris	Tris (hydroxymethyl) Aminomethane
Tris-HCl	Tris Hydrochloride
TZD	Thiozoladinedione
TZDs	Thiozoladinediones
U	Units
USDA	U.S. Department of Agriculture
UV	Ultraviolet
VLDL	Very low density lipoprotein

WAT	White adipose tissue
WC	Waist circumference
WHO	World Health Organization
WHR	Waist-hip ratio
4AAP	4 aminoantipyrine

Chapter One

Introduction

1.1 The Obesity pandemic and the Middle East

According to the World Health Organization (WHO), the world-wide prevalence of obesity is alarmingly increasing with almost 400 million adults reported to be obese in 2005, with an estimated increase to reach more than 700 million by the year 2015. Due to the worldwide-spread of obesity, it is now considered to have reached pandemic proportions; a fact that indicates a large health burden as obesity is an established independent risk factor for coronary artery disease (CAD), hypertension and diabetes mellitus (T2DM) (WHO, 2014).

It is ironic that, whereas malnourishment and starvation prevail in some poor regions in the world, overweight and obesity are alarmingly increasing in other regions that include both developed and developing countries. It is indeed unfortunate that some cultures including many people in the Saudi society, consider excess body weight, whether overweight or obesity, as being healthy. In the Western world however, many individuals believe that being overweight or obese increases one's risk to develop major chronic diseases. At the same time, most overweight or obese individuals in the Saudi society do not recognize the types of food that are needed for their nutritional or weight loss goals (Al-Nozha et al., 2005). It is well known that the net result of the balance between energy intake and energy expenditure determines an individual's body weight. If this balance is disturbed in favor of surplus in energy intake, the result will be excessive accumulation of fat that may impair health, which simply defines the states of overweight or obesity.

Although T2DM, obesity and CVD are strongly considered as components of an increasing global epidemic, there are still regions of the world where the

corresponding health implications have not been fully acknowledged. In comparison with other countries, only a few published studies have focused on the impact of T2DM and obesity in Saudi Arabia, although the social and economic implications are vast.

In a study conducted by Al Nozha and colleagues in Saudi Arabia over a 5-year period- between 1995 and 2000- examining 17,232 adult Saudi subjects (30-70 year old), from both genders and from selected households, it was observed that the crude prevalence of overweight (BMI = 25-29.9 kg/m^2) was 36.9%. In addition, overweight was found to be significantly more prevalent in males (42.4%) than females (31.8%; $p<0.0001$). The age-adjusted prevalence of obesity (BMI $\geq 30 kg/m^2$) was 35.5% with an overall prevalence of 35.6%, while the prevalence of severe (gross) obesity (BMI ≥ 40 kg/m^2) was 3.2%. Females were significantly more obese with a prevalence of 44% relative to males (26.4%; $p<0.0001$) (Al-Nozha et al., 2005; AlQuaiz et al., 2014).

Weight gain has been historically associated with wealth and prosperity in many traditional societies. This perception, coupled with the shift from fiber rich foods to a more "Western" diet, has contributed to the current health crisis facing the Saudi population. This acute change is clearly apparent not only in Saudi Arabia, but also across neighboring Gulf countries where the apparent "overnight wealth" has created an enormous health burden. The global study, REACH (Ohman, 2006), which evaluated data from 68,000 patients in 44 countries, revealed a significant difference in the potential burden of disease among the various nations examined. As far as the Middle East region is concerned, Musaiger and co-workers observed that within the last four decades, daily per capita fat content in the Middle Eastern diet

has tremendously increased, with a figure reaching 143.3% increase in Saudi Arabia. Moreover, the current situation has clear indications that this increasing trend would continue (Musaiger, 2002). These circumstances are by no means unique, as this trend is present in many other regions of the world. At present however, many regions worldwide lack sufficient reliable data on the current health status of the population, although they will be faced with the burden of obesity and associated diseases to manage in the future (Mathers et al., 2005).

Obesity and T2DM are strongly associated, where an estimated 80% of patients with T2DM are obese. This association is translated into a huge health-care cost burden on governments. Currently the WHO indicates that each individual with T2DM in Saudi Arabia costs the government about $800–1000 per month (WHO, 2011). As a result, with the recently-announced population of 25.7 million, and with approximately 30% of the population being diabetic, the actual annual treatment cost of T2DM in Saudi Arabia may greatly exceed the previous annual estimates of $7.7 billion (Alhyas, et al., 2011; Al-Daghri et al., 2011). It becomes obvious that, with the dramatic rise and cost associated with the current prevalence of T2DM and its associated complications, medication alone will not be sufficient to reduce the epidemic. Alternatively, a combination of physical activity, diet changes and medication are necessary to halt the epidemic.

Obesity and saturated-fat-rich-diets have long been associated with markedly increased insulin resistance and inflammation; both of which give rise to metabolic diseases, including T2DM (Mather et al., 2005; Al-Attas et al., 2014). Moreover, and despite the widespread use of statins to reduce circulating lipid levels, inflammation still persists in most subjects, and it is unclear what factors mediate the inflammatory

reactions. The International Obesity Taskforce has currently estimated that more than 312 million adults worldwide are obese (Haslam and James, 2005) and that the incidence of diabetes will rise by 32% in Europe and 72% in the USA by 2030 with the most dramatic projected increases (> 150%) predicted to be in the Middle East, India, Southeast Asia, Sub-Saharan Africa and Latin America (Hossain, 2007; Wild, 2004). Furthermore, the cardiovascular complications associated with obesity and T2DM could potentially overwhelm countries that are unprepared, either in management or prevention strategies. Thus, a better understanding of why a healthy diet is important and its impact on diabetic risk is clearly necessary.

1.2 Types of obesity

Etiologically, obesity is commonly classified into several subgroups; namely: monogenic obesity, syndromic obesity, and polygenic or common obesity (Herrera et al., 2010). The monogenic obesity is an autosomal disease where about 20 single gene disruptions have been described thus far. Phenotypically, it is manifested as morbid obesity without developmental delays (O'Rahilly, 2009). Interestingly, the role played by the leptin/melanocortin pathway in the central nervous system (CNS) in the regulation of whole-body energy homeostasis has been granted a critical position in all these mutation In these cases, obesity seems to be the result of increased appetite and reduced satiety (Coll et al., 2004; Coll and Yeo, 2013).

In contrast to monogenic obesity, syndromic obesity involves several genes, autosomal or X-linked, which manifest discrete genetic defects or chromosomal abnormalities. The phenotypic presentation of this subclass of obesity is characterized by clinically obese individuals who suffer from additional defects such as mental retardation, dysmorphic features and organ-specific developmental

abnormalities. Prader-Willi syndrome is one of the most well-known forms of syndromic obesity (Herrera et al., 2010).

Common obesity, as the name implies, defines the most common form of the disease, and it is the result of a complex interplay of genetic, environmental, and social factors that results in a long-term positive energy balance leading to prolonged storage of excess energy in adipose tissue. The genetic factors in this subclass, if exist, are known to be polygenic. Metabolic defects such as insulin resistance and increased risk of CVD are common consequences (Achike et al., 2011).

Obesity can also be secondary to systemic diseases; such as Cushing disease associated with trunk obesity, insulinoma resulting in frequent hypoglycemia which will in turn promote energy intake, and hypothyroidism manifested by a decrease in energy needs. In addition, binge eating disorder, a sedentary lifestyle, a high glycemic diet, and some medications such as psychotropic drugs, insulin, and corticosteroids, can all be considered etiological factors of obesity (Martinez et al., 2002; Al-Zoairy et al., 2013).

1.3 Measurement of Obesity

Epidemiological studies which were carried out just after World War II concluded that the best practical formulae to reflect the range of body weight indices was dividing the weight in kilograms by the square of height in meters [Weight (kg) / height (m^2)]. This ratio was called the Quetelet Index after Adolphe Quetelet (1796 – 1874), who was a Belgian social scientist and statistician and a pioneer in this kind of epidemiological studies. However, Quetelet Index was changed into Body Mass Index (BMI) by Ancel Keys in 1972 (Eknoyan, 2008)

which has become familiar to most people as a way to categorize the body weight into normal weight, overweight, or obese. Due to the broad range of body weight, the WHO has divided the BMI into categories as shown in the following table (Table 1.3.1). (http://www.who.int/bmi)

	BMI(kg/m^2)	
Classification	**Principal cut-off points**	**Additional cut-off points**
Underweight	**<18.50**	**<18.50**
Severe thinness	<16.00	<16.00
Moderate thinness	16.00 - 16.99	16.00 - 16.99
Mild thinness	17.00 - 18.49	17.00 - 18.49
Normal range	**18.50 - 24.99**	**18.50 - 22.99**
		23.00 - 24.99
Overweight	**≥25.00**	**≥25.00**
Pre-obese	25.00 - 29.99	25.00 - 27.49
		27.50 - 29.99
Obese	**≥30.00**	**≥30.00**
Obese class I	30.00 - 34.99	30.00 - 32.49
		32.50 - 34.99
Obese class II	35.00 - 39.99	35.00 - 37.49
		37.50 - 39.99
Obese class III	≥40.00	≥40.00

Table 1.3.1 Categorising humans based on body mass index (BMI) into different groups, underweight, normal weight, overweight or obese classes.

It is important to note that BMI and other similar anthropometric parameters as waist circumference (WC) and waist-hip ratio (WHR) are simple measurements that can give a relatively clear indicator of potential risk for obesity, and hence these measures are widely used by primary care physicians to assess and monitor their patients. It must be noted, however that these measures do not determine muscle mass: fat mass ratio; they only give an approximate idea concerning general total body weight. More accurate measurements of adipose tissue mass and distribution can be obtained by performing sophisticated analytical procedures; such as Computed Tomography (CT), Dual Energy X-ray Absorption (DEXA) and Magnetic

Resonance Imaging (MRI) (Bosello and Zamboni, 2000; Bozzetto et al., 2011; Gallagher et al., 2009; Kuk et al., 2005).

1.4 Consequences of obesity

As is the case with any ailment, obesity is a health condition that bears medical, psychological and economic consequences. The following sections will look at these aspects in more detail.

1.4.1 Medical

The scientific evidence of the medical consequences associated with obesity is rapidly mounting. It has been shown that obesity is linked to CVD such as myocardial ischemia and strokes (Arsenault et al., 2010; Lavie et al., 2009; Poirier et al., 2006). Obesity predisposes to T2DM (Dandona et al., 2005; Freemantle et al., 2008; Lazar, 2005; Mokdad et al., 2003), and is associated with musculoskeletal disorders such as osteoarthritis (Anandacoomarasamy et al., 2008; Krul et al., 2009; Wearing et al., 2006). Some medical research has also linked obesity to development of certain types of cancers such as endometrial, colon and breast cancers (Fleming et al., 2009; Rapp et al., 2005; Roberts et al., 2010; Sinicrope and Dannenberg, 2011; van Kruijsdijk et al., 2009).

1.4.2 Psychological

Obese individuals are often stigmatised and it has been often socially acceptable to become the centre of ridicule. Even the modern media and film-industry play a part in promoting this social attitude towards obese subjects. These social pressures manifest themselves in the form a range of psychological disorders

suffered by the obese individuals, including low-self-esteem, social isolation from the rest of the community, and even clinical depression. These psychological consequences often prelude to increased food intake as a compensatory measure for loneliness which creates a vicious cycle that revolves around obesity (Friedman et al., 2002; Gariepy et al., 2010; Pratt et al., 2007).

1.4.3 Economic

The world has started to realize the economic and financial implications of obesity. The WHO and governments of the industrialized world are feeling the burden of the increasing requirement to deal with obesity associated diseases; all of which presents a substantial burden in order to either treat current conditions or long-term look into prevention programmes. In England, the economic cost of treating obesity and its associated ailments was estimated to over four billion pounds in 2007 (FORESIGHT REPORT, 2006; DoH, 2008), whereas the estimates in the United States of America (USA) were over 75 billion dollars in 2000 (CDC, 2010).

1.5 Diabetes

Obesity predisposes to many conditions including T2DM. According to the WHO, T2DM is defined as “a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces” (WHO, 2014). Insulin aids target tissues such as liver, adipose tissue and muscle, to uptake and metabolically utilize glucose from the blood stream. Besides T2DM, other types of the disease exist, and depending on the cause, Diabetes Mellitus is mainly classified into three different types as follows:-

I. Type 1 diabetes (T1DM) where the pancreas does not produce sufficient insulin for blood glucose metabolism. This type of diabetes can only be controlled by life-long and daily administration of insulin.

II. T2DM where initially there is sufficient amount or even high amount of circulating insulin in the blood, but the target body tissues are not responsive enough to this circulating insulin. Controlled management of this type of diabetes ranges from lifestyle changes- physical activities and diet- to insulin sensitizers and agonists; and eventually insulin injections might be required.

III. Gestational diabetes is defined as a state of increased blood glucose during pregnancy. This type of diabetes is pregnancy-related and can be cured if managed properly during pregnancy.

There are also conditions which prelude to T2DM such as impaired glucose tolerance (IGT) and impaired fasting glycaemia (IFG); these two states form intermediaries, where blood glucose levels are bordering the cut-off points, between normality and diabetes. People with IGT and IFG are at higher risk than normal people in becoming diabetic in later life.

1.5.1 Prevalence of Diabetes:

T2DM is the most common form of the disease comprising about 90% of diagnosed cases of DM. According to the current estimates, there are more than 220 million diabetics worldwide and the number is rising annually (WHO, 2014). In England, the prevalence of diabetes has noted to be around 5.4% in 2009 whereas its prevalence in the USA has been estimated at about 7.8% in 2007 (NHS, 2009). Globally, the prevalence of diabetes show surprising results being most prevalent in developing countries.

In a recently published study by Al Daghri and colleagues (2011), the high prevalence of obesity coupled with T2DM among Saudis was highlighted. The study analysed demographic and anthropometric data obtained from a total of 9,149 Saudis (age range: 7-80 years) from both genders (5,357 males (58.6%) and 3,792 females (41.4%)). The overall prevalence of T2DM was 23.1% (95% confidence interval (95% CI) 20.47 to 22.15). This was age- and gender-related, as demonstrated in the age-adjusted prevalence of T2DM that was 31.6%, and by demonstrating a significant higher prevalence in males with an overall age adjusted prevalence of 34.7% (95% CI 32.6 to 35.4), than in females, with an overall age-adjusted prevalence of 28.6% (95% CI 26.7 to 29.3) ($P < 0.001$). Similarly, a high overall prevalence of obesity (31.1% (95% CI 30.1 to 32.0)) was shown among the studied individuals. Again, this was age- and gender-related, as demonstrated in the age-adjusted prevalence of obesity that was 40.0%, and by demonstrating a significant higher prevalence in females with an overall age adjusted prevalence of 36.5% (95% CI 35.1 to 37.83), than in males, with an overall age-adjusted prevalence of (25.1% (95% CI 23.7 to 26.3)) ($P < 0.001$). This study has also reported a high age-adjusted prevalence of hypertension (32.6% (95% CI 31.7 to 33.6)) and CAD (6.9% (95% CI 6.4 to 7.4) among the studied subjects (Figure 1.5.1.1).

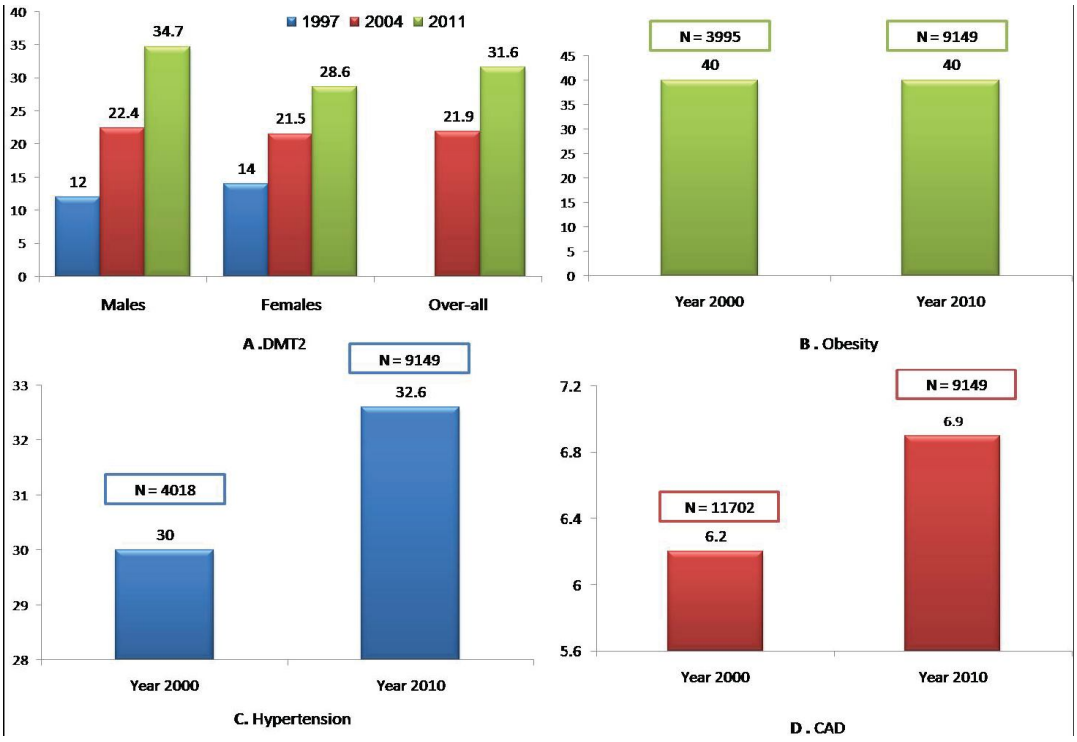


Figure (1.5.1.1) Trends in the prevalence of chronic noncommunicable diseases in the Kingdom of Saudi Arabia from 2000 to 2010. (A) Age adjusted prevalence of type 2 diabetes mellitus in Saudi Arabia according to gender (1997 (Al Daghri et al., 2010), 2004 (Al Nozha et al., 2004), 2011 (Al-Daghri et al., 2011)). (B) Obesity. (C) Hypertension. (D) Coronary artery disease. Previous estimates shown are from the central region (Riyadh) and do not include other regions.

1.5.2 Complications of Diabetes

As T2DM progresses, it starts affecting different body tissues resulting in a wide and variable range of complications that have been well established (Fowler, 2008; Mazzone et al., 2008; Moore et al., 2009). Subjects with T2DM have much higher risk of developing CVD (Fox et al., 2007; Kelly et al., 2009), neuropathy (Mert et al., 2010; Resnick et al., 2002), retinopathy (Fong et al., 2004; Bello et al., 2014) and nephropathy (Parving et al., 2001; Schena and Gesualdo, 2005; Bello et al., 2014). Each of these complications may result in detrimental consequences. For instance; in neuropathy, the patient with T2DM starts losing peripheral sensation in limbs, most commonly in the feet, with devastating consequences that may end up in

limb amputation; in diabetic retinopathy, the patient's eye sight begins to deteriorate and may culminate in blindness; and in nephropathy, progressive diabetes adversely affects the kidney functions resulting eventually in renal failure. Although it is evident that late stages of T2DM can be fatal, fatal consequences of T2DM can be triggered at any stage of the disease if it is uncontrolled such as in the case of developing diabetic ketoacidosis. Subjects with T2DM also have higher vulnerability to infections; and similar to obese subjects, patients with T2DM are prone to psychological depression (Lin et al., 2010).

1.5.3 Global Impact

Due to the increasing prevalence of T2DM and the wide range of associated complications, world economies expenditure on prevention and treatment is totalling billions of dollars (Yach et al., 2006; Zimmet et al., 2001; ADA, 2013). Therefore, there are many organisations and societies worldwide that are formed in response to the urgent need for advisory and research bodies to promote diabetes prevention and improve its treatment.

1.5.4 Obesity-Induced Diabetes or Diabesity

The link between obesity and diabetes is becoming ever evident as research progresses; which has prompted some researchers to coin a new term-Diabesity- elucidating this close link (Astrup and Finer, 2000; Freemantle et al., 2008; Farag and Gaballa, 2011). Although, obesity is a risk factor for developing T2DM, it should be noted that not all obese subjects will develop diabetes at later stage. Nevertheless, obesity is still the biggest single risk factor for T2DM. As mentioned earlier; obesity is a risk factor for CVD as well; and as such, morbid obesity coexists

with heart attacks, strokes, hypertension and T2DM. These associated factors led to the existence of a cluster of pathologies collectively termed metabolic syndrome or syndrome X (Bruce et al., 2011; Han and Lean, 2011; James et al., 2004; Weiss et al., 2004). WHO set five defining criteria for diagnosing metabolic syndrome. Those criteria were slightly simplified by the international diabetes federation (IDF) so that they become more practical and could be accommodated for use in the clinics by physicians (Table 1.5.4.1).

Criteria for diagnosis of metabolic syndrome:

1. ***Central obesity*** (waist circumference of ≥ 94 cm for European men and ≥ 80 cm for European women, with ethnicity-related specific values for other ethnic backgrounds)

In addition to any TWO of the following four factors:

2. **Raised triglyceride level**: (≥150 mg/dL or 1.7 mmol/L)
   Or specific treatment for this lipid abnormality

3. **Reduced high density lipoprotein, HDL**: (<40 mg/dL or 1.03 mmol/L in males)
   and (<50 mg/dL or 1.29 mmol/L in females)
   Or specific treatment for this lipid abnormality

4. **Raised blood pressure**: (systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg
   Or treatment of previously diagnosed hypertension

5. **Raised fasting plasma glucose, FPG**: (≥100 mg/dL or 5.6 mmol/L
   Or previously diagnosed type 2 diabetes

Table 1.5.4.1: The new International Diabetes Federation (IDF) definition, 2011

The recommendation from the IDF has been that three out of the five criteria should be present in a patient for the condition to be diagnosed as metabolic syndrome. However, one single criterion is central to the diagnosis of metabolic syndrome, namely increased body waist circumference; meaning this criterion must

always be one of the three factors (James et al., 2004; Bener et al., 2013). This emphasis on abdominal obesity highlights the instrumental role by which obesity plays the lead compared with other morbidities; one of which is T2DM. An increase in waist circumference has been shown to be a valid indicator of morbid obesity and a risk factor associated with other diseases such as CVD and T2DM, hence the current emphasis on the measurement of the increase in body waist circumference. Another important aspect of measuring the waist circumference is its practicality and ease of its use in the clinic (Despres and Lemieux, 2006; Grundy, 2004; Yoshimura N. et al., 2011).

Obesity-induced diabetes has been the theme of research for the last decade or so to elucidate the underlying mechanisms. The current answer to the question of how obesity causes T2DM directs us to the core of obesity, the role of adipose tissue. The widely accepted explanation is that chemical messengers secreted by adipose tissue interfere with the action of insulin in the target tissues leading to a phenomenon called insulin resistance (Bosello and Zamboni, 2000; Despres and Lemieux, 2006; Han and Lean, 2011; Weiss et al., 2004).

In such condition, peripheral tissues such as liver, muscle and adipose tissue become insensitive to the normal levels of circulating insulin in the bloodstream. This will result in elevated levels of blood glucose (hyperglycaemia). The target tissue's glucose starvation will stimulate new synthesis of glucose through the process of hepatic gluconeogenesis. The latter will increase the blood glucose even further, resulting in a vicious cycle. Adipose tissue in itself is a target tissue for insulin, and insulin resistance in adipose tissue may induce even more detrimental consequences on health such as, high levels of circulating free fatty acids (FFA),

increased triglycerides (TG; hypertriglyceridemia) and ectopic adiposity. Hypertriglyceridemia and high levels of circulating FFA add a compounding effect to insulin resistance by interfering with insulin action at the cellular level. Ectopic adiposity is the deposition of fat outside the adipose tissue such as liver, pancreas, muscle, heart and kidneys. It occurs when the adipose tissue is overloaded and excess fat is transferred to other sites for deposition. This condition exacerbates the pathologic effects of adipose tissue as it adversely affects the functionality of the other tissues which are not supposed to store such TG (Bosello and Zamboni, 2000; Yoshimura E. et al., 2011). For these reasons adipose tissue is the focus of on-going research.

A cause-effect relationship of obesity and insulin resistance is suggested by many groups and is detailed in several review articles. The increased mass of adipose tissue that occurs in obesity is believed to play a key role in predisposing the human body to systemic insulin resistance. This takes place via the dysregulation of multiple interconnected pathways and signals, for instance the inflammatory, endocrine, neural and cell regulatory pathways as illustrated in Figure 1.5.4.1 (Qatanani and Lazar, 2007; Cai et al., 2013).

In the following section, we will focus on the endocrine and inflammatory mechanisms.

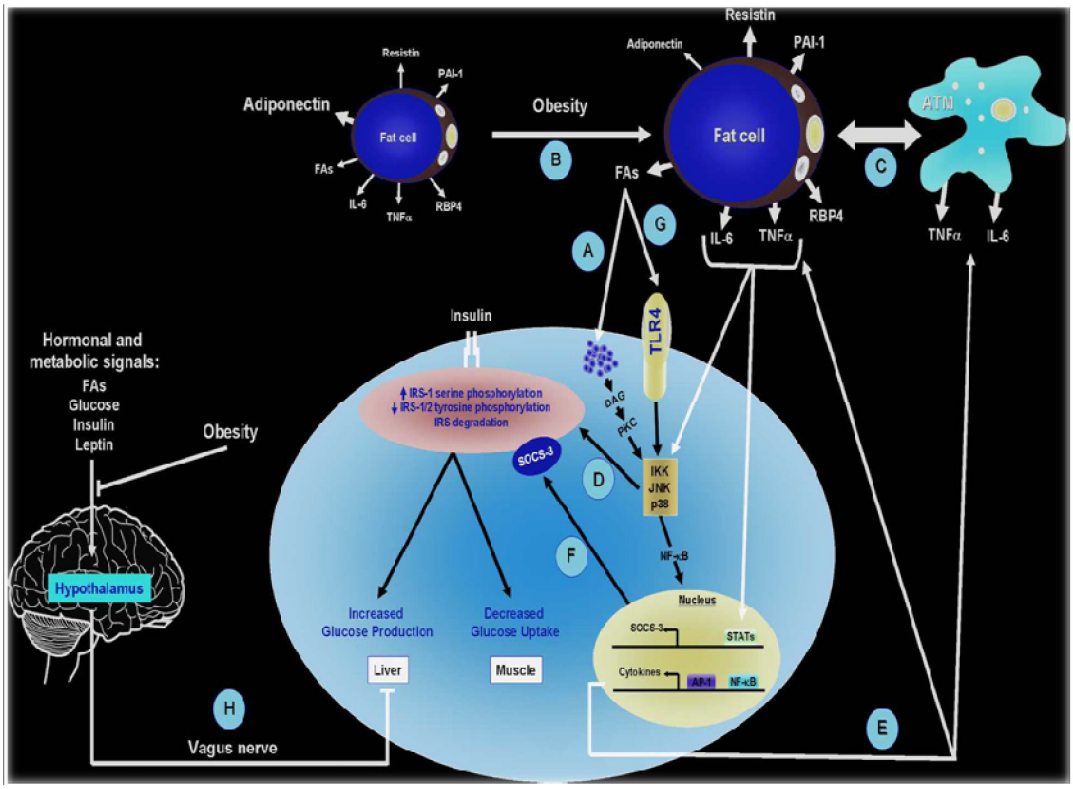


Figure (1.5.4.1): Obesity and insulin resistance relationship; the roles of inflammatory, endocrine, neuronal, and cell regulatory pathways. A: Obesity is associated with increase release of FAs which will act via increasing intracellular metabolites as DAG, the effects of which include activation of PKC, followed by other serine/threonine kinases that inhibit insulin signaling. B: Adipose tissue-released adipokines modulating insulin signaling markedly change in obesity. C: Obesity is an inflammatory state, associated with increase accumulation of adipose tissue macrophages (ATMs). ATMs will further augment the inflammatory state with the release of inflammatory mediators that modulate insulin signaling. D: Serine/threonine kinases that inhibit insulin signaling are affected by endocrine and inflammatory mediators. E: Further exacerbation of insulin resistance is mediated through the NF-κB-induced activation of inflammatory pathways. F: Adipokines-induced Suppressors Of Cytokines Signaling (SOCS) family proteins increase insulin resistance through alteration of phosphorylation pattern of Insulin Receptor Substrate (IRS)-1 and IRS-2, and increase IRS-1 and IRS-2 degradation. G: FAs are ligands for the Toll like Receptor (TLR) 4 and hence will activate the innate immune mechanisms. H: Insulin resistance is also augmented via central mechanisms acting through obesity associated modulation of the central response to hormones and nutrients. (Qatanani and Lazar, 2007).

1.6 Endocrine mechanisms

1.6.1 Glucose-Fatty acids (FAs) cycle

As mentioned earlier, obesity-associated increased adipose tissue mass due to hypertrophy of adipocytes and increase storage of TG will increase the fatty acids production into the circulation, i.e. FFA (McGarry, 2002; Boden, 2006; Har et al., 2013) (Figure 1.6.1.1). These have been strongly linked to insulin resistance and dysregulation of glucose uptake and metabolism. The hypertrophied adipocytes are resistant to the insulin action; and as such they will manifest increased hormone-sensitive lipase-induced lipolysis since the latter is normally inhibited by insulin. The increase amount of FFA will be taken up by the liver and the skeletal muscles, where they will compete with glucose as an energy source; this is referred to as imbalance in the glucose-FA (Randle) cycle with increased availability of FA and their oxidation products, and decrease utilization of glucose by cells. The FAs and potentially several metabolites including acyl-CoAs, ceramides, and diacylglycerol (DAG) act as signaling molecules activating protein kinases e.g. Protein Kinase C (PKC), Jun kinase (JNK), and the inhibitor of nuclear factor-κB (NF-κB) kinase-β (IKKβ). These kinases can then impair insulin signaling by increasing the inhibitory serine phosphorylation of insulin receptor substrates (IRS), the key mediators of insulin receptor signaling, leading to inhibition of insulin-mediated glucose uptake in the muscle and adipose tissue (Petersen and Shulman, 2006). In addition, the energy produced from FA oxidation will be utilized in the hepatic de novo synthesis of glucose (gluconeogenesis), since this pathway is energy-demanding and is now free from the normal suppression exerted by insulin. The increase in hepatic glucose production will obviously augment the hyperglycemia that is due to decrease glucose

uptake (Day and Bailey, 2011). However, the rate-limiting step for FA-induced insulin resistance is suggested by some to be the inhibited glucose uptake by peripheral cells of muscle and adipose tissue, rather than the disturbed intracellular glucose metabolism (Shulman, 2000). This accounts in part for the effect of lipotoxicity to impede the translocation of GLUT4 glucose transporters into the plasma membrane, reducing glucose transport. Since skeletal muscle accounts for > 70% of glucose disposal compared with about 10% into adipose tissue, the development of insulin resistance in muscle is a major feature of obesity-induced insulin resistance (Kahn et al., 2006; Arner and Langin, 2014).

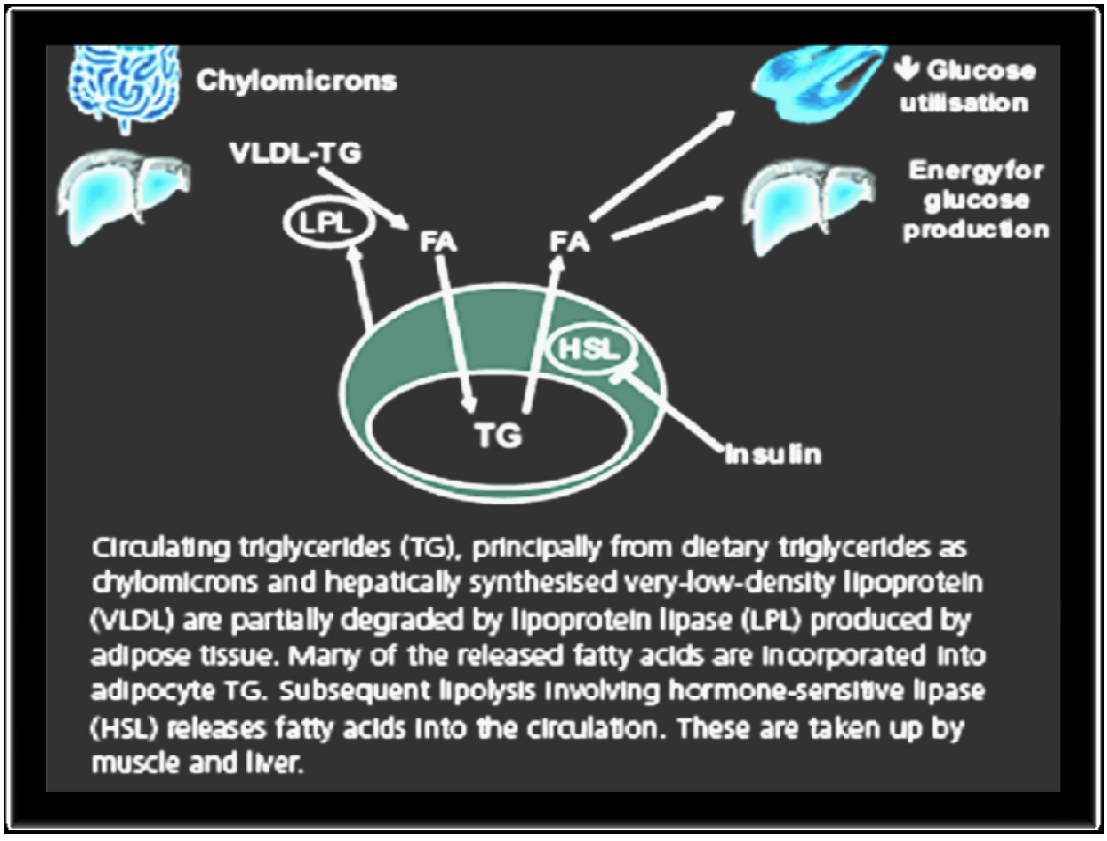


Figure (1.6.1.1) Factors affecting adipose depot: Circulating triglycerides (TG), principally from dietary triglycerides as chylomicrons and hepatically synthesized very-low-density lipoprotein (VLDL) are degraded by lipoprotein lipase (LPL) produced by adipose tissue. Many of the released fatty acids are incorporated into adipocyte TG. Subsequent lipolysis involving hormone-sensitive lipase (HSL) releases fatty acids into the circulation. These are taken up by muscle and liver (Day and Bailey, 2011).

1.7 Inflammatory mechanism

The concept of obesity as an inflammatory condition started more than 20 years ago, when Hotamisligil and colleagues demonstrated an overexpression of inflammatory cytokines in obesity (Hotamisligil et al., 1993). Adipose tissue cellular repertoire is known to be heterogeneous, with different types of cells including mature adipocytes, preadipocytes, immune cells, and endothelial cells. This is the base for the rapid and dynamic response of adipose tissue to changes in nutrients availability. For instance, adipocytes hypertrophy and hyperplasia take place in response to excess nutrients (Halberg et al., 2008). Furthermore, as a result of obesity, the adipose tissue will suffer from hypoxia due to insufficient blood supply; hypoxia in turn will lead to adipose tissue necrosis and macrophage infiltration (Cinti et al., 2005). Macrophages overproduction of pro-inflammatory mediators such as cytokines and chemokines is the underlying mechanism first for the localized inflammatory milieu prevailing in obese adipose tissue (autocrine and paracrine effects), which will then be propagated into a systemic inflammation associated with obesity metabolic comorbidities (endocrine effects). (Trayhurn and Wood, 2004; De Heredia et al., 2012; Wang B and Trayhurn P, 2006; Trayhurn, 2013).

1.8 Adipose Tissue: Heterogeneity and Functions.

The presence of adipose tissue in mammals is vital for their life, since it provides them with the fatty acids to be used in energy and heat production during fasting state and in postprandial periods. In fact, mammals have two types of adipose tissue with different functions, localization, and cellular composition; the white (WAT) and the brown (BAT) adipose tissue (Gesta et al., 2007).

Adult human body's adipose tissue is composed mainly of WAT; the function of which may be divided into two main areas: control of body metabolism and regulation of body's inflammatory and immunological response. WAT is the major source of FFA, which upon oxidation will provide the body with the high energy phosphate molecule adenosine triphosphate (ATP) during the process of coupled oxidative phosphorylation. This is vital for maintaining the energy homeostasis essential for driving various metabolic pathways. Furthermore, WAT plays a key role in the body's inflammatory and immunological response by secreting a plethora of active mediators; some are immune modulators, others are pro- or anti-inflammatory molecules (Wozniak et al., 2009; Juge-Aubry et al., 2005). These molecules will be discussed later in more details. Anatomically, WAT is found in different locations, mainly intra-abdominally for instance around the omentum, intestines, and abdominal organs; and subcutaneously as in the abdomen, buttocks, thighs, and other body locations (Gesta et al., 2007). Therefore, the WAT can be classified as subcutaneous, visceral, muscle, epicardial, peri-renal and peri-vascular. In fact, different WAT anatomical subgroups are thought to exert specific functions, since it was demonstrated that excess accumulation of adipose tissue in the upper part of the body, the so called android or central obesity represents a strong risk factor for certain inflammatory and metabolic diseases, while accumulation of adipose tissue in the lower part of the body, the so called gynoid or peripheral obesity represents a minimal risk for these diseases (Cancello and Clement, 2006; Gesta et al., 2007).

Similar to the variation in the anatomic locations of WAT, its cellular composition is also variable. The diverse cellular repertoire of WAT includes mature adipocytes, and a variety of other cells grouped under the description of stromo-

vascular fraction or SVF. The cells of SVF include preadipocytes, macrophages, lymphocytes, fibroblasts and endothelial cells (Gesta et al., 2007; Cancello and Clement, 2006; Subramanian and Ferrante, 2009; Park et al., 2012). As previously mentioned, the adipose tissue is the source of several active mediators, the sources of which are mainly the mature adipocytes, the preadipocytes, and the macrophages. These mediators can exert their action on the adipose tissue itself, the so called auto- or paracrine effects, or on other tissues, the so called endocrine effects (Wozniak et al., 2009; Juge-Aubry,2005; Subramanian and Ferrante, 2009; Fantuzzi, 2008;Lago et al., 2007; Lago et al., 2009; Chudek and Wiçcek, 2006). The macrophages are particularly known to be the source of various inflammatory molecules that are responsible for the low-grade chronic inflammatory state prevalent in obesity and its related metabolic disturbances (Wozniak et al., 2009; Cancello and Clement, 2006; Subramanian and Ferrante, 2009; Fantuzzi, 2008;Zeyda and Stulnig, 2007).

In contrast to WAT, BAT is not present in large amount in adult human body. However, recent Positron Emission Tomography (PET) studies have clearly demonstrated the presence of metabolically active BAT in specific locations in adult human bodies, such as the supra-clavicular, axillary, cervical and paraventral regions (Redinger, 2009; Frühbeck et al., 2001; Virtanen et al., 2009). The BAT depots in these sites were also found to be induced by cold and by autonomic nervous system activation, specifically the sympathetic system. Furthermore, and unlike WAT, BAT is mainly involved in thermogenesis and cold adaptation through uncoupling of oxidation and phosphorylation. Both WAT and BAT differ in appearance, composition, and cellular description. BAT possess smaller size adipocytes that are rich in mitochondria, the tissue as a whole is richer in its blood supply and hence in the autonomic nervous system regulation. These characteristics are responsible for

its brown colour. The unique function of BAT in heat vs. metabolic energy production has highlighted its potential role as a therapeutic target for obesity and related comorbidities (Frühbeck et al., 2007; Virtanen et al., 2009).

Obesity is usually associated with excess energy intake in the form of over nutrition. Visceral obesity in particular is associated with a state of disturbed metabolic homeostasis and stress (Karalis et al., 2009). Furthermore, in obesity a state of ongoing chronic inflammation exists, not only in the adipose tissue itself, but also in other metabolically active sites such as the liver and immune system (Cancello and Clement, 2006; Subramanian and Ferrante, 2009; Fantuzzi, 2008; Lago et al., 2007; Chudek and Wiçcek, 2006). Chronic inflammation is manifested by increased circulating levels of adipose tissue-derived hormone-like pro-inflammatory cytokines, collectively known as adipokines (Wozniak et al., 2009; Lago et al., 2007; Lago et al., 2009; Luft et al., 2013). In response to the obesity-associated stress, the hypothalamic-pituitary-adrenal axis is activated together with the autonomic nervous system, both central and peripheral components, to provide the body with adaptive responses (Chrousos and Gold, 1992). The result will be elevated levels of circulating glucocorticoids that will result in more proliferation and differentiation of fat cells, and hence more expansion to the adipose tissue. This will create a vicious cycle, especially with the prevalence of the chronic inflammatory state caused by the expanding WAT (Purnell et al., 2009). It is this vicious cycle between the inflammatory, the immune, and the endocrine system that will eventually precipitate the obesity associated comorbidities (Karalis at al., 2009; Hotamisligil and Erbay, 2008).

Obesity is accompanied by a drastic change in the prevailing environment in WAT. Under normal conditions, an anti-inflammatory milieu prevails in WAT and is maintained by the specific type of resident tissue macrophages, M2 macrophages, known to produce the enzyme arginase responsible for the inhibition of nitric oxide synthase, iNOS), and the anti-inflammatory cytokines interleukin (IL)-10 and IL-1Ra (Zeyda and Stulnig, 2007). This environment is protective against the development of obesity-related pathologies as insulin resistance (IR). Other factors exist that help maintaining the anti-inflammatory state, most notably are members of the nuclear receptor; peroxisome proliferator-activated receptors-(PPAR) (particularly PPAR-α and -γ) and liver X receptor- (LXR) families that are involved in nutrient transport and metabolism (Moller and Berger, 2003; Joseph et al., 2003; Pan et al., 2011). Recently, a new adipokine, the lipocalin-2 (LCN2) was demonstrated to contribute to the physiological anti-inflammatory status by upregulating PPARγ and increasing adiponectin secretion, while antagonizing TNF-α effect on gene expression related to inflammatory and metabolic genes in both adipocytes and macrophages. In addition, knocking down LCN2 expression was demonstrated to decrease the expression of PPARγ and its target genes, adiponectin, and leptin (Zhang et al., 2008).

On the other hand, in obesity the adipose tissue resident macrophages population manifests a polarization toward the CD11c-positive inflammatory type, the M1 macrophages, that release iNOS and several pro-inflammatory cytokines (Zeyda and Stulnig, 2007; Gordon, 2007; Lumeng et al., 2008). A crosstalk between adipocytes and macrophages takes place in obese WAT (Wozniak et al., 2009; Subramanian and Ferrante, 2009; Zeyda and Stulnig, 2007), together with cellular remodeling characterized by an increase in number (hyperplasia) and size

(hypertrophy) of adipocytes, macrophage infiltration, and fibrosis (Spalding et al., 2008; Bourlier et al., 2008; Henegar et al., 2008; Jernas et al., 2006; Maury et al., 2007; Skurk et al., 2007). Two factors are involved in inducing the adipocyte hypertrophy: increased fat storage in fully differentiated adipocytes and increased expression of proinflammatory mediators (Jernas et al., 2006; Maury et al., 2007; Skurk et al., 2007). More inflammation takes place when the hypertrophic adipocytes shift the immune balance towards the production of proinflammatory molecules (Bourlier et al., 2008; Cancello et al., 2006; Harman-Boehm et al., 2007; Sengenès et al., 2007; Weisberg et al., 2003; Blüher, 2013). It was shown that 20 % of the expansion in total fat mass is due to the visceral depot, while the remaining 80% is due to subcutaneous adipose tissue expansion (Mlinar and Marc, 2011).

The shift in the cytokines profile creates a tissue milieu responsible of the strong modification of the WAT macrophages pool from M2 type to M1 type (Zeyda and Stulnig, 2007; Gordon, 2007; Lumeng et al., 2008; Bourlier et al., 2008; Cancello et al., 2006; Harman-Boehm et al., 2007; Sengenès et al., 2007;Weisberg et al., 2003). Furthermore, while adipose tissue from lean individuals may preferentially secrete anti-inflammatory adipokines such as adiponectin, transforming growth factor β (TGFβ), interleukin 10 (IL-10), IL-4, IL-13, IL-1Ra and apelin; in obesity pro-inflammatory adipo-cytokines such as TNFα, IL-6, leptin, visfatin, resistin, angiotensin II and plasminogen activator inhibitor 1 (PAI-1) are released, as well as several other interleukins (Ouchi et al., 2011) coupled with a reduction in secretion of anti-inflammatory adipokines.

It would also appear that adipokines have different functions in normal-weight individuals and in the obese. In lean individuals, adipokines mediate

physiological functions while in states of metabolic disease the adipokines have altered effects, modulating insulin resistance either directly by affecting the insulin signaling pathway or indirectly via stimulation of inflammatory pathways. As previously mentioned, serine phosphorylation of IRS-1 by various adipokines directly or via inflammatory pathways including the JNK pathway and IKKβ/NF-kB pathway disrupts the insulin signalling pathways, possibly giving rise to insulin resistance (Pirola et al., 2004, Tilg & Moschen, 2008, Kalupahana et al., 2012).

1.9 The Adipokines

Adipokines are biologically active substances that, by definition, are released from adipose tissue, although not exclusively. Other tissues and organs, unrelated to adipose tissue were found to release some of these substances (Lago et al., 2007; Lago et al., 2009). The term adipokines includes hundreds of molecules encompassing more than 50 cytokines, chemokines, hormone-like substances and other effector molecules (Wozniak et al., 2009; Lago et al., 2007; Lago et al., 2009). A number of functions have been attributed to adipokines. Those functions are performed by adipokines through their local and systemic network-like effects regulating vital areas such as inflammation, insulin action and glucose homeostasis. They were shown to play a role in controlling appetite and satiety, glucose and lipid metabolism, inflammation and immune responses, and blood pressure regulation (Wozniak et al., 2009; Lago et al., 2007; Lago et al., 2009). Obviously, this intricate network system is altered in obesity, and as a consequence, is manifested as inflammation and defective metabolism not only in the adipose tissue, but also in the body as a whole.

Adipokine secretion is controlled by various mechanisms. A complex networking is involved that includes both extracellular and intracellular mediators. FFAs are among the primary extracellular factors involved in inducing the proinflammatory adipokines (Cnop, 2008). Chronic elevation of FFAs in obesity is a main contributing factor for what is called lipotoxity. This chronic elevation is due to the defective inhibitory effect of insulin on lipolysis, and the excessive consumption of dietary lipids (Ghanim et al., 2008; Di Zhao, 2013).

Innate immunity receptors, such as Toll-like receptor (TLR)-4 and -2, are expressed in WAT (particularly by adipocytes, preadipocytes, macrophages, and endothelial cells) and are involved in this obesity-related inflammatory process. Their expression is increased and induced in obese subjects (Ghanim et al., 2008; Vitseva et al., 2008). FFA and other molecules produced by hypoxic conditions during obesity activate these receptors, particularly TLR-4 (Ghanim et al., 2008; Vitseva et al., 2008). Hence, FFAs particularly via TLR-4 induce the pro-inflammatory adipokine production in adipocytes (Nguyen et al., 2005; Shi et al., 2006).

Lipopolysaccharide (LPS) is another factor able to activate TLR-4 (Cani et al., 2007a; Balistreri et al., 2009). A key source of LPS is the gut microbiota (Cnop, 2008). It is continually produced within the gut by death of Gram-negative bacteria and is absorbed into intestinal capillaries to be transported by lipoproteins (Cani et al., 2008; Leuwer et al., 2009). On the other hand, it has been observed that a high fat diet given to mice increases the proportion of gut LPS (Cani et al., 2008; Leuwer et al., 2009). These data indicate the gut microbiotia may have an important role in

the induction of chronic obesity-related inflammation (Cani et al., 2008). The role of LPS will be discussed further in details in the following sections.

In obese WAT the cells and the intracellular organelles are also exposed to increased stress, mainly as a result of metabolic overload (Hotamisligil and Erbay, 2008). In particular, mitochondria and the endoplasmic reticulum appear to be the most sensitive organelles to metabolic stressors. In addition, the development of hypoxic conditions in the expanded WAT during obesity results in an increased production of reactive oxygen species and the corresponding development of oxidative stress (Houstis et al., 2006; Trayhurn, 2013).

Signals mediated by both extracellular and intracellular factors culminate predominantly in the activation, principally via TLR-4 receptor, of NF-κB transcriptional factor, responsible for the production of inflammatory mediators, as well as the direct inhibition of insulin signaling (Ghanim et al., 2008;Vitseva et al., 2008; Shoelson et al., 2003).

Hence, NF-κB pathway represents the crucial and major factor responsible of obesity-induced inflammation. Other pathways also exist to amplify inflammation-mediated inhibition of insulin action, such as those mediated through the suppressor of cytokine signaling-(SOCS)-proteins and nitric oxide synthase iNOS (Rui et al., 2002; Mooney et al., 2001; Cruz et al., 2013) which produce nitric oxide (NO) that induces the degradation of IRS1 and iNOS activity results in reduced activity of Akt, a principal mediator of IRS signaling (Zeyda and Stulnig, 2009) (Figure 1.9.1).

Furthermore, obesity, and abdominal adiposity in particular, seems to accelerate ageing process. It has been demonstrated that obese women have

telomeres of 240 bp shorter than lean women of a similar age. In view of the hypothesis that telomere length *in vivo* represents cellular turnover and exposure to oxidative and inflammatory damage, this difference in telomere length between being lean and being obese might correspond to 8.8 years of ageing (Valdes et al., 2005; Cui et al., 2013).

The increased evidence on obesity, as a stronger predictor of metabolic diseases, has led to assess the mortality risk correlated with abdominal obesity (Baik et al., 2000; Carmienke et al., 2013). In 2008, a large European study reported that both general (BMI) and abdominal adiposity (WC; WHR) are strong predictors of mortality risk (Pischon et al., 2008).

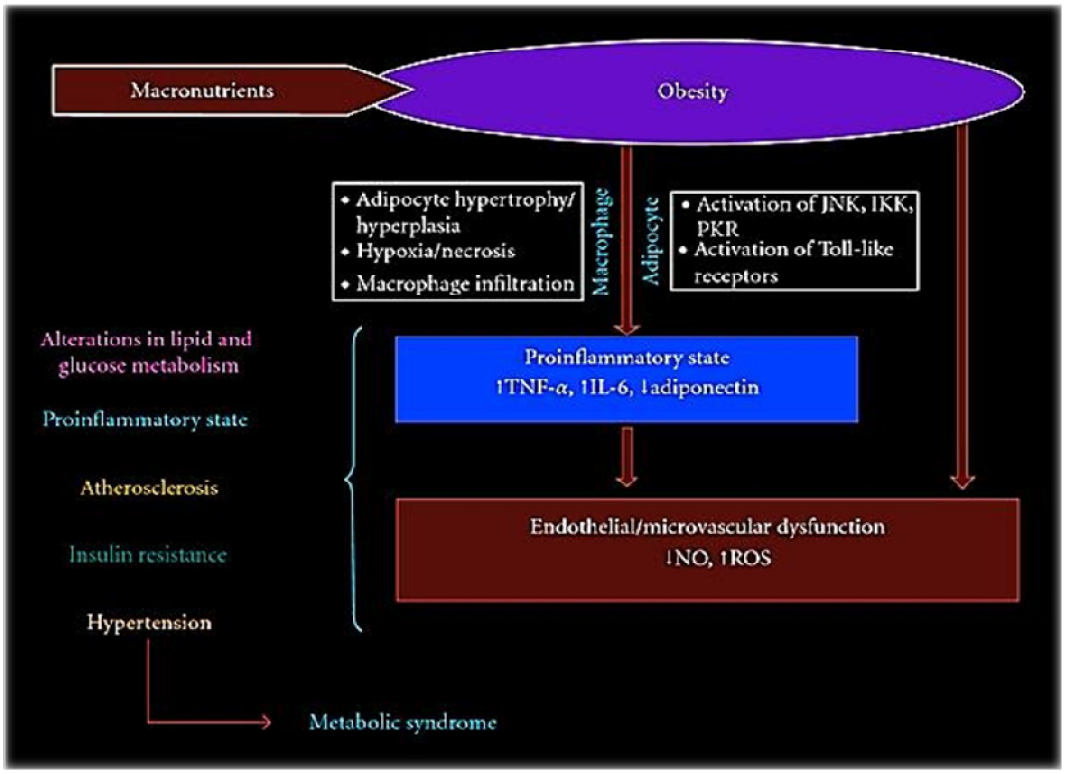


Figure (1.9.1) Simplified illustration for the negative impact and consequences of obesity showing the involved cells and the underlying molecular and cellular dysfunction. TNF-α: tumour necrosis factor alpha; IL-6 : interleukin 6; NO: nitric oxide; ROS: reactive oxygen species; JNK: c-jun N-terminal kinase; IKK : Inhibitor of k kinase; PKR: protein kinase R.(Emanuela et al., 2012)**.**

In the following section, the attention will particularly focus on adipokines involved in the obesity-related inflammatory disease.

1.9.1 Leptin

Leptin was one of the first proteins discovered to be secreted from adipose tissue, by the identification and sequencing of the *ob* gene from the *ob/ob* mouse (Zhang et al. 1994). Daily injection of leptin peptide in *ob/ob* mice resulted in a rapid reduction in food intake, body mass and percentage body fat but maintained lean muscle mass, increased energy expenditure and restored euglycaemia and reproductive function, confirming that it had an important role in energy homoeostasis and storage (Campfield et al., 1995; Holmström et al., 2013). However, leptin levels were noted to be increased in obese subjects, with little or no impact to regulate energy homoeostasis, which coined the well-established phrase 'leptin resistance' in obesity (Friedman & Halaas, 1998; Myers et al., 2010; Al Disi et al., 2010; Schwartz and Baskin, 2013). While this has dominated much of the literature on leptin, leptin was initially shown to have a pro-inflammatory function when studies observed that recombinant leptin-activated human T lymphocytes and

monocytes (Santos-Alvarez et al., 1999; Martín-Romero et al., 2000). More recently, leptin has also been shown to activate human B cells to secrete TNF-α, IL-6 and IL-10 (Agrawal et al., 2011). The pro-inflammatory nature of leptin has been noted in several studies, with i.v. injection of endotoxin inducing a sudden rise in leptin levels (Landman et al., 2003; Xiao et al., 2003). Furthermore, endotoxin-induced fever and anorexia in rats resulted in an increase in leptin levels as part of the inflammatory response (Sachot et al., 2004).

1.9.2 Tumor necrosis factor alpha (TNF-α.)

TNF-α is a pleiotropic pro-inflammatory cytokine originally thought to be secreted mainly by adipocytes, yet shown later to be secreted mainly by macrophages (Weisberg et al., 2003). In humans, both circulating and adipose tissue levels TNF-α are higher in obesity, and they decrease upon weight loss (Ambeba et al., 2013; Ziccardi et al., 2002). TNF-α promotes the secretion of other pro-inflammatory cytokines such as IL-6, and reduces anti-inflammatory cytokines such as adiponectin (Wang and Trayhurn, 2006). It can also induce adipocytes' apoptosis (Bennett et al., 2014). TNF-α induces insulin resistance in adipose tissue and muscle, but not in liver, through inhibition of tyrosine phosphorylation of insulin receptor and IRS-1, and thus inhibiting the insulin signaling pathway (Hotamisligil et al., 1994; Hotamisligil et al., 1996; Nowotny et al., 2013).

TNF-α levels were also found to positively correlate with other markers of insulin resistance (Hivert et al., 2008); nonetheless acute treatment with TNF-α inhibitor in obese subjects with type 2 diabetes reduced other systemic inflammatory

markers without reducing insulin resistance (Dominguez et al., 2005). More recently, assessment of anti-TNF-α inhibitor treatment, over the long term, given to subjects with metabolic syndrome, has shown to improve fasting blood sugar and also to increase adiponectin levels, confirming a role for TNF-α in obesity-related insulin resistance in humans (Stanley et al., 2011).

1.9.3 Interleulin-6 (IL-6)

Both macrophages and adipocytes of WAT are primary sources of circulating IL-6. IL-6 plays an important role in the regulation of whole-body energy homeostasis and inflammation. It was shown to inhibit the lipoprotein lipase activity both in vitro and in vivo. In addition, IL-6 was demonstrated to affect the appetite and the intake of energy by affecting the hypothalamus, and its receptor, IL-6 receptor, is shown to be expressed in several regions of the brain including the hypothalamus (Stenlöf et al., 2003).

Interestingly, IL-6 is demonstrated to have dual functions, depending on the tissue and metabolic state. In skeletal muscle, during exercise, it acts to increase glucose uptake resulting in muscle hypertrophy and myogenesis and AMPK mediated fatty acid oxidation, as well as having an anti-inflammatory effect (Starkie et al., 2003; Kelly et al., 2004). While in adipose tissue and hepatic tissue, IL-6 is shown to be a pro-inflammatory adipokine. It increases insulin resistance by up-regulating suppressor of cytokine signalling 3 (SOCS3), which in turn, impairs insulin-induced insulin receptor and IRS-1 phosphorylation (Senn et al., 2002, 2003; Rotter et al., 2003; Lukic et al., 2014). IL-6 is positively correlated with increasing body mass and plasma-FFAs (Fried et al., 1998), with reduction in circulating IL-6 following weight loss (Bastard et al., 2000; Ziccardi et al., 2002; Peters et al., 2013).

Moreover, IL-6 has been shown to be raised in subjects with T2DM and also increases the risk of future development of T2DM (Pradhan et al., 2001).

1.9.4 Adiponectin

Adiponectin is a hormone secreted by adipocytes that regulates energy homeostasis, body weight, lipid and glucose metabolism; increases insulin sensitivity and protects against chronic inflammation. Adiponectin levels have shown to be increased in weight loss studies, while its level decreased in animal models of obesity and insulin resistance (Liu M. and Liu F., 2010; Ohashi et al., 2014). Furthermore human analysis has shown that insulin resistance, hyperinsulinemia and the development of type 2 diabetes is associated with hypoadiponectinemia, independent of fat mass (Fumeron et al., 2004).

In a study designed by Su and colleagues (2011) to investigate plasma circulating pro-inflammatory cytokines, adiponectin and high-sensitive C-reactive protein (hsCRP) in young men with type 2 diabetes (divided into a non-obese group (NOYDM, body mass index, BMI $\leqq$ 25 kg/m^2, N = 23) and an obese group (OBYDM, BMI $\geqq$ 25 kg/m^2, N = 24). Twenty-four non-obese non-diabetic young men served as controls. Compared with controls, significantly greater HOMA-IR (7.9 ± 1.9 and 10.4 ± 2.5 vs. 1.5 ± 0.3, $P < 0.001$) and fasting insulin (103.3 ± 21.1 and 209.9 ± 24.4 vs. 48.5 ± 6.7 pmol/l, $P < 0.01$ and $P < 0.001$, respectively) were observed in NOYDM and OBYDM. There were significantly lower plasma adiponectin and higher hsCRP and TNF-α levels in OBYDM, whereas higher TNF-α and IL-6 were shown in NOYDM (Su et al., 2011).

Furthermore, adiponectin concentration was found to be positively associated with HDL-cholesterol level in type 2 diabetic Chinese subjects aged between 40-70 years. Adponectin concentration was the only main predictor of HDL-cholesterol level ($\beta = 0.321$, $p = 0.002$) after adjusting other factors for homogeneous type 2 diabetic subjects, this demonstrates that adiponectin concentration might be a valuable marker of atherosclerosis in type 2 diabetic patients (Hsu et al., 2012).

1.9.5 Resistin

Resistin is a cytokine with a molecular structure similar to adiponectin (Patel et al., 2004) and has a clear role in mice, affecting glucose homoeostasis and acting as a mediator of insulin resistance (Steppan et al., 2001, Schwartz & Lazar, 2011). However, its role in human adipose tissue has had a much more conflicted history (Nagaev& Smith 2001, Savage et al., 2001, McTernan CL et al., 2002; McTernan PG et al., 2006, Schwartz & Lazar, 2011). While initially considered not to be present in the adipocyte, subsequent studies have shown its presence and regulation (Nagaev& Smith, 2001; Savage et al., 2001; McTernan PG et al., 2002a, b; McTernan PG et al., 2003; Al-Daghri et al., 2005; Baker et al., 2006; Kusminski et al., 2007), although its role in humans appears more related to an inflammatory role than being an important factor regulating glucose metabolism.

In vitro studies in isolated abdominal subcutaneous adipocytes have shown an increase in resistin secretion following treatment of the adipocyte with endotoxin (LPS). In addition, treatment of adipocytes with recombinant human resistin increased release of IL6, TNFα as well as expression of TLR2, IKKβ and JNK, suggesting a possible role for resistin in pro-inflammatory mechanisms in the adipocyte via both the NF-κB and the JNK pathways (Kusminski et al., 2007).

1.9.6 New adipokines

The following sections represent some brief insights into recent additions to the adipokine family, which, akin to other adipokines, appear to impact on inflammation and insulin resistance (Table 1.9.6.1).

Apelin is a peptide that is produced in a wide range of tissues with positive effects on insulin sensitivity, glucose uptake and lipolysis in skeletal muscle as well as adipose tissue (Boucher et al., 2005; Dray et al., 2008; Yue et al., 2011; Attane´ et al., 2012). However, studies in humans have shown an increase in plasma apelin levels in obesity, morbid obesity, impaired glucose tolerance and T2DM with a reduction in apelin levels accompanying weight loss following diet or bariatric surgery. These findings suggest the presence of resistance to apelin, in a similar fashion to insulin and leptin (Heinonen et al., 2005; Li et al., 2006; Castan-Laurell et al., 2008, 2011; Erdem et al., 2008; Soriguer et al., 2009; Zhang Y et al., 2009). Apelin has also been shown to have a pro-inflammatory role with a close positive correlation demonstrated between apelin and TNFα levels, as well as other proinflammatory cytokines (Malyszko et al., 2008; Heinonen et al., 2009; Yu et al., 2012). Apelin expression also closely correlates with TNFα in adipose tissue of lean and obese individuals, and in vitro studies of cultured human adipose tissue explants show an up-regulation of apelin in response to TNFα (Daviaud et al., 2006). Further in vitro studies in human umbilical vein endothelial cells (HUVECs) suggest an increase in adhesion molecules (VCAM and ICAM) by apelin via the NF-κB and JNK pathways, further supporting its role as a pro-inflammatory adipokine (Lu et al., 2012).

Omentin is another new peptide that is produced in omental but not subcutaneous adipose tissue and exists in two isoforms, omentin 1 and omentin 2. Omentin 1 represents the predominant circulating form and positively affects insulin sensitivity, which is reduced in subjects with obesity and T2DM compared with lean subjects (de Souza Batista et al., 2007; Shibata et al., 2012). Omentin is thought to be an anti-inflammatory adipokine and acts by inhibiting TNF-α induced expression of adhesion molecules in endothelial cells by inhibiting the ERK/NF-kB pathway (Zhong et al., 2012), while in vascular smooth muscle cells omentin inhibits TNFα action via inhibition of p38 and JNK pathways (Kazamaet al., 2012). Taken together, this suggests that omentin may have a positive role to play to reduce inflammation in normal physiology.

Chemerin is a novel adipokine that has been shown to play a role in the regulation of adipogenesis and adipocyte metabolism (Goralski et al., 2007), as well as a role in glucose homoeostasis – as noted by studies on glucose intolerance in *ob/ob* and *db/db* mice (Ernst et al., 2010). In humans, however, chemerin seems to have a direct action on inflammation in adipocytes rather than glucose homoeostasis, as use of recombinant TNFα seems to induce chemerin secretion from adipocytes (Catala´net al., 2011, 2013). In other inflammatory cell types, such as macrophages, chemerin causes a pro-inflammatory action through increasing macrophage adhesion to VCAM-1 and fibronectin (Hart & Greaves, 2010). As such, in subsequently considered coronary artery disease patients, where inflammation had progressed, circulating chemerin levels were noted to be positively correlated with multiple markers of inflammation including TNFα, IL6, C-reactive protein (CRP), leptin and resistin, affirming its proinflammatory function (Lehrke et al., 2009). In separate studies of T2DM subjects at risk of CVD, analysis of their circulating chemerin

levels also revealed positive associations with inflammatory markers, including TNFα CRP, leptin and resistin (Weigert et al., 2010, Yu et al., 2012). These combined studies indicate a pro-inflammatory function for chemerin, which appears exacerbated in metabolic disease states.

Table 1.9.6.1: List of adipokines. Adapted and updated from Fruhbeck et al. (2001) and Kusminski et al. (2007)

Adipokine	Function/effect	Distribution	Effect of obesity	Evidence
Leptin	Satiety signal. Promotes increased energy expenditure	Secreted predominantly by WAT, Sc AT > Om AT. Also derives from BAT, skeletal muscle, stomach and plasma	↑In human obesity, correlates with BMI, ↓after fasting or weight loss	Meier & Gressner (2004) and Mantzoros et al. (2011)
Adiponectin	Improves energy homoeostasis, insulin sensitivity and glucose uptake. Anti-in ammatory properties	Secreted exclusively by adipocytes. mRNA and protein in Sc AT > Om AT. 2–3x greater secretion in females	↑In mouse models of obesity and insulin resistance (ob/ob and db/db). ↓In human obesity and T2DM.	Fisher et al. (2002), Spranger et al. (2003) and Whitehead et al. (2006)
TNFα	Reduces insulin secretion and insulin sensitivity. Stimulates lipolysis	Predominantly expressed by macrophages. Also expressed by WAT adipo-cytes, Sc AT > Om AT	↑After weight loss Correlates with BMI, ↑In human obesity: obese (2X) > lean. ↓ Adipose differentiation	Hotamisligil et al. (1993), Hube & Hauner (1999) and Tzanavari et al. (2010)
IL6	Affects glucose and lipid metabolism. Improves insulin sensitivity and glucose tolerance	35% of the basal supply is derived from WAT. Produced by macrophages, broblasts, endothelial cells and skeletal muscle	↑In morbidly obese patients. ↓After weight loss	Fried et al. (1998), Bastard et al. (2000) and Eder et al. (2009)

Resistin	Affects glucose metabolism and causes insulin resistance in rodents. In humans, it acts more as a pro-in ammatory cytokine	In rodents, secreted by WAT. In humans, secreted in macrophages and WAT	↑In human obesity, metabolic syndrome, T2DM and CVD	McTernan et al. (2002a, 2006) and Schwartz & Lazar (2011)
PAI-1	Potent inhibitor of brinolytic pathway	Expressed by Sc and Om AT. Positive correlation with abdominal adiposity	↑In human obesity, metabolic syndrome and T2DM	Shimomura et al. (1996) and Alessi et al. (2007)
RANTES	Pro-in ammatory			

CRP is another pro-inflammatory factor positively correlated with BMI in otherwise healthy individuals, and its elevation in obesity may be a consequence of IL-6 production. IL-6 is secreted by subcutaneous adipose tissue and increases hepatic CRP production. In addition, serum CRP is positively correlated with adipose tissue expression of IL-6, and IL-6 is necessary for human CRP gene expression in transgenic mice (Fontana et al., 2007; Bremer and Jialal, 2013).

1.10 Inflammatory characteristics of the gram negative bacteria's endotoxins (LPS)

The activation of the human adipose tissue immune cells may represent a mechanism for increased inflammation in that tissue (Haara et al., 2004; Blüher, 2013). Previously-published works have demonstrated that elevated circulating levels of the gut-derived bacteria endotoxins (the lipopolysaccharide; or LPS) may contribute to the increased activation of the innate immune pathway. LPS is a component of the outer cell wall of the gut-derived bacteria (Haara et al., 2004; Yoshimura E et al., 2011; Hui et al., 2012; Conde et al., 2011). The chemical composition of LPS comprises an antigen-O specific chain, a core region which represents a hetero-oligosaccharide, and a lipid A region that is highly conserved and representing the toxic part of the LPS (Osborn et al., 1964) (Figure 1.10.1).

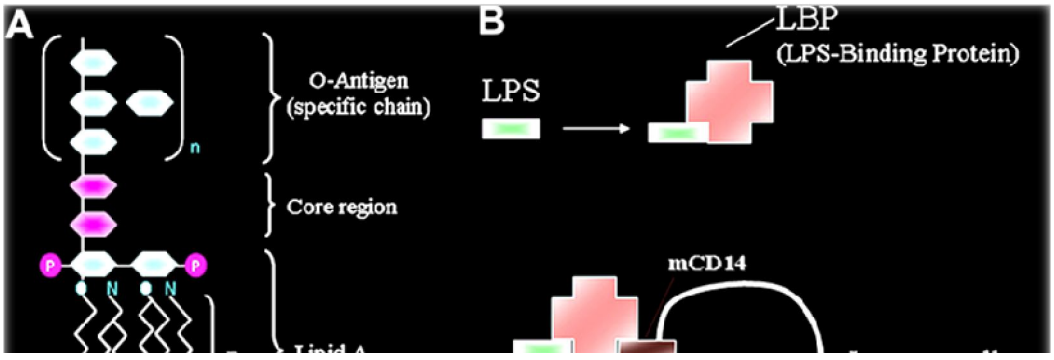


Figure (1.10.1) (A) General chemical structure of bacterial endotoxins. (B) Proinflammatory sequence of events taking place upon activation of an immune cell with LPS bound to LPS-binding protein. LPS: Lipopolysaccharide; LBP: Lipopolysaccharide-binding protein; mCD14: membrane cluster of differentiation 14; TLR4: toll-like receptor 4; MD2: myeloid differentiation protein-2; NFκB: nuclear factor κ B; IL-6: interleukin-6 (Laugerette et al., 2011a)

It is well documented that endotoxin stimulates the innate immunity pathway through the activation of the Toll like Receptors (TLRs) through several proteins, including the LPS-binding protein (LBP), CD14, myeloid differential protein 2 (MD2) and myeloid differentiation factor 88 (MyD88). This leads to intracellular activation of NF-κB and resulting pro-inflammatory cytokines (Creely et al., 2007; Baker et al., 2009; Youssef-Elabd et al., 2012). However, understanding of how gut-derived endotoxin affect metabolic function has changed in recent years, as studies have considered the direct impact of endotoxin as a systemic inflammatory insult. Based on the known strong affinity of endotoxin for chylomicrons (which are the lipoprotein particles responsible for dietary fat transportation across the intestinal wall), endotoxin is believed to cross the gastrointestinal mucosa coupled to these lipoproteins. Once in the circulation, endotoxin has been shown to mediate metabolic dysfunction in several tissues including adipose tissue, liver and muscle. In addition to the long-established list of metabolic risk factors including hyper triglyceridemia, reduced HDL-C and hyperglycemia; all are associated with insulin resistance; other mediators as LPS may prove relevant as well. Within this context, chronic low grade inflammation has been added to this list, coupled with obesity, insulin resistance and

an activated immune response in mediating several metabolic diseases (Ouchi et al., 2011).

In vitro studies, using human adipocytes have demonstrated the impact of adipose tissue on the immune response. For instance, endotoxin was demonstrated to stimulate TLRs and NF-κB inflammatory pathways, resulting in secretion of pro-inflammatory adipokines. Consequently, the body weight will be affected. (Creely et al., 2007; Dixon et al., 2008). Human body hosts gut microbiota which are considered a source of endotoxin. Due to the high affinity of endotoxin to chylomicrons, the gut-microbiota-induced endotoxin will be absorbed through these lipoprotein particles. However, monocytes, and in particular the hepatic Kupffer cells will rapidly remove the endotoxin. Therefore, under normal conditions, only small amounts of endotoxin will reach the systemic circulation due the protective role of the healthy liver. On the other hand, a compromised liver, due to ectopic fat deposition, has diminished capacity to remove the endotoxin, which can directly aggravate liver disease exacerbated by weight gain (Rao et al., 2004; Harte et al., 2010), leading to increased circulating endotoxin, or what is termed: metabolic endotoxemia to be distinct from the exogenous bacterial infection or sepsis-induced endotoxemia (laugerette, 2011a).

Thus, a combination of dietary lipoprotein patterns and an increase in circulating endotoxin mediate chronic low-grade systemic inflammation that could activate the TLR pathway to induce downstream insulin resistance. As lipoprotein patterns would appear to alter circulating endotoxin levels, recent studies have begun to evaluate this across different insulin resistance states to examine the impact of feeding. Interestingly, a single high-fat meal did alter endotoxin levels across the

different subgroups analyzed, where endotoxemia was ~20% more in the IGT subjects and obese groups relative to the non-obese control (NOC) group. On the other hand, subjects with T2DM experienced as much as 125% higher endotoxin levels than NOC; an effect that remains in the T2DM group even 4 hours after the meal (Harte et al., 2012). In addition, previous cross-sectional in vivo studies have shown that endotoxin appears to correlate with markers and conditions of insulin resistance, with endotoxin appearing to act as a predictive metabolic biomarker of T2DM (Dixon et al., 2008; Al-Attas et al., 2009; Miller et al., 2009; Harte et al., 2010; Pussinen et al., 2011).

Studies on experimental animals have also reported an association between endotoxin and insulin resistance. Similar to the results obtained through infusing endotoxin to those animals, feeding animals a high-fat diet has lead to the same effect (Cani et al., 2007a). Gut permeability has been shown to be affected by both insulin resistance and weight gain. (Brun et al., 2007; Harte et al., 2010; Ghanim et al., 2009). The capacity of endotoxin to affect the inflammatory pathways is strongly suggested by studies using either bolus infusion of endotoxin or the endotoxin derived from the gut secondary to dietary changes (Ghanim et al., 2009; Deopurkar et al., 2010).

Taken together, the in vivo and in vitro studies highlight the impact of endotoxin on the inflammatory pathways to promote secretion of pro-inflammatory adipocytokines to exacerbate the insulin-resistant state (Brun et al., 2007; Creely et al., 2007; Dixon et al., 2008; Al-Attas et al., 2009; Baker et al., 2009; Miller et al., 2009; Harte et al. 2010, 2012).

However, the gut-derived bacteria acts as a "primary insult" that activates the inflammatory state contributing to metabolic diseases as implicated by some clinical studies. Furthermore, cross sectional studies demonstrate elevation of endotoxin level in conditions such as obesity, coronary artery disease, T2DM and fatty liver disease. (Baker et al., 2009; Creely et al., 2007; Brun et al., 2007; Dixon et al., 2008; Al-Attas et al., 2009; Miller et al., 2009; Harte et al., 2010). In addition, these studies demonstrated a positive association between circulating endotoxin and WC, WHR, insulin levels, inflammatory cytokines and lipids, including total cholesterol, TG, and LDL cholesterol; and negative association with HDL cholesterol (Baker et al., 2009; Creely et al., 2007; Brun et al., 2007; Dixon et al., 2008; Al-Attas et al., 2009; Miller et al., 2009; Harte et al., 2010). The combined importance of dietary lipids and LPS in determining inflammatory risk may arise, since as previously mentioned endotoxin has a strong affinity for chylomicrons (Ghoshal et al., 2009; Amar et al., 2008; Moreno et al., 2010). As such, atherogenic risk and inflammation may arise as a result of increase in circulating level of endotoxin combined with dietary lipoprotein exacerbated by feeding patterns (Hall et al., 2009; Wyness, 2009). Therefore, a well-designed dietary intervention that results in altering the lipid profile may reduce endotoxin level and the arising inflammatory response. The dietary effect of high-SFA, high carbohydrate meal on circulating endotoxin levels have been explored in recent human studies, which found a substantial increase in circulating endotoxin in healthy subjects given a high-fat meal, in conjunction with markers of inflammation (as noted from mononuclear blood cells) (Cani et al., 2007b; Ghanim et al., 2009).

1.11 Links between high fat diets, inflammation and endotoxin

Several metabolic disorders are related to the glucose homeostasis and to the development of CVD (Alberti et al., 2005; Matareze et al., 2007). During the past decade, it became clear that a low-grade inflammation contributes to the development of the pathologies associated with obesity (Heilbronn and Campbell, 2008) which is consistent with many experimental and clinical studies that showed a cause effect relationship between inflammation and inflammatory signaling responses to the development of metabolic disorders associated with obesity. The analysis of the nutritional disorders associated with obesity reveals that the prolonged positive energy balance mostly through the ingestion of high-fat diet is associated with adverse health consequences of weight gain and obesity. However, the mechanism by which feeding high-fat diet promotes low grade inflammation is poorly understood. (Cani et al., 2009) (Figure 1.11.1).

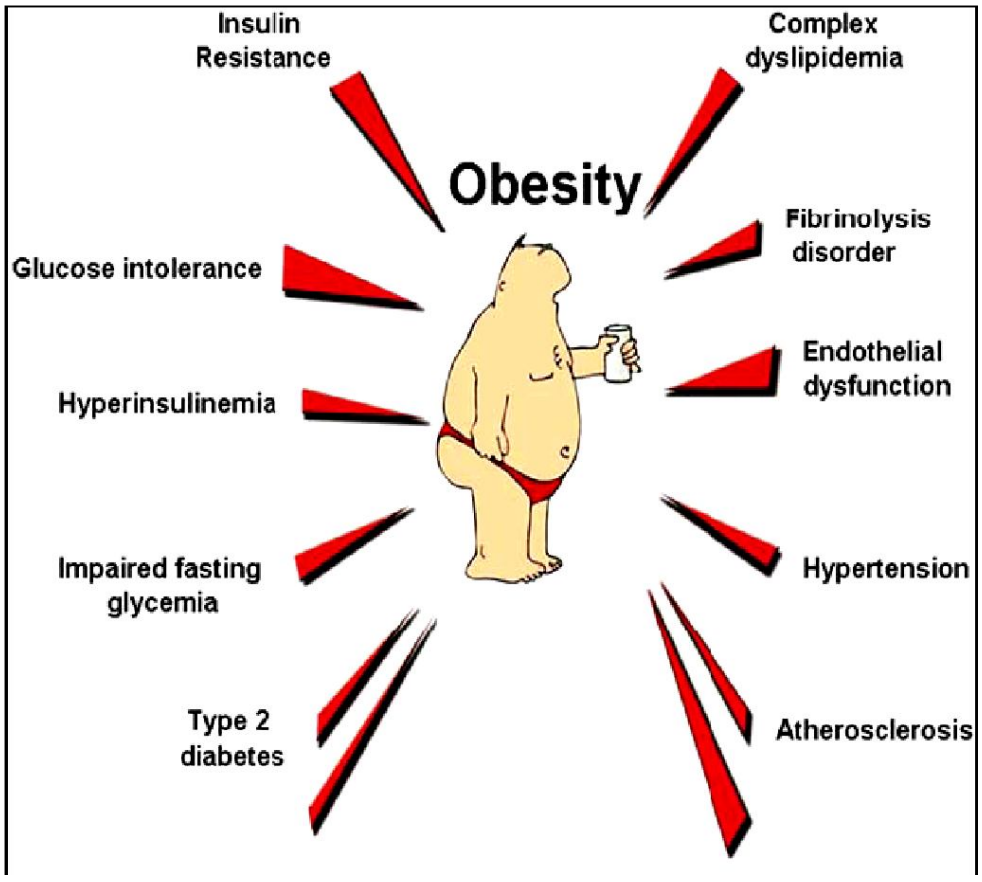


Figure (1.11.1) Obesity and associated metabolic disorders: Several metabolic disorders are associated with obesity related to glucose homeostasis and CVD that has been associated with low grade of inflammation (Cani et al., 2009).

The inflammatory Response to exogenous LPS can be affected by extrinsic factors such as diet, as shown in mice where their sensitivity to LPS injection has increased when submitted to high saturated fat and cholesterol diet (Huang et al., 2007). However, the effect of dietary fat in inducing the absorption of endogenous endotoxin with subsequent inflammatory response has been supported by recent studies.

The pioneering article by Cani et al. reported that a four week high fat diet in mice (72% energy as fat) increases plasma endotoxin levels (endotoxemia) in comparison with a control diet, and that chronic low-dose infusion of LPS leads to weight gain and insulin resistance (Cani et al., 2007a). In turn CD14-KO mice resisted to increased weight gain, endotoxemia and insulin resistance induced by a high fat diet (Cani et al., 2007a). Importantly, Shi et al. have also shown that TLR4-KO mice is protected from NFκB-induced inflammation and development of insulin resistance (Shi et al., 2006). These studies thus show a link between innate immunity and lipid-induced insulin resistance. (Figure 1.11.2).

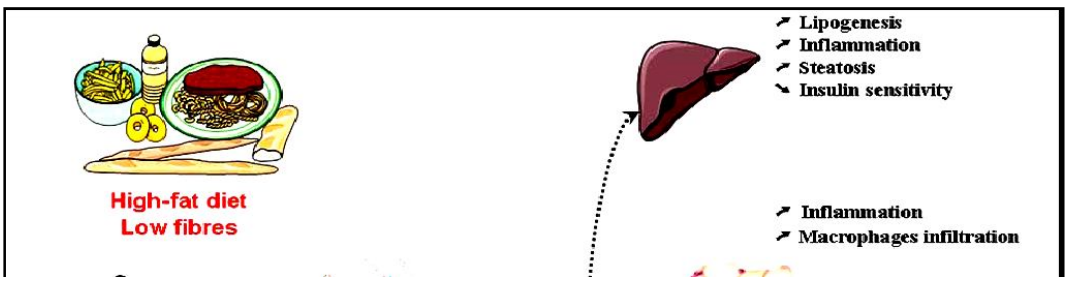


Figure (1.11.2) High-fat diet feeding changes gut microbiota, promotes metabolic endotoxemia and triggers the development of metabolic disorders, via a CD14/TLR4 dependent mechanism.
(1) High-fat diet feeding changes gut microbiota in a complex way and (2) specifically decreases *Bifidobacterium* spp. (3) This phenomenon is associated with a higher plasma LPS content (metabolic endotoxaemia), a LPS-dependent secretion of proinflammatory cytokines. High fat feeding and LPS promotes low-grade inflammation-induced metabolic disorders (insulin resistance, diabetes, obesity, steatosis, adipose tissue macrophages infiltration) (Cani et al., 2009).

Moreover, in mice fed a diet containing 35% energy as fat, plasma LPS was found to be lower as compared with counterparts fed a high-fat diet. (Amar et al., 2008). In humans, Amar et al. found a positive correlation between energy intake and endotoxemia which reveals a link between food intake and plasma endotoxin. (Amar et al., 2008).

Erridge and colleagues have studied this link on an acute basis, and demonstrated that an acute high fat bolus (50 g butter on toast) given to humans (lean to obese) who are occasional smokers was sufficient to transiently promote an increase in endotoxemia as early as 30 min after ingestion (Erridge et al., 2007). Since smoking might play a role in plasma endotoxin level via the LPS absorption in lungs (Hasday et al., 1999), and to correct for this possibility, Eridge and colleagues examined endotoxemia for 4 h under four different settings; namely with no meal,

with a high-fat meal, with no meal and 3 cigarettes, and lastly with a high-fat meal and 3 cigarettes. Their results demonstrate that fat was the only significant variable influencing postprandial endotoxemia (Erridge et al., 2007) (Figure 1.11.3).

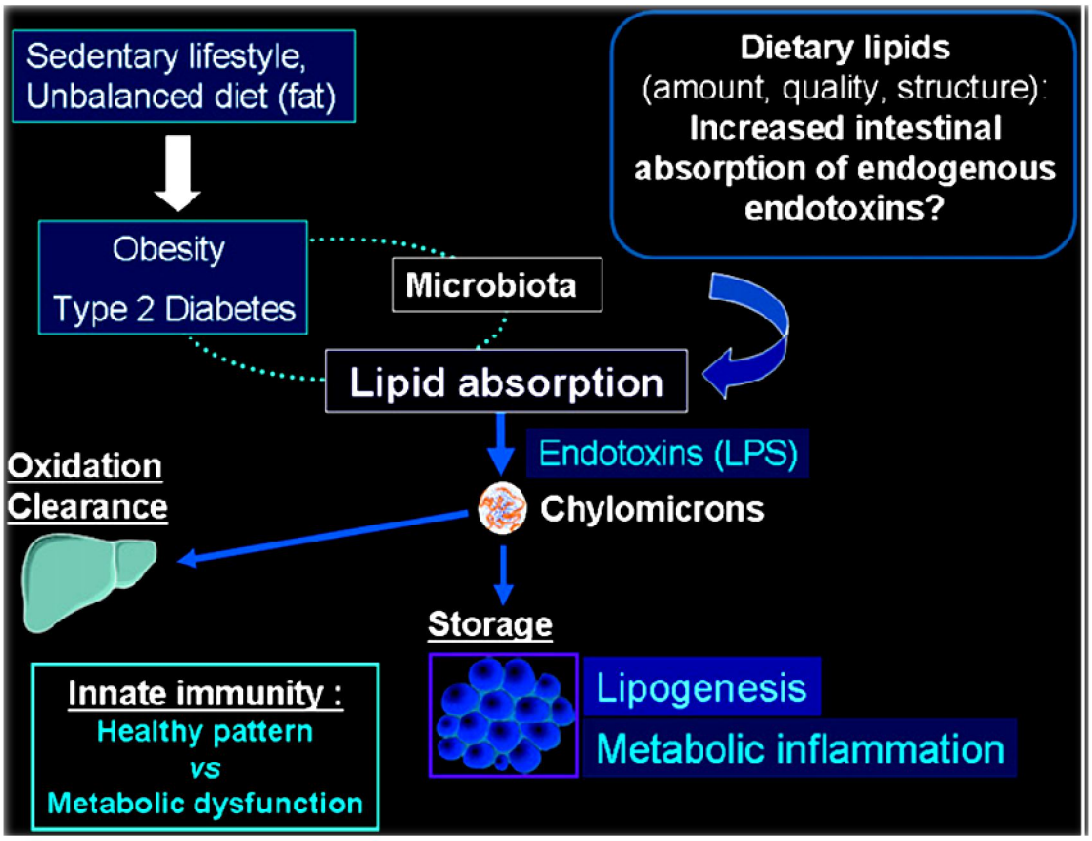


Figure (1.11.3) Possible impact of dietary lipids on postprandial lipid and LPS absorption and metabolic outcomes. Obesity and Type 2 Diabetes are characterized by altered profile of intestinal microbiota and by altered lipid metabolism. During lipid digestion, endotoxins from microbiota are absorbed along with lipids and can be vehicled by chylomicrons. In a healthy pattern, lipids are mostly oxidized and endotoxins are cleared by the liver. In a metabolic dysfunction pattern (obesity, type 2 diabetes), lipids are more oriented towards storage in the adipose tissue and more circulating endotoxins can contribute to generate low-grade inflammation (Laugerette et al., 2011a).

A recent study by Harte et al (2012) showed that exposure to a high-fat meal, as early as 1hr postprandial, elevates circulating endotoxin irrespective of metabolic state, this increase was more substantial in IGT and type 2 diabetes , which suggests that metabolic endotoxemia is exacerbated after high fat intake, and that in a compromised metabolic state such as type 2 diabetes, a continual snacking routine will cumulatively promote their condition more rapidly than in other individuals because of the greater exposure to endotoxin (Figure 1.11.4).

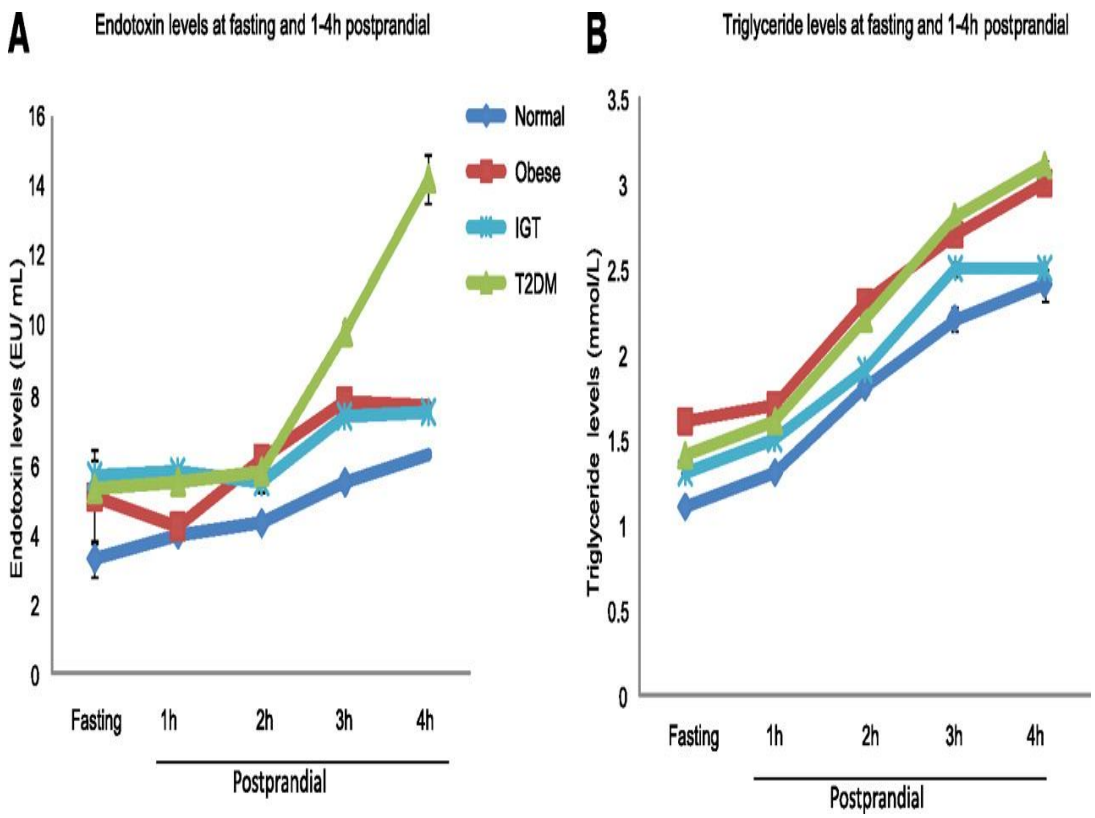


Figure (1.11.4) Changes in circulating endotoxin levels (A) and triglyceride levels (B) in NOC, IGT, obese, and type 2 diabetic (T2DM) subjects. Endotoxin and triglyceride levels were measured at baseline and then, after a high-SFA meal, at each hour postprandially over a 4-h duration. Each point on the graph represents the mean value for each cohort (± SEM) (Harte et al., 2012).

Overall, these results demonstrate that the repeated ingestion of single fat meal would trigger the increased endotoxemia. In fact, lipids and chylomicrons secretion is capable of promoting intestinal absorption of LPS from the gut microbiota. (Laugerette et al., 2011b; Ghoshal et al., 2009; Estadella et al., 2013), which could contribute to postprandial inflammatory responses (Lundman et al., 2007; Mehta et al., 2010) and thus to the onset and persistence of chronic low-grade inflammation**.**

A recent study by Lira et al (2012) showed the importance of life style changes (i.e. nutritional modification) in reducing the pro-inflammatory state in obese individuals. This study demonstrated that after interdisciplinary therapy,

endotoxinemia, pro-inflammatory status and insulin resistance were decreased. (Lira et al., 2012). Previous work published by the same group has shown that long-term therapy is effective in reducing body fat (particularly visceral fat), TNF-α and IL-6; and in increasing IL-10 and adiponectin. In addition, a positive correlation between pro-inflammatory cytokines (IL- 6 and TNF-α) and visceral fat was observed (Lira at al., 2011).

1.12 Benefits of Weight Loss

Weight loss occurs when energy expenditure exceeds energy intake. An energy deficit of 500-1000 kcal /day will result in a loss of ~1-2 pounds/week. As such moderate weight loss can improve insulin action, decrease fasting blood glucose concentrations and reduce the need for T2DM medications as well as improve other risk factors related to cardiovascular disease (Pi-Sunyer, 1993; Goldstein, 1992; Williams and Kelley, 2000; Torgerson et al., 2004). This has been affirmed by 'LOOK AHEAD' (Action for Health in Diabetes) a randomized clinical trial examining the long-term effects of life-style interventions on cardiovascular morbidity and mortality in 5,145 overweight or obese participants with T2DM. This trial has revealed that the magnitude of weight loss at 1 year was strongly ($P < 0.0001$) associated with improvements in glycaemia, blood pressure, triglycerides, and HDL cholesterol but not with LDL cholesterol ($P = 0.79$). Compared with weight-stable participants, those who lost 5 to <10% ([mean ±SD] 7.25±2.1 kg) of their body weight had increased odds of achieving a 0.5% point reduction in HbA1c (odds ratio 3.52 [95% CI 2.81–4.40]), a 5-mmHg decrease in diastolic blood pressure (1.48 [1.20 1.82]), a 5-mmHg decrease in systolic blood pressure (1.56 [1.27–1.91]), a 5 mg/dL increase in HDL cholesterol (1.69 [1.37–2.07]), and a 40

mg/dL decrease in triglycerides (2.20[1.71-2.83]). The odds of clinically signi cant improvements in most risk factors were even greater in those who lost 10–15% of their body weight (Wing et al., 2011).

1.13 Diet Modification

Dietary modification is considered the cornerstone in the management of T2DM. More than three out of every four adults with T2DM are at least overweight (Ali et al., 2013) and nearly half of individuals are obese (Nguyen et al., 2011). Due to this relationship between body weight (i.e., adiposity) and insulin resistance, weight loss has long been a recommended strategy for overweight or obese adults with diabetes (ADA, 2014) and several large scale studies have tried to assess the impact diet and exercise can have on health.

The Finnish Diabetes Prevention study (FDP) randomized 522 overweight subjects with IGT to usual care or diet and exercise recommendations. The dietary goals in the interventional group were a low fat-diet (<30% fat energy as fat) with, 10% saturated fatty acids (SFA) and dietary fiber >15g/1000Kcal. Participants in this group were instructed to increase physical activity to attain the target of weight loss to nearly 5% of baseline weight. The cumulative incidence of T2DM was 23% in the control group and 11% in the intervention group. Interestingly, risk reduction was directly proportional to the magnitude of lifestyle changes. Despite the success of many lifestyle trials, an important question remained to be answered. Weight loss appeared to be the driving force to reduce incidence of T2DM. Thus it is unclear whether diet with its macronutrient composition or exercise alone plays a significant role in preventing T2DM (Tuomilehto et al., 2001).

Salas-Salvadó and colleagues concluded -after reviewing epidemiologic and clinical trial evidences relating nutrients, foods and dietary to T2DM risk as well as the differential effects of carbohydrates and fat quantity and quality – that there is no universal dietary strategy to prevent T2DM or delay its onset. Furthermore, maintaining ideal body weight and consumption of the so- called prudent-diet (characterized by high intake of plant-based foods and lower intake of red-meat, sweets, high-fat dairy products and refined grains or a Mediterranean dietary pattern rich in olive oil, fruits and vegetables, including whole grains, pulses and nuts, low-fat dairy, and moderate alcohol consumption (mainly red wine)) appears as the best strategy to decrease T2DM risk, especially if dietary recommendations takes into account individual preferences, thus enabling long-time adherence (Salas-Salvadó, 2011). Furthermore, the low-fat eating pattern is one that has often been encouraged as a strategy to lose weight or to improve cardiovascular health within the U.S. In the Look AHEAD trial (Look Ahead Research Group, 2013), an energy reduced low-fat eating pattern was encouraged for weight loss, and individuals achieved moderate success (Pi-Sunyer et al., 2007). However, in a systematic review (Wheeler et al., 2012) and in four studies (Brehm et al., 2009; Davis et al., 2009; Guldbrand et al., 2012; Papakonstantinou et al., 2010) and in a meta-analysis (Kodama et al., 2009), lowering total fat intake did not consistently improve glycaemic control or CVD risk factors. The benefit gained from a low-fat diet appears to be more likely to occur when energy intake is also reduced and weight loss occurs (Pi-Sunyer et al., 2007; Look Ahead Research Group, 2013).

A variety of diets have been proposed to treat obesity. Although many different dietary approaches may result in short term weight loss, the limitation of most diets is the poor long-term compliance and weight regain. There is no “ideal”

conclusive eating pattern that is expected to benefit all individuals with diabetes (Wheeler et al., 2012).

Despite all these promising results, few studies have addressed the effects of dietary intervention and life style changes on dietary fat intake and endotoxin levels and their correlation with insulin resistance, therefore our study sought to establish whether a high-fat meal increased circulating endotoxin and inflammatory markers and whether this is altered in different metabolic disease states, as well as to study the effectiveness of dietary intervention through low fat, complex carbohydrate diets in decreasing inflammatory markers related to obesity among metabolically dysfunction states .

Currently we have very limited evidence in the field of metabolic disease regarding the importance of dietary intervention, what works, and how diets exacerbate inflammatory risk. However, this study, while challenging, has the appropriate infrastructure, including primary care networks, to provide novel and exciting data. The work detailed in this study will generate a large body of significant data with potential ramifications for clinical practice as we reassess the effects of feeding and dietary patterns. Based on pilot data, this study will highlight the factors that may increase our inflammatory risk, their temporal profile, and how they differ among NOCs, overweight and subjects with T2DM. We will also examine how, over time, a change in diet may reduce our inflammatory risk. As such we anticipate that a low fat diet will reduce inflammatory status impacting long-term pathological outcome such as CVD. Obviously, this is expected to have a fundamental impact on targeting therapeutic approaches for T2DM towards treating the inflammatory component of the disease, especially after verifying the results in a

larger patients population. Reducing the pathogenesis of T2DM by dampening the inflammatory response, which may also have an impact on insulin resistance status and general health of the individual, will have clear benefits. This could have profound effects on preventative T2DM management, as well as current T2DM care without excessive cost for the wider Saudi health economy.

1.14 Study hypothesis and Aims of the study

Our hypothesis is that gut-derived bacterial endotoxin, also known as lipopolysaccharide (LPS), may act as a potential mediator of inflammation in overweight and T2DM subjects, and that this can be altered acutely by feeding and metabolic status with long term metabolic effects.

To test this hypothesis; we will study the postprandial impact of lipids on circulating factors, such as endotoxin, that affect insulin sensitivity, glucose metabolism and diet may impact patients with and without type 2 diabetes. We will assess the role of potential mediators of chronic low level inflammation, such as saturated fatty acids (SFA), dietary changes and endotoxin. We will specifically examine the following:

1. The impact of a postprandial diet high in SFA on systemic inflammation, to establish that a high fat diet raises circulating endotoxin levels
2. The direct effect of diet on CVD risk factors to lower endotoxin levels and thus reduce inflammation on cardiometabolic risk.
3. How the different types of diet can influence insulin resistance and inflammation, postprandially, over time.

Chapter Two

Materials and Methods

2.1 Research methodology

Ethical approval had been granted by the Ethics Committee of King Saud University, Riyadh, Kingdom of Saudi Arabia, prior to the commencement of the research (**Appendix I**). The number of subjects recruited was based on the previous studies in this area (Ceriello et al., 2002; Dixon et al., 2008). A total of 214 female Saudi adults aged 18-50 years were recruited at the beginning of the study, 110 were excluded (54 subjects didn't meet the inclusion criteria while 56 refused to participate) (Figure 2.1.1). Finally a total of 92 female Saudi adults aged 18–50 years consisted of 18 healthy non-diabetic (ND) control subjects (n=18) (age 24.4±7.9; BMI 22.2 ± 2.2), overweight plus (overweight^{+}) subjects (n= 24) (age 32.0±7.8; BMI 28.5±1.5) and patients with T2DM (n= 50) (age 41.5±6.2; BMI 35.2±7.7), were recruited after informed consent was obtained from all the subjects who agreed voluntarily to participate in the study (**Appendix II**). Subjects were randomly selected from different regions of Riyadh city.

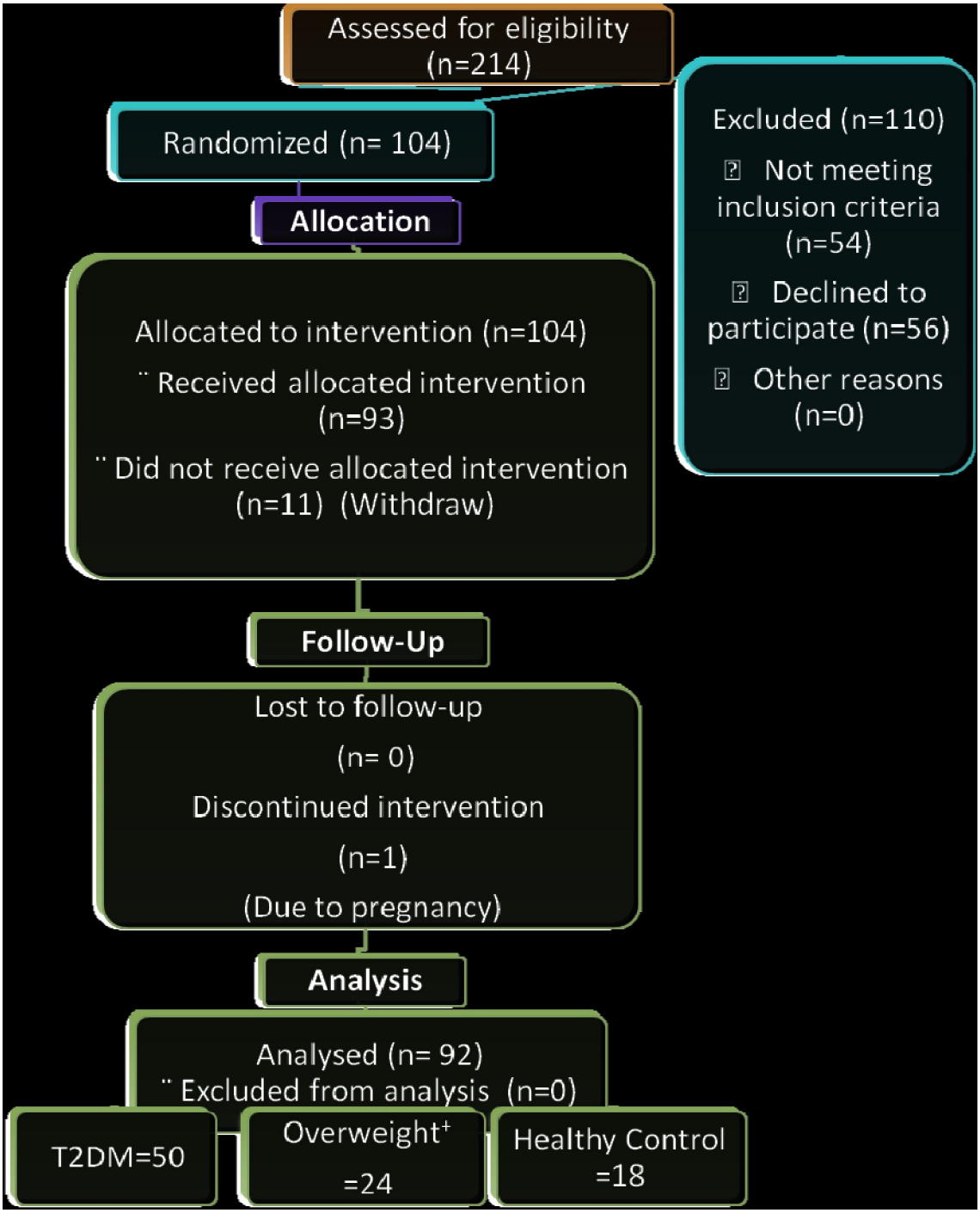


Figure 2.1.1 CONSORT (Consolidated Standards for Reporting of Trials) statement describes the flow of participants through our clinical trial

2.2 Medical screening

Subjects were medically screened at primary health care centers where a physician completed a medical examination of vital signs and clinical interview which include questions to determine eligibility based on inclusion and exclusion criteria, detailed health information obtained from all subjects using preset heath questionnaire. For collecting data for the study, a pre-coded questionnaire was designed. A pilot study was conducted to test the clarity of the questions with 10 participants. Some questions were modified, omitted or added to the questionnaire taking into the consideration the participants understanding. The questionnaire was judged for its face validity through review by 4 experts in nutrition, biochemistry and community health along with the research supervisors. The interview questionnaire which was previously used in large-scale epidemiologic survey in Saudi Arabia consists of socio-demographic data, past medical and treatment history, sleeping hours and level of physical activity (Al-Daghri et al, 2011) (**Appendix III**).

2.3 Exclusion & inclusion criteria

Patients with acute medical illness; malignancy, a history of cardiovascular disease (CVD) or those on chronic medication, as well as patients with hypertriglyceridemia (as steroids and lipid lowering drugs may affect the intervention trial), previous myocardial infarction to established CHD, and other chronic inflammatory disorders *e.g.* rheumatoid arthritis, crohn's disease, age under 18 and inability to give informed consent on grounds of competency.

All subjects were matched for age, BMI, and lipid profile as closely as possible. Only females, non-smokers, pre-menopausal, with a normal resting ECG

and blood pressure and with no history of vascular disease were selected. In addition, subjects with known long standing diabetes and receiving medication, or those with fasting glucose > 11 mmol/l, were excluded. Moreover subjects with a fasting triglycerides > 4 mmol/l were excluded from the study.

The infrastructure to undertake these studies forms part of an ongoing research program, and active facilities are available to facilitate this research. These studies were conducted over a three month period utilizing only women for this part of the study to increase the compliancy rate.

2.4 Anthropometry

Participating subjects were requested to return to their respective primary health care centre after an overnight fast (> 10 hours) for anthropometry and blood withdrawal. Anthropometric data was collected with emphasis on clinical markers of adiposity. These included height (cm) (to the nearest 0.5 cm), and weight (kg) (to the nearest 0.1 kg) from which BMI was calculated [weight (kg)/height (m^2)]. Waist (cm) and hips (cm) was also measured utilizing a standardized measuring tape in cm and its ratio was determined. Blood pressure (mmHg) was taken using a conventional mercury sphygmomanometer. Subjects were age and BMI matched as closely as possible. All these measurements were repeated post intervention at 3 months.

2.5 Clinical intervention (dietary regimen)

Patients were brought to the clinic two times over a three month period. For each visit the subjects had fasted overnight (12–14 hours) and blood samples were

collected pre and post meal. A standardized high-fat meal consists of whipping cream and contains 75g of fat, 5 g of carbohydrates, and 6 g of protein (Ceriello et al., 2002) was allocated to the subjects to drink it within 10 minutes. Baseline blood samples and post fat meal blood samples were taken over a 4 hour period (0, 1, 2, 3, 4 hours), with blood pressure noted. Subjects were divided according to the assigned dietary regimen into low fat and balanced diet groups (described in a later section). The same process was repeated after a 3 month period to repeat blood collection. Food frequency questionnaire was obtained from the subjects in each group at baseline and follow up visits.

All subjects were given health advice according to their assigned dietary regimen. Energy intake was set at the levels recommended by the dietary reference intake for subjects with low levels of physical activity at the same gender and age (DRI, 2008/2011).

All patients selected were provided with group information on the food pyramid, weight loss diets, food labels, fat free and low calorie foods, fast food, calories and nutritional composition, good nutritional choices in special occasions, functional foods, as well as health consequences of obesity and related diseases and some traditional Saudi cuisine that can be cooked with healthy low calorie, low fat choices. **(**Figure 2.5.1) shows the summary of the dietary recommendations.

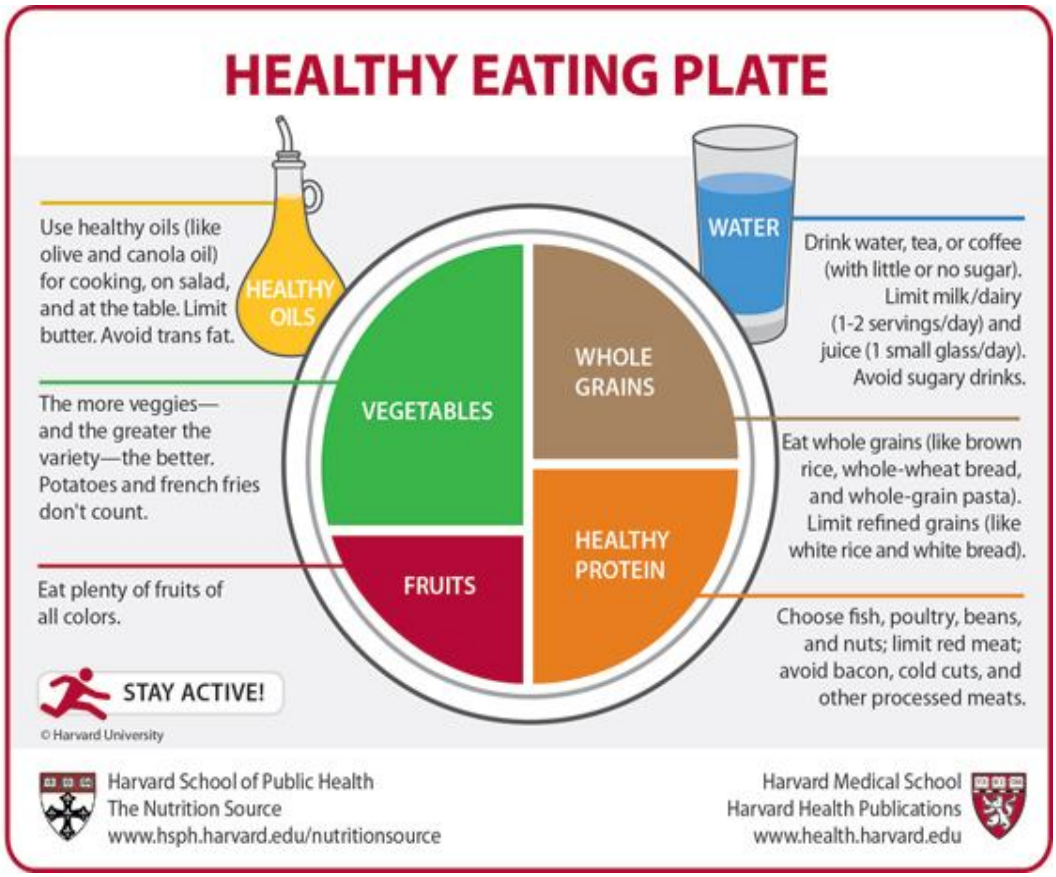


Figure 2.5.1 The healthy eating plate helps to create healthy & flavorful meals. Created by nutrition experts at Harvard School of Public Health in conjunction with Harvard Health Publications, The Healthy Eating Plate addresses key flaws in the U.S. Department of Agriculture's MyPlate (www.hsph.harvard/nutritionsource).

The monthly group sessions were interactive between the dietitian researcher and the subjects, allowing questions to be answered and explained. In addition, all patients received individual consultation during the intervention program, as well as printed dietary materials and handouts were designed and provided to all participants to go home according to their assigned dietary regimen to facilitate implementation of the dietary interventional program (**Appendix V**). To support their compliance health advice was confirmed by phone calls (once/week), messages and visits during the study if needed. Women were asked to record all food eaten daily for 3 months. Food records were collected by the dietitian and returned with feedback, weekly self-weighing and session attendance were tracked to promote adherence to the diet

intervention. In addition food frequency questionnaire (FFQ) which has been used previously (Al Disi et al., 2010) was the dietary tool that had been used to assess the food intake of the subjects prior and post the dietary intervention. The researcher interviewed the participants individually and the information was collected using a pre-designed questionnaire (**Appendix IV**) to assess the qualitative and the quantitative aspects of the food consumed by the participants over a period of 7 days. The FFQ included examining the intake around the five major food groups (starch & grains, meat & beans, dairy products, fruits and vegetables) along with the consumption of the junk food which are defined as heavily processed, highly palatable and hyper energetic and are often deprived of the vitamins and essential nutrients found in whole unprocessed foods (Bayol et al., 2007) since it poses the greatest risk to health and wellbeing (Blackburn and Gorge, 2001); energy–dense, nutrient-poor products were also included illustrated through the consumption of fat (cream, ghee and nuts), sweets (cakes, honey, jam, ice-creams, donuts and chocolates), salty snacks (pop-corn), soft drinks and caffeinated drinks (coffee, black & green tea).

Nutrient intake was calculated using USDA database (18^{th} - 21^{st} Ed, 2009, 2010) Program, as for the Saudi Arabic traditional dishes were analyzed using the Arabic food analysis program (version 1, 2006). The evaluation of the daily food intake was made by the means of the total energy and the total nutrient intake, the percentage of the total calories derived from fat, protein and carbohydrates.

Dietary nutrients values were compared with dietary reference intake (DRI) for specified age and gender for macronutrients (carbohydrates and protein) as well as for micronutrients (DRI, 2008/2011).

As for daily energy requirement (Kcal/day) was estimated using WHO equation (2005, 2007) (Sylvia Escott-Stump, 2011) according to age group and metabolic status. Dietary fat percentage has been estimated as 25% from daily energy requirement (Hooper et al., 2011) (**Appendix VI**).

As described earlier, subjects were divided into two groups according to their dietary regimen as follows:

1. Low fat diet group: fat is the most energy-rich of all energy-providing nutrients, therefore reducing fat intake in reducing total energy intake to induce weight loss. This diet defined as total fat intake < 30% of total energy intake and saturated fat intake <10%. Emphasizes vegetables, fruits, starches (e.g., breads/crackers, pasta, whole grains, starchy vegetables), lean protein, and low-fat dairy products (National Heart, Lung, and Blood Institute, 2005). Participants in this group were directed to avoid fried and processed foods and were provided with low fat recipes and substitutes (**Appendix V**).
2. Balanced diet (the composition of this diet was similar to the Mediterranean diet style, so-called prudent diet) which includes abundant plant foods (fruit, vegetables, breads, other forms of cereals, potatoes, beans, nuts, and seeds), fresh fruit as the typical daily dessert, olive oil as the principal source of fat, dairy products (principally cheese and yogurt), and fish and poultry consumed in low to moderate amounts, zero to four eggs consumed weekly, red meat consumed in low amounts, and wine consumed in low to moderate amounts, normally with meals. This diet is low in saturated fat (< or = 7-8% of energy), with total fat ranging from < 25% to > 35% of energy (Willett et al., 1995) (**Appendix V**).

- A third non-diabetic, non-obese group was proposed to serve as a reference or control group.

The following figure details schematically the different arms of the interventional trial proposed (Figure 2.5.2).

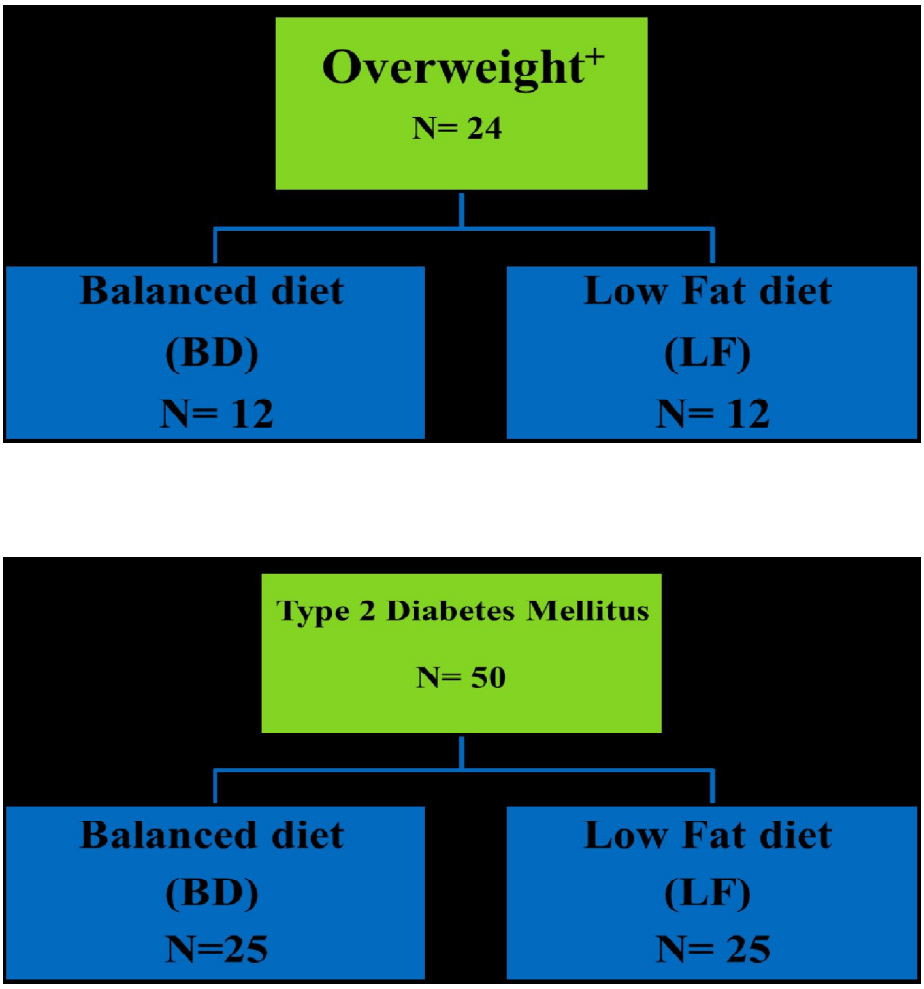


Figure 2.5.2 The different arms of the interventional trial proposed: Type 2 diabetes mellitus and overweight^{+} subjects were subdivided into two groups according to the assigned dietary regimen (LF = Low fat diet) group and (BD = Balanced diet) group

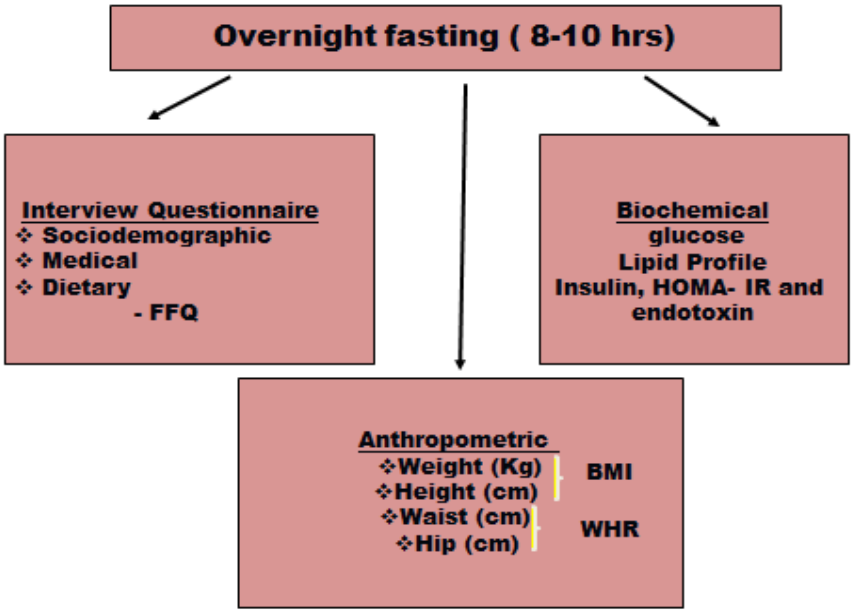


Figure 2.5.3 Summary of clinical intervention applied in the study: Data collected for the study includes: interview questionnaire, anthropometric measurements and biochemical parameters

2.6 Biochemical assessment

Fasting bloods were used to assess biochemical parameters which included: glucose, triglycerides, cholesterol, HDL, LDL, insulin, HOMA-IR and endotoxin.

Blood Sample Collection: These were collected with the patients in the fasting state using sterile vacutainer blood collection apparatus (BD). Whole blood, serum, EDTA plasma and fluoride tubes were collected from subjects. All samples were aliquoted and stored in –20^{o}C freezer facilities in preparation for subsequent analysis.

2.6.1 Laboratory Techniques

Serum samples were stored in a -20 ℃ freezer prior to analysis. Fasting glucose and lipid profiles were measured using a chemical analyzer. This

biochemical analyzer was calibrated routinely prior to the analysis of all serum samples using quality control samples provided by the manufacturer (ThermoFisher Scientific, Espoo, Finland). Serum free insulin concentrations were determined by electochemiluminescence method (ELICA) (COBAS E 411;Roche Diagnostics, Mannheim, Germany). Serum endotoxin was analyzed using a commercially available QCL-1000 LAL Endpoint Assay (Lonza, New Jersey, USA).

2.6.2 Insulin and HOMA-IR measurements

2.6.2.1 Insulin determination

Serum free insulin concentrations were determined by electro-chemiluminescence method (ECLIA) (COBAS E 411; Roche Diagnostics, Mannheim, Germany). The instruments were calibrated prior to analysis using quality control samples provided with the kits.

2.6.2.1.1 Test Principle

- Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: Insulin from 20 µL sample, a biotinylated monoclonal insulin-specific antibody, and a monoclonal insulin-specific antibody labeled with a ruthenium complex form a sandwich complex.

- 2nd incubation: After addition of streptavidin-coated microparticles, the complex became bound to the solid phase via interaction of biotin and streptavidin.

- The reaction mixture was aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode.

Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induced chemiluminescent emission which was measured by a photomultiplier.

- Results were determined via a calibration curve which is an instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

2.6.2.2 HOMA-IR measurements

- Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5 (Bonora et al, 2002).

2.6.3 Lipids assay

- Triglycerides are hydrolysed by lipase to glycerol and fatty-acids. The glycerol is phosphorylated to glycerol-3-phosphate, which then is oxidized to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine and 4-chlorophenol forming a quinoneimine dye. The absorbance of the formed color is measured at 510 nm **(**Figure 2.6.3.1). The results were calculated automatically by the Konelab analyzer using a calibration curve.

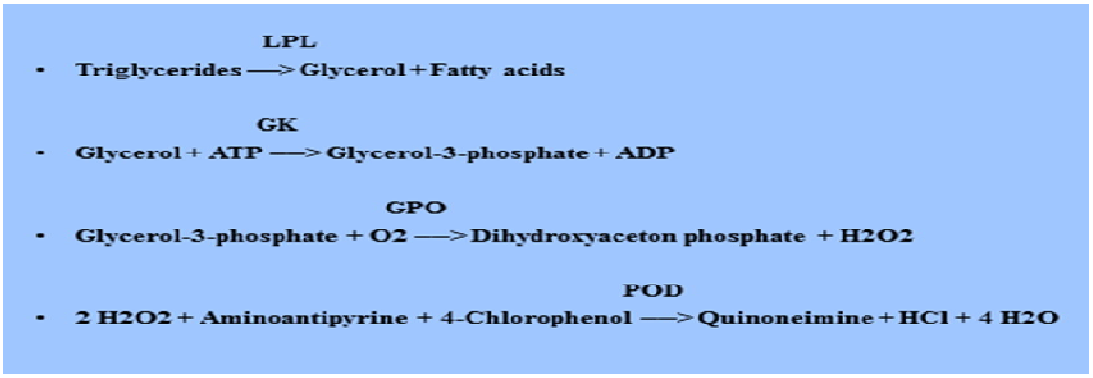


Figure 2.6.3.1 Triglyceride assay: Triglycerides are hydrolysed by lipoprotein lipase (LPL) to glycerol and fatty-acids. The glycerol is phosphorylated to glycerol-3-phosphate by Glycerokinase (GK), glycerol-3-phosphate is then oxidized to dihydroxyacetone phosphate and hydrogen peroxide mediated by Glycerol-3-phosphateoxidase (GPO). The hydrogen peroxide reacts with 4-aminoantipyrine and 4-chlorophenol forming a quinoneimine dye mediated by Glycerol-3-phosphateoxidase (GPO).

- Cholesterol esters are enzymatically hydrolysed by cholesterol esterase to cholesterol and free fatty acids. Free cholesterol, including that originally present, is then oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide combines with HBA and 4-aminoantipyrine to form a chromophore (quinoneimine dye) which may be quantitated at 500-550 nm. (Allain et al 1974) **(**Figure 2.6.3.2).

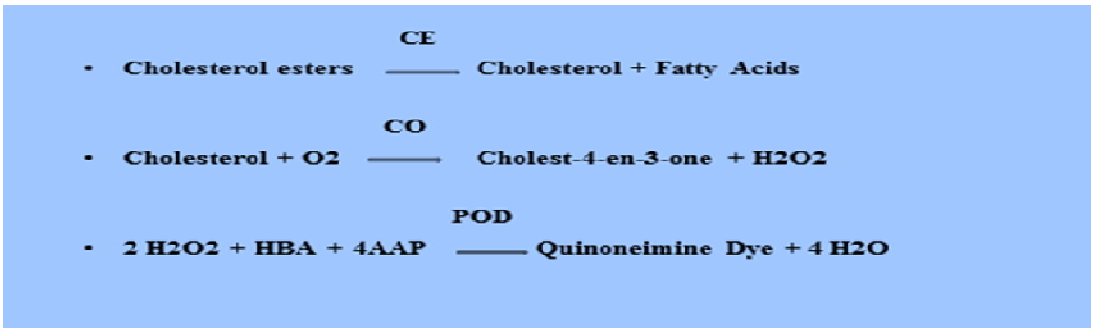


Figure 2.6.3.2 Cholesterol Esters test principle: cholesterol esters are enzymatically hydrolysed by cholesterol esterase (CE) to cholesterol and free fatty acids. Free cholesterol, including that originally present, is then oxidized by cholesterol oxidase (CO) to cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide combines with Hydroxybenzoic Acid (HBA) and 4-aminoantipyrine (4AAP) mediated by peroxidase (POD) to form a chromophore (quinoneimine dye).

- HDL-cholesterol test was undertaken as a homogeneous enzymatic colorimetric test, which was in the presence of magnesium sulfate, dextran sulfate which selectively forms water- soluble complexes with low density lipoprotein (LDL), very low density lipoprotein (VLDL) and chylomicrons, which are resistant to PEG modified enzymes. The cholesterol concentration of HDL-cholesterol was determined enzymatically by cholesterol oxidase coupled with PEG to the amino groups (approx. 40%) (Figure 2.6.3.3**)**. The results are calculated automatically by the Konelab analyzer using a calibration curve.

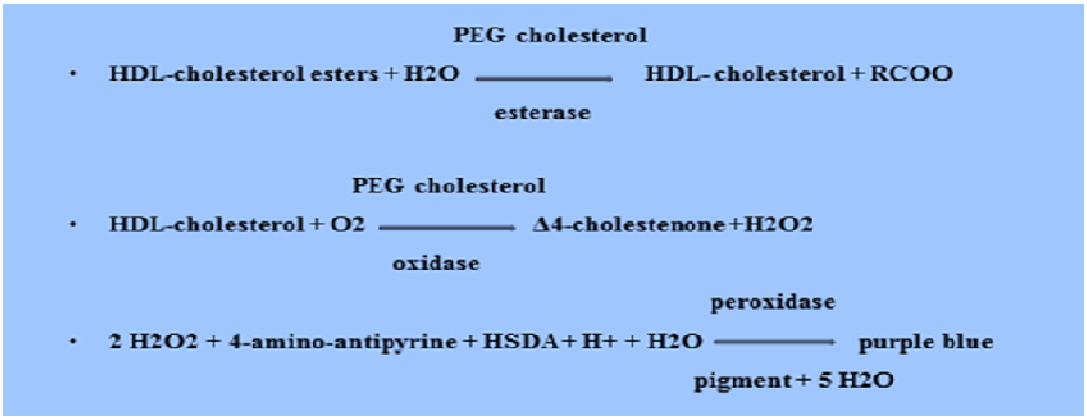


Figure 2.6.3.3 HDL- Cholesterol Esters assay: The method uses magnesium sulfate, dextran sulfate which selectively forms water- soluble complexes with low density lipoprotein (LDL), very low density lipoprotein (VLDL) and chylomicrons, which are resistant to PEG modified enzymes. The cholesterol concentration of HDL-cholesterol was determined enzymatically by cholesterol oxidase coupled with PEG to the amino groups.

- LDL-cholesterol was calculated by the following equation:

LDL = T. Cholesterol - HDL - (0.16* Triglyceride)

2.6.4 Glucose assay

To measure glucose concentration in human serum or plasma, this method employed glucose oxidase (GOD) and a modified Trinder colour reaction, catalysed

by the enzyme peroxidase (POD). Glucose was oxidised to D-gluconate by glucose oxidase with the formation of an equimolar amount of hydrogen peroxide. In the presence of peroxidase, 4-aminoantipyrine and phenol are oxidatively coupled by hydrogen peroxide to form a quinoneimine dye, coloured in red. The intensity of color in the reaction was measured at 510 nm and it was proportional to the glucose concentration in the sample (Figure 2.6.4.1).

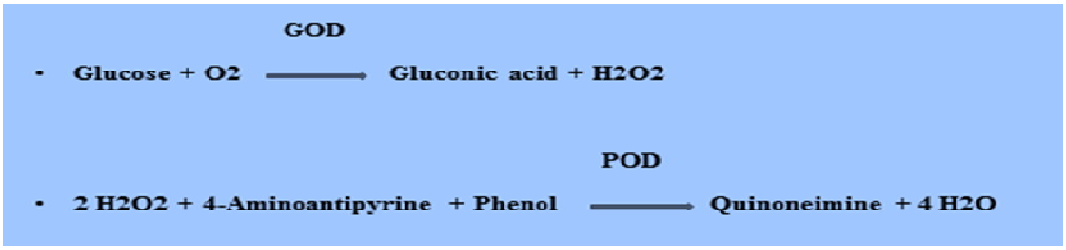


Figure 2.6.4.1 Glucose assay Glucose was oxidised to D-gluconate by glucose oxidase (GOD) with the formation of an equimolar amount of hydrogen peroxide. In the presence of peroxidase (POD), 4-aminoantipyrine and phenol are oxidatively coupled by hydrogen peroxide to form a quinoneimine dye, coloured in red.

2.6.5 Endotoxin assay

Endotoxin concentration was measured using a chromogenic kinetic Limulus amebocyte assay (LAL assay, BioWhitaker,Walkersville MD) which had been validated previously (Creely et al., 2007) (Figure 2.6.5.1).

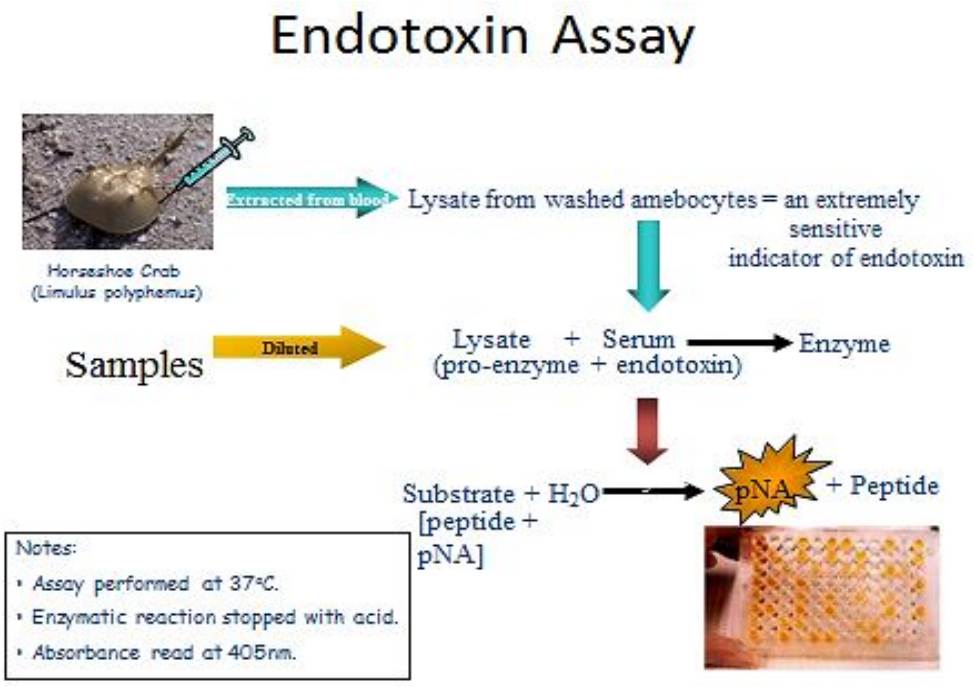


Figure 2.6.5.1 Endotoxin Assay: The Chromogenic Limulus Amebocyte Lysate (LAL) Test is a quantitative test for gram-negative bacterial endotoxin. A sample is mixed with the LAL supplied in the test kit and incubated at 37°C (±1°C) for 10 minutes. A substrate solution is then mixed with the LAL-sample and incubated at 37°C (±1°C) for an additional 6 minutes. The reaction is stopped with stop reagent. If endotoxin is present in the sample, a yellow color will develop. The absorbance of the sample can be determined spectrophotometrically at 405-410 nm

The use of LAL for the detection of endotoxin evolved from the observation by Bang that a Gram-negative infection of *Limulus polyphemus*, the horseshoe crab, resulted in fatal intra-vascular coagulation (Bang, 1956). Levin and Bang later demonstrated that this clotting was the result of a reaction between endotoxin and a clottable protein in the circulating amebocytes of Limulus (Levin and Bang, 1964a, 1964b). Following the development of a suitable anticoagulant for Limulus blood, Levin and Bang prepared a lysate from washed amebocytes which was an extremely sensitive indicator of the presence of endotoxin (Levin and Bang, 1968). Solum and Young, Levin and Prendergast have purified and characterized the clottable protein from LAL and have shown the reaction with endotoxin to be enzymatic (Solum, 1970; Solum, 1973; Young, Levin and Prendergast, 1972).

The present LAL method utilizes the initial part of the LAL endotoxin reaction to activate an enzyme which in turn releases p-nitroaniline (pNA) from a synthetic substrate, producing a yellow color.

2.6.5.1 Principle of the endotoxin assay

Gram-negative bacterial endotoxin catalyzes the activation of a pro-enzyme in the Limulus Amebocyte Lysate (LAL) (Young, Levin and Prendergast, 1972). The initial rate of activation was determined by the concentration of endotoxin present. The activated enzyme catalyzes the splitting of pNA from the colorless substrate Ac-lle-Glu-Ala-Arg-pNA. The pNA released was measured photometrically at 405-410 nm after the reaction was stopped with stop reagent. The correlation between the absorbance and the endotoxin concentration is linear in the 0.1-1.0 EU/ml range. The concentration of endotoxin in a sample was calculated from the absorbance values of solutions containing known amounts of endotoxin standard.

For assessment of endotoxin in the lab all areas were cleaned with an antibacterial reagent, microsol in conjunction with 70% ethanol. Additionally all reservoirs to contain the endotoxin assay reagents were bought sterilised and endotoxin free and all plastics utilised were suitable for endotoxin measurement. LAL water was used for preparation of all samples to be analysed and all solutions used in the experiments. A standard curve as was prepared each time using LAL water in sterile 15ml tubes-the standard curve in the range from 0-1 EU/ml or 0-10 EU/ml dependent on the dilution of the samples. Standards were 10, 5, 2.5, 1.0, 0.5, 0.25 and 0.1 EU/m. The plate was pre-warmed to 37ºC before the loading of the samples (app. 10 minutes). The endotoxin protocol as detailed below was followed:

2.6.5.2 Reagent preparation

Reagents were allowed to equilibrate to room temperature prior to use. Four standard endotoxin solutions were used as in the table below which shows the dilution scheme for the construction of these standards from the endotoxin supplied in the kit. The initial dilution from the endotoxin stock is 1/X, where X equals the concentration of the endotoxin vial. This yields an endotoxin solution containing 1.0 EU/ml. For example, if the potency was 23 EU/ml, the initial dilution was 1/23 or 0.1 ml of endotoxin stock into 2.2 ml of LAL Reagent Water (Table 2.6.5.2.1).

LAL Reagent Water	**Endotoxin Std. solution 1 EU/ml**	**Endotoxin stock solution**	**Endotoxin concentration EU/ml**
(X-1)/10 ml		0.1 ml	**1.0**
0.5 ml	0.5 ml		**0.5**
1.5 ml	0.5 ml		**0.25**
0.9 ml	0.1 ml		**0.1**

Table 2.6.5.2.1 Dilution scheme for the construction of standards from the endotoxin supplied in the kit (X = endotoxin concentration of the vial).

1. A solution containing 1.0 EU/ml endotoxin was prepared by diluting 0.1 ml of the endotoxin stock solution with (X-1)/10 ml of LAL reagent water in a suitable container, where X equals the endotoxin concentration of the vial. The solution was vigorously vortexed for at least 1 minute before proceeding.
2. 0.5 ml of this 1.0 EU/ml solution was transferred into 0.5 ml of LAL reagent water in a suitable container and labeled 0.5 EU/ml. This solution was vigorously vortexed for at least 1 minute before use.

3. 0.5 ml of the 1.0 EU/ml solution was transferred into 1.5 ml of LAL reagent water in a suitable container and labeled 0.25 EU/ml. This solution was vigorously vortexed for at least 1 minute before use.
4. 0.1 ml of the 1.0 EU/ml solution was transferred into 0.9 ml of LAL reagent water in a suitable container and label 0.1 EU/ml. This solution was vigorously vortexed for at least 1 minute prior to use.

2.6.5.3 Microplate endotoxin Method

1. To pre-equilibrate the microplate, the microplate was placed it the heating block adapter at 37℃ ± 1℃
2. While leaving the microplate at 37℃ ± 1℃, 50µl of sample or standard was carefully dispensed into the appropriate microplate well. Each series of determinations included a blank plus the four endotoxin standards ran in duplicate. The blank wells contained 50 µl of LAL reagent water instead of sample. All reagent additions and incubation times were identical.
3. At time T = 0 (zero), 50 µl of LAL was added to the first column of microplate wells as a multi-channel pipettor was used. Timing started as the LAL was added. Once the LAL has been dispensed into all microplate wells containing samples or standards, the microplate was removed from the heating block adapter and repeatedly tapped the side of the plate to facilitate mixing. Then, the plate was returned to the heating block adapter with the cover being replaced.
4. At T = 10 minutes, 100 µl of substrate solution was added (pre-warmed to 37℃ ± 1℃) by pipetting the substrate solution in the same manner as in Step 3, with a consistent pipetting rate. Once the substrate solution had been dispensed into all microplate wells, the microplate then removed from the heating block adapter

and repeatedly the side of the plate tapped to facilitate mixing. Then the plate was returned to the heating block adapter with the cover being replaced.

5. At T = 16 minutes, 100 µl of stop reagent was the added while maintaining the same pipetting order as in Steps 3 and 4. Once the stop reagent has been dispensed into all microplate wells, the plate was removed and repeatedly the side of the plate was tapped.
6. The absorbance of each microplate well was read at 405-410 nm (distilled water was used to adjust the photometer to zero absorbance).

2.6.5.4 Calculation of endotoxin concentration

The absorbance at 405-410 nm was linear in the concentration range of 0.1 to 1.0 EU/ml endotoxin. There are two methods to determine the endotoxin concentration of samples, graphic and calculator methods. For all samples it was important to subtract the mean absorbance of the blank from the mean absorbance value of the standards and samples then mean Δ absorbance could be calculated.

2.6.5.4.1 Graphic Method

The mean Δ absorbance for the four standards was plotted on the y-axis vs. the corresponding endotoxin concentration in EU/ml on the x-axis. A best fit straight line was drawn between these points to determine endotoxin concentrations of samples graphically (Figure 2.6.5.4.1).

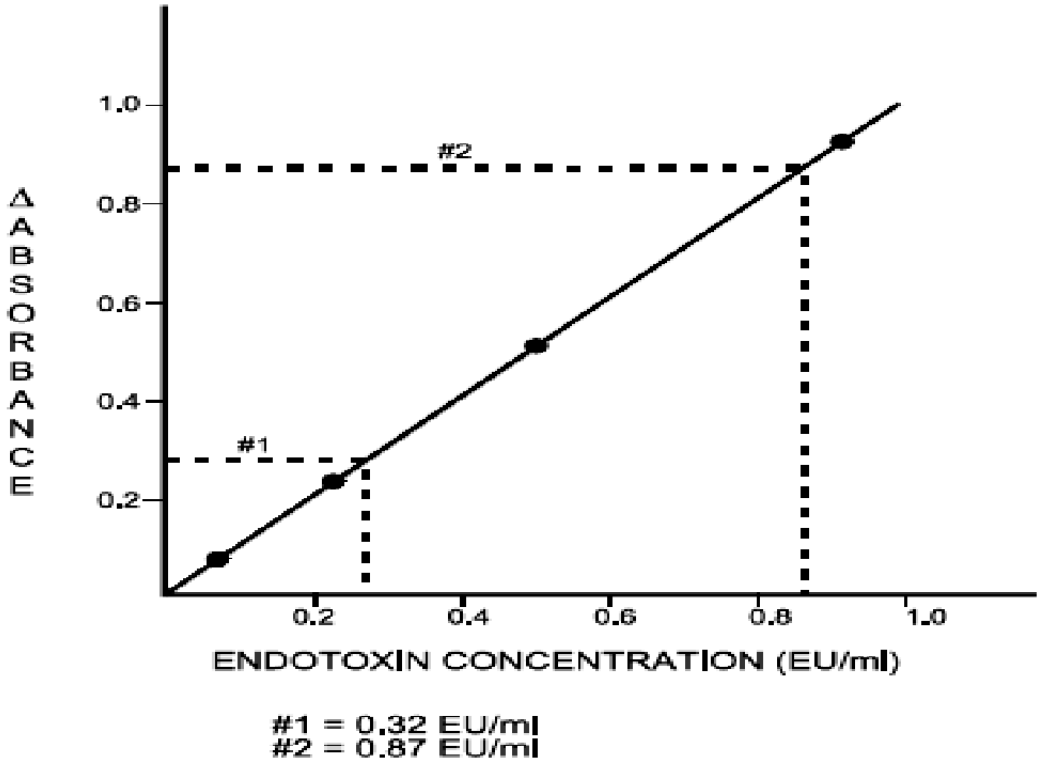


Figure 2.6.5.4.1 Graphic Method The mean Δ absorbance for the four standards was plotted on the y-axis vs. the corresponding endotoxin concentration in EU/ml on the x-axis. A best fit straight line was drawn between these points to determine endotoxin concentrations of samples graphically.

2.6.5.4.2 Calculator Method

A calculator equipped with linear regression capability can also be used. The mean Δ absorbance and the corresponding concentrations of the four standards were entered. The corresponding endotoxin concentration of the samples from their absorbance by linear regression was determined.

2.7 Data Analysis

Data was analyzed using SPSS version 16.5 (SPSS, Chicago, IL, USA). All continuous variables were presented as mean ± standard deviation. Frequencies were

expressed as percentages (%). With the exception of triglycerides, insulin and HOMA-IR, all other variables including the macronutrients were normally distributed. For comparison between groups (T2DM, overweight^{+} and control), Analysis of Variance (ANOVA) with post-hoc analysis and Kruskal-Wallis (for triglycerides, insulin and HOMA-IR) were used. Age and BMI were used as covariates. For comparison between pre- and post-intervention, paired T-test and Wilcoxon-Rank tests (for triglycerides, insulin and HOMA-IR) were used. For comparison within groups according to successive hours, repeated measures ANOVA (General Linear Model) with post-hoc analysis and Friedman's two-way analysis of variance (for triglycerides, insulin, HOMA-IR) were used. To determine associations between endotoxin and variables of interest post prandial, Spearman bivariate correlations and linear regressions were utilized. Significance was set at $p<0.05$.

G*Power was used for the calculation of sample size ascertaining difference between two dependent means (matched pairs). A sample size of N = 30 with α = 0.05 and effect size of 0.50 has a power of 0.75 to detect difference.

Chapter Three

Effects of high fat meal on metabolic endotoxemia amongst Saudi women with and without metabolic disease

3.1 Introduction

The nutritional transition and the rapid urbanization in the Middle East has introduced energy-dense, refined carbohydrates and increased saturated fat intake (Musaiger and Al-Hazzaa, 2012). This transition has paralleled the increased in life-style related chronic diseases such as type 2 diabetes mellitus (T2DM) (Al-Shoshan, 1992; Amuna and Zotor, 2008).

Whilst obesity represents the single most influential risk factor for T2DM, weight gain itself is a result of a complex interaction between genetic and environmental factors. Amongst the latter, a high fat diet appears involved in the increased occurrence of obesity and T2DM which are principal features of metabolic syndrome. The major metabolic consequence of high–fat diet is that effect on insulin action, where the regulatory mechanisms of body weight become impaired through lipotoxic effects (Magnan et al., 1999). In addition, obesity is also coupled with insulin resistance and associated with low grade chronic systemic inflammation (Wellen and Hotamisligil, 2005). Whilst trying to untangle the individual effects of obesity, insulin resistance and inflammation is difficult, previous models of diet-induced and genetic obesity has shown that adipose tissue presents an important source of pro-inflammatory cytokines such as tumor necrosis factor (TNF)- α (Hotamisligil et al., 1993; Weisberg et al., 2003), interleukin (IL)-1 (Hotamisligil et al., 1993; Weisberg et al., 2003), and IL-6 (Weisberg et al., 2003) during weight gain. These cytokines appear deleterious for muscle insulin action; for example, TNF-α has been shown to cause insulin resistance by increasing serine phosphorylation on insulin receptor substrate-1 (Hotamisligil et al., 1996), leading to

its inactivation. The consequent insulin resistant state will favour hyperinsulinaemia conditions and excessive hepatic and adipose tissue lipid storage.

However the triggering factors linking inflammation to high-fat diet–induced metabolic syndrome remains to be fully determined. However postprandial lipidaemia has emerged as a potential candidate following the discovery that ingestion of a high-fat meal causes systemic increases of a wide range of inflammatory mediators (Aljada et al., 2004; Blanco-Colio et al., 2000; van Oostrom et al., 2004; van Oostrom et al., 2003). Previous studies have shown that following a high-fat meal, there is an increased expression of nuclear transcription factor-κB (NF-κB) in leukocytes (Aljada et al., 2004; Blanco-Colio et al., 2000), a key transcription factor in the inflammatory cascade that regulates the transcription of numerous pro-inflammatory adipokines (Youssef-Elabd et al, 2012; Creely et al., 2007). To date, the cause of these postprandial inflammatory events remains poorly understood. One potential candidate factor that has arisen is bacterial endotoxin [lipopolysaccharide (LPS)], a potent inflammatory bacterial antigen that is present in large quantities in the human gut (Berg, 1996). Endotoxin circulates in the blood of healthy human subjects at low concentrations (between 1 and 200 pg/mL (Weidermann et al., 1999; Goto et al., 1994; Hasday et al., 1999; Boelke et al., 2001; Niebauer et al., 1999). However, Clinical studies have implicated gut-derived endotoxin as a "primary insult" to activate the inflammatory state, contributing to metabolic disease, with cross sectional data showing elevated systemic endotoxin levels in obesity, T2DM, coronary artery disease and fatty liver disease (Baker et al., 2009; Creely et al., 2007; Brun et al., 2007; Dixon et al., 2008; Al-Attas et al., 2009; Miller et al., 2009; Harte et al., 2010).

Endotoxin has an immediate impact on the innate immune pathway in human adipose tissue, acting via key receptors known as Toll-like receptors which leads to intracellular activation of NF-κB, leading to a rapid response within adipose tissue that may be exacerbated by increased adipose tissue mass (Lin et al., 2000; Kopp et al., 2009; Song et al., 2006; Shoelson and Goldfine, 2009; Wellen and Hotamisligil, 2005).

The importance of the dietary lipid intake and LPS in determining inflammatory risk may arise due to the strong affinity of endotoxin for chylomicrons (lipoproteins that transport dietary long-chain saturated fatty acids [SFAs]through the gut wall) as the endotoxin crosses the gastrointestinal mucosa (Ghoshal et al., 2009; Amar et al., 2008; Moreno-Navarrete et al., 2010). Recent human studies showed a substantial increase in circulating endotoxin among healthy individuals given a high-fat meal in conjunction with markers of inflammation (Cani et al., 2007a; Ghanim et al., 2009; Piya et al., 2013a; Piya et al., 2013b). Furthermore, Murine studies have identified an association between insulin resistance and endotoxin, with insulin resistance and weight gain both affecting gut permeability (Ghanim et al., 2009; Deopurkar et al., 2010).

However, it remains to be established whether the feeding pattern in Saudi subjects affects endotoxin absorption in different metabolic states, whether circulating levels of endotoxin and systemic lipid changes correlate together post-prandially, and whether this correlation is more apparent in insulin-resistant states.

Therefore, this chapter will establish whether a high-fat meal increased circulating endotoxin and whether this is altered in different metabolic disease states.

3.2 Research Design and Methods

The study comprised of 3 groups: healthy control subjects (n=18), overweight plus (overweight^{+}) subjects (n= 24) and patients with early onset of type 2 diabetes (n= 50).

All subjects were Saudi pre-menopausal females, randomly selected from different regions of Riyadh city, nonsmokers, with a normal resting ECG and blood pressure and with no history of vascular disease. In addition, subjects with known long standing diabetes and/or receiving anti-diabetic medication, those with fasting glucose levels >11 mmol/L, or with fasting triglycerides levels > 4 mmol/L were excluded from the study.

Screening fasted blood tests at baseline were performed both to qualify subjects for the study, and to assess glucose control; blood tests also examined lipid profiles. In addition, all subjects had their weight, height and waist and hip circumferences measured. Weight (in kilograms) was measured in light clothing to the nearest 0.1 kg. Height was measured using a digital stadiometer to the nearest centimeter. Waist circumference was measured at the level of the iliac crest at the end of normal respiration, and hip was measured at the widest circumference around the buttocks using measuring tape. Waist to hip ratio as well as BMI (were calculated. Blood samples were taken from the right or left antecubital vein in a sitting position. Blood pressure checked with a blood pressure monitor on the left arm. Ethical approval had been granted by the Ethics Committee of King Saud University, Riyadh, Kingdom of Saudi Arabia, prior to the commencement of the research and all patients gave written consent.

All research subjects (n = 92) with and without T2DM were given a high fat meal (standardized meal: 75 g fat, 5 g carbohydrate, 6 g protein) after an overnight fast of 12–14 h.

The cohort consisted of 92 Saudi women [18 non-diabetic (ND) control subjects (Age 24.4±7.9yr; BMI 22.2 ± 2.2kg/m^2), 24 overweight$^+$ subjects (Age 32.0±7.8yr; BMI 28.5±1.5Kg/m^2) and 50 overweight or obese T2DM patients (Age 41.5±6.2yr; BMI 35.2±7.7kg/m^2)]. Blood samples were drawn via cannula at baseline (0 h) and post-prandially (1, 2, 3, and 4 h), and endotoxin and lipid levels were measured.

3.2.1 *In vivo* assessment of the biochemical profile

On the assigned date, fasting blood samples were collected from participating subjects, and both plasma glucose and lipid profiles (triglycerides, total cholesterol, HDL and LDL) were determined using routine laboratory methods undertaken in the biochemistry laboratory. In brief, glucose was measured using a glucose oxidase method in an autoanalyzer (Konelab, Espoo, Finland). Serum free insulin concentrations were determined by electro-chemiluminescence method (COBAS-E-411; Roche Diagnostics, Mannheim, Germany). The instruments were calibrated prior to analysis using quality control samples provided with the kits. Homeostasis model assessment for insulin resistance (HOMA-IR) was then calculated for all patients using the HOMA formula: HOMA-IR = fasting insulin (mU/L) x plasma glucose (mmol/L)/22.5 (Bonora et al, 2002).

3.2.2 Analysis of circulating endotoxin

Serum endotoxin was analyzed using a commercially available QCL-1000 LAL End Point Assay (Lonza, Allendale, NJ). The assay, and the values given by the manufacturer for intra-assay coefficient of variation (CV) (3.9 ± 0.46%) and inter-assay CV (9.6 ± 0.75%), have been validated as detailed previously by the team (Creely et al., 2007).

3.3 Data Analysis

Data was analyzed using SPSS version 16.5 (SPSS, Chicago, IL, USA). All continuous variables were presented as mean ± standard deviation and were normalized prior to parametric analyses. For comparison between groups (T2DM, overweight and control), Analysis of Variance (ANOVA) with post-hoc analysis and Kruskal-Wallis (for triglycerides) were used. Age and BMI were used as covariates. For comparison between pre- and post-intervention, paired T-test was used. For comparison within groups according to successive hours, repeated measures ANOVA and Friedman's two-way analysis of variance (for triglycerides, insulin, HOMA-IR and endotoxin) were used. To determine associations between endotoxin and variables of interest post prandial, Spearman bivariate correlations was utilized. Significance was set at $p < 0.05$.

3.4 Results

3.4.1 General Characteristics of Subjects

A total of 92 subjects (N = 18 controls, N = 24 overweight^{+} group, and N = 50 T2DM group) were included in this cross-sectional study. Table 3.4.1.1 shows the general characteristics of subjects according to group, with the T2DM group having a mean duration of T2DM at 2.04 years. Furthermore, the T2DM subjects were significantly older as compared to both control ($p<0.001$) and overweight^{+} group ($p<0.001$), with the overweight^{+} group also being significantly older than the controls ($p=0.002$).

Table 3.4.1.1 Anthropometric and Metabolic Characteristics of Subjects According to Group

	Control	**Overweight^{+}**	**DMT2**	**P-Value**
N	18	24	50	
Age (years)	24.39 ± 7.92	32.04 ± 7.78*	41.50 ± 6.23*!	< 0.001
DM Duration (years)	--	--	2.04 (0-9)	
BMI (kg/m^2)	22.20 ± 2.21	28.54 ± 1.49*	35.24 ± 7.67*!	< 0.001
Waist (cm)	80.64 ± 7.23	95.75 ± 7.42*	112.3 ± 13. 43*!	< 0.001
Hip (cm)	98.69 ± 7.26	109.67 ± 5.01*	117.11 ± 11.59*!	< 0.001
WHR	0.82 ± 0.05	0.87 ± 0.05*	0.96 ± 0.07*!	< 0.001
Glucose (mmol/l)	4.81 ± 0.86	4.7 ± 0.41	7.9 ± 2.73*!	< 0.001
Triglycerides (mmol/l)#	1.03 ± 0.44	1.27 ± 0.8	1.9 ± 1.0*!	0.001
Total Cholesterol (mmol/l)	4.22 ± 0.71	4.5 ± 0.98	5.4 ± 1.07*!	0.003
HDL-Cholesterol (mmol/l)	1.29 ± 0.25	1.1 ± 0.4	0.96 ± 0.2*!	< 0.001
LDL-Cholesterol (mmol/)	2.76 ± 0.65	2.8 ± 0.67	3.66 ± 0.8*!	< 0.001

Note: Data presented as mean ± standard error; # denotes non-Gaussian distribution; P-values at extreme right denotes over-all significance according to group; "*" denotes significance as compared to controls; "!" denotes significance as compared to overweight^{+} group; p-value significant at < 0.05.

As expected, the T2DM group also had the highest anthropometric indices (BMI, waist and hip circumferences as well as WHR) than the overweight^{+} group and controls, with the overweight^{+} group being significantly higher than the controls in all indices as well. With regards to serum markers, the T2DM group as expected had the highest mean serum glucose levels (7.9±2.73mmol/l) than the overweight^{+} (4.7±0.41mmol/l, $p<0.01$) and controls (4.81±0.86mmol/l, $p<0.001$). The T2DM group also had the highest levels of serum triglycerides (1.9±1.0mmol/l), total cholesterol (5.4±1.07mmol/l) and LDL-cholesterol (3.66±0.8mmol/l), and the lowest in mean HDL-cholesterol (0.96±0.21mmol/l) than the overweight^{+} and controls (all $p<0.001$). Serum glucose and lipid levels of overweight^{+} and controls were not significantly different from one another (Table 3.4.1.1).

3.4.2 Effects of High Fat Meal in Different Groups

Table 3.4.2.1 highlights the glucose, insulin, HOMA-IR, lipids and endotoxin changes of subjects over time. In this section, the metabolic parameters are highlighted for each group as discussed below.

Table 3.4.2.1 Metabolic Changes pre and post a high fat-meal

	0 Hour	1 Hour	2 Hours	3 Hours	4 Hours
	GLUCOSE				
T2DM [N = 50]	7.9 ± 2.7	7.76 ± 2.49	7.52 ± 2.5*	7.29 ± 2.7 $^{*!§}$	7.0 ± 2.8 $^{*!§†}$
Overweight^{+}[N=24]	4.7 ± 0.4	4.6 ± 0.38	4.63 ± 0.40	4.6 ± 0.39	4.63 ± 0.7
Control [N = 18]	4.8 ± 0.86	5.1 ± 2.28	5.02 ± 1.68	4.76 ± 1.51	4.79 ± 1.6
	TRIGLYCERIDES#				
T2DM [N = 50]	1.9 ± 1.0	1.83 ± 0.7	2.4 ± 0.94$^{*!}$	2.7 ± 1.1$^{*!§}$	2.7 ± 1.3$^{*!§}$
Overweight^{+}[N=24]	1.3 ± 0.8	1.4 ± 0.8	1.7 ± 0.9$^{*!}$	2.0 ± 1.1$^{*!§}$	1.9 ± 1.3$^{*!§}$
Control [N = 18]	1.03 ± 0.43	1.17 ± 0.55	1.41 ± 0.91	1.44 ± 0.91	1.54 ± 1.0
	TOTAL CHOLESTEROL				
T2DM [N = 50]	5.4 ± 1.1	5.3 ± 1.0	5.4 ± 1.07	5.3 ± 1.08	5.4 ± 1.1
Overweight^{+}[N=24]	4.5 ± 1.0	4.5 ± 0.88	4.4 ± 0.8	4.4 ± 0.8	4.4 ± 1.0
Control [N = 18]	4.22 ± 0.71	4.06 ± 0.68	4.12 ± 0.65	4.1 ± 0.62	4.2 ± 0.72
	HDL-CHOLESTEROL				
T2DM [N = 50]	0.96 ± 0.21	0.96 ± 0.2	0.94 ± 0.19	0.91 ± 0.21$^{*!}$	0.89 ± 0.2$^{*!§†}$
Overweight^{+}[N=24]	1.20 ± 0.32	1.1 ± 0.32	1.1 ± 0.33	1.14 ± 0.4$^{*!}$	1.11 ± 0.4$^{*!§}$
Control [N = 18]	1.29 ± 0.25	1.25 ± 0.29	1.25 ± 0.28	1.21 ± 0.28	1.23 ± 0.28
	LDL-CHOLESTEROL				
T2DM [N = 50]	3.7 ± 0.8	3.6 ± 0.8	3.4 ± 0.9*	3.2 ± 0.9*	3.3 ± 0.9*
Overweight^{+}[N=24]	2.8 ± 0.7	2.7 ± 0.6	2.5 ± 0.6*	2.4 ± 0.6*	2.4 ± 0.6*
Control [N = 18]	2.72 ± 0.63	2.57 ± 0.58*	2.58 ± 0.55*	2.59 ± 0.55	2.66 ± 0.62
	Endotoxin				
T2DM [N = 50]	3.4 ± 0.8	3.0 ± 0.8	3.4 ± 0.9$^{!}$	3.5 ± 0.9$^{!}$	3.6 ± 0.9$^{!}$
Overweight^{+}[N=24]	3.0 ± 0.5	2.9 ± 1.4	3.5 ± 0.9	3.84 ± 1.6	3.5 ± 1.9
Control [N = 18]	1.54 ± 0.09	1.79 ± 0.10*	1.71 ± 0.8*	1.94 ± 0.17*	2.11 ± 0.2*
	Insulin#				
T2DM [N = 50]	11.7 ± 5.5	21.9 ± 17.7*	19.3 ± 12.6*	16.2 ± 12.3*	14.3 ± 8.2*
Overweight^{+}[N=24]	5.0 ± 3.4	15.6 ± 16.2*	16.9 ± 16.0*	13.1 ± 8.0*	10.3 ± 5.3*
Control [N = 18]	5.8 ± 0.64	10.3 ± 1.7	9.6 ± 3.4	8.2 ± 1.6	10.2 ± 2.6
	HOMA-IR#				
T2DM [N = 50]	3.7 ± 2.0	8.7 ± 12.0*‡	6.6 ± 6.0*‡	5.6 ± 5.4‡	3.9 ± 2.4
Overweight^{+}[N=24]	1.12 ± 0.7	3.5 ± 4.1*	3.9 ± 4.1*	2.8 ± 1.9*	2.1 ± 1.2
Control [N = 18]	1.3 ± 0.15	1.9 ± 0.4	2.1 ± 0.7*	1.9 ± 0.4	2.2 ± 0.6

Note: # denotes Non-Gaussian distribution; * denotes significance compared to 0 hour; ! denotes significance compared to 1; § denotes significance as compared to 2; † denotes significance as compared to 3; ‡ denotes significance as compared to 4; p significant at ≤ 0.05.

3.4.2.1 Post-Prandial Changes in Glucose Levels in Subjects with Different Metabolic States

In the T2DM group, mean glucose level was observed highest at 0 hour and subsequently decreased over time. Mean glucose level was lowest after 4 hours, and this was significantly lower than the rest of the individual hours ($p<0.01$). Consequently, the mean glucose level after 3 hours was also significantly lower than the previous 3 hours (hours 1, 2 and 3; $p< 0.05$). The mean glucose level at hour 2 was significantly lower than 0 hour ($p<0.05$). In both the control and overweight^{+} group, no significant differences were noted over time for glucose (Table 3.4.2.1.1). As expected, the mean glucose levels of the T2DM group was significantly higher than either the overweight^{+} or control group ($p< 0.01$) (Figure 3.4.2.1.1).

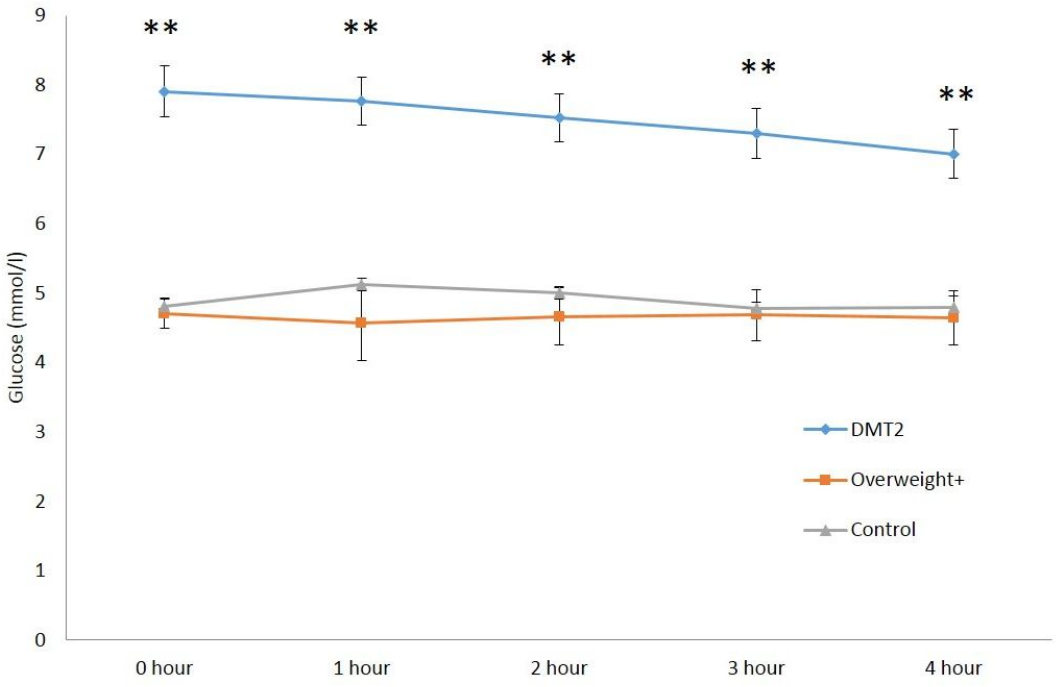


Figure 3.4.2.1.1 Baseline mean glucose levels adjusted for age and BMI according to group. ** denotes $p<0.01$.

3.4.2.2 Post-Prandial Changes in Triglyceride Levels in Subjects with Different Metabolic States

In contrast to glucose levels, the mean triglycerides for all groups followed an increasing trend over time. The mean triglyceride level in the T2DM group was highest after 3 and 4 hours, and this was significantly higher as compared to hours 0, 1 and 2 ($p<0.01$). In the overweight^{+} group, mean triglyceride level was highest at hour 3 followed by hour 4, and both were also significantly higher as compared to hours 0, 1 and 2 ($p<0.01$). In the control group, although similar increasing trend was noted over time, the mean triglyceride values were comparable to one another. Comparing all groups, the T2DM group had significantly higher mean triglyceride level as compared with either the overweight^{+} or control groups ($p<0.01$) (Figure 3.4.2.2.1).

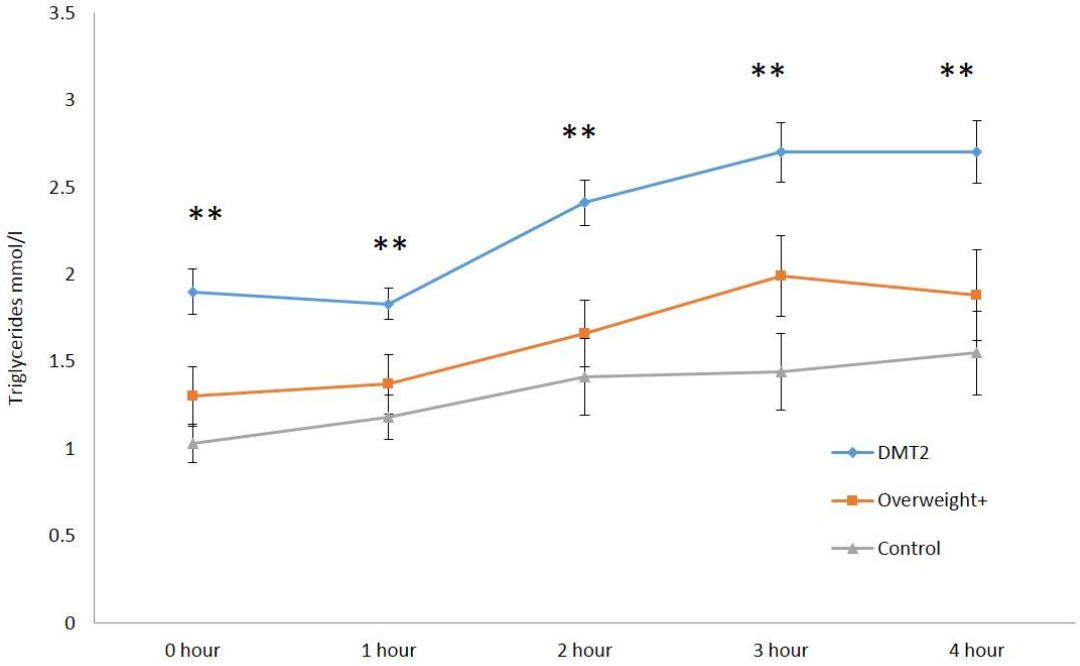


Figure 3.4.2.2.1 Baseline mean triglyceride levels adjusted for age and BMI according to group. ** denotes $p<0.01$.

3.4.2.3 Post-Prandial Changes in Total Cholesterol Levels in Subjects with Different Metabolic States

Across all groups, no significant trends were noted over time for cholesterol, and although the mean total cholesterol of the T2DM group was higher than overweight^{+} and control groups, the mean did not reach statistical significance (Figure 3.4.2.3.1).

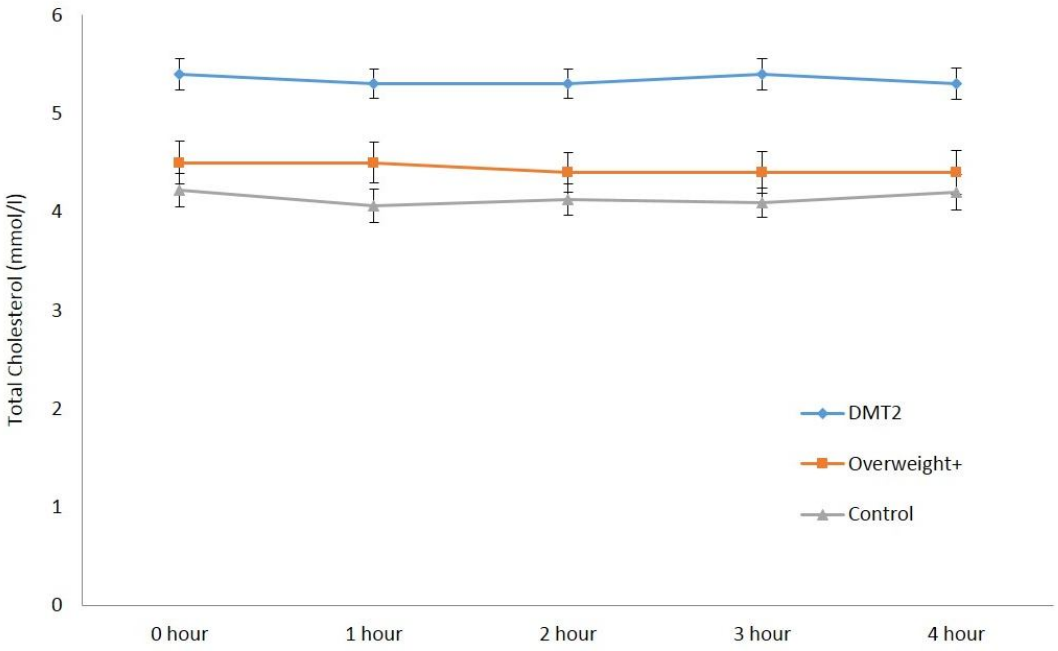


Figure 3.4.2.3.1 Baseline mean total cholesterol levels adjusted for age and BMI according to group.

3.4.2.4 Post-Prandial Changes in HDL-Cholesterol Levels in Subjects with Different Metabolic States

There was a decreasing trend in the mean HDL-cholesterol levels of both T2DM and overweight^{+} group over time post feeding, with no significant change in the control group. In the T2DM group, mean HDL-cholesterol was lowest after 4

hours, and this was significantly lower as compared to hours 0, 1, 2 and 3 ($p<0.01$). Mean HDL-cholesterol level at hour 3 was also significantly lower than hour 0 and 1 ($p<0.05$), and hour 2 significantly lower than hour 0 in the T2DM group ($p<0.05$). Similarly, the mean HDL-cholesterol in the overweight^{+} group was significantly lowest at hour 4 in comparison to hours 0, 1 and 2 ($p<0.01$). The next lowest was at hour 3, being significantly lower than hours 0 and 1 ($p<0.05$). While no observed changes were noted in the control group, their mean HDL-cholesterol levels were significantly higher than the T2DM and overweight^{+} groups ($p<0.01$) (Figure 3.4.2.4.1).

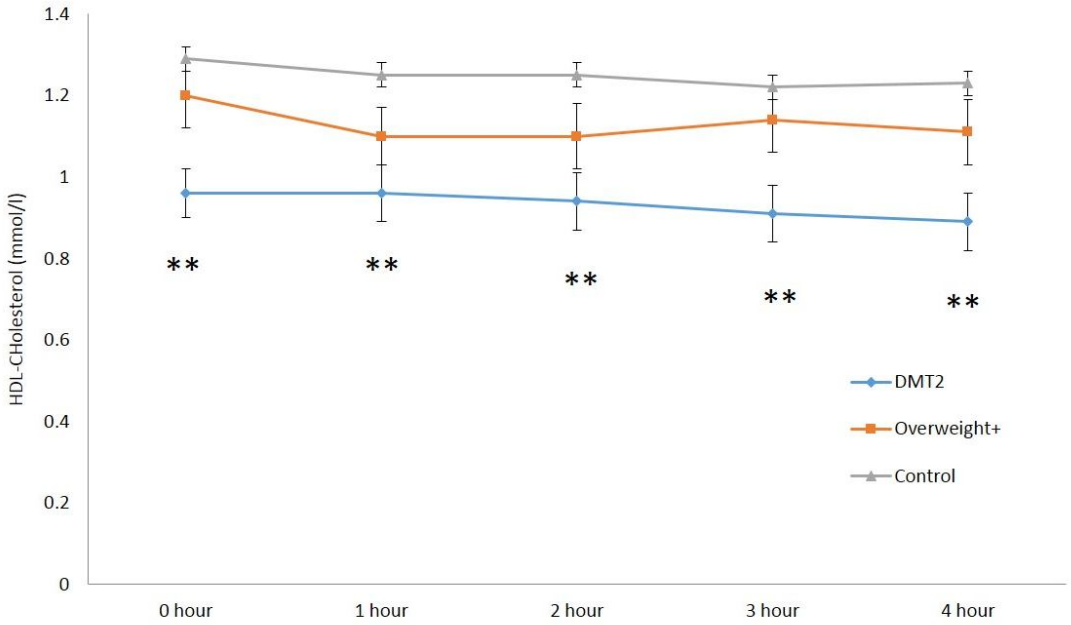


Figure 3.4.2.4.1 Baseline mean HDL-cholesterol levels adjusted for age and BMI according to group. ** denotes $p<0.01$.

3.4.2.5 Post-Prandial Changes in LDL-Cholesterol Levels in Subjects with Different Metabolic States

There was an apparent decreasing trend observed in the mean LDL-cholesterol levels in all groups post feeding, with mean LDL-cholesterol levels noted to be highest at 0 hour. In the T2DM group, mean LDL-cholesterol level at 0 hour was significantly higher as compared to succeeding hours 1, 2, 3 and 4 ($p<0.05$). In the overweight^{+} group, the 0 hour mean LDL-cholesterol was also significantly higher than hours 2, 3 and 4 ($p<0.05$). In the control group, the 0 hour mean LDL-cholesterol level was significantly lower than hours 1 and 2 only ($p<0.05$). T2DM had significantly higher mean LDL-cholesterol than both groups (Figure 3.4.2.5.1).

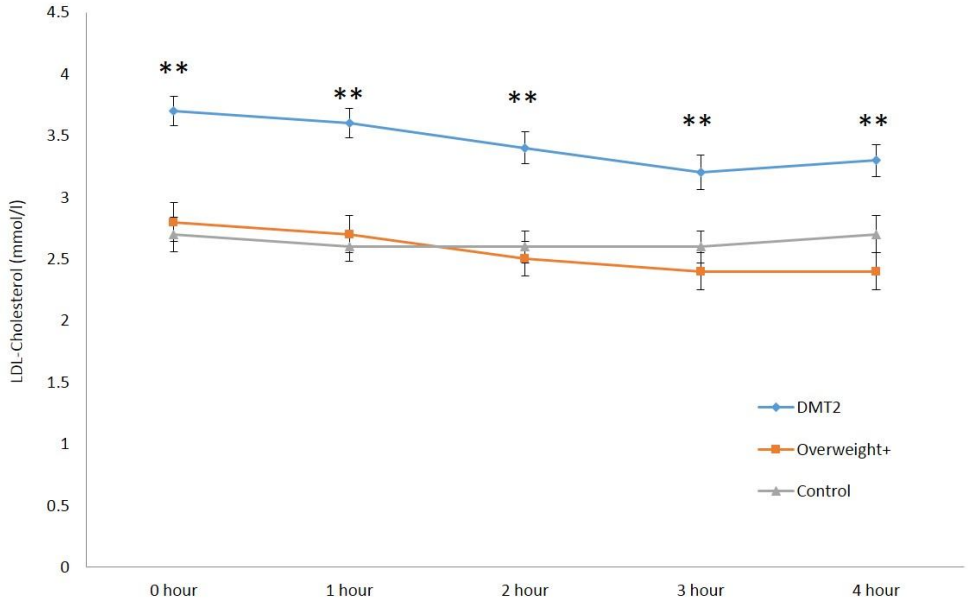


Figure 3.4.2.5.1 Baseline mean LDL-Cholesterol levels adjusted for age and BMI according to group.

3.4.2.6 Post-Prandial Changes in Endotoxin Levels in Subjects with Different Metabolic States

An increasing trend in mean endotoxin levels were observed in all groups and this trend is with respect to hour 0 compared to hour 4. The highest mean endotoxin levels were noted at hour 4 in all groups, which was statistically significant compared to hour 1 in the T2DM group and hour 0 in the control group ($p<0.01$). No significant changes were observed in the overweight^{+} group. T2DM and overweight^{+} groups had significantly higher endotoxin than controls (Figure 3.4.2.6.1).

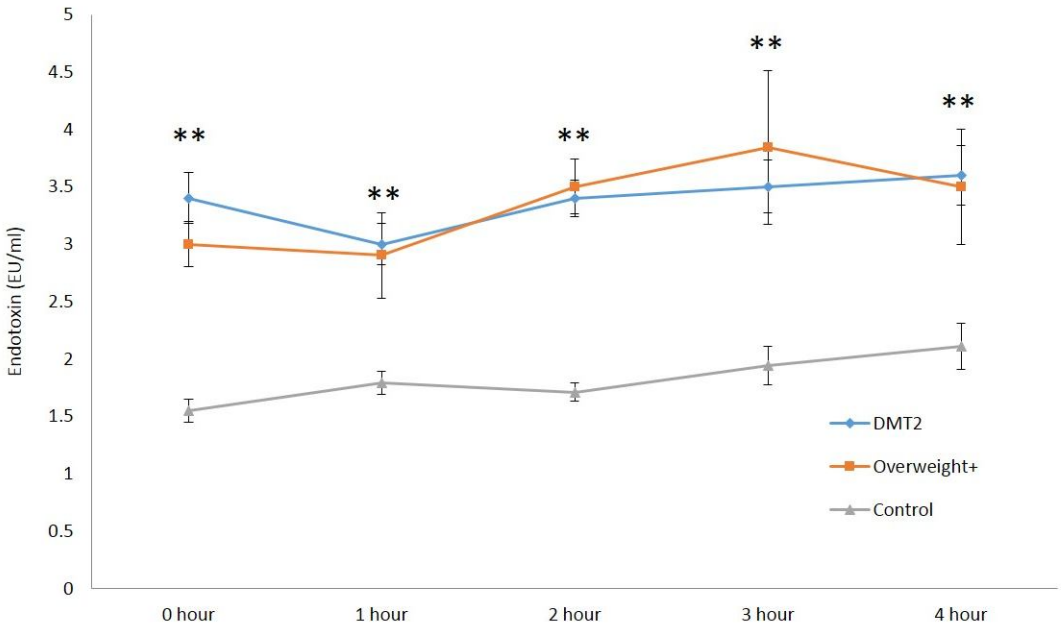


Figure 3.4.2.6.1 Baseline mean endotoxin levels adjusted for age and BMI according to group.

3.4.2.7 Post-Prandial Changes in Insulin levels in Subjects with Different Metabolic States

In the T2DM group, the highest mean insulin levels were noted at hours 1 and 2, decreasing at hours 3 and 4, and hour 0 being significantly lower as compared

to mean insulin levels across time ($p<0.01$). This pattern is similarly observed in the overweight^{+} and control group, with 0 hour having the lowest mean insulin level as compared to other hours. Across groups, the mean insulin level in the T2DM group is significantly higher in both the overweight^{+} and control groups ($p<0.01$) (Figure 3.4.2.7.1).

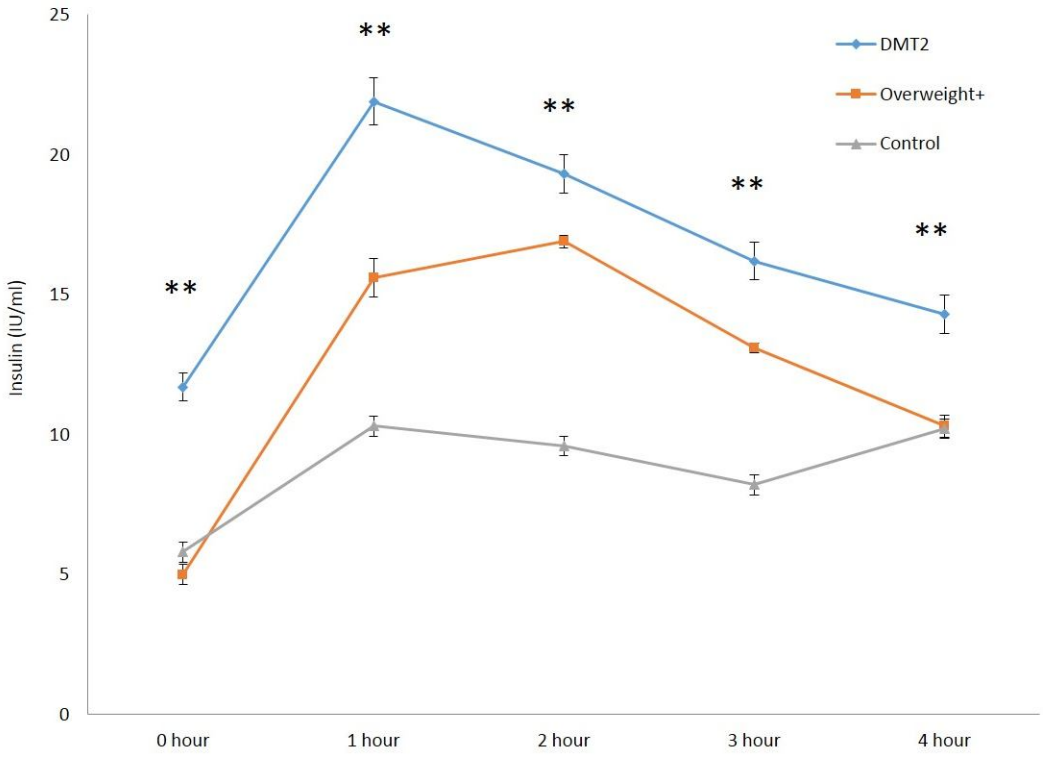


Figure 3.4.2.7.1 Baseline mean insulin levels adjusted for age and BMI according to group.

3.4.2.8 Post-Prandial Changes in HOMA-IR Levels in Subjects with Different Metabolic States

The mean HOMA-IR for all groups similarly followed the patterns observed in the mean insulin levels in all groups. In both the T2DM and overweight^{+} groups, HOMA-IR was lowest at hours 0 and 4, with the T2DM group showing the highest mean HOMA-IR at hour 1, and the overweight^{+} group at hour 2. There were no

significant changes observed in the control group. Across group, the mean HOMA-IR of the T2DM group was significantly higher than the overweight^{+} and control group ($p<0.01$) (Figure 3.4.2.8.1).

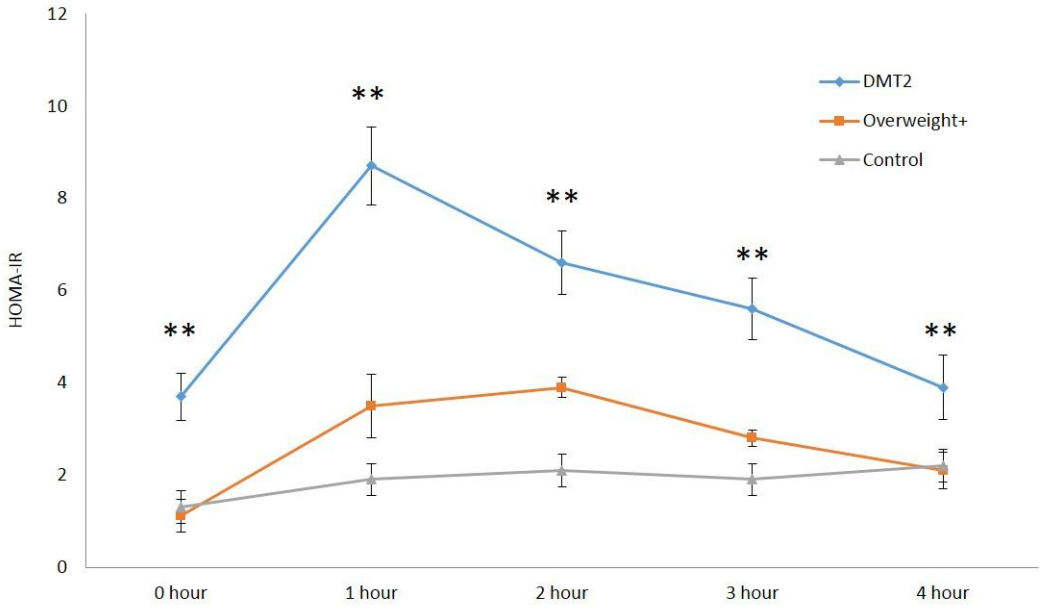


Figure 3.4.2.8.1 Baseline mean HOMA-IR levels adjusted for age and BMI according to group.

3.4.3 Associations of Metabolic Parameters to Endotoxin after High-Fat Meal

Table 3.4.3.1 shows how endotoxin is correlated to the cardiometabolic parameters in all groups across time. In all subjects, endotoxin is positively associated with LDL cholesterol ($R=0.38$; $p<0.05$) and this was observed 3 hours after high fat meal. In the T2DM group, endotoxin was significantly associated with triglycerides in 3 and 4 hours post-prandial high fat meal (R values 0.52 and 0.50; $p<0.05$, respectively). In the overweight^{+} subjects, endotoxin was highly associated with triglycerides ($R=0.63$; $p<0.05$) and total cholesterol ($R=0.71$; $p<0.05$) at zero hour. No endotoxin associations were observed in the control group.

Table 3.4.3.1 Bivariate Associations between Lipids, Glucose and Endotoxin

ALL SUBJECTS																									
Glucose					**Triglycerides**					**Total Cholesterol**					**HDL-Cholesterol**					**LDL-Cholesterol**				
0	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
-0.17	0.04	0.13	0.08	0.00	-0.13	0.10	0.14	0.22	0.20	0.16	-0.08	0.00	-0.07	-0.21	-0.16	0.15	-0.01	-0.08	-0.06	0.22	-0.06	0.05	**0.38**	-0.12
DMT2 SUBJECTS (N = 50)																								
-0.20	0.23	0.29	0.23	0.08	-0.12	0.32	0.26	**0.52**	**0.50**	0.18	0.04	0.02	0.0	-0.08	-0.28	-0.15	-0.14	-0.30	-0.05	0.30	0.02	0.005	-0.06	-0.19
OVERWEIGHT^{+} SUBJECTS (N = 24)																								
0.23	0.14	0.08	0.0	-0.18	**0.63**	0.04	0.08	0.26	0.25	**0.71**	-0.08	0.10	-0.13	-0.33	-0.13	0.41	0.13	-0.07	-0.18	0.36	0.14	0.43	**0.64**	0.28
CONTROL SUBJECTS (N = 18)																								
-0.36	0.15	0.22	0.24	0.14	-0.20	0.16	0.30	-0.14	-0.17	--	0.01	0.14	-0.05	-0.26	-0.28	-0.20	-0.39	-0.16	-0.31	-0.02	0.08	0.24	0.07	-0.09

Note: Data presented as coefficient (R); bold and red denotes significance at $p < 0.05$.

3.5 Discussion

In this chapter, the effects of a saturated fat meal on glucose, lipid, insulin and HOMA-IR and endotoxin levels of patients with T2DM, overweight^{+} and control were examined. This study highlighted even in the absence of high fat meal the presence of sub-chronic inflammation was apparent with noted difference in circulating endotoxin which was highest in the T2DM group, affirming prior studies that suggests that components of the gut microbiota, in particular endotoxin, are altered in the presence of insulin-resistant diseases such as T2DM (Shen et al, 2013). These studies also highlighted the impact of a high fat meal in Saudi women suggesting that endotoxin appears raised in the obese and T2DM states post feeding compared with the non-diabetic control subjects. Furthermore that at least in these cohorts the resulting increase in endotoxin appeared similar in both the overweight and T2DM states unlike previous studies in white Caucasians (Harte et al, 2012).

In addition to endotoxin these studies also considered the impact of elevated glucose and cholesterol levels as they represent other factors associated with sub-chronic inflammation, considered important in the development of coronary artery disease, T2DM and obesity. It has also been suggested that high fat meals regardless of the individual's metabolic status, also induce inflammatory changes (Herieka and Erridge, 2014; Harte et al, 2012). The changes observed in the lipid profile and insulin resistance after a high fat meal in this present study, resulted in lipidaemia, furthermore circulating triglyceride levels increased in all groups regardless of their metabolic status which appears consistent with previous studies (Wojczynski et al, 2011; Harte et al, 2012; Camargo et al, 2014; Meher et al, 2014). Analysis of the effect of a high saturated fat meal has been observed to cause deleterious changes in

the proteome level, in terms of DNA damage and pro-coagulant state (Camargo et al, 2013). The dramatic changes in triglycerides after a high fat meal has been studied in-depth using lipidomic methodologies and revealed that triglycerides response showed the most dynamic changes during post-prandial phase as compared to other lipids regardless of the individual's metabolic status and are dependent on the type of fat diet used (Bonham et al, 2013). Clinically this is relevant. As observed in the present study and other studies, higher post-prandial hypertriglyceridemia and hyperlipidemia are observed among patients with T2DM as compared to their healthier counterparts (Pirillo et al, 2014). Therefore dietary strategies defining the type of dietary fat within the diet appears important to lead to better management of the insulin response and increased fat oxidation both important features to reduce metabolic risk in overweight and T2DM patients (Munsters and Saris, 2014).

The current studies identifying that endotoxin is raised in patients with weight gain and T2DM following a high fat meal confirms and extends previous data on this area (Miller et al, 2009; Creely et al, 2007). Particularly elevated endotoxin levels amongst Middle-Eastern patients with T2DM are also consistent across ethnic groups such as African women (Hawkesworth et al, 2013), Chinese (Liu et al, 2013) and Caucasians (Monte et al, 2012). Whilst endotoxin is seen as important mediator of sub-clinical inflammation Piya and colleagues suggest that the gut flora appears to act as an essential determinant in the development of sub-chronic inflammation induced by obesity and T2DM, and that endotoxin may act as the systemic insult that triggers the inflammatory cycle (Piya et al, 2013). These current studies highlight that in Saudi women given a high fat meal both those with significant weight gain and T2DM appears to raise their endotoxin levels without a clear difference between the two groups in contrast to previous studies in white Caucasians (Harte et al 2012).

Whilst is it not clear why there should be a difference between the two groups in endotoxin levels post high fat feeding several explanation can be offered. Animal studies have shown that continuous infusion of endotoxin increases gut permeability, as does high-fat dietary feeding therefore one possible explanation for the difference between the two studies could be due to the dietary difference in fat consumption that the obese subjects eat in Saudi compared with the UK (Cani et al, 2007; Brun et al, 2007). Secondly from mouse model data it has been shown that in the *ob/ob* mice and *db/db* mice have an increased leaky gut which is thought to relate to the effect of insulin resistance of gut endothelium, as such whilst the Saudi women do not appear overly insulin resistant at baseline feeding increases this insulin resistant phenotype much more than observed with the control non-diabetic subjects (Cani et al, 2007; Brun et al, 2007). As such, Saudi women may have a more continual snack habit which may consequentially effect how they handle a high fat feed which could be different to the white Caucasians in the other study given a high fat feed. Furthermore ethnicity may clearly impact on individual sub-clinical inflammatory risk. Previous studies have shown that endotoxin can be stratified by gender and ethnicity therefore these data could help to explain why ethnicities may have a resulting high risk due to their post-feeding increase in endotoxin (Miller et al, 2009).

The different cardiometabolic changes across the group pre- and post-feeding showed that the T2DM group had significantly higher glucose, triglycerides, insulin and HOMA-IR as well as significantly lower HDL-cholesterol throughout the high fat challenge as compared to overweight^{+} and control subjects. Whilst these significant differences were expected since the baseline levels of T2DM subjects were already significantly different from other groups, the significant persistence of

dysmetabolism in the T2DM group highlights that baseline glycemic and lipid parameters tend to be exacerbated following fat load. Such an increased response may heighten the increased production of reactive oxygen species (ROS) which would further exacerbate existing oxidative stress and endothelial dysfunction secondary to impaired vasoreactivity, increased coagulation and vascular permeability (Tushuizen et al, 2005; Ceriello 2005). The postprandial increments in glucose and lipids observed in the present study confirms previous findings utilising women and even adolescents with T2DM as subjects in other ethnicities (Alssema et al, 2008; Schindhelm et al, 2007; Umpaichitra et al, 2004) and also reaffirms the requirement to effectively manage the postprandial glucose and lipid response in T2DM subjects through diet intervention to lower the increased CVD risk (Ceriello 2005; Charpentier et al, 2006; Leiter et al, 2005).

In conclusion, the present findings highlight the difference in glucose, lipids and endotoxin levels after exposure to a high-fat meal regardless of metabolic status. Although lipidaemia is more evident than endotoxemia, the present findings support previous observations that metabolic endotoxemia is exacerbated after a high fat intake. As such dietary interventions that can reduce the glycaemic and lipidomic response as well as provide a healthy gut microbiota, which may improve gut barrier function, appear important in Saudi patients with weight gain and/or T2DM.

Chapter Four

Effect of high-fat meal (pre-post) dietary intervention in subjects (T2DM, Overweight^{+} and Healthy subjects)

4.1 Introduction

Lipopolysaccharide (LPS, also known as endotoxin) has been identified as a triggering factor for the onset of insulin resistance, obesity and type 2 diabetes mellitus (T2DM), where normal circulating endotoxin levels were observed to be increased during the feeding state and decreased during fasting. In addition, studies in 4-week high-fat fed mice have shown an increase in metabolic endotoxemia (raised endotoxin levels associated in conditions of obesity, T2DM and CVD) coupled with intestinal microbiota changes, where the gram negative–to–gram positive ratio increased during high-fat feeding (Cani et al., 2007a), which collectively appeared to be normalised by dietary fibers (Cani et al, 2007b). Furthermore, in a rodent study where continuous subcutaneous infusion of endotoxin occurred in mice for 4 weeks, fasting glycaemia and insulinaemia and whole body, liver and adipose tissue weight gain were increased to a similar extent as mice on a high-fat fed diet. Furthermore markers of inflammation and liver triglyceride content were observed to increase with endotoxin infusion increased (Cani et al 2007a).

Several studies have shown that postprandial lipidaemia that emerges following ingestion of high-fat meal causes an increase in a wide range of inflammatory mediators (Aljada et al, 2004; Blanco-Colio et al, 2000; Van Oostrom et al, 2004; Van Oostrom et al, 2003; Piya et al., 2013a; Piya et al., 2013b). For instance, circulating leukocytes increase their expression of the activated form of nuclear transcription factor-κB (NF-κB) (Aljada et al, 2004; Blanco-Colio et al, 2000), and up-regulate several markers of leukocyte activation, such as CD11A,CD11B, and CD62L (Van Oostrom et al, 2004). Circulating neutrophil counts and plasma level of interleukin-8 also increase after high-fat meal but not

after water ingestion (Van Oostrom et al, 2003; Van Wijk et al, 2006). Low grade endotoxemia was suggested to contribute to such a post prandial inflammatory state (Erridge et al, 2007).

Evidence linking chronic endotoxemia to insulin resistance and T2DM has arisen from research studying the metabolic consequences of bariatric surgery. In a clinical study, where roux-en-Y gastric bypass (RYGB) surgery resulted in profound weight loss, that was accompanied resolution of T2DM in a majority of patients. Moreover, endotoxin, NF-κB DNA binding, TLR-4, TLR-2, CD14 expression, CRP, MMP-9, and MCP-1 were all observed to be significantly decrease after RYGB surgery. The mechanism underlying the resolution of insulin resistance and T2DM after RYGB was in part believed to be attributable to the reduction of endotoxemia and associated pro-inflammatory mediators (Monte et al, 2012).

Amar and colleagues conducted a dietary survey in 1015 subjects in France to evaluate the relation between plasma endotoxin and food intake. The participants were given a 3-day food record. Plasma endotoxin was measured in a subsample of 201 men. Moreover, to assess, under controlled conditions, the differential impact of various high-energy diets, plasma endotoxin concentrations were measured in mice fed a high fat or a high-carbohydrate diet over a 4-wk period. In humans, Plasma endotoxin concentration was positively correlated with fat and energy intake. Furthermore experimental rodent data showed that compared with the control mice, mice fed a high-energy diet also increased their plasma endotoxin. However, the increase in plasma endotoxin was blunted in mice fed high-carbohydrate diet as compared with mice fed a high-fat diet suggesting that fat is more efficient in

transporting bacterial endotoxin from the gut lumen into the bloodstream (Amar et al, 2008).

Lowering plasma endotoxin levels could be a potent strategy for the control of metabolic disease. The above described findings support the concept that the digestion of dispersed dietary lipids can enhance absorption of endogenous endotoxins. As such the long-term consequences of such postprandial endotoxemia in the context of high fat diets in humans, and the underlying mechanisms, could be important be remain to be further explored. Therefore, optimizing the quantity, composition, physicochemical properties and emulsification state of dietary fats can support possible strategies to limit postprandial endotoxemia, with the aim to lower the systemic inflammatory response.

To date few studies have addressed the effects on dietary intervention such as changing dietary fat intake, or life style changes and its impact on circulating endotoxin levels and their correlation with inflammation and insulin resistance. Therefore this chapter will examine the effectiveness of dietary intervention in decreasing inflammatory markers related to obesity among metabolically dysfunction states.

4.2 Research Design and Methods

This was a multi-center, randomized interventional study conducted at the primary care centers of the Riyadh city, involved 92 Saudi women, consisted of 18 healthy non-diabetic (ND) control subjects (n=18), overweight plus (overweight^{+}) subjects (n= 24) and patients with T2DM (n= 50).

As detailed earlier in chapter 2, only Saudi women who met the previously described inclusion criteria were recruited for this prospective diet intervention study. Anthropometric data and fasting blood samples were taken at pre- and 3 months post-intervention with glucose, lipid profile, insulin, HOMA-IR and endotoxin measured. Ethical approval had been granted by the Ethics Committee of King Saud University, Riyadh, Kingdom of Saudi Arabia, prior to the commencement of the research **(Appendix I)** and all patients gave written consent (**Appendix II**).

4.2.1 Clinical intervention (dietary regimen)

Patients were brought to the clinic two times over a three month-period. For each visit the subjects had fasted overnight (12–14 hours) and blood samples were collected pre and post meal at 4 time points. A standardized high-fat meal consists of whipping cream and contains 75g of fat, 5 g of carbohydrates, and 6 g of protein (Ceriello et al., 2002) was allocated to the subjects to drink it within 10 minutes. Baseline blood samples and post fat meal blood samples were taken through a cannula over a 4 hour period (0, 1, 2, 3, 4 hours), with blood pressure noted. The same process was repeated after a 3 month period to repeat blood collection. Food

frequency questionnaire was obtained from the subjects in each group at baseline and after 3 months of follow up visit.

Overweight^{+} and subjects with T2DM were instructed to follow a 500 kcal deficit diet to enhance weight loss of 5% or more. Energy intake was set at the levels recommended by the dietary reference intake for subjects with low levels of physical activity at the same gender and age (DRI, 2008/2011). Targeted macronutrient composition was 20%-30% fat, <10% of saturated fatty acids, 50%-60% carbohydrates, 15%-20% protein and at least 15g per 1000kcal fibres (**Appendix V**).

As described earlier in chapter 2, a 3 month dietary intervention was provided to overweight^{+} and subjects with T2DM, involving group and individual meetings to achieve and maintain weight loss through decreased caloric intake. Printed dietary materials and handouts were designed by the researcher and provided to all participants to go home according to their assigned dietary regimen to facilitate implementation of the dietary interventional program (**Appendix V**). To support their compliance health advice was followed by weekly phone calls, messages and visits during the study if needed. In addition, the food frequency questionnaire (FFQ) which has been used previously (Al Disi et al., 2010) was the dietary tool that has to assess the food intake of the subjects prior to and post the dietary intervention (**Appendix IV**). The researcher interviewed the participants individually and the information was collected using a pre-designed questionnaire to assess the qualitative and the quantitative aspects of the food consumed by the participants over a period of 7 days (**Appendix IV**).

Nutrient intake was calculated using USDA database (18^{th} - 21^{st} Ed, 2009, 2010) Program, as for the Saudi Arabic traditional dishes were analyzed using the

Arabic food analysis program (version 1, 2006). The evaluation of the daily food intake was made by the means of the total energy and the total nutrient intake, the percentage of the total calories derived from fat, protein and carbohydrates.

Dietary nutrients values were compared with dietary reference intake (DRI) for specified age and gender for macronutrients (carbohydrates and protein) as well as for micronutrients (DRI, 2008/2011).

As for daily energy requirement (Kcal/day) was estimated using WHO equation (Sylvia Escott-Stump, 2011) according to age group and metabolic status. Dietary fat percentage has been estimated as 25% from daily energy requirement (Hooper et al., 2011) (**Appendix VI**).

4.2.2 Biochemical assessment

All subjects were requested to submit an over-night fasting blood samples (12-14 hours fasting) from which the different metabolic parameters were assessed which included: Fasting blood glucose, complete lipid profile, insulin, HOMA-IR and endotoxin. These parameters were measured at baseline for all the groups and repeated after 3 months period for overweight^{+} and T2DM subjects.

4.2.2.1 Blood Sample Collection

These were collected with the patients in the fasting state using sterile vacutainer blood collection apparatus (BD). Whole blood, serum, EDTA plasma and fluoride tubes were collected from subjects. All samples were aliquoted and stored in –20^{o}C freezer facilities in preparation for subsequent analysis.

4.2.2.2 In *vivo* assessment of the biochemical profile

On the assigned date, fasting blood samples were collected from participating subjects, and both plasma glucose and lipid profiles (Triglycerides, total cholesterol, HDL and LDL) were determined using routine laboratory methods undertaken in the biochemistry laboratory. In brief, glucose was measured using a glucose oxidase method in an auto-analyzer (Konelab, Espoo, Finland). Serum free insulin concentrations were determined by electro-chemiluminescence method (COBAS-E-411; Roche Diagnostics, Mannheim, Germany). The instruments were calibrated prior to analysis using quality control samples provided with the kits. Homeostasis model assessment for insulin resistance (HOMA-IR) was then calculated for all patients using the HOMA formula: HOMA-IR = fasting insulin (mU/L) x plasma glucose (mmol/L)/22.5 (Bonora et al, 2002).

4.2.2.3 Analysis of circulating endotoxin

Serum endotoxin was analyzed using a commercially available QCL-1000 LAL End Point Assay (Lonza, Allendale, NJ). The assay, and the values given by the manufacturer for intra-assay coefficient of variation (CV) (3.9 ± 0.46%) and interassay CV (9.6 ± 0.75%), have been validated in the biochemistry laboratory at university hospital Coventry and Warwickshire, as detailed previously. (Creely et al, 2007).

Endotoxin concentration was measured using a chromogenic kinetic Limulus amebocyte assay (LAL assay, BioWhitaker, Walkersville MD) which had been

validated previously (Creely et al., 2007), which is a quantitative test for Gram-negative bacterial endotoxin. Gram-negative bacterial endotoxin catalyzes the activation of a proenzyme in the LAL, and the initial rate of activation is directly determined by the concentration of endotoxin. The activated enzyme catalyzes the splitting of p-nitroaniline (pNA) from the colorless substrate Ac-lle-Glu-Ala-Arg-pNA, and the released pNA was measured photometrically at 405–410 nm following termination of the reaction. The correlation between the absorbance and the endotoxin concentration was linear in the range of 0.1–1.0 EU/ml. For the purposes of this study, all samples were run in duplicate within the same plate; therefore, no interassay variability was observed in this study.

4.3 Data Analysis

Data was analyzed using SPSS version 16.5 (SPSS, Chicago, IL, USA). All continuous variables were presented as mean ± standard deviation and were normalized prior to parametric analyses. For comparison between groups, univariate general linear model (GLM) was used with Bonferroni post-hoc comparisons. Age and BMI were used as covariates. For comparison between pre- and post-intervention, paired T-test was used for normally distributed variables and Wilcoxon Signed Ranks test for insulin and HOMA-IR. Bivariate linear regression analysis was used to determine associations between endotoxin variables of interest. Significance was set at $p<0.05$.

4.4 Results

4.4.1 Effects of 500kcal deficit/day in the anthropometric variables of Overweight^{+} and T2DM Subjects

After 3 months of dietary intervention for both the overweight^{+} and T2DM groups, anthropometric variables were reassessed and significant improvements were observed in both groups as noted in terms of significantly lower mean follow-up BMI as compared to baseline (Overweight^{+} Group baseline: 28.54±1.49kg/m^2 versus follow-up: 27.95±2.25 kg/m^2, $p<0.05$; T2DM group baseline: 35.24±7.67kg/m^2 versus follow-up: 35.04±8.07kg/m^2, $p<0.05$) and significantly lower mean hip circumference as well (Overweight^{+} group baseline: 109.67±5.01cm versus follow-up: 108.07±4.07cm, $p<0.05$; T2DM group baseline: 112.3±13.43cm versus follow-up: 109.21±12.71cm, $p<0.01$). Moreover, no significant changes were observed in the mean waist circumference group over time in the overweight^{+} group, yet a significantly higher WHR was noted after follow-up (baseline: 0.87±0.05 versus follow-up: 0.88±0.06, $p<0.05$). Contrary to the overweight^{+} group, the T2DM group showed a more favourable response to the dietary intervention as noted by the significantly lower mean waist circumference (baseline: 112.3±13.43cm versus follow-up: 109.21±12.71cm, $p<0.01$) and WHR (baseline: 0.96±0.07cm versus follow-up: 0.93±0.06cm, $p<0.01$) after 3 months follow-up (Table 4.4.1.1).

Table 4.4.1.1 Anthropometric Changes over Time According to Group

	Overweight^{+}		T2DM	
N	24		50	
Age (years)	32.04 ± 7.78		41.50 ± 6.23	
DM Duration (years)	--		2.04 (0-9)	
	Baseline	**Post-Intervention**	**Baseline**	**Post-Intervention**
BMI (Kg/m^2)	28.54 ± 1.49	27.95 ± 2.25 *	35.24 ± 7.67	35.04 ± 8.07*
Waist (cm)	95.75 ± 7.42	95.87 ± 7.78	112.3 ± 13. 43	109.21 ± 12.71 **
Hip (cm)	109.67 ± 5.01	108.07 ± 4.07 *	117.11 ± 11.59	116.7 ± 13.18 **
WHR	0.87 ± 0.05	0.88 ± 0.06 *	0.96 ± 0.07	0.93 ± 0.06 **

Note: P-values at extreme right denote significance for baseline comparisons according to group; * denotes significance at $p < 0.05$ as compared to visit 1; ** denotes significance at $p < 0.01$ as compared to visit 1.

4.4.2 Effects of 500kcal deficit/day in the cardiometabolic variables and endotoxin levels in Overweight^{+} and T2DM Subjects

Fasting serum glucose, insulin, lipids and endotoxin were also quantified in all subjects after the 3-month dietary intervention. In the T2DM group, there was a significant decrease in fasting glucose (baseline 7.9±2.7mmol/l versus follow-up 7.0±1.8mmol/l; $p<0.05$), triglycerides (baseline 1.9±1.0mmol/l versus follow-up 1.6±0.8mmol/l; $p<0.05$), total- (baseline 5.4±1.1mmol/l versus follow-up 4.6±1.0mmol/l; $p<0.01$) and LDL-cholesterol (baseline 3.7±0.8mmol/l versus follow-up 2.9± 0.8mmol/l; $p<0.01$) in follow-up as opposed to baseline. A significant increase was also found in HDL-cholesterol (baseline 0.96±0.2mmol/l versus follow-up 1.01±0.2mmol/l; $p<0.01$) in the T2DM group. No significant changes were noted in fasting insulin, HOMA-IR and endotoxin levels in the T2DM group over time.

In the overweight^{+} group, a significant decrease was observed in total-[baseline 4.5±1.0mmol/l versus follow-up 3.7±0.8mmol/l; p<0.01] and LDL-cholesterol [baseline 2.8±0.7mmol/l versus follow-up 2.2±0.6mmol/l; p<0.01] overtime. However, a significant increase was observed in fasting insulin [baseline 5.0±3.4IU/ml versus follow-up 12.0±6.6IU/ml; p<0.05] in follow-up as compared to baseline. No changes were observed in the fasting glucose, HOMA-IR and endotoxin levels overtime (Table 4.4.2.1).

Table 4.4.2.1 Metabolic Changes over Time According to Group

	Overweight^{+}		**T2DM**	
N	24		50	
Age (years)	32.04 ± 7.78		41.50 ± 6.23	
DM Duration (years)	--		2.04 (0-9)	
	Baseline	**Post-Intervention**	**Baseline**	**Post-Intervention**
Glucose (mmol/l)	5.15 ± 3.5	4.65 ± 2.85	7.9 ± 2.73	7.0 ± 1.79*
Insulin (IU/ml)#	5.0 ± 3.4	12.7 ± 6.6*	11.7 ± 5.5	10.8 ± 6.0
HOMA-IR#	1.1 ± 0.7	0.93 ± 1.9	3.7 ± 2.0	3.1 ± 2.2
Triglycerides (mmol/l)	1.27 ± 0.8	1.4 ± 0.5**	1.9 ± 1.0	1.6 ± 0.8*
Total Cholesterol (mmol/l)	4.5 ± 0.98	3.7 ± 0.8**	5.4 ± 1.07	4.6 ± 0.96**
HDL-Cholesterol (mmol/l)	1.1 ± 0.4	0.91 ± 0.15*	0.96 ± 0.2	1.01 ± 0.2**
LDL-Cholesterol (mmol/)	2.8 ± 0.67	2.2 ± 0.6**	3.66 ± 0.8	2.9 ± 0.8**
Endotoxin	3.0 ± 0.5	3.27 ± 1.1	3.4 ± 0.9	3.2 ± 1.1

Note: P-values at extreme right denote significance for baseline comparisons according to group;
* denotes significance at p<0.05 as compared to visit 1; ** denotes significance at p<0.01 as compared to visit 1.

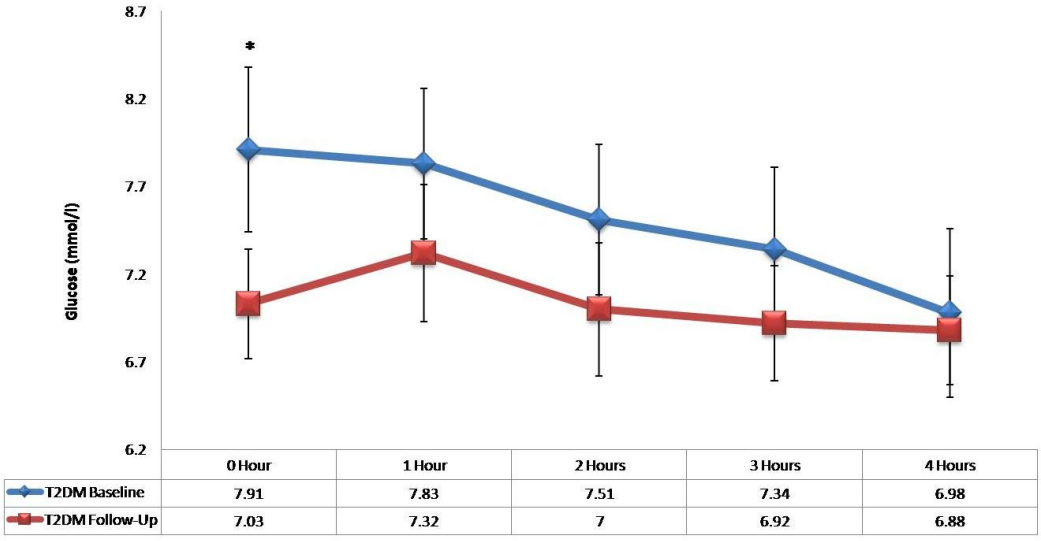
4.4.3 Post-prandial effects of high fat intake in the variables measured at baseline and follow-up in the T2DM group

Comparisons between post-prandial fat intake at baseline and 3-month follow-up visits in the T2DM are shown in Table 4.4.3.1. Differences at 0 hour were previously mentioned in tables 4.4.1.1 and 4.4.2.1. For HOMA-IR, a significant difference was noted at 2 hours post-prandial ($p<0.05$) with the follow-up visit being significantly lower than baseline. For total- and LDL cholesterol levels, significant differences persisted in baseline and follow-up values through-out hours 1-4 ($p<0.05$), with 0-hour having stronger significance ($p<0.01$). Endotoxin values at follow-up 2 hour post-prandial was also significantly lower than baseline post-prandial of the same hour ($p<0.05$). The rest of the comparisons per hour were not significantly different from one another (Table 4.4.3.1). Linear figures were plotted to show trends in baseline and follow-up post-prandial values for glucose (Figure 4.4.3.1), triglycerides (Figure 4.4.3.2), total cholesterol (Figure 4.4.3.3), HDL-cholesterol (Figure 4.4.3.4), LDL-cholesterol (Figure 4.4.3.5), endotoxin (Figure 4.4.3.6), insulin (Figure 4.4.3.7) and HOMA-IR (Figure 4.4.3.8).

Table 4.4.3.1 Post-Prandial Fat Meal Changes in Glucose, Insulin and HOMA-IR, Lipids and Endotoxin in T2DM Subjects at Baseline and 3 Months Follow Up

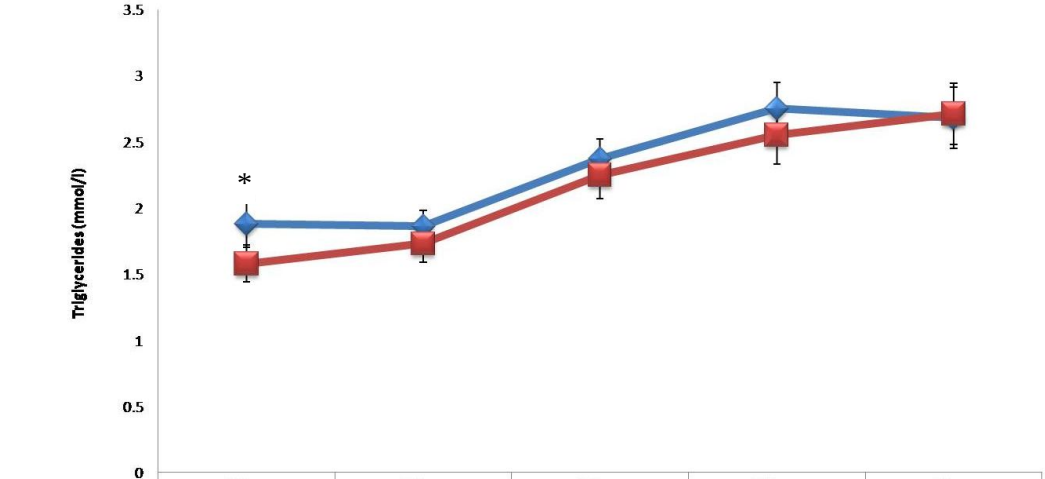
	0 Hour		**1 Hour**		**2 Hours**		**3 Hours**		**4 hours**	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
Glucose	7.9 ± 2.7	7.0 ± 1.8*	7.8 ± 2.5	7.3 ± 2.2	7.5 ± 2.5	7.0 ± 2.2	7.3 ± 2.7	6.9 ± 1.9	7.0 ± 2.8	6.9 ± 1.8
Insulin#	11.7 ± 5.5	10.8 ± 6.0	21.9 ± 17.7	15.8 ± 8.5	19.3 ± 12.6	14.9 ± 9.9	16.2 ± 12.3	15.5 ± 9.0	14.3 ± 8.2	13.5 ± 7.3
HOMA-IR#	3.7 ± 2.0	3.1 ± 2.2	8.7 ± 12.0	5.3 ± 3.3	6.6 ± 6.0	4.7 ± 3.8*	5.6 ± 5.4	4.8 ± 2.7	3.9 ± 2.4	3.9 ± 2.3
Triglycerides#	1.9 ± 1.0	1.6 ± 0.8*	1.8 ± 0.7	1.7 ± 0.8	2.4 ± 0.9	2.2 ± 1.0	2.7 ± 1.1	2.6 ± 1.2	2.7 ± 1.3	2.7 ± 1.3
Total Cholesterol	5.4 ± 1.1	4.6 ± 1.0**	5.3 ± 1.0	4.5 ± 1.0*	5.4 ± 1.1	4.6 ± 1.0*	5.3 ± 1.1	4.6 ± 1.0*	5.4 ± 1.1	4.7 ± 1.0*
HDL-Cholesterol	0.96 ± 0.2	1.0 ± 0.2**	0.96 ± 0.2	0.98 ± 0.2	0.94 ± 0.2	0.97 ± 0.2	0.91 ± 0.2	0.92 ± 0.2	0.89 ± 0.2	0.9 ± 0.2
LDL-Cholesterol	3.7 ± 0.8	2.9 ± 0.8**	3.6 ± 0.8	2.8 ± 0.9*	3.4 ± 0.9	2.6 ± 0.8*	3.2 ± 0.9	2.6 ± 0.8*	3.3 ± 0.9	2.6 ± 0.8*
Endotoxin	3.4 ± 0.9	3.2 ± 1.1	3.0 ± 0.9	3.0 ± 1.0	3.4 ± 0.9	3.0 ± 1.0*	3.5 ± 1.2	3.2 ± 0.7	3.6 ± 1.5	3.4 ± 1.3

Note: Data presented as mean ± standard deviation; * denotes significance at 0.05 level; ** denotes significant at 0.01 level.



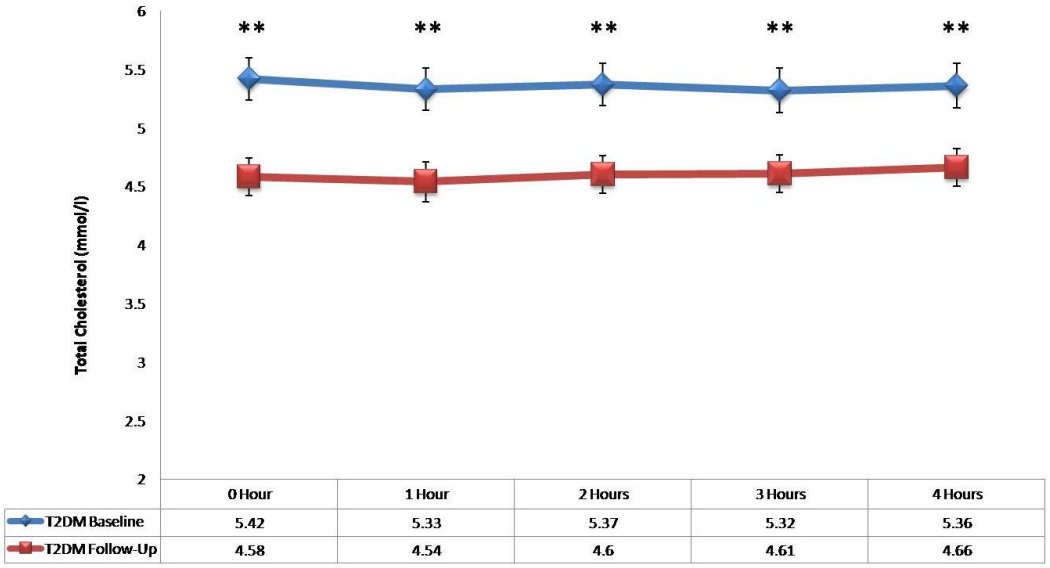
	0 Hour	1 Hour	2 Hours	3 Hours	4 Hours
T2DM Baseline	7.91	7.83	7.51	7.34	6.98
T2DM Follow-Up	7.03	7.32	7	6.92	6.88

Figure 4.4.3.1 Mean glucose levels pre- and post-intervention (T2DM group). * denotes p-value < 0.05.



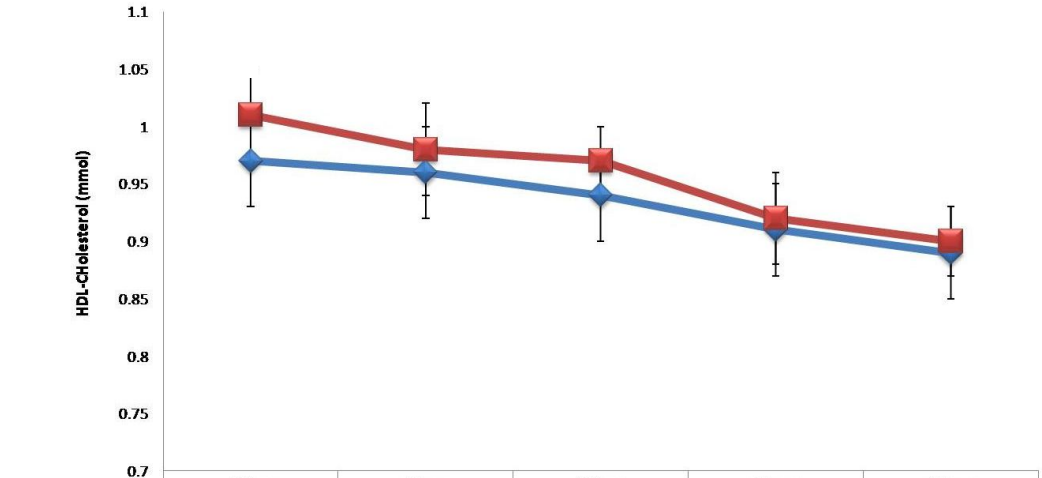
	0 Hour	1 Hour	2 Hours	3 Hours	4 Hours
T2DM Baseline	1.88	1.86	2.37	2.75	2.68
T2DM Follow-Up	1.58	1.73	2.25	2.55	2.71

Figure 4.4.3.2 Mean triglyceride levels pre- and post-intervention (T2DM group).



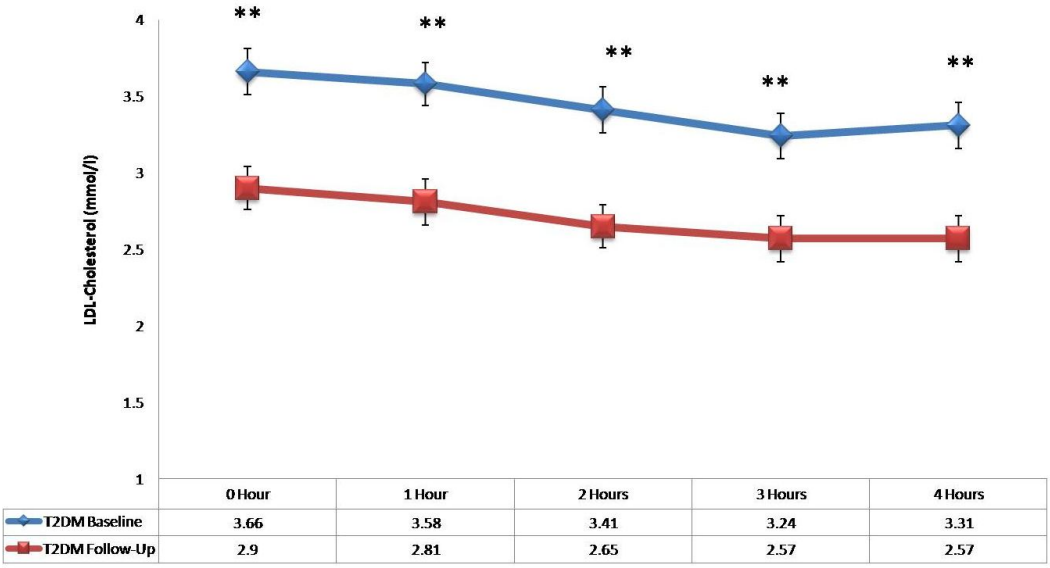
	0 Hour	1 Hour	2 Hours	3 Hours	4 Hours
T2DM Baseline	5.42	5.33	5.37	5.32	5.36
T2DM Follow-Up	4.58	4.54	4.6	4.61	4.66

Figure 4.4.3.3 Mean total cholesterol levels pre- and post-intervention (T2DM group). ** denotes p-value < 0.01.



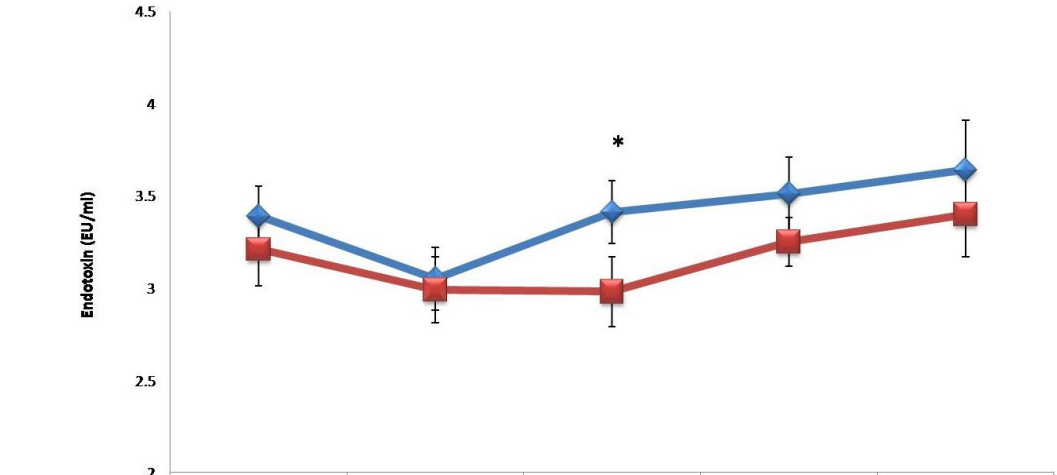
	0 Hour	1 Hour	2 Hours	3 Hours	4 Hours
T2DM Baseline	0.97	0.96	0.94	0.91	0.89
T2DM Follow-Up	1.01	0.98	0.97	0.92	0.9

Figure 4.4.3.4 Mean HDL-cholesterol levels pre- and post-intervention (T2DM group).



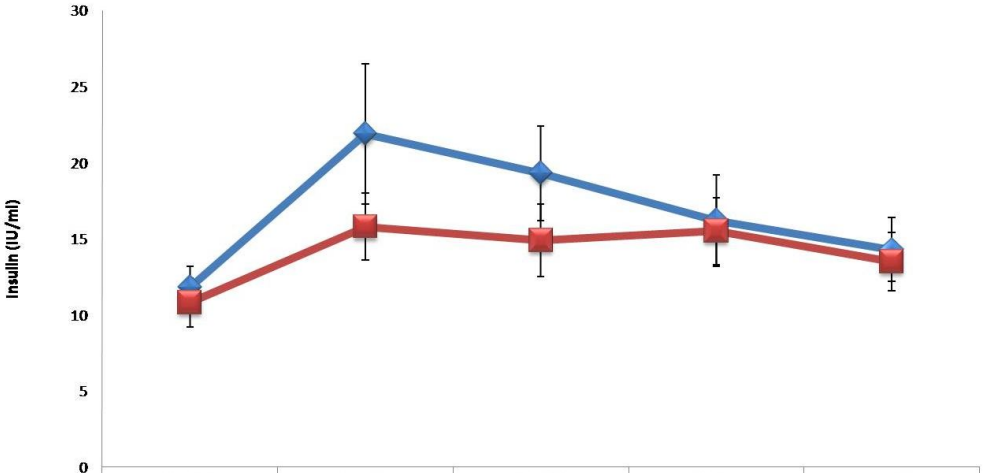
	0 Hour	1 Hour	2 Hours	3 Hours	4 Hours
T2DM Baseline	3.66	3.58	3.41	3.24	3.31
T2DM Follow-Up	2.9	2.81	2.65	2.57	2.57

Figure 4.4.3.5 Mean LDL-cholesterol levels pre- and post-intervention (T2DM group). ** denotes p-value < 0.01.



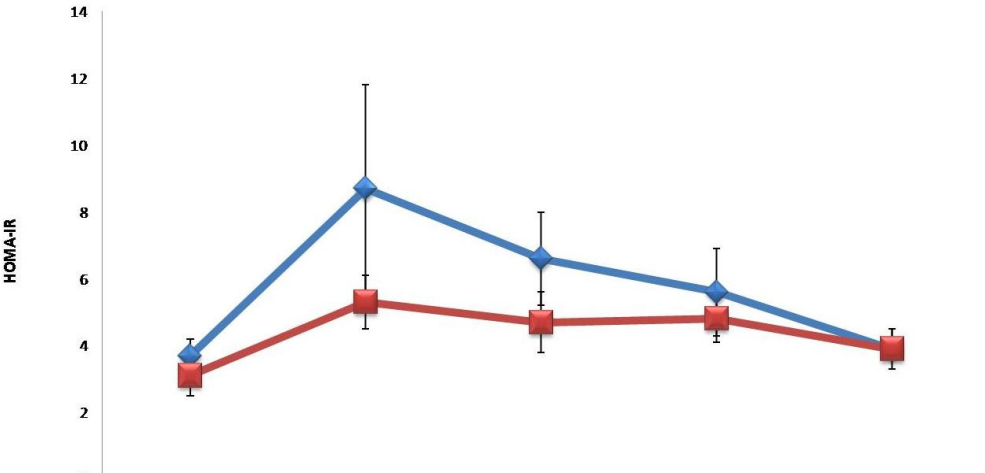
	0 Hour	1 Hour	2 Hours	3 Hours	4 Hours
T2DM Baseline	3.39	3.05	3.41	3.51	3.64
T2DM Follow-Up	3.21	2.99	2.98	3.25	3.4

Figure 4.4.3.6 Mean endotoxin levels pre- and post-intervention (T2DM group). * denotes p-value < 0.05.



	0 Hour	1 Hour	2 Hours	3 Hours	4 Hours
T2DM Baseline	11.8	21.9	19.3	16.2	14.3
T2DM Follow-Up	10.8	15.8	14.9	15.5	13.5

Figure 4.4.3.7 Mean insulin levels pre- and post-intervention (T2DM group).



	0 Hour	1 Hour	2 Hours	3 Hours	4 Hours
T2DM Baseline	3.7	8.7	6.6	5.6	3.9
T2DM Follow-Up	3.1	5.3	4.7	4.8	3.9

Figure 4.4.3.8 Mean HOMA-IR pre- and post-intervention (T2DM group).

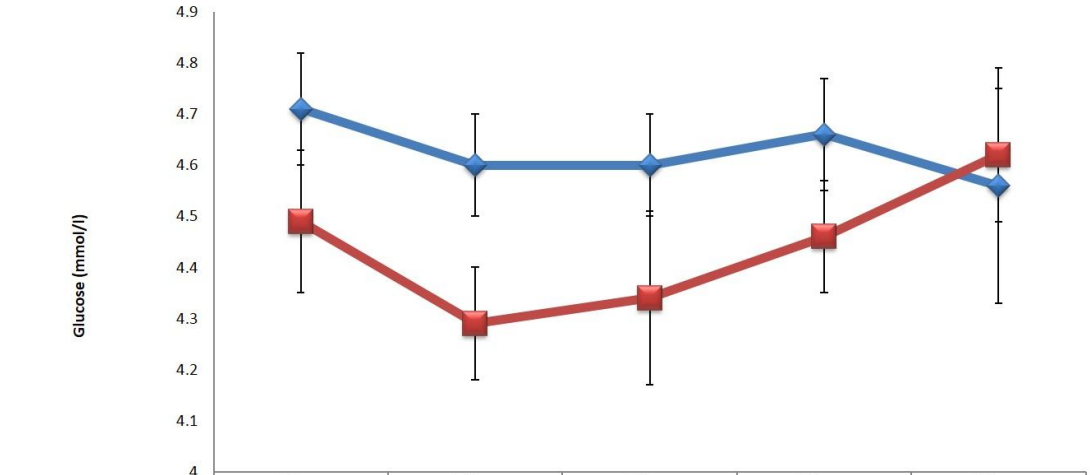
4.4.4 Post-prandial effects of high fat intake in the variables measured at baseline and follow-up in the Overweight^{+} group

Comparisons between post-prandial fat intake at baseline and follow-up visits in the overweight^{+} group are shown in Table 4.4.4.1. Differences at 0 hour were previously mentioned in Tables 4.4.1.1 and 4.4.2.1. Glucose levels were significantly lower at 1 hour follow-up than baseline 1 hour post-prandial ($p<0.05$). Similar to the T2DM group, a significantly lower total- and LDL-cholesterol values were observed in follow-up across all hours ($p<0.05$) as compared to baseline. Contrary to the T2DM group however, a significantly lower HDL-cholesterol values were also noted in the follow-up and TG was significantly higher at baseline ($p<0.05$). The rest of the variables were not significantly different from one another. Linear figures were plotted to show trends at baseline and follow-up post-prandial values for glucose (Figure 4.4.4.1), triglycerides (Figure 4.4.4.2), total cholesterol (Figure 4.4.4.3), HDL-cholesterol (Figure 4.4.4.4), LDL-cholesterol (Figure 4.4.4.5), endotoxin (Figure 4.4.4.6), insulin (Figure 4.4.4.7) and HOMA-IR (Figure 4.4.4.8).

Table 4.4.4.1 Post-Prandial Fat Meal Changes in Glucose, Insulin and HOMA-IR, Lipids and Endotoxin in Overweight^{+} Subjects at Baseline and 3 Months Follow Up

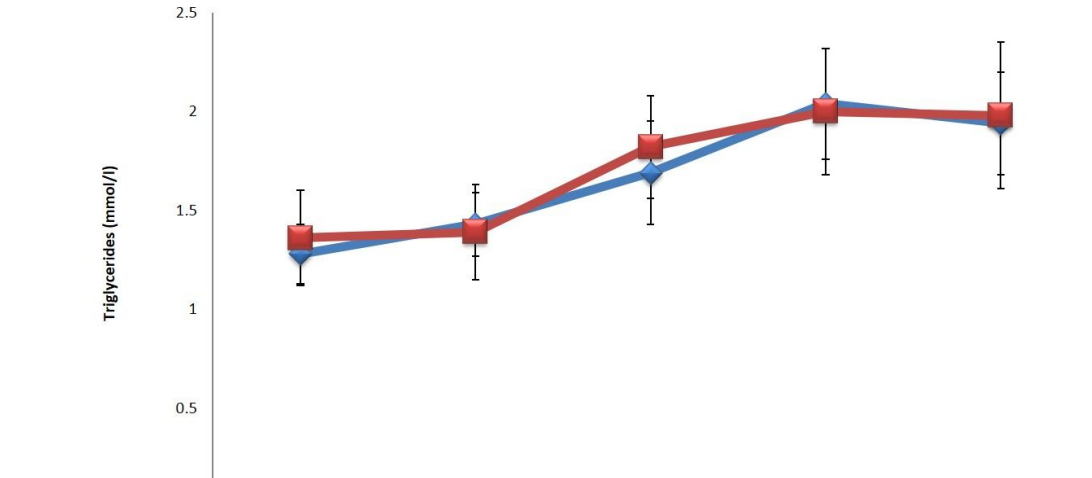
	0 Hour		**1 Hour**		**2 Hours**		**3 Hours**		**4 hours**	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
Glucose	5.15 ± 3.5	4.65 ± 2.8	4.6 ± 0.4	4.3 ± 0.4*	4.6 ± 0.4	4.3 ± 0.6	4.6 ± 0.4	4.5 ± 0.4	4.6 ± 0.8	4.6 ± 0.5
Insulin#	5.0 ± 3.4	12.7 ± 6.6*	15.6 ± 16.2	20.4 ± 20.4	16.9 ± 16.0	19.0 ± 15.3	13.1 ± 8.0	11.4 ± 7.4	10.3 ± 5.3	12.5 ± 7.7
HOMA-IR#	1.1 ± 0.7	0.93 ± 1.9	3.5 ± 4.1	4.2 ± 4.3	3.9 ± 4.1	4.3 ± 3.6	2.8 ± 1.9	2.4 ± 1.7	2.1 ± 1.2	2.8 ± 2.0
Triglycerides#	1.3 ± 0.8	1.4 ± 0.5	1.4 ± 0.8	1.4 ± 0.6	1.7 ± 0.9	1.8 ± 0.9	2.0 ± 1.1	2.0 ± 1.0	1.9 ± 1.3	2.0 ± 0.9
Total Cholesterol	4.5 ± 1.0	3.7 ± 0.8*	4.5 ± 0.9	3.6 ± 0.8*	4.4 ± 0.8	3.4 ± 0.9*	4.4 ± 0.8	3.5 ± 0.8*	4.4 ± 1.0	3.6 ± 0.8*
HDL-Cholesterol	1.2 ± 0.3	0.9 ± 0.2*	1.1 ± 0.3	0.9 ± 0.2*	1.1 ± 0.3	0.82 ± 0.2*	1.1 ± 0.4	0.8 ± 0.2*	1.1 ± 0.4	0.8 ± 0.2*
LDL-Cholesterol	2.8 ± 0.7	2.2 ± 0.6*	2.7 ± 0.6	2.1 ± 0.6*	2.5 ± 0.6	1.9 ± 0.5*	2.4 ± 0.6	1.8 ± 0.5*	2.4 ± 0.6	1.9 ± 0.5*
Endotoxin	3.0 ± 0.5	3.2 ± 1.1	2.9 ± 1.4	3.1 ± 1.3	3.5 ± 0.9	3.2 ± 1.3	3.8 ± 1.6	3.3 ± 0.8	3.5 ± 1.9	3.5 ± 1.7

Note: Data presented as mean ± standard deviation; * denotes significance at 0.05.



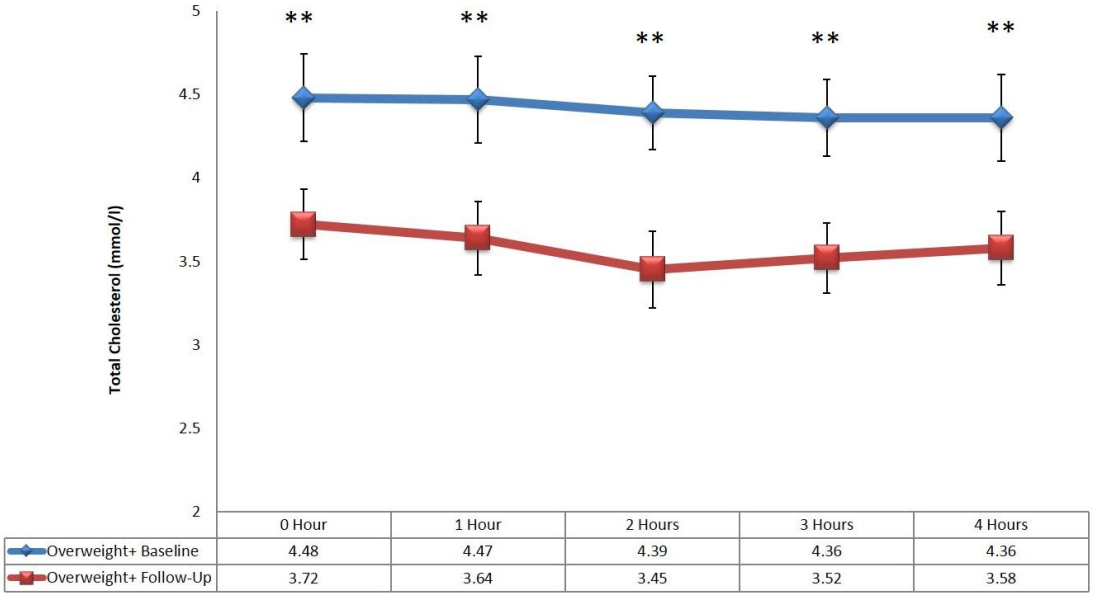
	0 Hour	1 Hour	2 Hours	3 Hours	4 Hours
Overweight+ Baseline	4.71	4.6	4.6	4.66	4.56
Overweight+ Follow-Up	4.49	4.29	4.34	4.46	4.62

Figure 4.4.4.1 Mean glucose levels pre- and post-intervention (Overweight^{+} group).



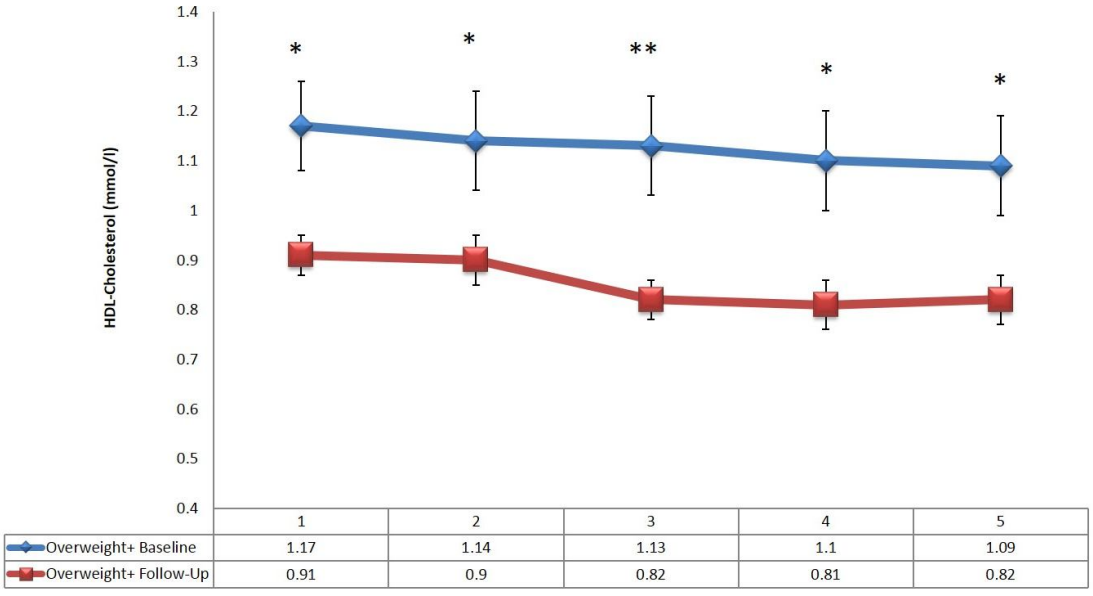
	0 Hour	1 Hour	2 Hours	3 Hours	4 Hours
Overweight+ Baseline	1.28	1.43	1.69	2.04	1.94
Overweight+ Follow-Up	1.36	1.39	1.82	2	1.98

Figure 4.4.4.2 Mean triglyceride levels pre- and post-intervention (Overweight^{+} group).



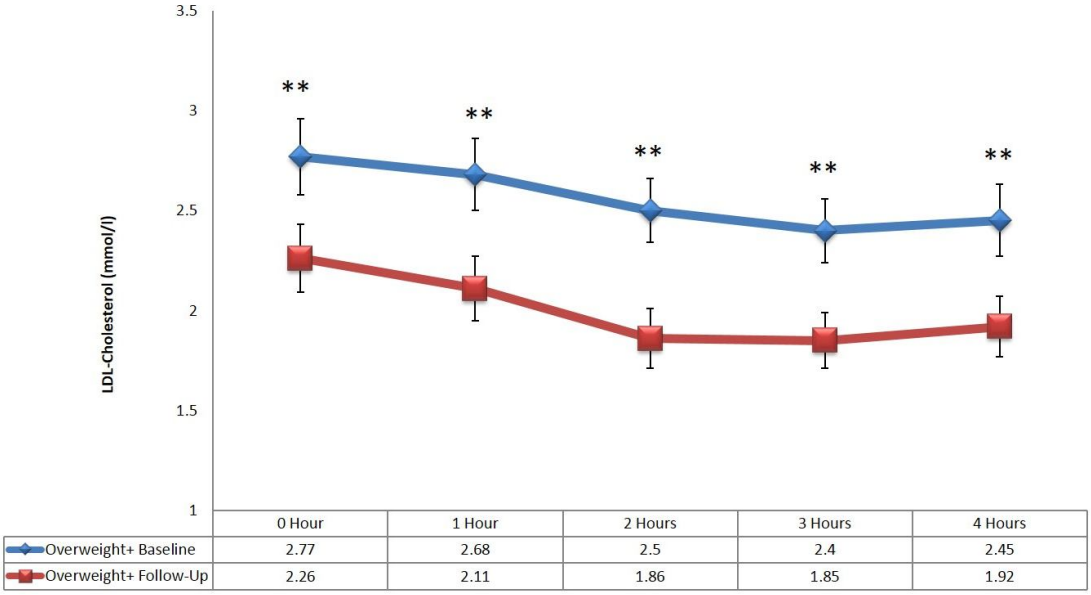
	0 Hour	1 Hour	2 Hours	3 Hours	4 Hours
Overweight+ Baseline	4.48	4.47	4.39	4.36	4.36
Overweight+ Follow-Up	3.72	3.64	3.45	3.52	3.58

Figure 4.4.4.3 Mean total cholesterol levels pre- and post-intervention (Overweight^{+} group). ** denotes p<0.01.



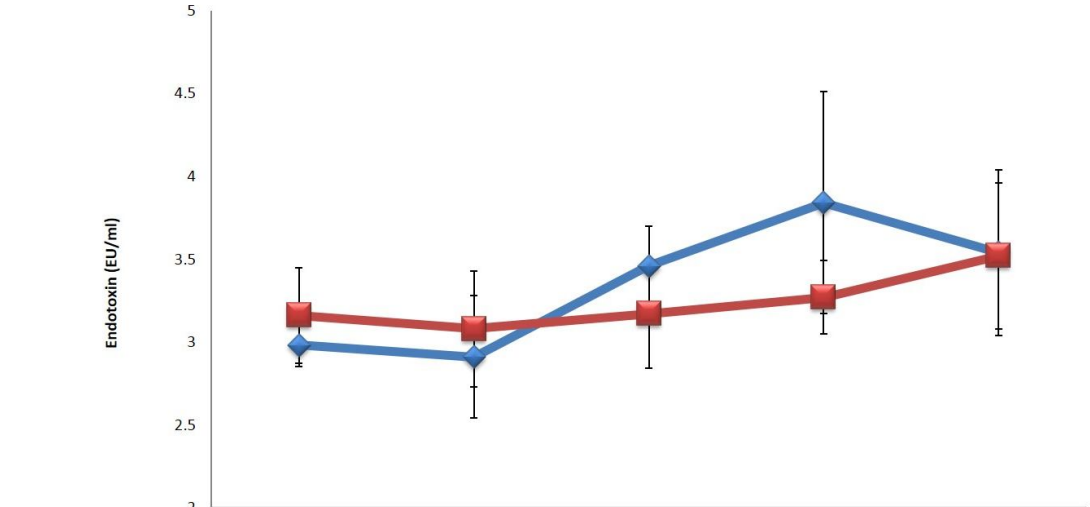
	1	2	3	4	5
Overweight+ Baseline	1.17	1.14	1.13	1.1	1.09
Overweight+ Follow-Up	0.91	0.9	0.82	0.81	0.82

Figure 4.4.4.4 Mean HDL-cholesterol levels pre- and post-intervention (Overweight^{+} group). * denotes p<0.05; ** denotes p<0.01.



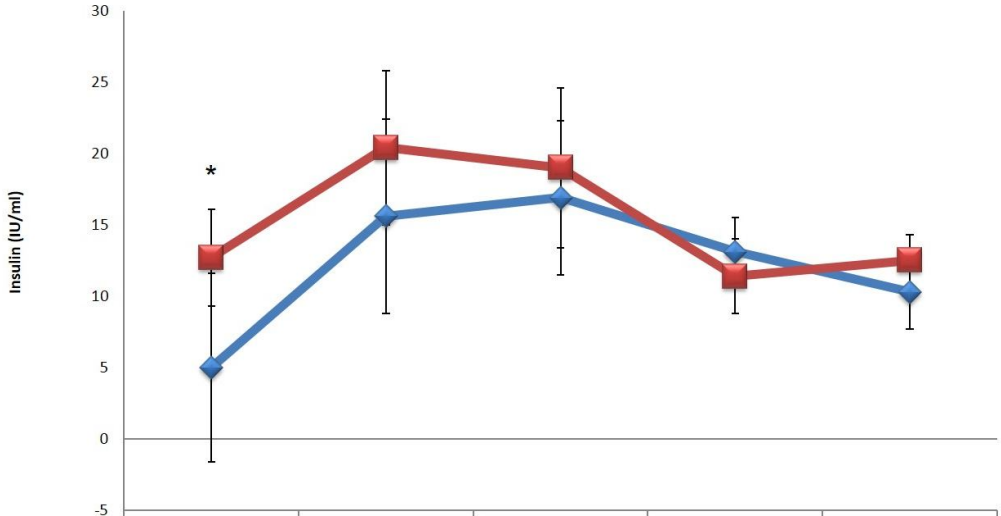
	0 Hour	1 Hour	2 Hours	3 Hours	4 Hours
Overweight+ Baseline	2.77	2.68	2.5	2.4	2.45
Overweight+ Follow-Up	2.26	2.11	1.86	1.85	1.92

Figure 4.4.4.5 Mean LDL-cholesterol levels pre- and post-intervention (Overweight^{+} group). ** denotes $p<0.01$.



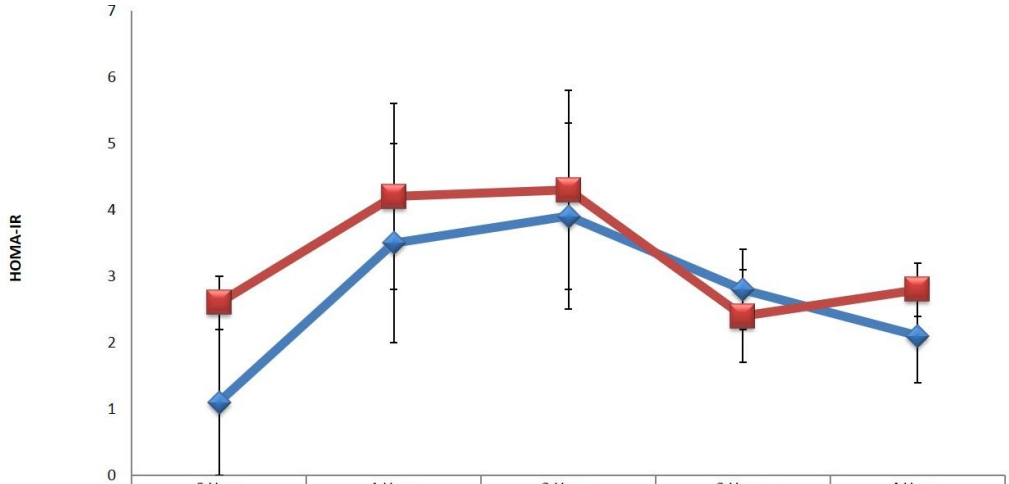
	0 Hour	1 Hour	2 Hours	3 Hours	4 Hours
Overweight+ Baseline	2.98	2.91	3.46	3.84	3.54
Overweight+ Follow-Up	3.16	3.08	3.17	3.27	3.52

Figure 4.4.4.6 Mean endotoxin levels pre- and post-intervention (Overweight^{+} group).



	0 Hour	1 Hour	2 Hours	3 Hours	4 Hours
Overweight+ Baseline	5	15.6	16.9	13.1	10.3
Overweight+ Follow-Up	12.7	20.4	19	11.4	12.5

Figure 4.4.4.7 Mean insulin levels pre- and post-intervention (Overweight^{+} group). * denotes $p<0.05$



	0 Hour	1 Hour	2 Hours	3 Hours	4 Hours
Overweight+ Baseline	1.1	3.5	3.9	2.8	2.1
Overweight+ Follow-Up	2.6	4.2	4.3	2.4	2.8

Figure 4.4.4.8 Mean HOMA-IR pre- and post-intervention (Overweight^{+} group).

4.4.5 Mean fold changes in endotoxin values

Figure 4.4.5.1 shows that there has been a modest increase in circulating endotoxin in both T2DM and overweight^{+} group at 0 hour after the dietary intervention, albeit these changes were not significant. Despite the non-significance, comparing the mean fold changes in both groups showed that the T2DM group were lower for the rest of the hour series than the overweight^{+} group with the exception of 0 hour.

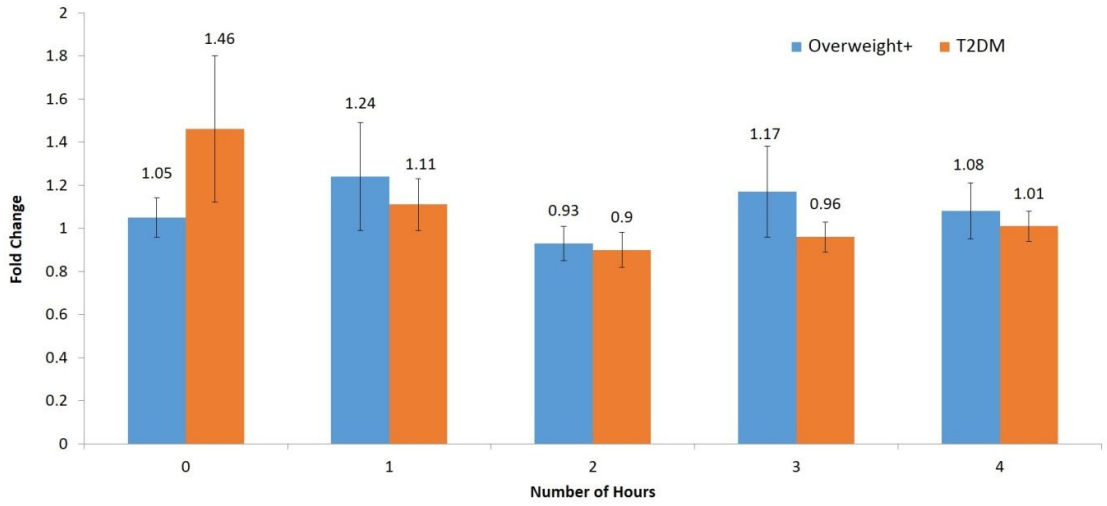


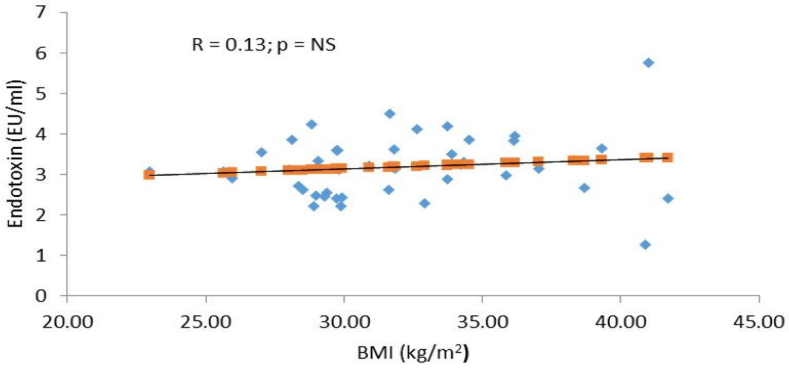
Figure 4.4.5.1 Mean fold change in endotoxin levels in overweight^{+} and T2DM groups

4.4.6 Associations of Endotoxin to Cardiometabolic Parameters Measured at Baseline

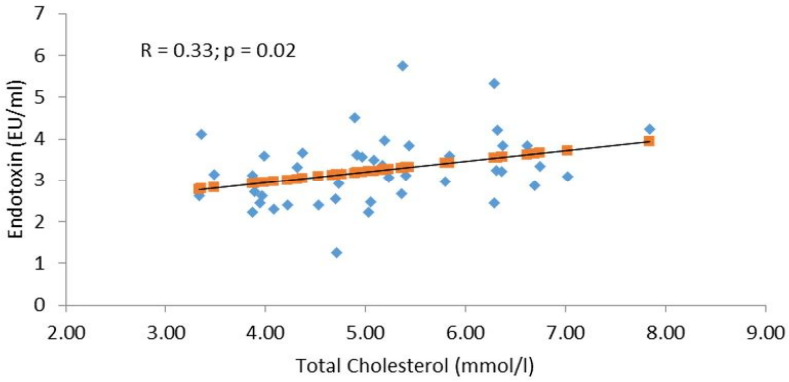
Figures 4.4.6.1 and 4.4.6.2 show the baseline associations of endotoxin in all subjects. Endotoxin was positively and significantly associated with both total- and LDL-cholesterol (R values 0.33 and p=0.02, respectively). A near significant association was also observed between triglycerides and endotoxin (R=0.28; p=0.06). No significant difference was noted between BMI and endotoxin. Worthy

to note was the inverse yet non-significant association between endotoxin and HDL-cholesterol (R=-0.15; p=NS). Upon stratification, endotoxin was found to be significantly associated with BMI in the T2DM group (R=0.38; p=0.03) (not shown in Figure). The rest of the endotoxin associations with other parameters in both T2DM and overweight^{+} group were not significant.

A. BMI
(R = 0.13; P = NS)



B. T. Cholesterol
(R = 0.33; P = 0.02)



C. LDL Cholesterol
(R = 0.33; P = 0.02)

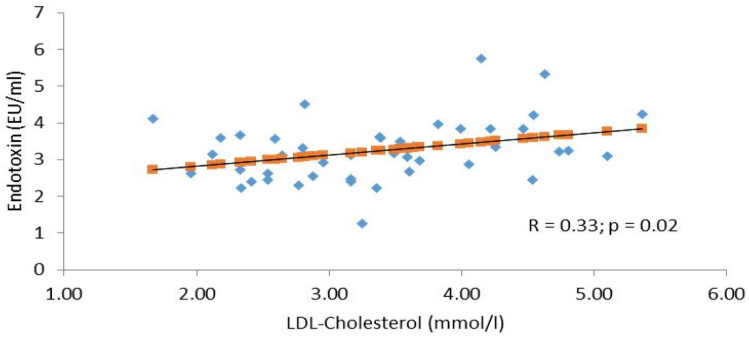
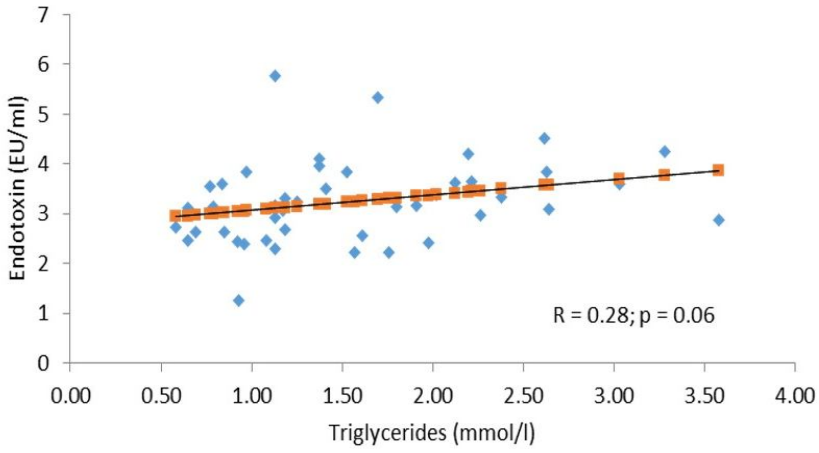


Figure 4.4.6.1 Baseline Associations of Endotoxin in All Subjects versus A. BMI, B. Total Cholesterol, C. LDL-Cholesterol

D. Triglycerides
(R = 0.28; P = 0.06)



E. HDL-Cholesterol
(R = -0.15; P = NS)

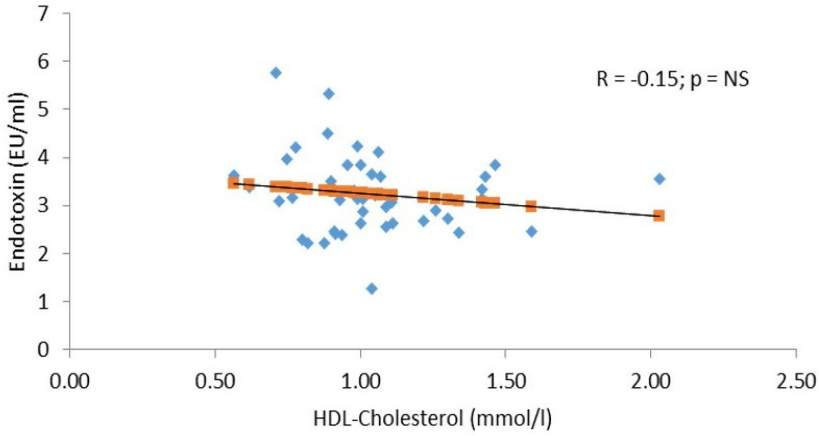
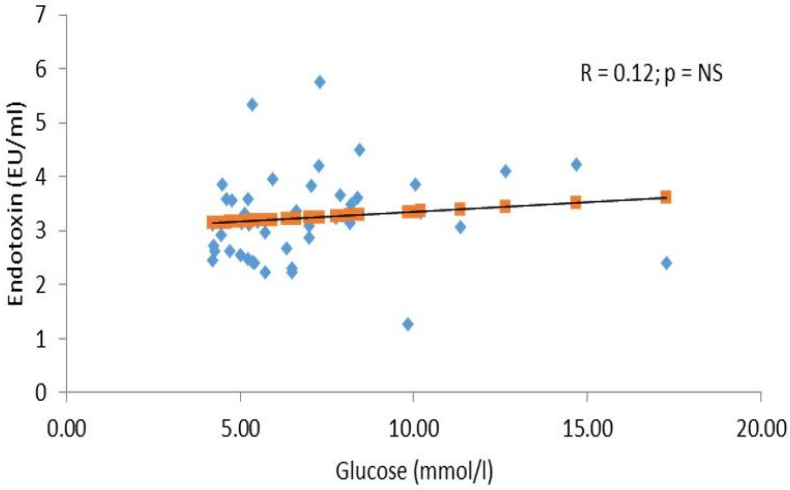


Figure 4.4.6.2 Baseline Associations of Endotoxin in All Subjects versus D. LogTriglycerides and E. HDL-Cholesterol

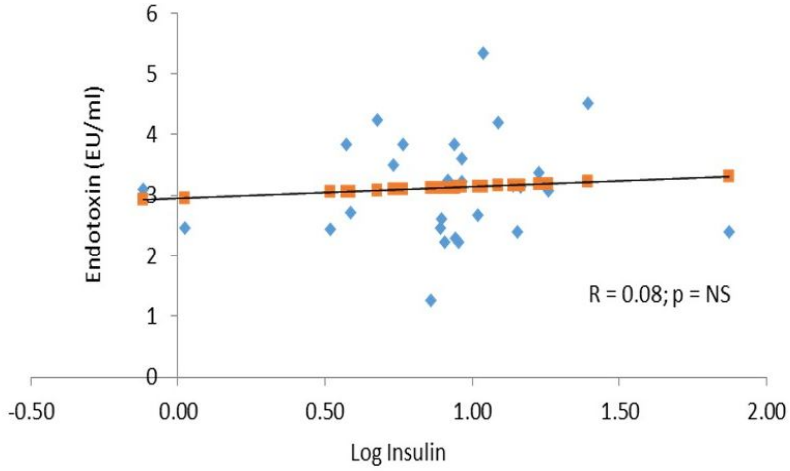
4.4.7 Associations of Endotoxin to Glycaemic parameters Measured at Baseline

Figure 4.4.7.1 shows no significant associations between endotoxin and fasting glucose, log insulin and log HOMA-IR in all subjects. When split according to groups, no associations were elicited (data not included).

A. Glucose
(R = 0.12; P = NS)



B. Log Insulin
(R = 0.08; P = NS)



C. Log HOMA-IR
(R = 0.10; P = NS)

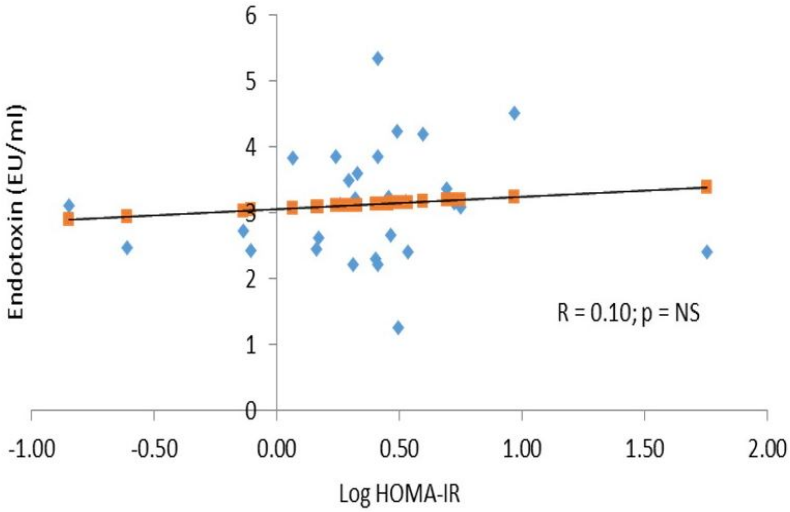


Figure 4.4.7.1 Baseline Associations of Endotoxin in All Subjects versus A. Glucose, B. log Insulin and C. log HOMA-IR

4.4.8 Associations of Endotoxin to BMI and Lipid parameters Measured at Follow-Up

Figure 4.4.8.1 shows no significant associations between endotoxin, BMI and lipids after follow-up visit. A borderline significant association between endotoxin and triglycerides was noted (R=0.29; p=0.07). This borderline significance persisted in the overweight^{+} group after stratification (R=0.52; p=0.08). No other significant associations were elicited for the rest of the lipids in both groups (not included in Figure).

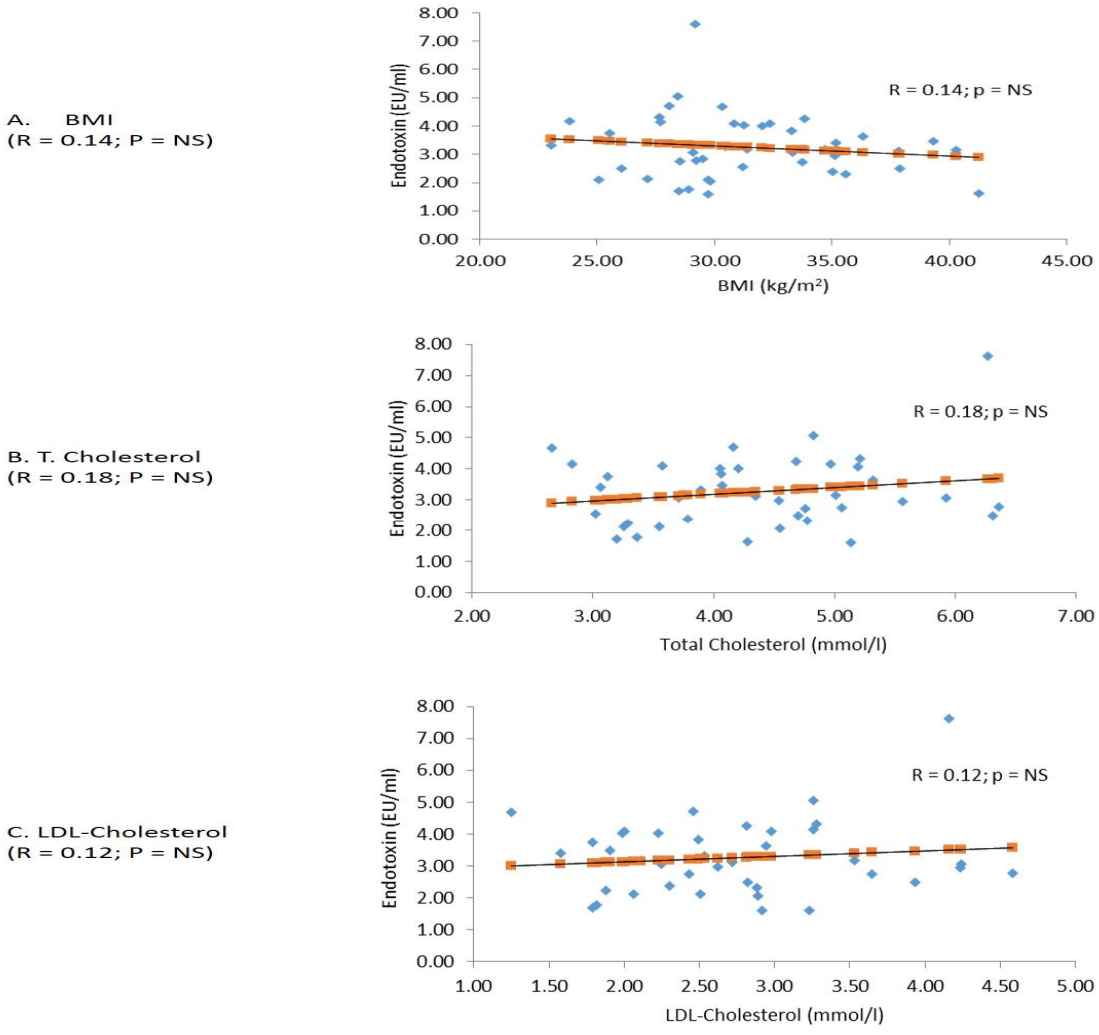


Figure 4.4.8.1 Follow-Up Associations of Endotoxin in All Subjects versus A. BMI, B. Total Cholesterol, C. LDL-Cholesterol

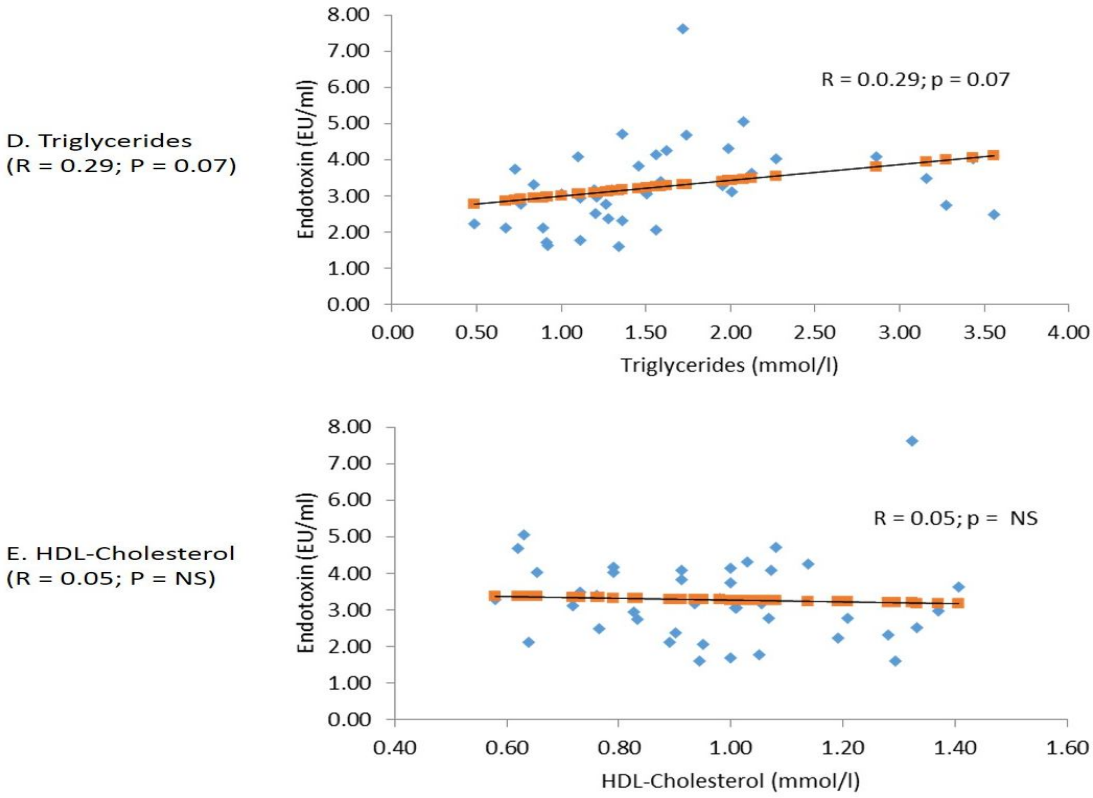
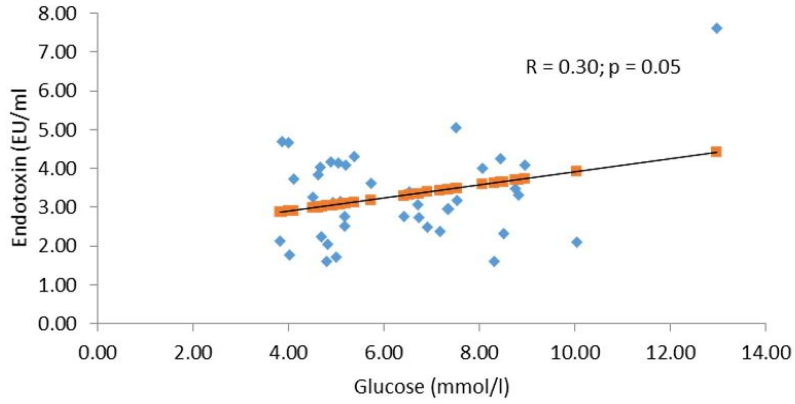


Figure 4.4.8.2 Follow-Up Associations of Endotoxin in All Subjects versus D. LogTriglycerides and E. HDL-Cholesterol

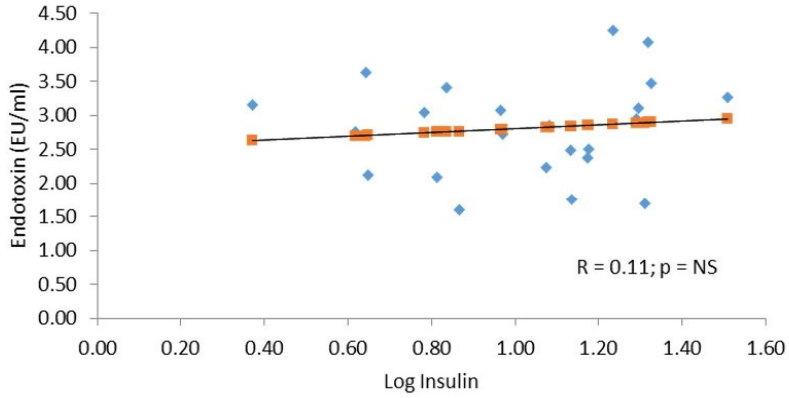
4.4.9 Associations of Endotoxin to Glycaemic parameters Measured at Follow-Up

Figure 4.4.9.1 shows the associations of endotoxin to glycemic parameters after 3 months dietary intervention. In all groups, a significant positive association was observed between endotoxin and glucose (R=0.30; p=0.05) while no associations were observed between endotoxin, log insulin and log HOMA-IR. After splitting into groups, the association of endotoxin was apparent only in the T2DM group (R=0.49; p=0.008), as well as the near significant association between endotoxin and log HOMA-IR (R=0.45; p=0.07) (not shown in Figures). The rest of the associations of endotoxin post-intervention were unremarkable.

A. Glucose
(R = 0.30 ; P = 0.05)



B. Insulin
(R = 0.11 ; P = NS)



C. HOMA-IR
(R = 0.26 ; P = NS)

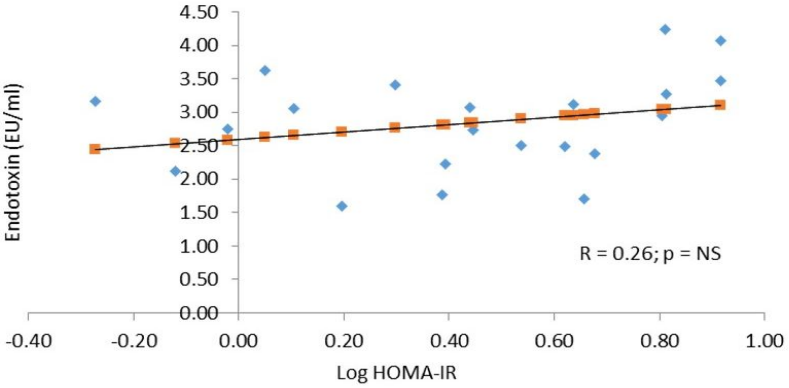


Figure 4.4.9.1. Follow-Up Associations of Endotoxin in All Subjects versus A. Glucose, B. Insulin, C. Homa-IR

4.5 Discussion

This current chapter has highlighted the differences observed in lipids, glycaemic and endotoxin profiles of both the overweight^{+} and T2DM groups after the dietary intervention. The cardiometabolic changes in post-prandial high fat intake between baseline and follow-up and the associations of endotoxin to these variables will also be evaluated.

The dietary intervention of 500 kcal deficit/day was observed to be beneficial in the reduction of total- and LDL cholesterol levels in both the T2DM and overweight^{+} groups. In addition, fasting glucose and HDL-cholesterol levels also improved in the T2DM group. Endotoxin levels remained stable in both groups. These favourable changes observed can be attributed to the over-all caloric restriction and improved quality of diet introduced in both cohorts.

Previous studies with caloric restriction diets have consistently shown to be beneficial in the prevention and management of T2DM (Ley et al, 2014). Our results are in agreement with several recent clinical trials and intervention studies that also observed significant improvements in cardiovascular disease risk factors, particularly total- and LDL-cholesterol owing to a 3-month diet-induced weight loss (Brekke et al, 2014; Mateo-Gallego et al, 2014). Given that the duration of the dietary intervention is short, advice has been dutifully given to the subjects to consistently maintain the type of beneficial diet introduced to them in the study. Adoption to the type of diet used in the present study, even in the absence of exercise (Bekkouche et al, 2014), has been proven to be more effective, if not modestly beneficial, in the longer term (Thompson et al, 2014; Bhattarai et al, 2013; Rees et al, 2013; Gotsis et al, 2014). The concomitant improvement in the glucose levels of the T2DM group

and not the overweight^{+} group further confirms that the type of intervention used appears to have glucose-lowering affects that appears favourable for the T2DM population (Juanola-Falgarona et al, 2014).

The post prandial effects between groups were anticipated, since the metabolic and BMI-status of subjects are important factors on how the subjects respond to high fat meals (Schwander et al, 2014). Nevertheless and with the exception of isolated and select variables, the effects of post-prandial high fat intake was fairly consistent for both the baseline and follow up visits in both the overweight^{+} and T2DM groups. This can be partially explained by the uniform dose of high fat meal given at baseline and follow-up. The more improved health benefits in weight and metabolic status in the T2DM group could be explained by a greater compliance to the intervention given.

It is important to highlight the associations of endotoxin in the variables measured in the present study. In the previous chapter it was observed that endotoxin levels were significantly lower in the control group than both the overweight^{+} and T2DM groups, and that endotoxin levels were comparable for both the overweight and T2DM groups, suggesting that perhaps the overweight^{+} group had impaired insulin resistance. In this chapter we noted a significant association between endotoxin and BMI, particularly in the T2DM group, a group which falls well within the obese category. This association confirms the sub-clinical inflammation existing in obese patients and the role of endotoxins in exacerbating low-grade inflammation and insulin resistance (Cani et al, 2012). While observational studies are inconsistent in showing an association between BMI and endotoxin, this has not been the case for obese subjects. Mechanisms that may highlight the association with increasingly

adiposity may occur through changes in gut permeability during weight gain, in which patients have a “leakier” gut mucosa in obese subjects than those with insulin resistance (Piya et al, 2013). Furthermore differences in the microbiota composition between obese and non-obese individuals (Boroni-Moreira et al, 2012), and the post prandial inflammation induced by high fat diets have been observed (Herieka and Erridge, 2014). These same mechanisms may also partially explain the other borderline significant associations seen in the study such as the association between endotoxin, triglycerides, total and LDL-cholesterol in all subjects, and the association of endotoxin and insulin resistance in T2DM subjects. The higher number of associations to the T2DM group as compared to the overweight^{+} group also reinforces the role of endotoxemia in insulin resistance.

One of the clear caveats in the present study is the small sample size which, with increase may produce stronger associations between endotoxin and the variables measured. As it stands, the borderline significance elicited are best affirm previous studies. Further investigations with the inclusion of more inflammatory markers may provide insights on how a diet-induced weight loss programme may improve inflammatory status and gut permeability.

In summary, a 500 kcal deficit/day, in the absence of exercise, for 3 months, resulted in improved glucose levels and lipid profile which is more obvious in the T2DM group than the overweight^{+} group. Endotoxin levels in both groups, while stagnant even after dietary intervention, were associated with lipid profile, particularly total and LDL-cholesterol in both groups; and with BMI and insulin resistance in the T2DM group, reinforcing the exacerbating role of endotoxemia in obese and insulin resistant subjects. Lastly, the effects of a high fat meal did not

confer many changes in the post-prandial evaluation of cardiometabolic parameters of both groups at baseline and follow-up assessments suggesting the importance of a low fat diet to improve cardiometabolic health. Further studies are warranted to assess the protective effects of the present dietary intervention to reduce subclinical inflammation in the longer-term.

Chapter Five

The Effectiveness of Dietary Intervention among Type 2 Diabetes Mellitus, Overweight plus (overweight^{+}) and lean Saudi Women

5.1 Introduction

If the prevalence of type 2 diabetes mellitus (T2DM) continues to increase at the current rate, the global burden of this disease will swell between from 171 million in 2000, to 366 million patients in 2030 (Wild et al., 2004). In 2011, Al-Daghri and colleagues revealed that the age-adjusted prevalence of T2DM amongst urban Saudis was 31.6%, with another 10.2% having impaired fasting glucose (IFG) (Al-Daghri et al., 2011), confirming yet again that Saudi Arabia has one of the highest prevalence of T2DM on a global scale. If left ignored, this already heavy burden will overwhelmingly affect the nation's limited resources in the near future, as half of the population will be deemed handicapped with the presence of T2DM.

5.1.1 Effectiveness of Medical Nutrition Therapy in Treatment and Prevention of Diabetes

The effectiveness of medical nutritional therapy (MNT) in the management of T2DM has been well established (Franz et al., 2008). MNT is defined as a "nutritional diagnostic, therapy, and counseling services for the purpose of disease management, which are furnished by a registered dietitian or nutrition professional" (U.S. Department of Health, 2001). Furthermore, the American Diabetes Association (ADA) currently recommends that "*individuals who have pre-diabetes or diabetes should receive individualized MNT as needed to achieve treatment goals, provided by registered dietitian familiar with components of MNT for diabetes*" (ADA, 2009). MNT interventions, both short and long term, were found to be beneficial in improving metabolic and behavioral outcomes. Pastors et al. summarized that "randomized, controlled nutrition therapy outcome studies have documented

decreases in [A1C] of ~1% in newly diagnosed type 1 diabetes mellitus (T1DM), 2% in newly diagnosed T2DM and 1% in T2DM with an average duration of 4 years." (Pastors et al, 2002).

To achieve an effective MNT, it is important to explore the nutritional status and estimate the food intake of the general population. Most methods for measuring food intake have limitations that must be taken into account to determine which is most appropriate for the study aims. Self-reporting of food intake can lead to a certain degree of measurement error that may bias the relationship between nutrient and/or food intake and the disease of interest. Suggested errors of measurement can stem from variability within subject in food intake, difficulties in remembering which and how much food was consumed (Fernández-Ballart et al., 2010).

Food frequency questionnaires (FFQ) are widely accepted and most frequently used method to assess food intake in large epidemiological studies which associate diet with chronic diseases because they provide a convenient assessment of the habitual dietary intake of an individual over a long period of time without altering usual food habits and are relatively inexpensive when self-administered (Willett, 1998).

5.1.2 Eating Patterns and Weight loss

Studies designed to reduce excess body weight have used a variety of energy-restricted eating patterns with various macronutrient intakes and occasionally included a physical activity component and ongoing follow-up support. Studies achieving the greatest weight losses, 6.2 kg and 8.4 kg, respectively, included the Mediterranean-style eating pattern (Esposito et al., 2009) and a study testing a

comprehensive weight loss program that involved diet (including meal replacements) and physical activity (Pi-Sunyer et al., 2007).

Dietary patterns are influenced by food availability, perception of healthfulness of certain foods and by the individual's preferences, culture, religion, knowledge, health beliefs, and access to food and resources (e.g., budget/income) (Jones-McLean et al., 2010), these factors should be considered when individualizing eating pattern recommendations. Unfortunately there are limited studies in Saudi Arabia and the Middle-East in general, with regards to prospective dietary interventions. To fill this gap, we aim to examine the effect of comprehensive MNT and dietary intervention using energy deficit diet (500Kcal/day deficit) among Saudi women with various metabolic states to develop strategies that enhance adherence and to determine if certain nutritional approaches promote greater adherence than others. To the best of our knowledge this is the first study to investigate the influence of hypocaloric diet among adult Saudi women with various metabolic states.

5.2 Research design and methods

5.2.1 Site and Duration of the Study

This is a multi-center, randomized interventional study conducted at the primary care centers of the Riyadh city, involved 92 Saudi women, consisted of 18 healthy control subjects (n=18), overweight plus (overweight^{+}) subjects (n= 24) and patients with early onset of T2DM (n= 50).

5.2.2 Selection of the Volunteers and Ethics Approval

5.2.2.1 Inclusion/Exclusion Criteria

Participants were eligible if they were women aged between 18-50 years, non-smokers, pre-menopausal, with a normal resting ECG and blood pressure and with no history of vascular disease. Candidates were excluded if they have acute medical illness; malignancy, a history of cardiovascular disease (CVD) or those on chronic medication, as well as patients with hypertriglyceridemia (as steroids and lipid lowering drugs may affect the intervention trial), previous myocardial infarction to established CHD, and other chronic inflammatory disorders e.g. rheumatoid arthritis, crohn's disease, age under 18 and inability to give informed consent on grounds of competency. In addition, subjects with known long standing diabetes and receiving medication, or those with fasting glucose > 11 mmol/l or/and fasting triglycerides > 4 mmol/l were excluded from the study.

5.2.2.2 Ethical approval

Ethical approval was granted by the Ethics Committee of King Saud University, Riyadh, Kingdom of Saudi Arabia, prior to the commencement of the research (**Appendix I**).

5.2.3 Medical Screening

Subjects were medically screened at primary health care centers where a physician completed a medical examination of vital signs and clinical interview which include questions to determine eligibility based on inclusion and exclusion criteria, detailed health information obtained from all subjects using preset heath questionnaire. For collecting data for the study, a pre-coded questionnaire was designed. The interview questionnaire which was previously used in large-scale epidemiologic survey in Saudi Arabia consisted of socio-demographic data, past medical and treatment history, sleeping hours and level of physical activity (Al-Daghri et al, 2011) (**Appendix III).**

5.2.4 Clinical Assessment

Anthropometric measurements with emphasis on clinical markers of adiposity were assessed. These measurements included height (cm) and weight (kg) from which BMI will be calculated [weight (kg)/height (m^2)]. Waist (cm) and hips (cm) were also measured and its ratio was determined. Blood pressure (mmHg) was taken using the conventional mercurial sphygmomanometer. All these measurements were repeated after 3 months period.

5.2.5 Biochemical Assessment

All subjects were requested to submit an over-night fasting blood samples (12-14 hours fasting) from which the different metabolic parameters were assessed which included: Fasting blood glucose and complete lipid profile. These parameters were measured at baseline and after 3 months (3 months study duration).

5.2.5.1 Blood Sample Collection

These were collected with the patients in the fasting state using sterile vacutainer blood collection apparatus (BD). Whole blood, serum, EDTA plasma and fluoride tubes were collected from subjects. All samples were aliquoted and stored in –20^{o}C freezer facilities in preparation for subsequent analysis.

5.2.5.2 Laboratory Techniques

Serum samples were stored in a -20 ℃ freezer prior to analysis. Fasting glucose and lipid profiles were measured using a chemical analyzer. This biochemical analyzer was calibrated routinely prior to the analysis of all serum samples using quality control samples provided by the manufacturer (ThermoFisher Scientific, Espoo, Finland).

5.2.6 Clinical Intervention (Dietary Regimen)

To enhance weight loss of 5% or more, participants were prescribed a 500 Kcal deficit energy diet less than their daily recommended dietary allowances which were set at the levels recommended by the dietary reference intake for subjects with

low levels of physical activity at the same gender and age (DRI, 2008/2011). Targeted macronutrient composition was 20%-30% fat, <10% of saturated fatty acids, 50%-60% carbohydrates, 15%-20% protein and at least 15g per 1000kcal fibres (**Appendix V**).

As described earlier in chapter 2, participants with T2DM (n= 50) and overweight^{+} subjects (n= 24) received group and individualized dietary consultation. This was provided by the dietician researcher at baseline visit followed by monthly group sessions. Topics discussed include dietary regimen plan, healthy eating habits, general dietary guidelines, goal setting, modeling, social support, and relapse prevention and management. Subjects in the dietary intervention group were instructed to keep a written record of their daily food intake using a designed sheet developed by the researcher (**Appendix VII**) to be collected by the end of the study period of intervention.

Dietary interventional program prescribed to selected subjects was initiated during 3 month period consisted of baseline in-person counselling sessions lasting 1 hour with subsequent dietician phone calls and messages (once/week). In addition, printed dietary materials and handouts were designed by the researcher and provided to all participants to go home according to their assigned dietary regimen to facilitate implementation of the dietary interventional program (**Appendix V**). Food frequency questionnaire (FFQ) which has been used previously (Al Disi et al., 2010) was the dietary tool to assess the food intake of the subjects pre- and post the dietary intervention (**Appendix IV**).

Nutrient intake was calculated using USDA database (18th - 21st Ed, 2009, 2010) Program, as for the Saudi Arabic traditional dishes were analyzed using the

Arabic food analysis program (version 1, 2007). The evaluation of the daily food intake was made by the means of the total energy and the total nutrient intake, the percentage of the total calories derived from fat, protein and carbohydrates.

Dietary nutrients values were compared with dietary reference intake (DRI) for specified age and gender for macronutrients (carbohydrates and protein) as well as for micronutrients (2008/2011).

As for daily energy requirement (Kcal/day) was estimated using WHO equation (2005, 2007) (Sylvia Escott-Stump, 2011) according to age group and metabolic status. Dietary fat percentage has been estimated as 25% from daily energy requirement (Hooper et al., 2011) (**Appendix VI**).

5.3 Data Analyses

Data was analyzed using SPSS, version 16.5 (SPSS Inc, Chicago, IL, USA). Data were presented as mean±standard deviation for all continuous variables. Analysis of variance (ANOVA) was done to compare differences between control, overweight^{+} and T2DM group. Paired T-test was done to assess difference between baseline and follow-up visits in both the overweight and T2DM groups. Spearman correlation was done to determine associations between macronutrient intake and all parameters measured in all groups at baseline and follow-up. Significance was set at $p<0.05$.

5.4 Results

5.4.1 Baseline Comparisons in Anthropometry in all Groups

Table 5.4.1.1 shows the comparison of controls, overweight^{+} and T2DM groups according to anthropometry as well as observed changes between overweight^{+} and T2DM groups over time. Across all groups, T2DM patients had the highest measures in terms of mean BMI, waist and hip circumferences and WHR. It was noted that 40% of the T2DM subjects were receiving metformin whilst the rest where on diet alone advice.

Table 5.4.1.1 Anthropometric Characteristics According to Groups

	Control	**Overweight^{+}**	**T2DM**
N	18	24	50
Age (years)	24.39 ± 7.92	32.04 ± 7.78**	41.50 ± 6.23**
DM Duration (years)	--	--	2.04 (0-9)
Metformin Use	--	--	40% (20/50)
BMI (kg/m^2)	22.20 ± 2.21	28.54 ± 1.49**	35.24 ± 7.67**
Waist (cm)	80.64 ± 7.23	95.75 ± 7.42**	112.3 ± 13. 43**
Hip (cm)	98.69 ± 7.26	109.67 ± 5.01**	117.11 ± 11.59**
WHR	0.82 ± 0.05	0.87 ± 0.05**	0.96 ± 0.07**

Note: Data presented as mean ± standard deviation; * denotes significance at 0.05 level; ** denotes significance at 0.01 level.

5.4.2 Baseline Comparisons in the Dietary Intake of all Groups

The overweight^{+} group had significantly higher carbohydrate consumption and total calories than both T2DM and control ($p<0.01$), and this was also evident in the % DRI (Figure 5.4.2.1). No significant differences were observed in the protein and fat intake in all groups (Table 5.4.2.1) (Figures 5.4.2.2 and 5.4.2.3, respectively). On the other hand, the control group had the highest intake (Table 5.4.2.1) and % DRI (Figure 5.4.2.4) of fiber followed by the overweight^{+} group and lowest from the T2DM group at baseline ($p = 0.003$). Total caloric intake and % DRI (Figure 5.4.2.5) was significantly higher in the overweight^{+} group than T2DM and control ($p = 0.011$).

Table 5.4.2.1 Dietary Intake Characteristics According to Groups

	Control	**Overweight^{+}**	**T2DM**
N	18	24	50
Carbohydrates			
Grams	94.0 ± 5.5	210.3 ± 9.2**	192.7 ± 12.9**
Calories	375.8 ± 22.0	841.1 ± 36.8**	770.9 ± 51.6**
Protein			
Grams	64.4 ± 5.5	66.1 ± 5.2	59.3 ± 3.9
Calories	257.7 ± 22.0	264.4 ± 20.9	237.3 ± 15.6
Fats			
Grams	67.3 ± 7.3	93.8 ± 5.9	84.3 ± 4.0
Calories	606.0 ± 65.8	844.3 ± 53.1	758.5 ± 36.0
Fiber			
Grams	18.5 ± 1.7	13.1 ± 2.2*	12.9 ± 0.6**
Total Calories			
Kilocalories	1239.6 ± 95.9	1949.7 ± 63.7**	1766.7 ± 61.8*

Note: Data presented as mean ± standard deviation; * denotes significance at 0.05 level; ** denotes significance at 0.01 level.

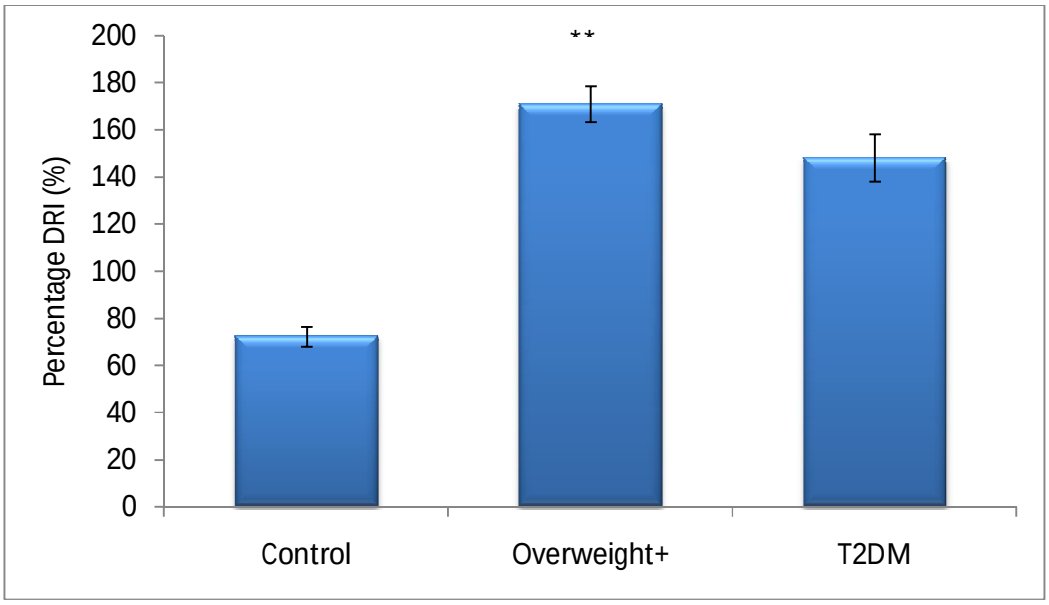


Figure 5.4.2.1 Percentage DRI (%) in carbohydrate intake according to groups at baseline; ** denotes significance compared to control.

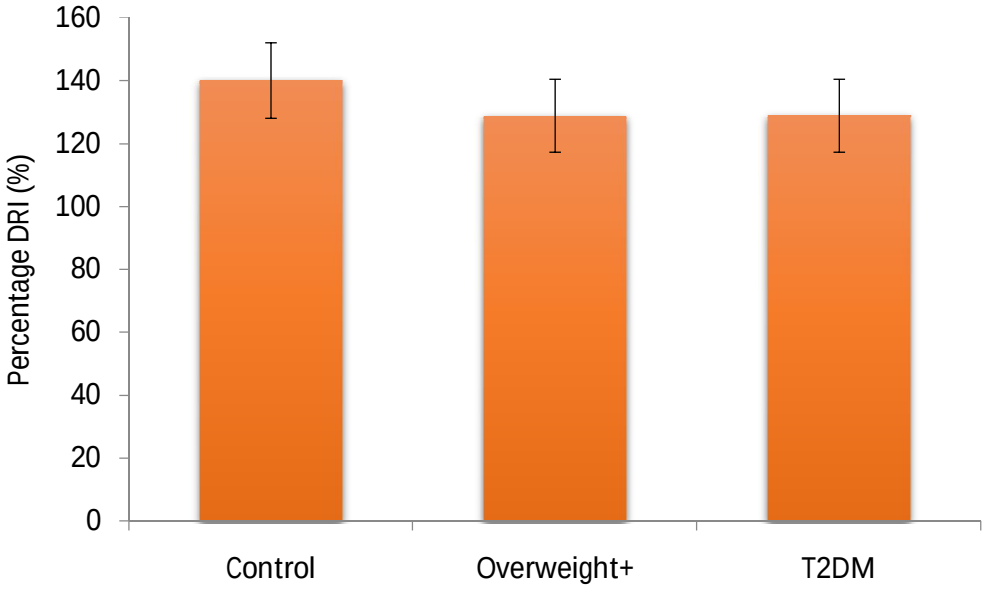


Figure 5.4.2.2 Percentage DRI (%) in protein intake according to groups at baseline.

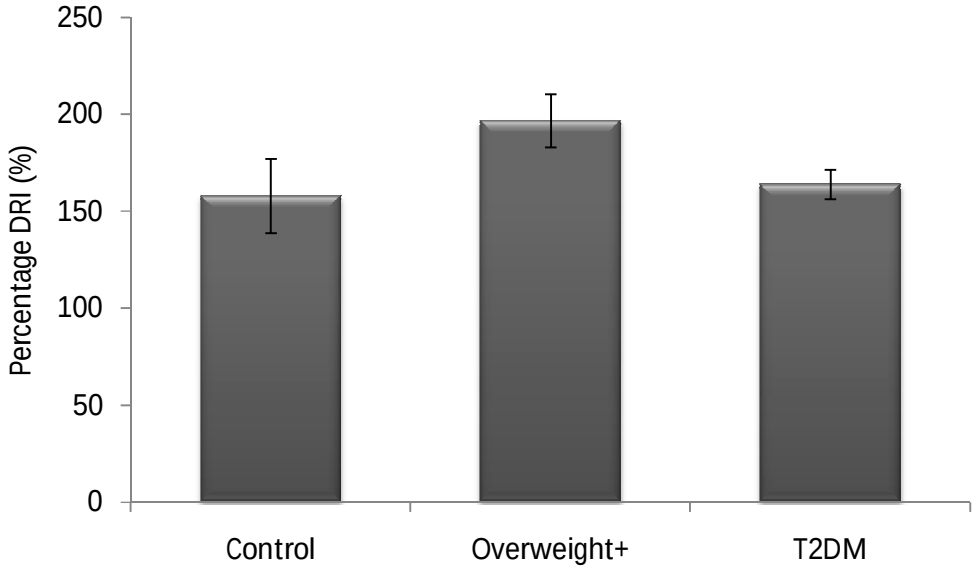


Figure 5.4.2.3 Percentage DRI (%) in fat intake according to groups at baseline.

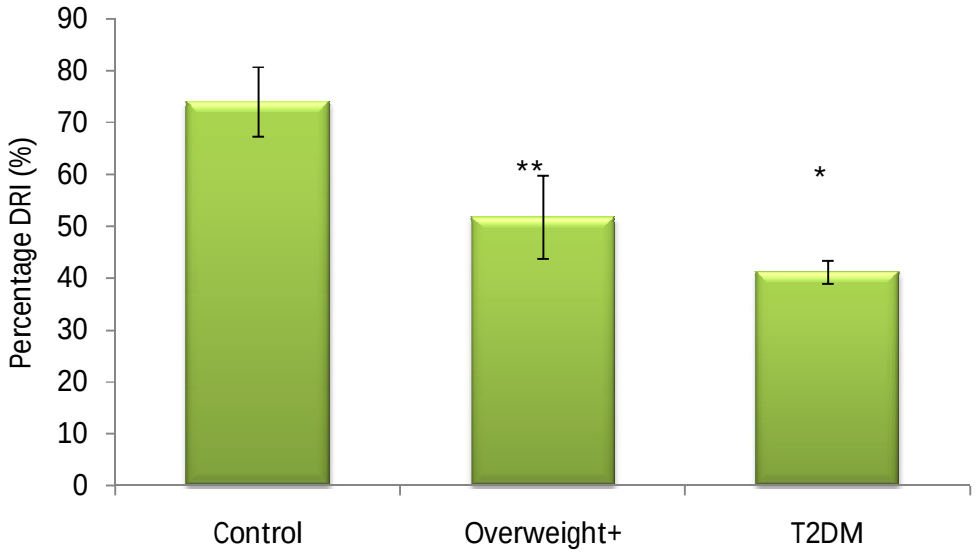


Figure 5.4.2.4 Percentage DRI (%) in fibre intake according to groups at baseline; * denotes significance compared to control at 0.05 level; ** denotes significance compared to control at 0.01 level.

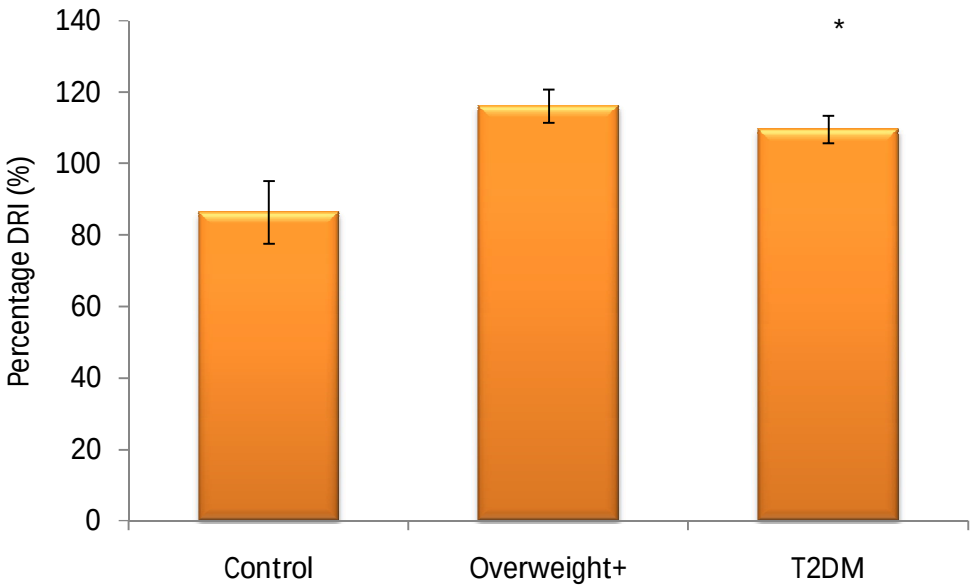


Figure 5.4.2.5 Percentage DRI (%) in total caloric intake according to groups at baseline; * denotes significance compared to control at 0.05 level.

5.4.3 Changes in Anthropometry after 3 Months Follow-Up

Prospectively, significant improvements were observed in both the overweight^{+} and T2DM group in terms of decreased mean BMI [Overweight^{+} Group 28.54±1.49kg/m^2 versus 27.95±2.25kg/m^2, $p<0.05$; T2DM group 35.24±7.67kg/m^2 versus 35.04±8.07kg/m^2, $p<0.05$] and hip circumference [Overweight^{+} group 109.67±5.01cm versus 108.07±4.07cm, $p<0.05$; T2DM group 112.3±13.43cm versus 109.21±12.71cm, $p<0.01$] at follow-up visit as compared to baseline. Only the T2DM group showed significant improvements in waist circumference (112.3±13.43cm versus 109.21±12.71cm, $p<0.01$) and WHR (0.96±0.07cm versus 0.93±0.06cm, $p<0.01$) after 3 months follow-up. In the overweight^{+} group, there was no significant difference in the waist circumference group over time, while a significantly higher WHR was noted after follow-up (0.87±0.05 versus 0.88±0.06, $p<0.05$) (Table 5.4.3.1).

Table 5.4.3.1 Anthropometric Changes in Overweight^{+} and T2DM Groups Overtime

	Overweight^{+}		**T2DM**	
N	24		50	
Age (years)	32.04 ± 7.78		41.50 ± 6.23	
DM Duration (years)	--		2.04 (0-9)	
	Baseline	**Post-Intervention**	**Baseline**	**Post-Intervention**
BMI (kg/m^2)	28.54 ± 1.49	27.95 ± 2.25*	35.24 ± 7.67	35.04 ± 8.07*
Waist (cm)	95.75 ± 7.42	95.87 ± 7.78	112.3 ± 13. 43	109.2 ± 12.7**
Hip (cm)	109.67 ± 5.01	108.07 ± 4.07*	117.11 ± 11.59	116.7 ± 13.18**
WHR	0.87 ± 0.05	0.88 ± 0.06*	0.96 ± 0.07	0.93 ± 0.06**

Note: Data presented as mean ± standard deviation; * denotes significance at 0.05 level; ** denotes significance at 0.01 level.

5.4.4 Changes in Dietary Intake after 3 Months Follow-Up

In the overweight^{+} group, significant improvements were observed after 3 months intervention in terms of decreased carbohydrate intake (210.3±9.2 versus 181.7±8.8; $p<0.01$), increased protein intake (66.1±5.2 versus 71.5±5.3; $p<0.05$), decreased fat intake (93.8±5.9 versus 92.6±9.4; $p<0.05$) and decreased total caloric consumption (1949.7±63.7 versus 1326.8± 165.5; $p<0.01$) (Table 5.4.4.1). Similar significant improvements were also noted in the T2DM group in 2 macronutrients, namely carbohydrate intake (192.7±12.9 versus 163.9±11.2; $p<0.01$) and decreased fat intake (84.3±4.0 versus 61.5±2.9; $p<0.01$). A significant decrease in protein (59.3±3.9 versus 45.4±3.1; $p<0.01$) and total caloric intake (1766.7±61.8 versus 1391.0±49.3; $p<0.01$) were also observed in the T2DM group. There was a modest increase in fiber intake in both the overweight^{+} and T2DM group, though it did not reach statistical significance (Table 5.4.4.1).

Table 5.4.4.1 Dietary Changes in Overweight^{+} and T2DM Groups Overtime

	Overweight^{+}		T2DM	
N	24		50	
	Baseline	**Post-Intervention**	**Baseline**	**Post-Intervention**
Carbohydrates				
Grams	210.3 ± 9.2	181.7 ± 8.8**	192.7 ± 12.9	163.9 ± 11.2**
Calories	841.1 ± 36.8	726.8 ± 35.3	770.9 ± 51.6	655.7.0 ± 44.8
Protein				
Grams	66.1 ± 5.2	71.5 ± 5.3*	59.3 ± 3.9	45.4 ± 3.1**
Calories	264.4 ± 20.9	286.3 ± 21.2	237.3 ± 15.6	181.5 ± 12.4
Fats				
Grams	93.8 ± 5.9	92.6 ± 9.4*	84.3 ± 4.0	61.5 ± 2.9**
Calories	844.3 ± 53.1	833.4 ± 84.6	758.5 ± 36.0	553.8 ± 26.1
Fiber				
Grams	13.1 ± 2.2	18.9 ± 4.3	12.9 ± 0.6	14.0 ± 0.9
Total Calories				
Kilocalories	1949.7 ± 63.7	1326.8 ± 165.5**	1766.7 ± 61.8	1391.0 ± 49.3**

Note: Data presented as mean ± standard deviation; * denotes significance at 0.05 level; ** denotes significance at 0.01 level.

5.4.5 Associations of macronutrients to Cardiometabolic Indices

Table 5.4.5.1 highlights the significant baseline and post-intervention associations of the macronutrients to the anthropometric and lipid indices measured across groups. In the control group, carbohydrate intake had significant negative effects on LDL-cholesterol ($R = -0.52$; $p<0.05$). Moving on to the overweight^{+} group, a significant association was observed in baseline HDL-cholesterol and protein intake (baseline $R = 0.64$; $p<0.01$). Finally in the T2DM group, baseline carbohydrate intake was significantly associated with BMI ($R = 0.30$; $p<0.05$. The rest of the coefficients for all groups were non-contributory (Table 5.4.5.1). Figure 5.4.5.1 shows the mean fold changes in the BMI and WHR of both the overweight^{+} and T2DM group.

Table 5.4.5.1 Association of Dietary Intake (grams) to Cardiometabolic Indices

	Control				Overweight+				T2DM			
	CHO	**CHON**	**FATS**	**Kcal**	**CHO**	**CHON**	**FATS**	**Kcal**	**CHO**	**CHON**	**FATS**	**Kcal**
BMI												
Baseline	0.30	-0.01	-0.04	0.14	0.34	0.10	-0.08	0.01	**0.30***	0.16	-0.04	0.21
Post-Intervention	--	--	--	--	0.21	0.01	0.10	0.30	0.17	0.03	-0.08	0.10
Waist												
Baseline	0.08	0.10	-0.02	0.07	-0.04	-0.17	0.26	-0.10	0.23	0.16	0.03	0.17
Post-Intervention	--	--	--	--	0.12	0.14	0.35	0.35	0.15	-0.07	0.08	0.14
Hips												
Baseline	0.28	0.10	-0.02	0.07	-0.01	0.32	0.05	-0.10	0.26	0.05	0.01	0.21
Post-Intervention	--	--	--	--	0.28	0.21	0.30	0.38	0.12	-0.14	0.03	0.08
WHR												
Baseline	-0.13	0.03	0.22	-0.20	-0.03	-0.12	0.30	0.19	0.05	-0.20	-0.06	-0.02
Post-Intervention	--	--	--	--	-0.14	-0.20	0.13	-0.04	0.07	-0.08	-0.07	0.03
Glucose												
Baseline	-0.20	-0.27	0.14	-0.04	0.39	0.43	-0.22	0.11	-0.17	-0.04	0.09	-0.05
Post-Intervention	--	--	--	--	-0.02	-0.35	0.43	0.21	-0.12	0.03	0.05	-0.09
Triglycerides												
Baseline	0.04	0.19	0.31	0.32	0.12	-0.03	0.04	0.21	-0.18	-0.01	-0.12	-0.13
Post-Intervention	--	--	--	--	-0.03	-0.24	0.21	0.35	-0.18	0.18	-0.23	-0.32
HDL-Cholesterol												
Baseline	-0.10	0.02	-0.29	-0.36	0.33	**0.64****	-0.38	0.13	-0.13	0.21	0.06	0.02
Post-Intervention	--	--	--	--	0.08	0.03	0.42	0.20	0.31	-0.23	-0.21	0.13
LDL-Cholesterol												
Baseline	**-0.52***	-0.30	0.36	-0.29	0.18	-0.06	0.15	0.18	0.10	-0.09	0.25	0.15
Post-Intervention	--	--	--	--	0.27	-0.26	0.08	0.29	0.07	-0.13	0.21	0.02
Total Cholesterol												
Baseline	-0.46	-0.25	-0.27	-0.42*	0.34	0.18	0.12	0.38	0.08	-0.07	0.23	0.14
Post-Intervention	--	--	--	--	0.20	-0.32	0.20	0.31	0.08	-0.23	0.18	-0.02

Note: Data presented as coefficient (R); * denotes significance at 0.05 level; P-value significant at $p < 0.05$.

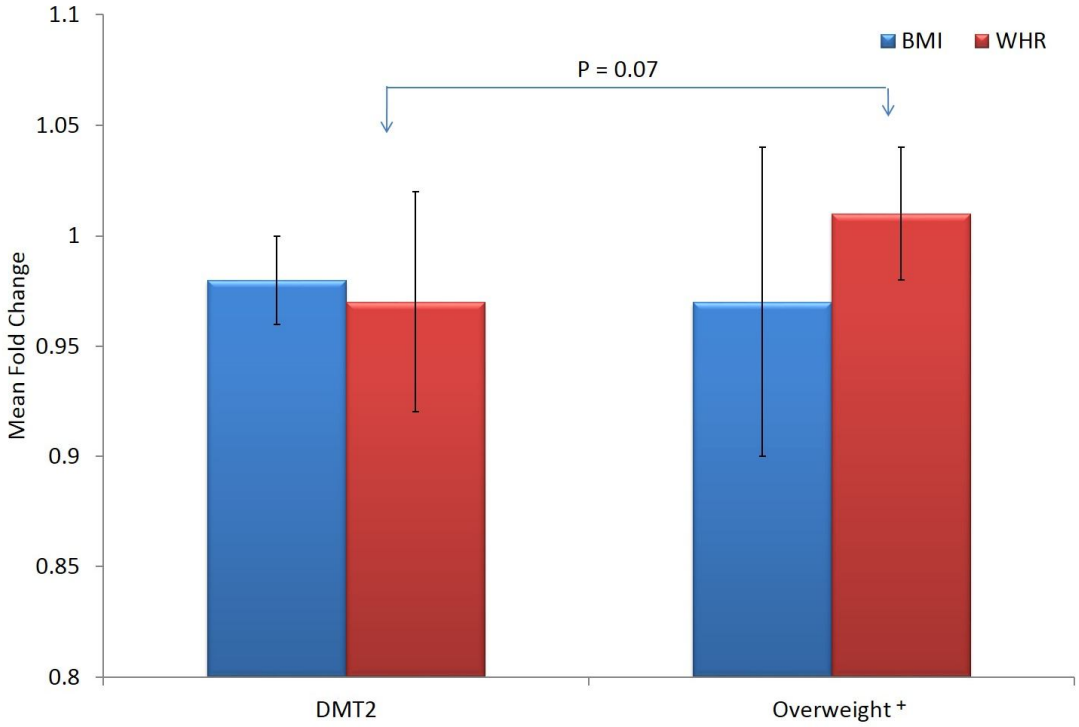


Figure 5.4.5.1 Mean fold changes in BMI and WHR in Overweight^{+} Patients and the T2DM group

5.5 Discussion

The main finding in the present study is that significant changes in the macronutrient consumption (500 kcal/day deficit) in both the overweight^{+} and T2DM groups during the 3-month dietary intervention led to favorable effects in both anthropometric and cardiometabolic indices of both groups, although improvements were more pronounced in the T2DM group. These findings highlight the importance of diet in the prevention and control of T2DM, even in the absence of modified physical activity. Previous work by Salas-Salvado and colleagues has recommended that the best strategy to accompany maintenance of ideal body weight is the implementation of the "prudent diet", which is similar to the dietary intake used in the present study, characterized by an increased intake of plant-based food groups that are generally recommended for health promotion, and a decreased intake of red meat and its products, sweets, high-fat dairy, refined grains and/or a Mediterranean diet known for its high olive oil content, fruits and vegetables, including whole grains, pulses, nuts and low-fat dairy minus the moderate alcohol and red wine consumption because of religious restrictions (Salas-Salvado et al, 2011; Lazarou et al, 2012, ADA, 2012). The Mediterranean diet has received special attention because of its ability to reduce cardiovascular risk and its complications aside from the already known prevention of other major non-chronic communicable diseases (Bonnaciio et al, 2012). It has also been proven effective even as a single intervention (the absence of physical activity modification) in altering a favourable metabolic profile (Kontogianni et al, 2012) since it is known that pre-diabetic states already manifest several abnormal anthropometric, lipid and adipocytokine (leptin) profile (Al-Daghri et al, 2007).

The significant associations elicited between macronutrient intake and the different cardiometabolic parameters in the present study are worthy of mention. Carbohydrate intake was negatively associated with LDL-cholesterol in the control group, suggesting first, that these significant associations may grossly reflect which among the groups were more adherent and compliant to the dietary intervention used, and second, that there are several types of carbohydrates that provide beneficial metabolic effects when consumed in regulated amounts. The level of adherence to healthy dietary patterns influences the risk of T2DM, with the highest adherence having the lowest risk (Alhazmi et al, 2013). In the present cohort, it seemed that whilst both the overweight^{+} and T2DM groups significantly improved their dietary intake, the significant improvements were more pronounced in the T2DM subjects, which also corresponded with more significant improvements in anthropometric parameters elicited after follow-up. The most likely reason for this discrepancy in response among groups is that some of the T2DM subjects were on metformin monotherapy, an established standard care drug for T2DM known to decrease food consumption and induce weight loss (Lee and Morley, 1998). This metformin mono-therapy advantage of the T2DM group may have boosted the effects of a 500 kcal/day deficit with apparent results as early as 3 months as observed in the present study. As for the second point, Song and colleagues demonstrated that in the Korean population, refined-grain and white rice were observed to be associated with metabolic syndrome in women only (Song et al, 2014). The same refined-grain intake also increases risk of metabolic syndrome in the South Asian population (Radhika et al, 2009). In our study, whole-grain intake was encouraged as the main carbohydrate source since this has been consistently proven to be beneficial among

patients with T2DM in various studies conducted using the Middle-Eastern population (Al-Khudairy et al, 2013, Esmaillzadeh et al, 2005, Issa et al, 2011).

The authors acknowledge several limitations. Despite the significant associations elicited between macronutrient intake and anthropometrics measures, the results should still be interpreted with caution, as these were based on self administered food frequency questionnaires, which are highly subjective. The findings cannot be applied to men with T2DM since gender is a confounder and differences in dietary intake and pattern have been recently demonstrated (Leblanc et al, 2014). The lack of follow-up in the lean control group also limits the findings to the overweight^{+} and T2DM groups in terms of the beneficial effects following dietary intervention.

In summary, this study showed that a 500 kcal/day deficit over a 3-month period dietary is clinically beneficial among adult Saudi females with T2DM than the overweight^{+} subjects that seem to swap their fat diet for more protein that didn't translate to a better health impact. Longer prospective studies are needed taking into consideration other subgroups, such as those with pre-diabetes, to determine whether the dietary intervention alone can reduce progression of T2DM among high-risk adult Arabs.

Chapter Six

Effect of Diet type (low-fat vs balanced diet [prudent diet]) on weight loss, metabolic status and endotoxin among type 2 diabetes and overweight plus (overweight^{+}) Saudi Women

6.1 Introduction

6.1.1 Nutrition therapy for the management of diabetes

Life-style modifications are effective tools to prevent type 2 diabetes Mellitus (T2DM), and weight loss is critical to success (Bantle et al., 2008). The impact of healthy, calorie-restricted diet together with physical activity on subjects with impaired glucose tolerance, which is a pre-diabetic state, have been studied in 5 clinical trials (Pan et al., 1997; Tuomilehto et al., 2001; Kosaka et al., 2002; Knowler et al., 2002; Ramachandran et al., 2006). The risk reductions reported in these trials were between 30 and 70%. Moreover, and in four of these studies (Pan et al., 1997; Tuomilehto et al., 2001; Kosaka et al., 2002; Knowler et al., 2002) T2DM rates were lowered in relation to weight loss whereas in the Indian trial lifestyle modification was successful despite no weight loss. Observational studies have also shown that diets rich in red meat, processed foods, refined grains and sweets increase diabetes risk, while diets low in red meat and whole-fat dairy products and rich in vegetables are associated with decreased risk of diabetes (Kastorini and Panagiotakos, 2009). Generally, a low-fat diet (LFD) has been recommended to patients with T2DM to achieve weight loss (Becker et al., 2008) particularly, low dietary saturated fat has been recommended for that purpose such as the Mediterranean diet (Margetts, 2003; Bantle et al., 2008).

The classical Mediterranean diet (MedDiet), includes high intake of legumes, vegetables, fruits, olive oil, grains and nuts, moderate intake of sea food and wine, and low intake of red meat, processed foods, and whole-fat dairy products is largely considered a healthy dietary pattern (Martínez-González et al., 2009). It was recently

demonstrated that MedDiet acheives better glycemic control and delayed the need for T2DM medication in newly diagnosed T2DM patients compared with the LFD (Esposito et al., 2009).

Further evidence on the benefit of MedDiets over LFD has obtained in the Prevención con Dieta Mediterránea (PREDIMED) study, a large nutrition interventional trial for primary cardiovascular prevention in individuals at high cardiovascular disease (CVD) risk. In this trial, 418 non-diabetic subjects aged 55-88 years were randomly assigned to education on a LFD (control group) or to one of two MedDiets, supplemented with either free virgin olive oil (1 liter/week) or nuts (30 g/day). No advice on physical activity was given to the study's participants. The primary end-point was diabetes incidence diagnosed by the 2009 American Diabetes Association (ADA) criteria. The study results demonstrated that MedDiet -without calorie restriction-seem to be effective in the prevention of diabetes in subjects at high CVD risk (Salas-Salvadó et al., 2011b). This was in line with results from other research groups demonstrating that MedDiets seem more effective in inducing clinically relevant long-term reduction in CVD risk factors and inflammatory mediators as compared to LFD (Nordmann et al., 2011).

In another community-based study looking at the differential effect of 3 types of diets; a low carbohydrate Mediterranean (LCM), a traditional Mediterranean (TM), and the 2003 ADA diet on the CVD risk factors in 259 diabetic patients (with their mean age = 55 years, and mean BMI = 31.4 kg/m^2 over a duration of 12-months, Elhayany and colleagues (2010) have demonstrated that this dietary intervention was effective in improving most modifiable cardiovascular risk factors (fasting plasma glucose, HbA1c and triglyceride (TG) levels) in all the dietary

groups. While only the LCM improved HDL levels and was superior to both the ADA and TM in improving glycaemic control (Elhayany et al., 2010).

Dietary Approaches to Stop Hypertension (DASH) diet has been proposed in T2DM patients as well since CVD complications are the most frequent problem among those patients (Kalofoutis et al., 2007). This diet, which is rich in fruits, vegetables, whole grains, low-fat dairy products and low in saturated fat, total fat, cholesterol, refined grains and sweets with sodium intake of 2,400 mg/day (Buse et al., 2007), has demonstrated beneficial effects on cardiometabolic risks amongst T2DM patients (Azadbakht et al., 2011).

From the above brief discussion on various diet studies, it is apparent which dietary intervention should be undertaken for overweight and T2DM patients remains a matter of choice which is further hampered by the fact that studies that compare the effectiveness of various diets are frequently limited by short follow-up period and high dropout rates.

As described in earlier chapters, diet can affect the inflammatory response to exogenous lipopolysaccharide (LPS) as supported by recent studies showing that high fat diet can result in increased endotoxemia. The latter can in turn be triggered by repeated ingestions of single high fat meals with subsequent inflammatory response (Harte et al., 2012; Laugerette et al., 2009; Ghoshal et al., 2009; Amar et al., 2008; Cani et al., 2007; Erridge et al., 2007). LPS has been implicated as a potent inducer of tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6). LPS also reduces adiponectin. In normal conditions, as in healthy individuals, the absorbed endotoxin is rapidly removed by monocytes, particularly resident Kupffer cells within the liver. Emerging evidence has indicated that chronic endotoxemia plays a

role in insulin-resistant state and obesity (Al-Attas et al., 2009; Creely et al., 2007; Pimentel et al., 2012). However to date no previous study has explored the role of different types of dietary regimens as possible strategies to reduce postprandial endotoxin absorption and/or the metabolic consequences as regard low-grade inflammation amongst T2DM and overweight subjects. Therefore this study will examine the importance of dietary intervention, what might work, and how diets may impact on inflammatory risk.

6.2 Research Design and Methods

A total of 74 Saudi women aged between 18-50 years were enrolled in a randomized interventional study at primary care centers of different regions of Riyadh city. Fifty subjects were with type 2 diabetes mellitus, and 24 subjects were overweight plus (overweight^{+}). All subjects were matched for age, BMI, and lipid profile as closely as possible. Only females, non-smokers, pre-menopausal, with a normal resting ECG and blood pressure and with no history of vascular disease were selected. In addition, subjects with known long standing diabetes and receiving diabetes medication, or those with fasting glucose > 11 mmol/L, were excluded. Moreover subjects with a fasting triglycerides > 4 mmol/L were excluded from the study. Ethical approval had been granted by the Ethics Committee of King Saud University, Riyadh, Kingdom of Saudi Arabia, prior to the commencement of the research (**Appendix I**), and each subject signed an informed consent form prior to being included in the study (**Appendix II**).

6.2.1 Medical screening

Subjects were medically screened at primary health care centers where a physician completed a medical examination of vital signs and clinical interview which included questions to determine eligibility based on inclusion and exclusion criteria. Detailed health information was obtained from all subjects using preset heath questionnaire which was previously used in large-scale epidemiologic survey in Saudi Arabia (Al-Daghri et al, 2011)**.** The interview questionnaire consists of socio-demographic data, past medical history, treatment history, sleeping hours and level of physical activity (**Appendix III**).

6.2.2 Anthropometric measurements

After signing an informed consent form, each participating subject was requested to return to her respective primary health care centre after an overnight fast (12-14 hours) two times over a three months-period (the study duration). Anthropometry and blood withdrawal were collected in the first visit. Anthropometry included height (to the nearest 0.5 cm), weight (to the nearest 0.1 kg), waist and hip circumference measurements utilizing a standardized measuring tape in cm. Systolic and diastolic blood pressure measurements were taken, as well as BMI (calculated in kg/m^2). These measurements were repeated in the second visit, after 3 months.

6.2.3 Dietary interventional program

Subjects were divided according to the assigned dietary regimen into low fat and balanced diet groups (described in a later section). In addition, food frequency questionnaire (FFQ) was the dietary tool that had been used to assess the subjects' food intake prior and post the dietary intervention (i.e. at baseline and follow up visit).

All subjects were given health advice according to their assigned dietary regimen. Energy intake was set at the levels recommended by the dietary reference intake (DRI) for female subjects with low levels of physical activity at the same age (DRI, 2008/2011).

The dietary intervention program was delivered with a combination of group, individual, and telephone contacts. Participants received monthly group sessions as

well as 1 individualized consultation at baseline visit. The group sessions were interactive between the dietitian researcher and the subjects, allowing questions to be answered and explained. Main topics discussed were: healthy eating pattern, low calorie and low fat choices, importance of fruits, vegetables and fiber, functional foods, information on food pyramid, goal setting and relapse prevention and management. Women in the intervention group were given an individualized dietary consultation according to their assigned dietary regimen (**Appendix V**). Subsequent dietician contacts (phone calls and messages) occurred every week during the study to support their compliance. The researcher interviewed the participants individually and the information was collected using a pre-designed FFQ questionnaire (**Appendix IV**) to assess the qualitative and the quantitative aspects of the food consumed by the participants over a period of 7 days (Al-Disi et al., 2010).

Nutrient intake was calculated using Food Processor USDA (18^{th} - 21^{st} Ed, 2009, 2010) Program, as for the Saudi Arabic traditional dishes were analyzed using the Arabic food analysis program (version 1, 2006). The evaluation of the daily food intake was made by means of the total energy and the total nutrient intake, as well as the percentage of the total calories derived from fat, protein and carbohydrates. Dietary nutrients values were compared with DRI for specified age and gender for macronutrients (carbohydrates and protein) as well as for micronutrients (2008/2011). Daily energy requirement (Kcal/day), were estimated using WHO equation (2005, 2007) (Sylvia Escott-Stump, 2011) according to age group and metabolic status. Dietary fat percentage has been estimated as 25% from daily energy requirement (Hooper et al., 2011) (**Appendix VI**).

6.2.3.1 Prescribed Dietary regimen

The project dietitian enrolled participants and randomly allocated them to their assigned dietary regimen as follows, because this was a dietary intervention, patients were not blinded.

1. Low fat diet group: fat is the most energy-rich of all energy-providing nutrients, therefore reducing fat intake results in reducing total energy intake to induce weight loss. This diet defined as total fat intake < 30% of total energy intake and saturated fat intake <10%. This diet emphasizes on the intake of vegetables, fruits, starches (e.g., breads/crackers, pasta, whole grains, starchy vegetables), lean protein, and low-fat dairy products (National Heart, Lung, and Blood Institute, 2005). Participants in this group were directed to avoid fried and processed foods and were provided with low fat recipes and substitutes.
2. Balanced diet (the composition of this diet was similar to the Mediterranean diet style, so-called prudent diet) which includes abundant plant foods (fruits, vegetables, breads, other forms of cereals, potatoes, beans, nuts, and seeds), fresh fruit as the typical daily dessert, olive oil as the principal source of fat, dairy products (principally cheese and yogurt), and fish and poultry consumed in low to moderate amounts, zero to four eggs consumed weekly, red meat consumed in low amounts, and normally with meals. This diet is low in saturated fat (≤7-8% of energy), with total fat ranging from < 25% to > 35% of energy (Willett et al., 1995).

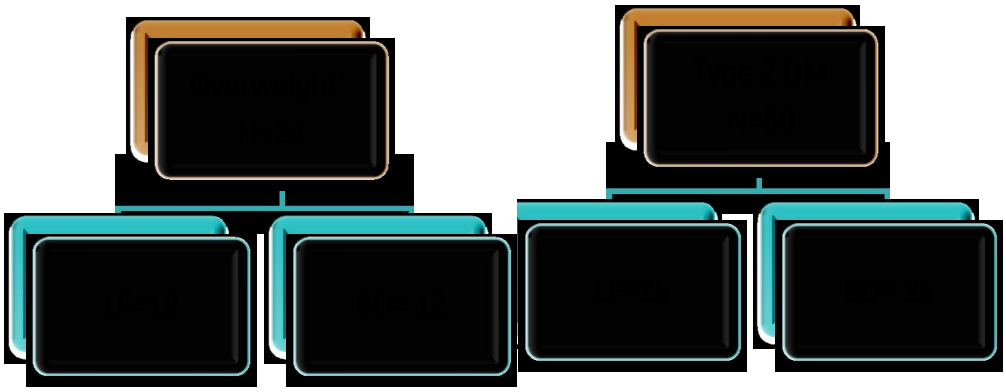


Figure 6.2.3.1.1 The different arms of the interventional trial proposed: T2DM and overweight^{+} subjects were subdivided into two groups according to the assigned dietary regimen (LF = Low fat diet) group and (BD = Balanced diet) group**.**

6.2.4 Biochemical assessment

All subjects were requested to submit an over-night fasting blood samples from which the different metabolic parameters were assessed. Serum samples were stored in a -20 °C freezer prior to analysis. Fasting glucose and lipid profiles were measured using a chemical analyzer (Konelab, Vantaa, Espoo). Fasting insulin was determined using by electrochemiluminescence method (ECLIA) (COBAS E 411; Roche Diagnostics, Mannheim, Germany). The instruments were calibrated prior to analysis using quality control samples provided with the kits. Serum Endotoxin was analyzed using a commercially available QCL-1000 LAL Endpoint Assay (Lonza, New Jersey, USA). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting insulin (µU/mL) × fasting glucose (mmol/L)/22.5 (Bonora et al, 2002). These parameters were measured at baseline and after 3 months (3 months study duration).

6.3 Data Analysis

Data was analyzed using SPSS version 16.5 (SPSS, Chicago, IL, USA). All variables were presented as mean ± standard deviation. For comparison between baseline and post-intervention, paired T-test (for normally distributed variables) and Wilcoxon signed-rank tests (for insulin, TG and HOMA-IR) were used. Bivariate correlation was used to test associations between macronutrients and cardiometabolic risk factors. Significance was set at $p<0.05$.

6.4 Results

6.4.1 Effects of Low Fat versus Balanced Diet in the Anthropometric and Clinical Parameters of the T2DM Group

Table 6.4.1.1 shows the comparison between low fat and balanced diet interventions in the T2DM group. In the low fat diet program, significant improvements were noted in the anthropometric measures in terms of significantly lower weight [baseline 85.4±3.1kg versus follow-up:84.1±3.2kg; $p<0.05$], BMI [baseline 34.5±4.9kg/m^2 versus follow-up: 34.0±5.0kg/m^2; $p<0.05$], waist circumference [baseline 115.1±11.9cm versus follow-up: 110.0±12.1cm; $p<0.01$], and WHR [baseline 0.98±0.01 versus follow-up: 0.95±0.1; $p<0.05$] in the follow-up as compared to baseline. Furthermore, with the exception of HDL-cholesterol which modestly increased after follow-up, all the measured lipids, including glucose, decreased after 3 months of intervention. These improvements were most notable in the total cholesterol [baseline 5.4±1.2mmol/l versus follow-up: 4.7±1.1mmol/l; $p<0.05$] and LDL-cholesterol levels [baseline 3.7±0.9mmol/l versus follow-up: 3.1±0.9mmol/l; $p<0.05$]. The rest of the differences were not significant.

In contrast, only modest improvements in all parameters were observed among the T2DM subjects under the balanced diet program. Only two lipid parameters achieved statistical significance, namely total [baseline 5.3±0.9mmol/l versus follow-up: 4.5±0.8mmol/l; $p<0.01$] and LDL-cholesterol [baseline 3.5±0.8mmol/l versus follow-up: 2.8±0.7mmol/l; $p<0.01$], after comparing baseline and follow-up values (Table 4.6.1.1).

Table 6.4.1.1 Differences in Anthropometric and Clinical Parameters According to Diet Type (T2DM Group)

Parameters	Low Fat Diet N = 25		Balanced Diet N = 25	
	Baseline	**Post-Intervention**	**Baseline**	**Post-Intervention**
Weight (kg)	85.36 ± 3.11	84.11 ± 3.18*	79.41 ± 12.4	78.7 ± 11.6
BMI (kg/m^2)	34.51 ± 4.94	34.01 ± 5.0*	33.8 ± 4.63	33.53 ± 4.47
Waist (cm)	115.1 ± 11.9	110.03 ± 12.1**	110.53 ± 14.9	106.81 ± 10.7
Hips (cm)	117.9 ± 11.9	116.03 ± 12.55	115.47 ± 10.4	115.5 ± 10.55
WHR	0.98 ± 0.08	0.95 ± 0.07*	0.96 ± 0.08	0.93 ± 0.06
Glucose (mmol/l)	7.51 ± 2.58	6.6 ± 1.2	8.63 ± 2.83	7.73 ± 2.02
Insulin (IU/ml)#	11.1 ± 5.0	8.1 ± 5.3	12.8 ± 6.8	13.4 ± 6.6
HOMA-IR#	3.2 ± 1.6	2.2 ± 1.5	4.5 ± 2.4	4.3 ± 2.5
Triglycerides (mmol/l)#	2.04 ± 1.32	1.77 ± 0.92	1.78 ± 0.77	1.5 ± 0.71
Total Cholesterol (mmol/l)	5.44 ± 1.24	4.72 ± 1.11*	5.29 ± 0.91	4.48 ± 0.81**
HDL-Cholesterol (mmol/l)	0.95 ± 0.26	0.98 ± 0.25	0.98 ± 0.18	1.01 ± 0.24
LDL-Cholesterol (mmol/l)	3.71 ± 0.91	3.06 ± 0.94*	3.5 ± 0.75	2.79 ± 0.69**
Endotoxin (EU/ml)	3.3 ± 0.72	3.3 ± 0.73	3.3 ± 1.0	3.3 ± 1.4

Note: Data presented as mean ± standard deviation; # not normally distributed; * denotes significance at 0.05 level; ** denotes significance at 0.01 level.

6.4.2 Effects of Low Fat versus Balanced Diet in the Dietary Intake of the T2DM Group

Table 6.4.2.1 shows the differences in the macronutrient intake of T2DM subjects assigned in low fat versus balanced diet. In the low fat diet, there were strong significant decreases in the follow up visit than baseline in terms of carbohydrate [baseline 182.1±72.9g versus follow-up: 148.7±60.0g; $p<0.01$], protein [baseline 60.9±19.7g versus follow-up: 45.8±14.9g; $p<0.01$], and dietary fat [baseline 83.2±23.4g versus follow-up: 57.6±14.7g; $p<0.01$] intakes as well as total calories [baseline 1720.5±396.7kcal versus follow-up: 1295.9±294.1kcal; $p<0.01$]. Similar significant differences were also observed in the balanced diet group, except

that the significantly lower carbohydrate intake [baseline 220.2±89.2g versus follow-up: 183.3±73.6g; $p<0.05$] after 3 months was statistically weaker than the significance achieved by the low fat diet group in the same macronutrient. In both groups, there were no statistically significant differences in fibre intake in baseline and follow-up. Worthy to note however was the modest increase in the post intervention fibre intake of T2DM subjects from the low fat diet group as compared to baseline [baseline 13.4±3.4g versus follow-up: 14.2±6.1g; p=NS] (Table 6.4.2.1).

Table 6.4.2.1 Differences in Dietary Intake According to Diet Type (T2DM Group)

Parameters	Low Fat Diet N = 25		Balanced Diet N = 25	
	Baseline	**Post-Intervention**	**Baseline**	**Post-Intervention**
Carbohydrates				
Grams	182.1 ± 72.9	148.7 ± 60.0**	220.2 ± 89.2	183.3 ± 73.6*
% DRI	140.1 ± 56.1	114.4 ± 46.1**	165.9 ± 29.6	149.2 ± 36.8*
Protein				
Grams	60.87 ± 19.7	45.75 ± 14.9**	63.3 ± 28.2	44.6 ± 22.6**
% DRI	132.3 ± 42.9	99.5 ± 32.4**	137.5 ± 61.3	96.9 ± 49.1**
Fats				
Grams	83.19 ± 23.4	57.6 ± 14.69**	89.3 ± 24.3	66.0 ± 20.6**
% DRI	194.3 ± 50.8	134.0 ± 32.4**	137.5 ± 61.3	96.9 ± 49.1**
Fibre				
Grams	13.4 ± 3.4	14.2 ± 6.1	13.9 ± 3.5	13.7 ± 3.7
% DRI	53.7 ± 13.7	56.9 ± 6.3	55.7 ± 14.2	54.8 ± 14.7
Total Calories				
Kilocalories	1720.5 ± 396.7	1295.9 ± 294.1**	1937.5 ± 323.0	1505.9 ± 271.9**
% DRI	115.4 ± 23.0	86.2 ± 18.1 **	117.9 ± 26.3	98.7 ± 19.9**

Note: Data presented as mean ± standard error; * denotes significance at 0.05 level; ** denotes significance at 0.01 level.

6.4.3 Effects of Low Fat versus Balanced Diet in the Anthropometric and Clinical Parameters of the Overweight^{+} Group

Table 6.4.3.1 shows the comparison between low fat and balanced diet interventions in the overweight^{+} group. With 24 overweight subjects, 12 subjects were assigned for each dietary intervention. In the low fat diet program, significant improvements were most noted in the total [baseline 4.8±0.9mmol/l versus follow-up: 3.9±0.8mmol/l; $p<0.05$] and LDL-cholesterol levels [baseline 3.0±0.4mmol/l versus follow-up: 2.5±0.6mmol/l; $p<0.05$]. The rest of the differences were not significant. Other observed, but not statistically significant changes include modest decreases in weight [baseline 73.8±0.9kg versus follow-up: 71.9±4.4kg; p=NS], BMI [baseline 28.6±0.6kg/m^2 versus follow-up: 27.8±2.8kg/m^2; p=NS], triglycerides [baseline 1.8±1.2mmol/l versus follow-up: 1.7±0.6mmol/l; p=NS] and HDL-cholesterol [baseline 1.2±0.4mmol/l versus follow-up: 0.9±0.2mmol/l; p=NS] (Table 6.4.3.1).

The overweight^{+} subjects in the balanced diet group had a more favourable outcome anthropometrically than their low fat diet counterparts in terms of improved weight [baseline 68.9±5.7kg versus follow-up: 67.0±5.8kg; $p<0.05$], BMI [baseline 28.5±1.6kg/m^2 versus follow-up: 27.7±2.1kg/m^2; $p<0.05$] and hip circumference [baseline 111.6±4.8cm versus follow-up: 107.9±2.9cm; $p<0.01$] after 3 months of intervention. In terms of glucose and lipid measures, both the low fat diet and balanced diet are similar with respect to significantly lower total [baseline 4.3±1.0mmol/l versus follow-up: 3.7±0.8mmol/l; $p<0.05$] and LDL-cholesterol [baseline 2.7±0.8 versus follow-up: 2.2±0.6; p-value<0.05] in the follow-up as

compared to baseline. The rest of the changes in lipids, including glucose, were m o d e s t .

Table 6.4.3.1 Differences in Anthropometric and Clinical Parameters According to Diet Type (Overweight^{+} Group)

Parameters	Low Fat Diet N = 12		Balanced Diet N = 12	
	Baseline	**Post-Intervention**	**Baseline**	**Post-Intervention**
Weight (kg)	73.82 ± 0.94	71.92 ± 4.36	68.87 ± 5.7	67.0 ± 5.8*
BMI (kg/m^2)	28.58 ± 1.0	27.8 ± 2.8	28.48 ± 1.57	27.73 ± 2.08*
Waist (cm)	94.3 ± 7.9	94.3 ± 8.9	99.12 ± 7.78	95.94 ± 8.28
Hips (cm)	110.5 ± 5.6	109.2 ± 6.1	111.56 ± 4.8	107.88 ± 2.94**
WHR	0.86 ± 0.07	0.87 ± 0.07	0.89 ± 0.04	0.89 ± 0.06
Glucose (mmol/l)	4.78 ± 0.47	4.57 ± 0.55	4.67 ± 0.43	4.49 ± 0.52
Insulin (IU/ml)#	5.3 ± 3.6	4.6 ± 2.4	5.0 ± 3.4	4.7 ± 3.3*
HOMA-IR#	1.1 ± 0.8	0.93 ± 0.6	1.1 ± 0.7	0.93 ± 1.89
Triglycerides (mmol/l)#	1.81 ± 1.16	1.66 ± 0.62	0.95 ± 0.41	1.08 ± 0.35
Total Cholesterol (mmol/l)	4.84 ± 0.86	3.91 ± 0.85*	4.32 ± 1.05	3.71 ± 0.76*
HDL-Cholesterol (mmol/l)	1.22 ± 0.42	0.88 ± 0.19	1.16 ± 0.33	0.98 ± 0.05
LDL-Cholesterol (mmol/l)	3.0 ± 0.42	2.48 ± 0.55*	2.73 ± 0.79	2.24 ± 0.61*
Endotoxin (EU/ml)	3.1 ± 0.47	3.18 ± 0.53	2.9 ± 0.5	2.5 ± 0.82

Note: Data presented as mean ± standard deviation; # not normally distributed * denotes significance at 0.05 level; ** denotes significance at 0.01 level.

6.4.4 Effects of Low Fat versus Balanced Diet in the Dietary Intake of the Overweight^{+} Group

Table 6.4.4.1 shows the differences in the macronutrient intake of overweight^{+} subjects under low fat versus balanced diet intervention. In the low fat diet, there were strong significant decreases in the follow up visit than baseline in terms of fibre intake [baseline 8.2±1.7g versus follow-up: 6.1±2.3g; $p<0.01$] as well as total calories [baseline 1921.2±167.6kcal versus follow-up: 1629.4±166.7kcal;

p<0.01]. A significantly lower fat intake post intervention was also observed [baseline 94.8±14.9g versus follow-up: 75.0±17.4g; p<0.05]. Both carbohydrate and protein intake were lower in follow up than baseline, but did not achieve statistical significance. In the balanced diet group, a lower but not significant difference was noted in the carbohydrate, protein and fat intake in follow-up than baseline. In contrast to the low fat diet group, which had significantly lower fibre intake post intervention, a modest increase in the fibre intake was noted in the balanced diet group. Only total calories achieved a statistically lower value in follow-up than baseline [baseline 2023.6±365.2kcal versus follow-up: 1713.9±461.6kcal; p<0.05] (Table 6.4.4.1).

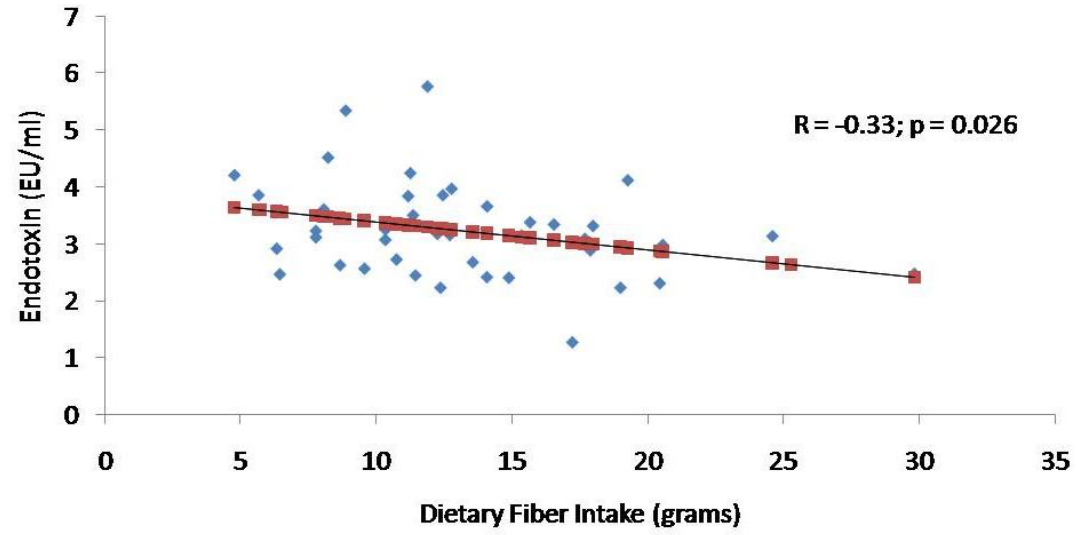
Table 6.4.4.1 Differences in Dietary Intake According to Diet Type (Overweight^{+} Group)

Parameters	Low Fat Diet N = 12		Balanced Diet N = 12	
	Baseline	**Post-Intervention**	**Baseline**	**Post-Intervention**
Carbohydrates				
Grams	198.9 ± 42.7	179.1 ± 34.2	215.6 ± 38.4	194.0 ± 47.8
% DRI	153.1 ± 32.9	137.8 ± 26.3	165.9 ± 29.6	149.2 ± 36.8
Protein				
Grams	68.0 ± 29.5	59.5 ± 19.2	62.0 ± 20.1	53.4 ± 15.9
% DRI	147.86 ± 64.2	129.4 ± 41.8	134.8 ± 43.8	116.2 ± 34.6
Fats				
Grams	94.8 ± 14.9	75.0 ± 17.44*	101.4 ± 32.5	80.4 ± 33.6
% DRI	173.76 ± 33.86	136.2 ± 28.7*	134.8 ± 43.8	116.2 ± 34.6
Fibre				
Grams	8.2 ± 1.69	6.13 ± 2.29**	12.3 ± 9.4	13.1 ± 8.6
% DRI	32.7 ± 6.77	24.6 ± 9.2**	49.3 ± 37.9	52.5 ± 34.4
Total Calories				
Kilocalories	1921.2 ± 167.6	1629.4 ± 166.7**	2023.6 ± 365.2	1713.9 ± 461.6*
% DRI	101.9 ± 9.7	86.3 ± 7.7**	117.9 ± 26.3	99.6 ± 28.6*

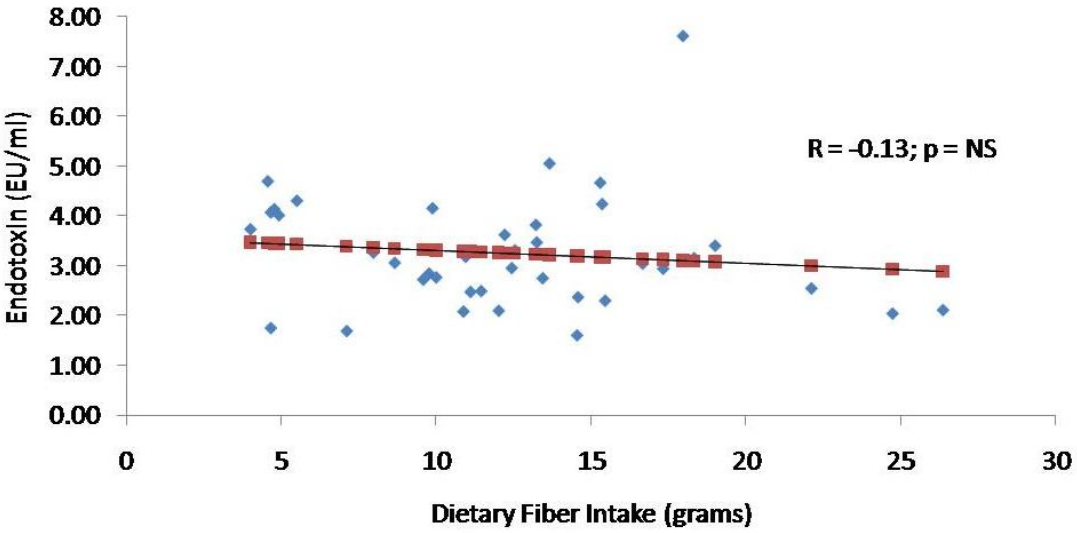
Note: Data presented as mean ± standard error; * denotes significance at 0.05 level; ** denotes significance at 0.01 level.

6.4.5 Associations of macronutrient intake to variables measured post intervention

Bivariate associations were examined in both the T2DM and overweight^{+} group to determine existing correlations between the macronutrient intake, anthropometrics, lipid profiles, glucose and endotoxin. A significant inverse association between endotoxin and dietary fibre intake was observed in all subjects at baseline (R=-0.34; p=0.024) but was lost after follow-up (Figure 6.4.5.1). Furthermore, a near significant positive association was noted between dietary fibre intake and HDL-cholesterol also at baseline, but not after intervention (Figure 6.4.5.2). These associations were lost after stratification to T2DM and overweight^{+}. No other associations were elicited.

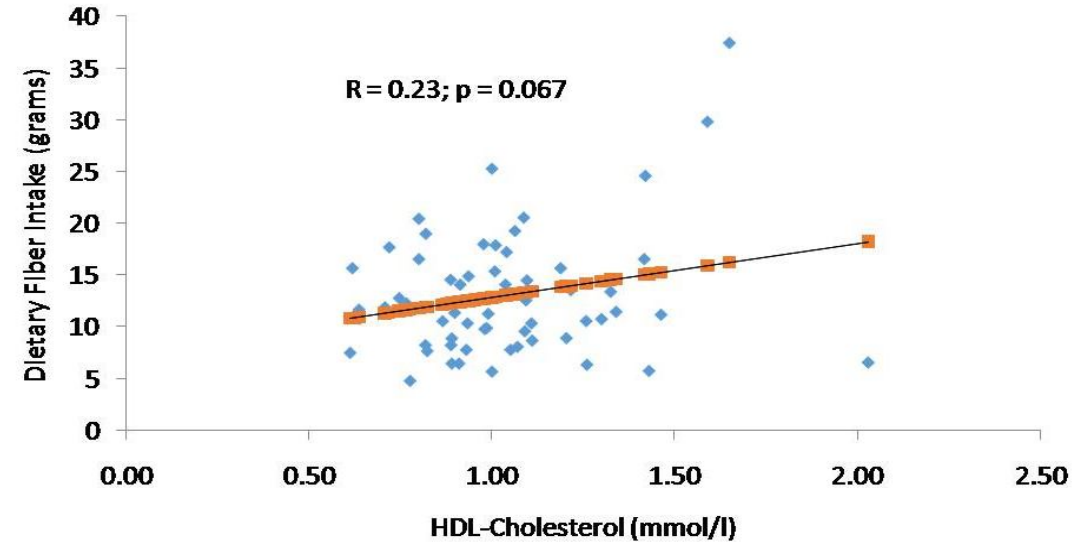


A. Baseline

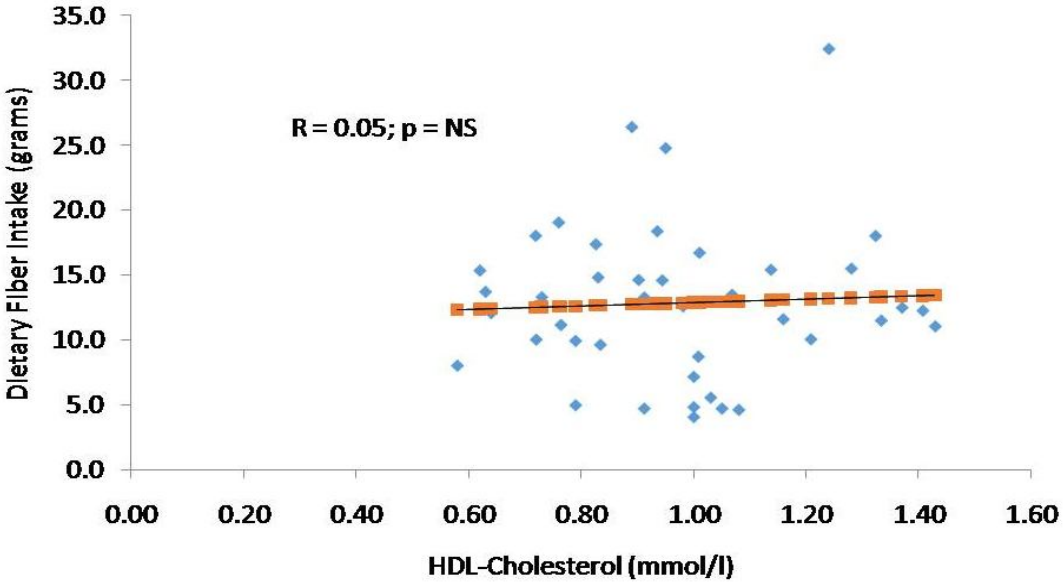


B. Post Intervention

Figure 6.4.5.1 Inverse association between endotoxin and dietary fibre intake at baseline (A) and post intervention (B)



A. Baseline



B. Post Intervention

Figure 6.4.5.2 Positive association between dietary fibre intake and HDL-Cholesterol at baseline (A) and post intervention (B)

6.5 Discussion

In this chapter the effects of two types of 3-month dietary interventions (low fat versus balanced diet) were examined to determine the effects on anthropometric, lipids, glucose and endotoxin profile of overweight^{+} and T2DM patients. Upon comparing baseline and follow-up results, it appeared that T2DM patients had a more favourable response to the low fat as opposed to the balanced diet, while the overweight^{+} group seemed to benefit more from the balanced diet rather than the low fat diet. The more improved metabolic profile in the T2DM group receiving the low fat diet may have been the result in part due to significant weight loss, a finding not observed in the balanced diet group. Hypocaloric diets aimed at reducing dietary fat intake has been shown to sustain a modest but significant weight loss, which in turn may reduce the pathogenesis of T2DM complications such as cardiovascular diseases (Swinburn et al, 2001; Hooper et al, 2012). However, while the intervention in the study itself implied "low fat diet", the significant caloric restrictions was not limited to dietary fat alone, since significantly lower differences were also observed in carbohydrates and proteins and total caloric intake, which, cumulatively, improved the over-all metabolic profile of T2DM patients in this group. This is important to note since reductions in dietary fat intake with no change in total caloric intake, for example, is an ineffective weight loss strategy (Walker and Parker, 2014).

On the same premise weight loss also led to a better cardiometabolic profile in the overweight^{+} group and although the group benefited from both dietary interventions, the improvements were more apparent with the balanced diet intervention. A well-balanced diet, on its own, has been shown to induce fat loss in both animal and human studies (Gerbaix et al, 2013; Di Daniele et al, 2013).

Furthermore, while the low fat diet intervention in the present study significantly reduced the total caloric intake post intervention, this did not translate to significant weight loss. One explanation for the lack of weight loss may be due to changes in dietary fibre intake, which significantly decreased in the low fat group after 3 months. Dietary fibre is important for weight loss, since it increases total energy expenditure by providing the added thermic heat in foods (Higgins, 2014). Furthermore, recently identified genetic variants that influence weight loss such as TCF7L2 have been shown to be modulated by dietary fibre intake (Haupt et al, 2010; Heni et al, 2012). Several meta-analyses studies also showed an independent association between dietary fibre intake and coronary heart disease risk, with a dose dependent cardioprotective effect (Wu et al, 2014; Pereira et al, 2014). Another notable finding from this current study was the inverse association between endotoxin and dietary fibre intake in all subjects at baseline. Dietary fibre has been shown to improve intestinal barrier and decrease bacterial translocation secondary to endotoxinaemia in animal studies (Hou et al, 2010; Wan et al, 2010; Sherry et al, 2010). In humans, a high fibre diet has been shown to prevent increases in endotoxin levels, most likely through the modulation of certain gut hormones which in turn, strengthens the intestinal wall (Weickert and Pfeiffer, 2008). Compared to a high fat meal challenge, which causes acute rise in circulating endotoxin levels, a high fibre diet does not seem to alter endotoxin concentrations (Ghanim et al, 2009; Harte et al, 2012). Aside from the protective effect of dietary fibre against endotoxinaemia, the present study also noted a borderline significant association between HDL-cholesterol and dietary fibre intake. This confirms the well-established benefits of increased fibre consumption in improving certain blood lipid components and even

T2DM, secondary to its viscous and gel forming properties (Weickert and Pfeiffer, 2008; Isken et al, 2010; Nutall, 1993).

In both the T2DM and overweight^{+} groups, the first metabolic risk factors to improve were total and LDL-cholesterol independent of weight loss. These favourable changes in cholesterol levels affirm other similar short term intervention studies (Milsom et al, 2014; Chen et al, 2009; Kim et al, 2012). It has been well known that plasma lipids are subject to fairly rapid changes depending on the dietary fatty acid intake in energy-balance states, with decreases in total and LDL-cholesterol apparent for active weight loss and improvements HDL-cholesterol depending on energy restriction duration as well as type of dietary fat (Noakes and Clifton, 2000).

The present study was limited by the small sample size. Several modest improvements including HDL-cholesterol and other anthropometric measures may have been more apparent if a larger cohort was used. The findings of the study are also limited to women who were overweight^{+} and/or had T2DM. As such, findings may not be applicable to men and children. How the type of diet restriction affects other markers (inflammation, aging, immunity) aside from the conventional biomarkers used in the present study warrant future investigations.

Nevertheless and as it stands, there is scarcity of information with regards to dietary intervention studies among patients with T2DM in Saudi Arabia and in the Gulf region in general. Thus, findings from this chapter have significant strengths and clinical implications, especially in the kingdom of Saudi Arabia where the prevalence of T2DM and obesity is high (Al-Daghri et al, 2011).

In conclusion, a 3-month low fat diet without exercise resulted to a more favourable metabolic profile in the T2DM group, while a balanced diet, again without exercise, seems to be more favourable amongst the overweight^{+} group, suggesting that a uniform weight loss strategy through dietary interventions may not apply in all high risk groups. A customized approach may prove effective in the over-all aim of T2DM prevention in this population. Further studies on a larger scale, taking into consideration other populations such as men, high-risk children and the elderly are needed to identify which dietary interventions will prove most beneficial.

Chapter 7

Final Discussion

7.1 Discussion

The increasing importance of endotoxin (metabolic endotoxinaemia) as a mediator of sub-chronic inflammation amongst patients with obesity and insulin resistance has been gaining supportive evidence over the past few years. Metabolic endotoxinaemia appears a causal factor in obesity- and insulin resistance-related inflammatory states (Boroni Moreira and de Cassia Goncalves Alfenas, 2012), and this factor appears to be regulated through diet and dietary interventions that can change the gut microbiota and endotoxin over load (Kemp, 2013). Furthermore, there is also increasing evidence that suggests that the endotoxin binding protein, known as the lipoprotein-binding protein, which is found both systemically and at the surface of adipocytes, when coupled with endotoxin can promote inflammation in insulin-resistance states and enhance adipose tissue dysfunction (Liu et al, 2014; Moreno-Navarrete et al, 2011; Moreno-Navarrete et al, 2013). As such the ability to change microbiota appears key and previous studies recommend exploration of diet and nutrient intake which improve the microbial imbalance known as dysbiosis, which is observed in patients with obesity and T2DM. To date however, there are limited studies focusing on the effects of dietary interventions on circulating endotoxin levels in either animal (Oz et al, 2013; Luyer et al 2004) or human studies (Lira et al, 2012, Xiao et al, 2014). Consequently and to the best of our knowledge, population-based studies utilising the Arab population, who are at heightened metabolic risk, as not been undertaken before and represents a novel and urgent study.

From early investigations of the dietary intervention that was undertaken for this thesis it was observed that the cardiometabolic parameters of adult Saudi women

per se was significantly different from one another, and that this was largely dependent on their current metabolic state. From the analysis of the patients it was observed the anthropometric measurements, glucose and lipids were higher in T2DM subjects than overweight and control subjects secondary to the underlying insulin-resistance and metabolic syndrome present in these subjects (Boden and Laakso, 2004). It has also been observed that the aforementioned risk factors appear to impact on intestinal permeability, translating to a weakened epithelial barrier function in the T2DM subjects and to some extent, the overweight group, leading to a significantly higher circulating endotoxin capability as compared to control subjects as noted in other studies as well (de Kort et al, 2011).

Furthermore following a standardized high fat meal, post prandial lipidaemia was evident in all groups but more so for the overweight^{+} and T2DM group, confirming previous studies on high fat meal's exacerbating effects in the lipid profiles of at-risk groups (Madhu et al, 2008; Kumar et al, 2010) secondary to the lower peripheral clearance of high fat meals in patients with obesity and insulin resistance as compared to lean healthy subjects (Lambert and Parks, 2012). Post prandial endotoxemia was also evident in the T2DM and overweight^{+} groups compared with the control group suggesting there is a heightened sub-inflammatory risk following a high fat meal in Arab women in a similar fashion so the previous studies on South Asian women with and without obesity and T2DM (Harte et al, 2012). The findings from the early results chapter reinforce previous findings on metabolic endotoxemia, its associations to known cardiometabolic indices and its response to a high fat meal challenge. To the best of our knowledge, these observations are the first of its kind in the Middle-Eastern region. Clinical implications of these findings are therefore far-reaching, especially in a region where

existing culture and traditions are already favorable to the development of non-chronic communicable diseases, and only made worse by the rapid industrialization and burgeoning rise of fast food chains.

In the subsequent chapters of the thesis it was observed how a 3-month caloric restriction diet can induced beneficial cardiometabolic effects in the overweight^{+} and T2DM subject groups. Whilst is was already anticipated that there would be weight loss on such a diet it was interesting to observe how dietary modifications could also improve the cardiometabolic health of subjects (Wygant et al, 2014; Soare et al, 2014; Speakman and Mitchell 2011). Although the study may seem relatively simple in outlook there is a severe lack of dietary intervention studies in the region, specifically in Saudi Arabia, where most prospective studies focus on the effects of health during the holy month of Ramadan which has nothing to do with caloric restriction (Aljabnoor et al, 2014; Al-Mendalawi, 2011; Khatib and Shafagoj 2004). Furthermore most other studies in the region appear to provide review analysis or cross-sectional studies on the dietary habits of the population (Al-Muammar et al, 2014; Al-Khudairy et al, 2013; Al-Daghri et al, 2013). Thus these studies were undertaken to provide the first pieces of evidence that such dietary intervention can work in a Saudi culture to fill the gaps in existing knowledge. Interestingly, the responses of both the T2DM and overweight^{+} groups varied depending on the type of dietary intervention provided (low fat versus balanced-diet), with the low fat diet being more favorable in the T2DM group and the balanced diet more compatible with the overweight group, suggesting a tailored strategy in the management of these metabolic conditions in the Middle-Eastern population.

Further findings also explored the significance of dietary components, such as protein and carbohydrate on health which observed that fibre contents appeared to be important. These current studies highlighted the significant inverse relationships between dietary fibre, HDL-cholesterol and circulating endotoxin. Dietary fibre has been previously observed to strengthen the intestinal barrier and inhibit endotoxin release in the circulation in animal studies (Hou et al, 2010; Wan et al, 2010) and is known to be protective against a plethora of gastronintestinal diseases in humans (Otles and Ozgoz, 2014). Inulin, which is a form of dietary fibre, was observed to improve metabolic endotoxemia and other inflammatory markers in women with T2DM (Dehghan et al, 2014; Poughassem et al, 2013), and increased dietary fibre intake through supplementation on its own or in addition to caloric restriction can induce beneficial weight loss (Reichert et al, 2014). The ability of dietary fibre to induce weight loss is due its properties to increase production of glucagon-like peptide (GLP-1) and peptide YY (PYY), hormones that induce satiety, as well as its ability to decrease both energy and fat intake (Lattimer and Haub, 2010), making it clinically useful as a non-pharmacologic intervention in improving cardiovascular health in obese and T2DM patients (Fujii et al, 2013). Taken together, it appears that the promotion of dietary fibre intake in the overweight, obese and T2DM population in the Middle East has a potential role in decreasing risk of metabolic endotoxemia and improving their over-all cardiometabolic conditions.

7.2 Limitations of the Current Studies

Some of the caveats have already been mentioned in the previous chapters, however the subjects used in these studies were all women this was due to religious and cultural reasons that prohibit the student from interacting with the opposite gender. Hence, the findings may not be applicable to overweight and T2DM Arabic men. Furthermore whilst adipose tissue is important in the inflammation response and the inflammatory gene expression could have changed over a 3 month dietary intervention study, no adipose tissue biopsies were obtained. This lack of adipose tissue biopsies was in part as there was a lack of previous Arab studies to determine whether the dietary intervention would work successfully in a Saudi environment, Furthermore it was considered that an additional procedure may have significantly impacted on subject recruitment and jeopardized the principal study objectives. As such, there are limited mechanistic insights from the studies undertaken; although subsequent studies could examine this aspect which was felt to be beyond the scope of this thesis. The lack of control subjects in the intervention study could be considered another limitation as we could not assess the direct effect with the control group at three months. Nevertheless it was deemed inappropriate for the control healthy lean group to go on such a diet by the ethics committee. With respect to the methods, the validity of the technique used for the quantification of endotoxin (LAL assay) has been previously questioned despite being the most widely used assay for this type of investigation and validated in the labs on my thesis research; however as with all studies, findings should be interpreted with caution (Creely et al, 2007; Hansen et al, 2013). Lastly, although the dietary questionnaires used have been previously utilized in several publications from the group, it has not been validated and this may still introduce bias in the results. Nevertheless, the studies conducted

have considerable merits in being the first dietary intervention study in the Middle East focusing on metabolic endotoxemia among women with varying metabolic states.

7.3 Future Directions

This thesis has highlighted the clinical importance of diet and nutrition as an important strategy in the reduction of obesity and T2DM in the women of Middle Eastern region. As one of the first dietary interventional studies performed in the Arab region, the thesis suggests that despite the lack of readily definable portion sizes due to the buffet style of eating in Saudi there are opportunities to undertake future successful prospective investigations. One of these studies could extend the time the Saudi women were studied for, as due to time restrictions the current thesis studies only examined diet over a 3 month duration with avoidance of Ramadan noting the second study would have to involve Ramadan as part of the study time. Furthermore the current studies were only undertaken in women and may not be applicable to men or the same success might not have been achieved. Therefore now with this baseline study it would be of interest to see whether men show the same levels of benefit over the 3 month period. Furthermore the effect of a high fat oral challenge should be investigated in Arab men subjects as well to see gender-specific differences in cardiometabolic parameters, including endotoxin. It would also be important to investigate in detail how different nutritional compositions in the diet can alter endotoxin levels and the gut microbiota in general which in turn will lead to over-all improvement in the cardiovascular health of at risk patients. Future interventional clinical trials are warranted to serve as compelling evidence of the role of subtle dietary intervention to improve health and lower the economic burden of

metabolic disease in the Saudi health system. Finally, the exploration on the roles of dietary fibre appears important as well as other products such as probiotics that can promote beneficial bacterial floras, improve gut barrier integrity lowering systemic metabolic endotoxinaemia. Subjects that can undertake subtle dietary interventions to begin to lower metabolic risk could have a large impact on health. Such studies may also help patients to reduce food intake and boost the already clear benefits of caloric restriction observed in the present thesis. A clinical trial on the use of probiotics in an Arab population has just begun and the results of this will be interested and may support further ways to promote metabolic health in Saudi (Alokail et al, 2013).

7.4 Conclusion

This current thesis undertaken exclusively on Saudi Arabian women with varying metabolic states, has sought to expand our knowledge and understanding on how metabolic endotoxemia differs in the presence of T2DM, obesity and weight loss compared to lean, non-T2DM subjects. Also these studies have examined how an acute high fat oral challenge exacerbates cardiometabolic and inflammatory conditions of at risk groups, and how a 3-month caloric restriction in the absence of exercise and other pharmacologic interventions may still confer beneficial weight loss that translates to immediate improvement in cardiometabolic health. Whilst it is clear that longer prospective studies, with the inclusion of other populations such as men and children at risk are necessary to reinforce the present findings, the current observations from this present study offer tremendous clinical potential in the over-all aim of curbing the prevailing obesity and T2DM epidemic engulfing the Middle East region.

Appendices

Appendix I: Ethical Approval

بسم الله الرحمن الرحيم

Kingdom of Saudi Arabia
Ministry of Higher Education
College of Medicine
& King Khalid Univ. Hospital

المملكة العربية السعودية
وزارة التعليم العالي
جامعة الملك سعود
كلية الطب
ومستشفى الملك خالد الجامعي

25.10.1431
Date 04.10.2010 التاريخ : No.: 10/2761/IRB الرقم :

Dr. Assim Abdulaziz Alfadda
Associate Professor
Department of Internal Medicine

Subject: **Project No. 10-173**
"A dietary interventional study moderating fat intake in Saudi subjects with metabolic disease"

Dear Dr. Alfadda,

Thank you for your letter dated 27.09.2010 in response to the IRB comment raised in meeting no. 9 held on 25 Rajab 1431 (07 July 2010). The Board reviewed your response and found that you have adequately fulfilled the requirements; the above-mentioned project is now approved.

Wishing you success in your research.

Sincerely yours,

Prof. Ahmed S. BaHammam
Chairman
Institutional Review Board
College of Medicine

Received.
12/10/10

Appendix II: Consent form

Title of the Research Project
A dietary interventional moderating fat intake in Saudi subjects with metabolic disease.

You are being asked to participate voluntarily in a Research Study. If you decide to take part in this study, please sign this consent form and return it.
STUDY PURPOSE: to study the effect of fat rich meal, diet and dietary supplement on inflammatory biomarkers among Saudi subjects with metabolic disease.
STYDY PLAN: Informed consent will be obtained from all participants. Subjects will be grouped into one of the following: Type 2 diabetes (on diet alone), overweight subjects and insulin resistant group. These subjects will eat, on different days: (1) a high-fat meal (whipping cream) and (2) glucose solution. Blood samples will be drawn at 0,1,2,3, and 4 hours (5 times), and the following biomarkers will be assayed: endotoxin, blood glucose level, plasma lipid level, insulin resistance.
BENEFITS: The result of this study may help you to improve your life style by adapting a healthy dietary regimen that is considered a mean to reduce the risk of metabolic disease and their complications. In addition, in the future with God's will the patients will benefit from the knowledge acquired.
SIDE EFFECT: There are no side effects. Your participation in this study does not have any further risks or discomfort to you. If you are diabetic; the meal provided may increase the blood glucose level which will be controlled by the researcher.
REFUSEL: If you refuse to participate, in this study will be kept confidential. The results of this research may be published, however your identity will never be revealed.
APPROVAL: I fully understand the information and the consent form; therefore I agree to participate in this study. In addition, I do not mind using the all the components of the samples obtained from me in this and in future studies by the investigators in the Obesity Research Center and their collaborators. This form has been explained to the participant by one of the investigators before his signature.
I sign freely and voluntarily. A copy has been given to me.
Investigator or Associate:
Dr.
Signature Date:
Patient Name:
Signature Date:
Witness Names:
Signature Date:

If you have any further concerns or questions, you can contact Dr. Assim A. Al-Fadda (email: aalfadda@ksu.edu.sa)

Appendix III: Interview Questionnaire

Serial No. :……...			**Date: / / 201**		
National ID: ……			**Name: ……..**		
Sex: ( ) M ( ) F			**Age: ……**		
Birth Date: / / Place: ……			**Telephone: ……..**		
Marital status	Single ☐	Married ☐	Divorced ☐	Widowed ☐	Child ☐
*If you married is your wife or husband your relative?					
No ☐			Yes ☐		
Relative Degree 1st degree ☐ 2nd degree ☐					

Socio-economic status	**Annual income**	**Job**	**Education**
	No income ☐	Government ☐	Uneducated ☐
	5000 SAR > ☐	Private ☐	Pre. College ☐
	1000 – 5000 SAR ☐	Retired ☐	College ☐
	10000 – 20000 SAR ☐	No work ☐	High Edu. ☐
	20000 SAR< ☐		

Family history					
Diabetes 1st degree ☐			**Diabetes 2nd degree** ☐		
Father ☐	Mother ☐	Brother/Sisters ☐	Uncle ☐	Grandpa/ma ☐	Grandchildren ☐
Hypertension ☐	Hyperlipidemia ☐	Asthma ☐	Obesity ☐	CHD ☐	Cancer: …… ……
Others: _____					

Subject Medical history					
CHD ( )	HTN ( )	( ) Dyslipidemia	( ) Diabetes	( ) Asthma	( ) Cancer
( ) Liver Disease	( ) Kidney Disease	Others: _____			

Smoking: (adults):				
( ) Smoker	( ) Sheshan	( ) Ex-Smoker		
# of packs/day _____	# of packs/day _____	Duration (years): ……	Years quitted: ……...	( ) Never smoked

Examination:				
Wieght _____ (cm)	Height _____ (cm)	Waist _____ (cm)	Hip _____ (cm)	SAD _____ (cm)
Blood Pressure: /				

Type of Diabetes:					
Type 1 Diabetes ☐	Type 2 Diabetes ☐	IGT ☐	GDM ☐	Other types ☐	Duration:_____ years

Complications:	DKA ☐ Per year……		☐ Hyperglycemia Per year……		
	Nephropathy ☐	Retinopathy ☐	Nephropathy ☐	Vasculopathy ☐	Laser ☐

Foot ulcer ☐	Dialysis ☐	Cataract ☐	IHD ☐	CVA ☐	PVD ☐
Handicap:	Blindness ☐				
Amputation ☐	Stroke ☐	Dialysis ☐	Heart failure ☐	Deafness ☐	Muteness ☐
Treating Physician:					
General practitioner ☐	Internist ☐			Endocrinologist ☐	
Physical activity (adults):	( ) Yes			( ) No	
If yes, please answer the following questions:					
Frequency of physical activity:					
Daily ☐	3-4 times a week ☐	1-2 times a week ☐	Few times a month ☐	Once a month ☐	
Types of activity			**Frequency (minutes)**		
Hard physical exercise (e.g. Running, bicycling……..)					
High physical exercise (Tennis, Handball,)					
Middle (1) physical exercise (Volleyball walking, house cleaning……)					
Middle (2) physical exercise (Table tennis, housekeeping,)					
Low physical exercise (Normal walking, ironing, dish washing,)					
No. of sleeping hours ( )	( ) separated		( ) connected		
For female Subjects Only					
Age at Menarche_____					
Years of Menopause (For menopausal only): _____					
Are you pregnant?	[ ] Yes	[ ] No	[ ] week		
Age at 1st pregnancy: _____					

History of Hormone Replacement Therapy (HRT):		[ ] Yes	[ ] No
History of GDM		[ ] Yes	[ ] No
History of mammogram		[ ] Yes	[ ] No
History of Breast Lesions	[ ] Yes	[ ] No	(Specify)_____
History of Breast Surgery	[ ] Yes	[ ] No	Date / /
Breast Examination	[ ] Never	[ ] Occasional	[ ] Regular

List of Medications **(Place a check on all medications used by the subject)**	
Anti-diabetic Agents	[ ] Warfarine
[ ] Insulin – insulin analogues Statins	[ ] Heparin
[ ] Tolbutamide – Orinase	[ ] Clopidogrel
[ ] Tolazamide – Tolinase	[ ] Amiodarone – Amiodarone, Cordarone
[ ] Chlorpropramide – Diabenese	[ ] Disopyramide – Rythmodan
[ ] Glipizide – Glucotrol	[ ] Flecainide acetate – Apo – Flecainide
[ ] Glyburide – Micronase, Diabeta	[ ] Mexiletine – Novo – Mexiletine
[ ] Glimepiride – Amaryl	[ ] Procainamide – Procan
[ ] Gliclazide – Diamicron	[ ] Propafenone – Rhythrnol, Nu, Apo, Gen, PMS
[ ] Repaglinide	[ ] Digoxin
[ ] Acarbose	[ ] Clonidine
[ ] Metformin – Repaglinide Thiazolidinediones	[ ] Methyldopa
[ ] Pioglitazone – Actos	[ ] Diazoxide
[ ] Rivoglitazone	[ ] Hydralazine
[ ] Rosiglitazone – A vandia	[ ] Isosarbide dinitrate
[ ] Troglitazone	[ ] Nitroglycerin
Anti-Hyperlipidemics	[ ] Prazosin, Terazosin, Dozazosin
[ ] Atorvastatin – Lipitor, Torvast	[ ] Atenolol, Acebutolol, Bisoprolol, Labetalol, Metaoprolol, Nadolol, Oxprenolol, Pndolol, Propranolol, Sotalol, Timolol
[ ] Cerivastatin – Lipobaby	[ ] Amlodipine, Felodipine, Nifedipine
[ ] Fluvastatin – Lescol, Lescol XL	[ ] Diltiazem, Verapamil
[ ] Lovastatin – Mevacor, Altocor, Altoprev	[ ] Captopril, Benazepril, Enalapril, Cilazapril, Perindopril, Quinapril, Ramipril, Lisinopril
[ ] Mevastatin	[ ] Candesartan, Irbesartan, Losartan, Telmisartan, Valsartan
[ ] Pitavastatin – Livalo, Pitava	[ ] Spironolactone

[ ] Pravastatin – Pravachol, Selektine, Lipostat	[ ] Hydrochlorothiazide
[ ] Rosuvastatin – Crestor	[ ] Furosemide
[ ] Simvastatin – Zocor, Lipex	[ ] Pentoxyphylline
[ ] Simvastatin + Ezetimibe – Vytorin	***Other (Please specify)***
[ ] Atorvastatin + Amlodipine – Caduet	_____ _____
[ ] Simvastatin + Niacin – Simcor	_____ _____
[ ] Cholestyramine	_____
[ ] Colestipol	
[ ] Benzafibrate, fenofibrate	
[ ] Gemfibrozil	
Cardiovascular drugs	
[ ] Aspirin	

Appendix IV: Food frequency questionnaire

Please circle the appropriate number to the number of times the consumption of food or drink daily or weekly, and tick the box when you choose rarely.

Food Item	Daily	Weekly	Rarely	Weight (gm)
White bread	1 2 3	1 2 3 4 5 6	☐	
Brown bread	1 2 3	1 2 3 4 5 6	☐	
Rice	1 2 3	1 2 3 4 5 6	☐	
Pasta	1 2 3	1 2 3 4 5 6	☐	
Potatoes	1 2 3	1 2 3 4 5 6	☐	
Algreesh	1 2 3	1 2 3 4 5 6	☐	
Alsliq	1 2 3	1 2 3 4 5 6	☐	
Alqursan	1 2 3	1 2 3 4 5 6	☐	
Almarquq	1 2 3	1 2 3 4 5 6	☐	
Lamb	1 2 3	1 2 3 4 5 6	☐	
Beef	1 2 3	1 2 3 4 5 6	☐	
Chicken	1 2 3	1 2 3 4 5 6	☐	
Burger	1 2 3	1 2 3 4 5 6	☐	
Pizza	1 2 3	1 2 3 4 5 6	☐	
Fried chicken	1 2 3	1 2 3 4 5 6	☐	
French Fries	1 2 3	1 2 3 4 5 6	☐	
Sausage	1 2 3	1 2 3 4 5 6	☐	
Fish	1 2 3	1 2 3 4 5 6	☐	
Tuna	1 2 3	1 2 3 4 5 6	☐	
Shrimp	1 2 3	1 2 3 4 5 6	☐	
Liver	1 2 3	1 2 3 4 5 6	☐	
Eggs	1 2 3	1 2 3 4 5 6	☐	
Cheese	1 2 3	1 2 3 4 5 6	☐	
Milk	1 2 3	1 2 3 4 5 6	☐	
Buttermilk	1 2 3	1 2 3 4 5 6	☐	
Yoghurt	1 2 3	1 2 3 4 5 6	☐	
Beans	1 2 3	1 2 3 4 5 6	☐	
Lentils	1 2 3	1 2 3 4 5 6	☐	
Chickpeas	1 2 3	1 2 3 4 5 6	☐	
Nuts	1 2 3	1 2 3 4 5 6	☐	
Apples	1 2 3	1 2 3 4 5 6	☐	
Dates	1 2 3	1 2 3 4 5 6	☐	
Watermelon	1 2 3	1 2 3 4 5 6	☐	
Oranges	1 2 3	1 2 3 4 5 6	☐	
Bananas	1 2 3	1 2 3 4 5 6	☐	
Melon	1 2 3	1 2 3 4 5 6	☐	
Grapes	1 2 3	1 2 3 4 5 6	☐	
Mango	1 2 3	1 2 3 4 5 6	☐	
Pear	1 2 3	1 2 3 4 5 6	☐	
Tangerine	1 2 3	1 2 3 4 5 6	☐	
Apricot	1 2 3	1 2 3 4 5 6	☐	
Strawberries	1 2 3	1 2 3 4 5 6	☐	

Dried fruits	1 2 3	1 2 3 4 5 6	☐
Tomatoes	1 2 3	1 2 3 4 5 6	☐
Cucumber	1 2 3	1 2 3 4 5 6	☐
Lettuce	1 2 3	1 2 3 4 5 6	☐
Watercress	1 2 3	1 2 3 4 5 6	☐
Zucchini	1 2 3	1 2 3 4 5 6	☐
Eggplant	1 2 3	1 2 3 4 5 6	☐
Orange Juice	1 2 3	1 2 3 4 5 6	☐
Other Juices	1 2 3	1 2 3 4 5 6	☐
Soft drinks	1 2 3	1 2 3 4 5 6	☐
Black Tea	1 2 3	1 2 3 4 5 6	☐
Green Tea	1 2 3	1 2 3 4 5 6	☐
Coffee	1 2 3	1 2 3 4 5 6	☐
Chocolate	1 2 3	1 2 3 4 5 6	☐
Chilled desserts	1 2 3	1 2 3 4 5 6	☐
Popcorn	1 2 3	1 2 3 4 5 6	☐
Cake	1 2 3	1 2 3 4 5 6	☐
Doughnuts	1 2 3	1 2 3 4 5 6	☐
Grain corn	1 2 3	1 2 3 4 5 6	☐
Unsweetened grain corn	1 2 3	1 2 3 4 5 6	☐
Biscuits	1 2 3	1 2 3 4 5 6	☐
Honey	1 2 3	1 2 3 4 5 6	☐
Jam	1 2 3	1 2 3 4 5 6	☐

Appendix V: Diets

Low Fat Diet

1000 calories (25% fat, 27 g of fat)

Breakfast

- One egg or 30 g cheese or three spoons of beans
- Half a loaf of Arabian bread or two slices of bread
- Coffee or tea without sugar

Snack

- One serving of fruits or half a cup of fruit juice without sugar

Lunch

- 60 g of boiled or grilled meat or fish or chicken without the skin and fat
- 3 spoons of rice or macaroni gursan or greeish or cooked potato
- Half a cup of boiled vegetables
- Cup of fresh vegetables salad
- Half a spoon of oil
- Coffee or tea without sugar

Snack

- One serving of fruits or half a cup of fruit juice without sugar

Dinner

- 30 g of boiled or grilled meat or fish or chicken without the skin and fat
- 3 spoons of rice or macaroni gursan or greeish or cooked potato
- A cup of boiled vegetables
- Fresh vegetables salad
- Coffee or tea without sugar

Bed-time snack

- One cup of low-fat milk, butter milk or yogurt without sugar

1200 calories (25% fat 35 g of fat)

Breakfast

- One egg or 30 g of cheese or 3 spoons of beans
- Half a loaf of Arabian bread or two slices of bread or half a cup of cornflakes
- One cup of low-fat milk, butter milk or yogurt without sugar
- Coffee or tea without sugar

Snack

- One serving of fruits or half a cup of fruit juice without sugar

Lunch

- 60 g of boiled or grilled meat or fish or chicken without the skin and fat
- 3 spoons of rice or macaroni gursan or greeish or cooked potato
- Half a cup of boiled vegetables
- Cup of fresh vegetables salad
- Half a spoon of oil
- Coffee or tea without sugar

Snack

- One serving of fruits or half a cup of fruit juice without sugar

Dinner

- 60 g of boiled or grilled meat or fish or chicken without the skin and fat
- 3 spoons of rice or macaroni gursan or greeish or cooked potato

- Half a cup of boiled vegetables
- Fresh vegetables salad
- One serving of fruits or half a cup of fruit juice without sugar
- Coffee or tea without sugar

Bed-time snack

- 3 pieces of tea biscuit or shaboura

1400 calories (25% fat 37 g of fat)

Breakfast:

- One serving of fruits or half a cup of fruit juice without sugar
- One egg or 30 g of cheese or 3 spoons of beans
- Half a loaf of Arabian bread or two slices of bread or half a cup of cornflakes
- One cup of low-fat milk, butter milk or yogurt without sugar
- Coffee or tea without sugar

Snack:

- 3 pieces of tea biscuit or shaboura

Lunch

- 60 g of boiled or grilled meat or fish or chicken without the skin and fat
- 6 spoons of rice or macaroni gursan or greeish or cooked potato
- Half a cup of boiled vegetables
- Cup of fresh vegetables salad
- Half a spoon of oil
- Coffee or tea without sugar

Snack

- 3 dates with coffee

Dinner

- 60 g of boiled or grilled meat or fish or chicken without the skin and fat
- 3 spoons of rice or macaroni gursan or greeish or cooked potato
- Fresh vegetables salad
- One serving of fruits or half a cup of fruit juice without sugar
- Coffee or tea without sugar

Bed-time snack

- One serving of fruits or half a cup of fruit juice without sugar
- Half a cup of low-fat milk, or yogurt

1600 calories (25% fat 45 g of fat)

Breakfast:

- One cup of low-fat milk
- 1 slice of cheese or 3 spoons of beans or 1 boiled egg twice a week or a piece of liver hand size once a week
- Quarter loaf of Arabian bread medium size or one slice of bread or half of white bread

Snack

- One serving of fruits or half a cup of fruit juice without sugar

Lunch

- One cup of butter milk, or half cup of low fat yogurt
- 60 g of boiled or grilled meat or fish or chicken without the skin and fat (hand size)

- 6 spoons of rice or macaroni gursan or greeish or cooked potato or quarter loaf of brown bread
- A cup of cooked vegetables
- A plate of fresh vegetables unsliced
- One serving of fruits or half a cup of fruit juice without sugar

Snack

- 3 dates with coffee

Dinner

- One cup of milk or butter milk, or half cup of low fat
- One serving of fruits or half a cup of fruit juice without sugar
- 2 slices of cheese or 6 spoons of beans or 60 g of meat or chicken without fat
- Cup of cooked vegetables without fat
- Quarter loaf of Arabian bread
- Coffee or tea without sugar

Bed-time snack

- One serving of fruits or half a cup of fruit juice without sugar
- Quarter loaf of brown bread or one slice of toast or half slice of white bread
- One slice of low fat cheese or one cup of low fat milk

1800 calories (25% fat 52 g of fat)

Breakfast

- One cup of low-fat milk
- 1 slice of cheese or 3 spoons of beans or 1 boiled egg twice a week or a piece of liver hand size once a week
- Quarter loaf of Arabian bread medium size or one slice of bread or half of white bread
- One serving of fruits or half a cup of fruit juice without sugar

Snack

- 3 pieces of tea biscuit or shaboura

Lunch

- One cup of butter milk, or half cup of low fat yogurt
- 60 g of boiled or grilled meat or fish or chicken without the skin and fat (hand size)
- 6 spoons of rice or macaroni gursan or greeish or cooked potato without fat or quarter loaf of brown bread
- A cup of cooked vegetables without fat
- A plate of fresh vegetables unsliced
- One serving of fruits or half a cup of fruit juice without sugar
- One spoon of oil

Snack

- 3 dates with coffee

Dinner

- One cup of milk or butter milk, or half cup of low fat
- One serving of fruits or half a cup of fruit juice without sugar
- 2 slices of cheese or 6 spoons of beans or 60 g of meat or chicken without fat
- Cup of cooked vegetables without fat
- half loaf of Arabian bread
- Coffee or tea without sugar
- One spoon of oil

Bed-time snack

- One serving of fruits or half a cup of fruit juice without sugar
- Quarter loaf of brown bread or one slice of toast or half slice of white bread
- One slice of low fat cheese or half cup of low fat milk

2000 calories (25% fat 57 g of fat)

Breakfast

- One cup of low-fat milk
- 1 slice of cheese or 3 spoons of beans or 1 boiled egg twice a week or a piece of liver hand size once a week
- Half loaf of Arabian bread medium size or one slice of bread or half of white bread
- One serving of fruits or half a cup of fruit juice without sugar

Snack

- 3 pieces of tea biscuit or shaboura

Lunch

- One cup of butter milk, or half cup of low fat yogurt
- 60 g of boiled or grilled meat or fish or chicken without the skin and fat (hand size)
- 6 spoons of rice or macaroni gursan or greeish or cooked potato without fat or quarter loaf of brown bread
- A cup of cooked vegetables without fat
- A plate of fresh vegetables unsliced
- One serving of fruits or half a cup of fruit juice without sugar
- One spoon of oil

Snack

- 3 dates with coffee

Dinner

- One cup of milk or butter milk, or half cup of low fat yogurt
- One serving of fruits or half a cup of fruit juice without sugar
- 2 slices of cheese or 6 spoons of beans or 60 g of meat or chicken without fat
- Cup of cooked vegetables without fat
- half loaf of Arabian bread
- Coffee or tea without sugar
- One spoon of oil

Bed-time snack:

- One serving of fruits or half a cup of fruit juice without sugar
- Half loaf of brown bread or one slice of toast or half slice of white bread
- One slice of low fat cheese or half cup of low fat milk

Balanced Diet

1000 calories (30% fat 32g of fat)

Breakfast

- One egg or 30 g cheese or three spoons of beans
- Half a loaf of Arabian bread or two slices of bread
- Coffee or tea without sugar

Snack

- One serving of fruits or half a cup of fruit juice without sugar

Lunch

- 60 g of boiled or grilled meat or fish or chicken without the skin and fat
- 3 spoons of rice or macaroni gursan or greeish or cooked potato
- Cup of fresh vegetables
- Half a spoon of oil
- Coffee or tea without sugar

Snack

- One serving of fruits or half a cup of fruit juice without sugar

Dinner

- 30 g of boiled or grilled meat or fish or chicken without the skin and fat
- 3 spoons of rice or macaroni gursan or greeish or cooked potato
- Half a cup of boiled vegetables
- Fresh vegetables salad
- Half a spoon of oil
- Coffee or tea without sugar

Bed-time snack

- One cup of low-fat milk, butter milk or yogurt without sugar

1200 calories (30% fat 30 g of fat)

Breakfast

- One serving of fruits or half a cup of fruit juice
- One egg or 30 g of cheese or 3 spoons of beans
- Half a loaf of Arabian bread or two slices of bread or half a cup of cornflakes
- One cup of low-fat milk, butter milk or yogurt without sugar
- Coffee or tea without sugar

Snack

- One serving of fruits or half a cup of fruit juice without sugar

Lunch

- 60 g of boiled or grilled meat or fish or chicken without the skin and fat
- 3 spoons of rice or macaroni gursan or greeish or cooked potato
- Half a cup of boiled vegetables
- Cup of fresh vegetables salad
- 1 spoon of oil
- Coffee or tea without sugar

Snack

- 3 dates with coffee

Dinner

- 60 g of boiled or grilled meat or fish or chicken without the skin and fat
- 3 spoons of rice or macaroni gursan or greeish or cooked potato
- Half a cup of boiled vegetables
- Fresh vegetables salad
- 1 spoon of oil
- Coffee or tea without sugar

Bed-time snack

- 11/2 pieces of tea biscuit or shaboura

1400 calories (30% fat 47 g of fat)

Breakfast

- One egg or 30 g of cheese or 3 spoons of beans
- Half a loaf of Arabian bread or two slices of bread or half a cup of cornflakes
- One cup of low-fat milk, butter milk or yogurt without sugar
- Coffee or tea without sugar

Snack

- One serving of fruits or half a cup of fruit juice without sugar

Lunch

- 60 g of boiled or grilled meat or fish or chicken without the skin and fat
- 3 spoons of rice or macaroni gursan or greeish or cooked potato
- Half a cup of boiled vegetables
- Cup of fresh vegetables salad
- 1 spoon of oil
- One serving of fruits or half a cup of fruit juice without sugar
- Coffee or tea without sugar

Snack

- 3 pieces of tea biscuit or shaboura

Dinner

- 60 g of boiled or grilled meat or fish or chicken without the skin and fat
- 3 spoons of rice or macaroni gursan or greeish or cooked potato
- Fresh vegetables salad
- One serving of fruits or half a cup of fruit juice without sugar
- Coffee or tea without sugar

Bed-time snack

- One serving of fruits or half a cup of fruit juice without sugar
- Half a cup of low-fat milk, or yogurt

1600 calories (30% fat 52 g of fat)

Breakfast:

- One cup of low-fat milk
- 1 slice of cheese or 3 spoons of beans or 1 boiled egg twice a week or a piece of liver hand size once a week
- Quarter loaf of Arabian bread medium size or one slice of bread or half of white bread

Snack:

- One serving of fruits or half a cup of fruit juice without sugar

Lunch

- One cup of butter milk, or half cup of low fat yogurt
- 60 g of boiled or grilled meat or fish or chicken without the skin and fat (hand size)
- 6 spoons of rice or macaroni gursan or greeish or cooked potato or quarter loaf of brown bread
- A cup of cooked vegetables
- A cup of fresh vegetables unsliced
- 2 spoon of oil

Snack

- 3 dates with coffee

Dinner

- One cup of milk or butter milk, or half cup of low fat
- 2 slices of cheese or 6 spoons of beans or 60 g of meat or chicken without fat
- 1 Cup of cooked vegetables without fat
- Half loaf of Arabian bread
- Coffee or tea without sugar

Bed-time snack

- One serving of fruits or half a cup of fruit juice without sugar
- Quarter loaf of brown bread or one slice of toast or half slice of white bread
- One slice of low fat cheese or one cup of low fat milk

1800 calories (30% fat 62 g of fat)

Breakfast

- One cup of low-fat milk
- 1 slice of cheese or 3 spoons of beans or 1 boiled egg twice a week or a piece of liver hand size once a week
- Quarter loaf of Arabian bread medium size or one slice of bread or half of white bread
- One serving of fruits or half a cup of fruit juice without sugar

Snack

- 11/2 pieces of tea biscuit or shaboura

Lunch

- One cup of butter milk, or half cup of low fat yogurt
- 60 g of boiled or grilled meat or fish or chicken without the skin and fat (hand size)
- 6 spoons of rice or macaroni gursan or greeish or cooked potato without fat or quarter loaf of brown bread
- A cup of cooked vegetables without fat
- A cup of fresh vegetables unsliced
- 2 spoons of oil

Snack

- 3 dates with coffee or One serving of fruits or half a cup of fruit juice without sugar

Dinner

- One cup of milk or butter milk, or half cup of low fat
- One serving of fruits or half a cup of fruit juice without sugar
- 2 slices of cheese or 6 spoons of beans or 60 g of meat or chicken without fat
- 1 Cup of cooked vegetables without fat
- half loaf of Arabian bread
- Coffee or tea without sugar
- 2 spoons of oil

Bed-time snack

- One serving of fruits or half a cup of fruit juice without sugar
- One slice of low fat cheese or half cup of low fat milk

2000 calories (30% 67 g of fat)

Breakfast

- One cup of low-fat milk

- 1 slice of cheese or 3 spoons of beans or 1 boiled egg twice a week or a piece of liver hand size once a week
- Half loaf of Arabian bread medium size or one slice of bread or half of white bread
- 2 serving of fruits or a cup of fruit juice without sugar

Snack

- 3 pieces of tea biscuit or shaboura

Lunch

- One cup of butter milk, or half cup of low fat yogurt
- 60 g of boiled or grilled meat or fish or chicken without the skin and fat (hand size)
- 6 spoons of rice or macaroni gursan or greeish or cooked potato without fat or quarter loaf of brown bread
- A cup of cooked vegetables without fat
- A cup of fresh vegetables unsliced
- 2 spoons of oil

Snack

- 3 dates with coffe

Dinner

- One cup of milk or butter milk, or half cup of low fat yogurt
- One serving of fruits or half a cup of fruit juice without sugar
- 2 slices of cheese or 6 spoons of beans or 60 g of meat or chicken without fat
- Cup of cooked vegetables without fat
- half loaf of Arabian bread
- Coffee or tea without sugar
- 2 spoons of oil

Bed-time Snack

- One serving of fruits or half a cup of fruit juice without sugar
- Quarter loaf of brown bread or one slice of toast or half slice of white bread
- One slice of low fat cheese or half cup of low fat milk

Food Exchange list

Altenative	Alte 1	Alte 2	Alte 3	Alte 4	Alte 5	Alte 6	Alte 7	Alte 8	General notes
Dairies group	1 cup low fat milk	1 cup low fat yogurt	1 cup low fat buttermilk	1/3 cup skimmed dried milk	11/4 skimmed milk	3/4 whole fat milk			Drink low fat diary instead of whole fat one
Meat group	30g cooked meat	30g cooked fish	30g cooked chicken	1/4 can lite tuna	3 egg whites	30g low fat cheese	2tsp low fat labnah	1 tsp peanut butter	Use lean meat before cooking Boiled or grilled meat more likely than fried one
Fruits group	1 medium orange	1/2 banana	1 small apple	1 slice watermelon	1/2 cup unsweetened juice	2-3 dates	15 grapes	2 tsp raisins	Fresh fruits better than canned fruits or juices
Vegetables group	1 cup fresh vegetables or 1/2 cup cooked vegetables								Eat lots of fresh vegetables it has lower calories
Carb group	1 slice brown toast or 1/4 arabic bread	1/2 cup cornflakes	4-6 unsweetened biscuits	6 tsp cooked rice	1/2 cup cooked pasta	1 potato	1/3 cup beans or lentil	1/2 cup peas	Carbs with lots of fiber slow down the absorption of blood sugar
Fat group	1 tsp oil	2 tsp lite mayonnaise	2 tsp tahini	10 small olives	20 peanuts	6 almonds			Use vegetables oils like olive oil instead of animal fats like ghee and butter in cooking and in small amounts

- Keep your body weight in normal range
- Eat 3 meals and 2 snacks
- Eat your specified quantities and do it on time
- Avoid eating much sugar like honey, jam, and soft drinks
- Dowhat suit you of physical exercise
- You are allowed to drink tea, coffee and soft drink but without sugar
- Look at the alternative table to replace any kind of food from your diet

Appendix VI: Tables

WHO EQUATIONS (2007)

MALE	FEMALE	AGE
22.7 w + 495	22.5 w+499	3-9 years
17.5 w+651	12.2 w+746	10-17 years
15.3 w + 679	14.7 w+496	18-29 years
11.6 w + 879	8.7 w + 829	30-60 years
13.5 w + 487	10.5 w + 596	>60 years old

Activity factor

1.2	Confined to bed
1.3	Low activity
1.61	Average activity
2	Highly active

Appendix VII

Daily Food Record

Date:			Name :				
			Daily intake goal:				
Dear participants kindly write what you eat to become aware of what and how much you are eating							
Menu	**Sunday**	**Monday**	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**
Breakfast							
Snack							
Lunch							
Snack							
Dinner							
Snack							

Appendix VIII - List of publications and abstracts

- **Al-Disi D,** Al-Daghri N, Khanam L, Al-Othman A, Al-Saif M., Sabico S, Chrousos G**.** Subjective sleep duration and quality influence diet composition and circulating adipocytokines and ghrelin levels in teen-age girls. **Endocr J** 2010; 57(10):915-923.

- Metcalfe D, Harte AL, Aletrari MO, Al-Daghri NM, **Al-Disi D**, Tripathi G, McTernan PG. Does endotoxaemia contribute to osteoarthritis in obese patients? **Clin Sci** 2012; 123:627-634.

- Harte AL, Tripathi G, Piya MK, Barber TM, Clapham JC, Al-Daghri N, **Al-Disi D**, Kumsaiyai W, Saravanan P, Fowler AE, O'Hare JP, Kumar S, McTernan, PG. NFκB as a potent regulator of inflammation in human adipose tissue, influenced by depot, adiposity, T2DM status, and TNFα. **Obesity** 2013; 21:2322–2330. doi: 10.1002/oby.2033

- **Al-Disi D**, Al-Daghri N, Khan N, Alsaif M, Alfadda A, Sabico S, Tripathi G, McTernan PG. A 3-month low fat diet leads to significant lipid profile improvement in obese T2DM Saudi subjects, without substantial weight loss, and the capacity to manage a damaging high-fat meal challenge more appropriately post intervention. **Endocrine Abstracts** 2014; 34 P223.

- **Al-Disi D**, Al-Daghri NM, Khan N, Al-Fadda A, Sallam R, Al-Seif M, Sabico S, Tripathi G, McTernan PG. A 3-Month Balanced Diet with Complex Carbohydrate Improves Cardiometabolic Profile, Metabolic Endotoxaemia, and the Capacity to Manage a Damaging High-Fat Meal Challenge More Appropriately in Obese T2DM Subjects. P-752, **ADA** 2014.

- **Al-Disi D**, Al-Daghri NM, Khan N, Al-Fadda A, Sallam R, Al-Seif M, Tripathi G, McTernan PG. Positive influence of a 3-month dietary intervention on cardiometabolic health in Saudi women with or without type 2 diabetes mellitus. Submitted to **Journal of Nutrition** (MS 2014/199281).

List of Poster Presentations

The following abstract has been selected as one of the top 40 scoring Young Endocrinologist's posters at Society for Endocrinology BES 2014
24-27 March 2014 - The ACC Liverpool, UK

A 3-month low fat diet leads to significant lipid profile improvement in obese T2DM Saudi subjects, without substantial weight loss, and the capacity to manage a damaging high fat meal challenge more appropriately post intervention

Dara Al-Disi[1,2], Nasser M. Al-Daghri[1], Nasiruddin Khan[1], Mohammad Alsaif[1], Assim Alfadda[1], Shaun Sabico[1,2], [1]Reem Sallam, [2]Lucia Martinez de la Escalera, Gyanendra Tripathi[2], Philip McTernan[2]
[1]King Saud University, Riyadh, Saudi Arabia; [2]Warwick Medical School, Coventry, UK

Warwick Medical School

Background

Current evidence highlights that dietary cholesterol, trans-fatty acids and saturated fatty acids (SFAs) are all known to increase the levels of systemic atherogenic lipoproteins and cardiovascular disease. The aim of this study was to observe the direct effect of dietary change, via a calorie-restricted diet on (1) cardio-metabolic profile and (2) a high-fat meal challenge pre- and post 3-month intervention.

Methods

T2DM subjects (Saudi female, age:41.50±6.2yrs, BMI:35.24±7.67kg/m², n=50) were given a high-fat meal pre- and post- calorie restricted diet (3 months; 500 kcal deficit/day, balanced diet with complex carbohydrate). Baseline (0 hr) and post-prandial sera (1-4 hr) were taken from subjects, anthropometric and biochemical data was collated at both time points.

Results

On baseline comparison of pre- and post-diet interventions, there were modest reductions in anthropometric data, BMI ($p<0.05$), waist ($p<0.001$) and waist to hip ratio (WHR; $p<0.001$). Baseline HDL-cholesterol increased significantly ($p<0.001$) whilst LDL- and total cholesterol were significantly reduced (pre-total cholesterol: 5.16 ±0.04 vs post-total cholesterol: 4.74 ± 0.75; pre-LDL cholesterol: 3.85 ± 1.07 vs post-LDL cholesterol: 3.44 ± 0.81, $p<0.05$). The findings also showed significant changes in the effects of high fat meal intake on the metabolic profile pre- and post-diet intervention. At 4hr post-prandially, post-dietary intervention, HDL-cholesterol was 5.6% higher than pre-diet, whilst LDL- and total cholesterol were 12.7% and 9.3% lower, respectively, than at the 4hr equivalent pre-diet ($p<0.01$).

Table 1. Subject s' Characteristics at Pre- and Post Intervention

N	50	
Age (years)	41.50 ± 6.23	
DM Duration (years)	2.04 (0-9)	
	Baseline	**Post-Intervention**
BMI (kg/m²)	35.24 ± 7.67	35.04 ± 8.07*
Waist (cm)	112.3 ± 13. 43	109.21 ± 12.71 **
Hip (cm)	117.11 ± 11.59	116.7 ± 13.18 **
WHR	0.96 ± 0.07	0.93 ± 0.06 **
Glucose (mmol/l)	7.69 ± 2.45	6.84 ± 1.84 **
Triglycerides (mmol/l)	1.79 ± 0.89	1.54 ± 0.82 **
Total Cholesterol (mmol/l)	5.16 ± 1.04	4.74 ± 0.85 *
HDL-Cholesterol (mmol/l)	0.94 ± 0.21	1.06 ± 0.22 **
LDL-Cholesterol (mmol/)	3.85 ± 1.07	3.44 ± 0.81*

Note: Data presented as mean ± SD; * denotes significance at 0.05 level; ** denotes significance at 0.01 level

Figure 1. Mean HDL-Cholesterol Levels Pre- and Post Intervention

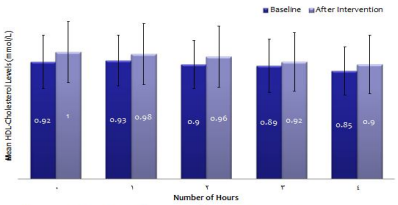


Figure 2. Mean LDL-Cholesterol Levels Pre- and Post Intervention

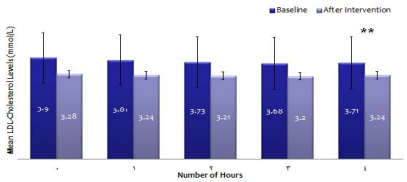
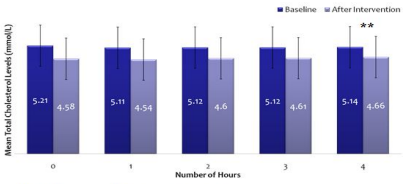


Figure 3. Mean Total Cholesterol Levels Pre- and Post Intervention



Note: ** denotes significance at 0.01 level

Conclusion

Caloric restriction has been observed to be beneficial among T2DM patients in terms of weight loss, and improved cardiometabolic profile [1-2]. These findings suggest that lipid mediators associated with increased cardiometabolic risk can be quickly reversed as a result of a balanced diet, in T2DM subjects without substantial weight loss. As a result, the body is able to cope with the occasional high-fat meal insult, whilst still maintaining a reduced long-term CVD risk. As such, this is a diet that patients with T2DM may be able to adhere to more successfully, longer-term.

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3. Soare, et al. Benefits of caloric restriction for cardiometabolic health, including type 2 diabetes mellitus risk. Diabetes Metab Res Rev 2014; 30 S 1-41-7

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A 3-month Balanced-Diet with Complex Carbohydrate Improves Cardiometabolic Profile, Metabolic Endotoxemia, and the Capacity to Manage a Damaging High Fat Meal Challenge More Appropriately in Obese T2DM Subjects

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Background

Current evidence highlights that dietary cholesterol, trans-fatty acids and saturated fatty acids (SFAs) are all known to increase the levels of systemic atherogenic lipoproteins and cardiovascular disease. The aim of this study was to observe the direct effect of dietary change, via a calorie-restricted diet on (1) cardio-metabolic profile and (2) a high-fat meal challenge pre- and post 3-month intervention.

Methods

T2DM subjects (Saudi female, age:41.50±6.2yrs, BMI:35.24±7.67kg/m², n=50) were given a high-fat meal pre- and post- calorie restricted diet (3 months; 500 kcal deficit/day, balanced diet with complex carbohydrate). Baseline (0 hr) and post-prandial sera (1-4 hr) were taken from subjects, anthropometric and biochemical data including endotoxin was collated at both time points.

Results

From baseline comparison of pre- and post-diet interventions, there were modest reductions in anthropometric data, BMI ($p<0.05$), waist ($p<0.001$) and waist to hip ratio (WHR; $p<0.001$). Baseline HDL-cholesterol increased significantly ($p<0.001$) whilst LDL- and total cholesterol as well as endotoxin were reduced (pre-total cholesterol: 5.16 ±0.04 vs post-total cholesterol: 4.74±0.75; pre-LDL cholesterol: 3.85±1.07 vs post-LDL cholesterol: 3.44±0.81, $p<0.05$; pre-endotoxin: 3.3±0.81 vs post-endotoxin: 3.19±1.1). The findings also showed significant changes in the effects of high-fat meal intake on the metabolic profile pre- and post-diet intervention. At 4hr post-prandially, post-dietary intervention, HDL-cholesterol was 5.6% higher than pre-diet, whilst LDL- and total cholesterol were 12.7% and 9.3% lower, respectively, than at the 4hr equivalent pre-diet ($p<0.01$).

Table 1. Subjects' Characteristics at Pre- and Post Intervention

N	50	
Age (years)	41.50 ± 6.23	
T2DM Duration (years)	2.04 (0-9)	
	Pre-Intervention	Post-Intervention
BMI (kg/m²)	35.24 ± 7.67	35.04 ± 8.07*
Waist (cm)	112.3 ± 13.43	109.21 ± 12.71**
Hip (cm)	117.11 ± 11.59	116.7 ± 13.18**
WHR	0.96 ± 0.07	0.93 ± 0.06**
Glucose (mmol/l)	7.69 ± 2.45	6.84 ± 1.84 **
Triglycerides (mmol/l)	1.79 ± 0.89	1.54 ± 0.82**
Total Cholesterol (mmol/l)	5.16 ± 1.04	4.74 ± 0.85 *
HDL-Cholesterol (mmol/l)	0.94 ± 0.21	1.06 ± 0.22 **
LDL-Cholesterol (mmol/l)	3.85 ± 1.07	3.44 ± 0.81*
Endotoxin	3.3 ± 0.81	3.19 ± 1.1

Note: Data presented as mean ± SD; * denotes significance at 0.05 level; ** denotes significance at 0.01 level

Figure 1. Mean HDL-Cholesterol Levels Pre- and Post Intervention

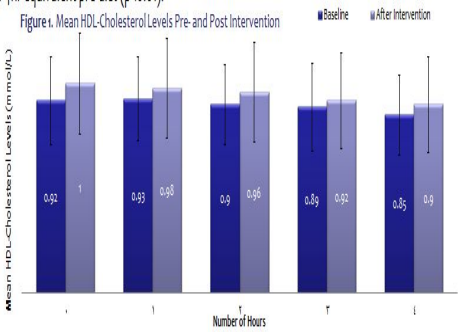


Figure 2. Mean LDL-Cholesterol Levels Pre- and Post Intervention

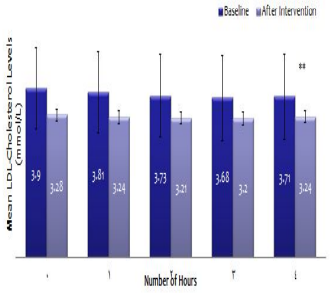
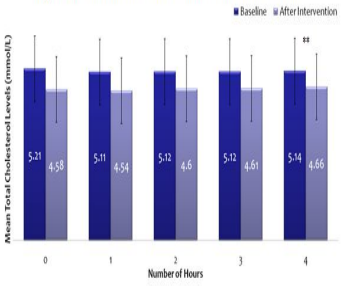
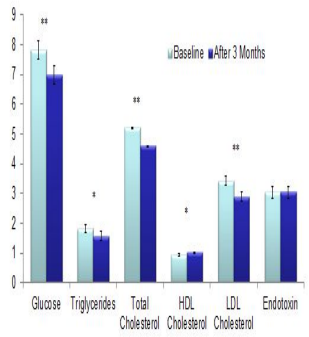


Figure 3. Mean Total Cholesterol Levels Pre- and Post Intervention



Note: ** denotes significance at 0.01 level

Figure 4. Glucose, Lipids and Endotoxin Changes Over Time



Note: * denotes significance at 0.05 level; ** denotes significance at 0.01 level

Conclusion

Caloric restriction has been observed to be beneficial among T2DM patients in terms of weight loss, and improved cardiometabolic profile [1-2]. These findings suggest that lipid mediators associated with increased cardiometabolic risk can be quickly reversed as a result of a balanced diet, in T2DM subjects with minimal weight loss. As a result, the body is able to cope with the occasional high-fat meal insult, whilst still maintaining a reduced long-term CVD risk. As such, this is a diet that patients with T2DM may be able to adhere to more successfully, longer-term.

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