METABOLIC AND LIFESTYLE PROFILING OF OVERWEIGHT FEMALE RUNNERS COMPARED TO LEAN COUNTERPARTS: EXPLORING THE IMPLICATIONS AND CAUSES OF THEIR ELEVATED BODY WEIGHT

by

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DECLARATION

I, **David Leith**, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Onwards and upwards!

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LIST OF ABBREVIATIONS

24HR	24 Hour Diet Recall
3DR	3 Day Diet Record
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
ASAT	Abdominal Subcutaneous adipose Tissue
ATP	Adenosine Triphosphate
ATP III	Adult Treatment Panel III
BD	Body Density
BF%	Body Fat Percentage
BMI	Body Mass Index
CoA	Coenzyme A
Cpm	Counts per minute
CRP	C-Reactive-Protein
CVD	Cardiovascular disease
DXA	Dual Energy X-ray Absorptiometry
FFA	Free Fatty Acids
FFQ	Food Frequency Questionnaire
FI	Fasting Insulin
FPG	Fasting Plasma Glucose
GAUC	Glucose Area Under the Curve
GI	Glycaemic Index

GLUT4	Glucose Transporter 4
GWAS	Genome Wide Association Studies
HbA1c	Glycated Haemoglobin
HDL	High-Density-Lipoprotein
HOMA-IR	Homeostatic model assessment of insulin resistance
HPA	Hypothalamic pituitary axis
HRmax	Maximum Heart Rate
IAAT	Intra-abdominal Adipose Tissue
IAUC	Insulin Area under the Curve
IDF	International Diabetes Federation
IDL	Intermediate-Density-Lipoprotein
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
LCHF	Low-Carbohydrate, High-Fat
LDL	Low-Density-Lipoprotein
LIPA	Light Physical Activity
MetS	Metabolic Syndrome
MHO	Metabolically Healthy Overweight / Obese
MPA	Moderate Physical Activity
MRC	Medical Research Council
MRI	Magnetic Resonance Imaging
MVPA	Moderate-to-Vigorous Physical Activity
n-3	Omega 3

n-6	Omega 6
NAFLD	Non-alcoholic fatty liver disease
NCD(s)	Non-communicable disease(s)
NCEP	National Cholesterol Education Programme
NHANES	National Health and Nutrition Examination Survey
OGTT	Oral Glucose Tolerance Test
PAL	Physical Activity Level
PPAR	Peroxisome proliferator-activated receptor
PPGR(s)	Postprandial Glucose Response(s)
PSQ	Perceived Stress Questionnaire
PSQI	Pittsburgh Sleep Quality Index
PTRS	Peak Treadmill Running Speed
RER	Respiratory Exchange Ratio
RMR	Resting Metabolic Rate
ROS	Reactive Oxygen Species
RPE	Rating of Perceived Exertion
SED	Sedentary
SNP(s)	Single Nucleotide Polymorphism(s)
SNS	Sympathetic Nervous System
T2D	Type 2 Diabetes
TC	Total Cholesterol
TEE	Total Energy Expenditure
TFEQ	Three Factor Eating Questionnaire

TG	Triglycerides
TNF-α	Tumour Necrosis Factor α
US	United States of America
WASO	Wake After Sleep Onset
WHO	World Health Organisation
VAT	Visceral Adipose Tissue
VLCHF	Very low carbohydrate, high fat
VLDL	Very-low-density-lipoprotein
VPA	Vigorous Physical Activity

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ABSTRACT

INTRODUCTION. There appears to be an emerging phenotype of recreational runners who are overweight despite being regularly active. This conflicts with the common perception that exercise protects against weight-gain, and it may be caused by underlying insulin-resistance. Alternatively, recent research has brought attention to metabolically healthy obese (MHO) individuals, who have increased adiposity but no commonly associated metabolic abnormalities, such as insulin-resistance, hypertension, dyslipidaemia and systemic inflammation. This study aimed to determine whether overweight (OW, BMI ≥ 25 $kg.m^{-2}$) female runners were at risk of developing metabolic pathology and compare the findings to lean (LN, BMI < 23 $kg.m^{-2}$) counterparts. A secondary aim was to explore potential inherent or lifestyle factors that may have predisposed or contributed to weight-gain in OW runners.

METHODS. Twenty (10 OW, 10 LN) female recreational runners (years of running experience 7.1 ± 4.4 OW; 8.0 ± 3.7 LN) matched for mean age (38.7 ± 4.6 OW; 37.7 ± 4.3 LN), current mileage in $km.week^{-1}$ (42.0 ± 10.9 OW; 44.5 ± 12.1 LN) and running calibre expressed as energy expenditure ($kcal.min^{-1}$) in their most recent half-marathon (9.0 ± 1.1 OW; 9.2 ± 1.1 LN) were recruited for this study. Body fat percentage (BF%) was determined using DXA. Participants completed questionnaires about health history, lifestyle and eating habits and validated questionnaires concerning recent sleep and stress. Their diet was recorded using 3-day diet records and analysed using the South African Food Data System (Medical Research Council of South Africa). Habitual sleep and physical activity were quantified using 7-day actigraphy (Actiwatch 2) and accelerometry (Actigraph GTX3+) respectively. Blood pressure and resting metabolic rate were measured after an overnight fast. Blood samples were analysed for cardio-metabolic parameters and an Oral Glucose Tolerance Test was performed for insulin-sensitivity.

RESULTS. OW exhibited a greater body weight (74.4 ± 6.4 kg OW; 59.4 ± 7.8 kg LN, $p < 0.001$) but similar fat-free-mass (49.4 ± 5.6 kg OW; 45.4 ± 5.9 kg LN) to the LN group. OW had a higher BF% (32.1 ± 3.9 OW; 21.8 ± 3.9 LN, $p < 0.0001$), and systolic (118 ± 10 mmHg OW; 107 ± 5 mmHg LN, $p < 0.05$), but not diastolic (72 ± 6 mmHg OW; 68 ± 4 mmHg LN) blood pressure. There was no difference between groups in serum uric acid, alanine aminotransferase, % HbA1c, total cholesterol, HDL-cholesterol, triglycerides or free-fatty-acids. OW had higher levels of C-reactive protein (1.30 ± 0.97 $mg.L^{-1}$ OW; 0.59 ± 0.35 $mg.L^{-1}$ LN, $p < 0.05$), total cholesterol / HDL-cholesterol (2.70 ± 0.40 OW; 2.30 ± 0.42 LN, $p < 0.05$) and LDL-cholesterol

(2.99 ± 0.65 mM OW; 2.43 ± 0.72 mM LN, $p < 0.05$), but these were within normal ranges. IDL-cholesterol constituted a significantly greater proportion of total cholesterol in OW compared to LN, but HDL- and LDL- cholesterol sub-fraction distributions were similar. Indices of hepatic (HOMA-IR, 1.06 ± 0.51 OW; 0.86 ± 0.24 LN), and whole-body (Matsuda, 7.84 ± 2.46 OW; 9.16 ± 2.28 LN) insulin-sensitivity were variable and similar between groups. Total area-under-the-curve of the OGTT insulin response tended to be higher in OW ($p = 0.08$). Two OW runners had insulin-resistance (Matsuda < 5); but no participants had the metabolic syndrome. RMR (kcal.kg FFM^{-1}.day^{-1}) was lower in OW (29.5 ± 2.1 OW; 31.6 ± 2.3 LN, $p < 0.05$), but there were no significant differences in lifestyle factors (diet, physical activity, sleep and stress). Total energy intake in kcal.day^{-1} (1928 ± 354 OW; 2166 ± 489 LN) and % macronutrient composition as Protein/Fat/Carbohydrate/Alcohol (20/44/33/3 OW; 16/43/36/5 LN) were both similar between groups. OW and LN also exhibited similar activity in steps.day^{-1} (10 742 ± 3552 OW; 12 073 ± 3273 LN) and percentage accelerometer wear-time spent in Sedentary/Light/Moderate-Vigorous physical activity (75/14/11 OW; 72/15/13 LN). Both groups attained *circa* 7 hours.$night^{-1}$ of sleep, with good sleep onset latency (7.3 ± 5.8 minutes OW; 5.8 ± 3.5 minutes LN) and sleep efficiency (91.6 ± 4.4% OW; 90.7 ± 2.8% LN), and they reported reduced to average levels of recent stress.

DISCUSSION. OW runners presented with greater mean adiposity than LN counterparts, but the two groups were not as distinct as anticipated. OW runners did present with greater metabolic risk according to some traditional risk factors, including inflammation, systolic blood pressure, LDL-C and total cholesterol. However, the first three were within normal ranges and the clinical relevance of the latter is questionable. It was, therefore, concluded that on average the OW group was not at metabolic risk. Only two OW runners and no LN runners were insulin-resistant according to indices derived from the OGTT. These findings may primarily reflect the insulin-sensitising effects of regular exercise and the consequent fitness of the OW runners. Appetite-dysregulation is speculated to have played an integral role in their prior weight-gain. We did not identify any lifestyle discrepancies that could have explained this weight-gain. The cross-sectional nature of this study made it difficult to assess past behaviour during weight-gain, and inter-individual variation was considerable. In combination with the small sample size, these factors limited the generalisability of the results. Future exploration of the 'overweight-runner' phenotype is warranted to clarify the mechanisms of weight-gain in habitual runners and consequent lifestyle changes that may promote meaningful weight-loss.

1.) INTRODUCTION

1.1.) The Obesity Problem

The prevalence of obesity has skyrocketed in recent decades to the point that it now affects at least 600 million people and is considered a global epidemic[1]. As of 2013, overweight and obesity (Body Mass Index or BMI greater than or equal to 25 $kg.m^{-2}$) afflicted 37% of men and 38% of women worldwide, having already surpassed the number of people suffering from undernutrition in 2000[1]. Most pertinently, South Africa appears to be amongst the most obese nations worldwide, and South African women in particular, currently exhibit a striking prevalence of 69% overweight and 42% obesity[1]. Paradoxically, obesity was once considered a concern of affluent first-world countries, yet more recently, low-to-middle-income countries (such as South Africa) have experienced the most alarming increases in obesity, such that many of these countries now face concurrent burdens of obesity and undernutrition[2,3]. For example, the National Income Dynamics Study found that 45% and 37% of South African households with a stunted or underweight child, respectively, had at least one obese adult [4].

Obesity typically presents with increased risk for chronic non-communicable diseases (NCDs)[2,3]. It also has adverse impacts on work productivity, employment, transportation and the environment, and places an immense burden on both individual finances as well as the national healthcare system and economy[1,5]. In fact, a 2014 McKinsey Global Institute report placed obesity as the third greatest social burden, accounting for an estimated $2 trillion in resources (almost equivalent to that of smoking or armed violence, war and terrorism)[6]. In a recent study of 70 000 members of a South African medical aid scheme, moderate obesity was associated with 11% greater medical expenditure, which was comparable to that of past or current smokers[7]. Further, despite the adoption of multi-level strategies, it has proven difficult to halt the progression of obesity and associated diseases[3], and the majority of the global adult population is predicted to be obese by 2030[8]. Therefore, it seems imperative to better understand the obesity pathology and develop more effective public health strategies to secure a healthier outlook and economic security for future generations.

As shall be explored in this review, the aetiology of obesity is multi-factorial, encompassing complex gene-environment-lifestyle interactions that may differ subtly from one individual to another. The complexity is compounded by peculiar metabolic phenotypes in which obesity and normally-associated diseases develop independently. This will be explored with particular reference to the emerging paradox of athletic individuals who gain weight and/or develop metabolic illness despite following advice to exercise regularly. It is important to better understand the mechanisms that differentiate these phenotypes, so as to better inform health guidelines that take cognisance of such discrepancies.

1.2.) Obesity and obesity-associated disease

Obesity has been defined as an excess accumulation of fat in adipose tissue to the extent that health may be impaired[9]. Although typically recognised on the basis of a BMI greater than or equal to 30 $kg.m^{-2}$, this has proven contentious given that BMI is unable to distinguish between muscle mass ('fat-free-mass') and fat mass[10]. In fact, recent evidence suggests that individuals with a high BMI and elevated muscle mass, may be protected from premature mortality relative to individuals with lower BMI[11]. Given that upper-body obesity, indicative of greater abdominal or visceral fat, has been most commonly associated with disease risk,[12] waist circumference (greater than or equal to 80 cm for females) has become a more popular construct for diagnosing obesity and consequent health risk in a clinical setting [10].

Obesity rarely exists in isolation and affected individuals typically present with metabolic abnormalities, including but not limited to, hypertension, hyperglycaemia, insulin-resistance, dyslipidaemia, endothelial dysfunction and systemic inflammation[13,14]. This co-existence of multiple metabolic disturbances was first recognised in the 1920s by the Swedish physician Eskil Kylin, and was subsequently linked in the 1940s to the appearance of abdominal obesity in the same patients[12]. The clustering of disturbances has since become associated with increased risk for type 2 diabetes (T2D) and cardiovascular disease (CVD)[12]. Although each abnormality increases risk for chronic disease, none in isolation increases risk to the same extent as presenting with multiple disturbances simultaneously[14]. The term 'metabolic syndrome' (MetS) was coined to describe this

clustering of T2D and CVD risk factors, which by inference are likely to share a common underlying pathophysiology[14].

There are various definitions of the MetS, which differ in the relative emphasis placed on its four central features (central obesity, insulin-resistance, atherogenic dyslipidaemia and endothelial dysfunction)[14]. The World Health Organisation (WHO) was first to develop its MetS definition in 1998, at a time when insulin-resistance was thought to be the salient defect[15]. Consequently, the WHO criteria stipulate that insulin-resistance is an absolute requirement for MetS[15]. This may be identified by impaired fasting glucose (≥ 6.1 $mmol.l^{-1}$ in plasma glucose) or impaired glucose tolerance (≥ 7.8 $mmol.l^{-1}$ at the 120 minute blood draw during a 75 gram Oral Glucose Tolerance Test, OGTT). WHO also requires any two of central obesity (by a waist: hip ratio ≥ 0.80 in women or a BMI ≥ 30 $kg.m^{-2}$), dyslipidaemia by either elevated serum triglycerides (TG, ≥ 150 $mg.dl^{-1}$) or reduced serum High-Density-Lipoprotein-cholesterol (HDL-C, < 39 $mg.dl^{-1}$ in women), or hypertension (blood pressure ≥ 140 / 90 mmHg)[15]. The National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) criteria, as updated in 2005 by the American Heart Association, has been considered more clinically applicable since it does not make an absolute requirement that assumes an underlying cause[16]. ATP III requires the presence of three of the following five criteria: elevated waist circumference (≥ 88 cm), hyperglycaemia (fasting plasma glucose ≥ 5.6 $mmol.l^{-1}$), TG ≥ 150 $mg.dl^{-1}$, HDL-C < 50 $mg.dl^{-1}$ in women or hypertension (≥ 130 / 85 mmHg)[16]. Finally for the purpose of this review, the International Diabetes Federation (IDF) published new criteria in 2005 that required obesity to be present (by population-specific waist circumference cut-points), in addition to any two of the following: abnormal TG or HDL-C, elevated blood pressure or impaired fasting glucose, as per the thresholds set out by ATP III[17]. The latter has received criticism for having considered obesity as opposed to insulin-resistance as the primary pathophysiology[18]. Regardless, abdominal obesity and insulin-resistance have both been hypothesised to be the primary regulators of MetS. The exact mechanisms by which they foster related metabolic pathologies are complex and require further delineation. For example, whilst weight-loss interventions in the context of MetS have benefitted other risk factors, other research has found obese persons who do not present with metabolic complications ('Metabolically Healthy Obesity'), and normal-weight individuals who unexpectedly present with insulin-resistance, hypertension and dyslipidaemia[18]. Therefore, it seems likely that insulin-resistance is the primary defect

underlying MetS and links increased adiposity to metabolic illness in persons predisposed to obesity[18].

1.3.) Insulin-resistance as the underlying pathology

Insulin is released into systemic circulation by pancreatic beta cells, primarily in response to carbohydrate ingestion[19]. Insulin is crucial to blood glucose homeostasis by stimulating the rapid uptake of postprandial blood glucose into insulin-dependent peripheral tissues[19]. This includes adipose tissue, but most glucose is taken up by skeletal muscle, where it may be oxidised or stored as glycogen[19,20]. At the cellular level, insulin binds to insulin receptors at the plasma membrane, which stimulates the glucose transporter (GLUT4) to be translocated to the membrane via the 'IRS-1 – PI3K – Akt' enzymatic pathway[20]. GLUT4 translocation is required for the uptake of glucose into the cell[20]. At the liver, when exogenous glucose is ingested, insulin acts to potently suppress glucose output from glycogenolysis and partially suppresses (approximately 20%) gluconeogenesis[21,22], significantly reducing endogenous glucose production. Additionally, insulin promotes fat synthesis and storage in both adipose tissue and the liver, and inhibits both adipose tissue lipolysis (fat breakdown) and fat oxidation in the muscle[19]. Insulin also exerts important physiological functions at the endothelium (increased nitric-oxide dependent vasodilation and capillary recruitment), brain (neuronal development, eating behaviour and cognitive functioning) and heart (increased myocardial blood flow and cardiac contractility) and therefore plays a central role in regulating overall metabolic and cardiovascular homeostasis[23]. In the insulin-resistant condition, however, certain tissues appear to experience a reduced response to the insulin signal[18].

Although there is no consensus as to the exact pathogenesis of insulin-resistance, it has been acknowledged that different tissues develop insulin-resistance at different times.[18] Additionally, while some insulin pathways develop resistance, other pathways in the same tissue may remain insulin-sensitive[18,24]. The primary defects initiating metabolic pathology appear to be an inability of insulin to suppress endogenous glucose production from the liver, and impaired insulin-stimulated glucose uptake in skeletal muscle (and to a lesser extent in adipose tissue), especially for glycogen synthesis[24,25]. The former promotes fasting

hyperglycaemia, whilst the latter reduces glucose tolerance considerably, since skeletal muscle normally accounts for 80% of whole-body glucose disposal[24]. In such cases, regular carbohydrate ingestion, combined with increased hepatic glucose output, causes a relative hyperglycaemia, for which pancreatic beta cells attempt to compensate by secreting more insulin[12,20]. This pattern promotes the development of chronically elevated blood insulin (known as 'hyperinsulinaemia') and metabolic pathology[20].

Early in the MetS pathophysiology, adipose tissue itself appears to develop insulin-resistance[12]. Most importantly, the normal inhibition of lipolysis becomes impaired, which increases the breakdown of stored TG to free-fatty-acids (FFA) that enter into circulation[24]. The fat synthesis pathway, however, remains insulin-sensitive and in the context of prolonged insulin exposure, promotes fat storage and adipocyte hypertrophy[24]. In turn, abdominal adiposity may exacerbate the insulin-resistant condition via several mechanisms[26]. Firstly, expanding adipose tissue becomes infiltrated with pro-inflammatory monocyte-derived macrophages[24]. These secrete pro-inflammatory 'adipocytokines', such as tumour-necrosis-factor-α (TNF-α) and interleukin-6 and reduce the secretion of the anti-inflammatory, insulin-sensitising hormone adiponectin[24]. Consequently, insulin-resistance and obesity have been commonly linked to a chronic low-grade pro-inflammatory state[18,27]. TNF- α and interleukin-6 , appear to impair skeletal muscle insulin-signalling by activating certain serine-kinase enzymes (e.g. c-Jun N-terminal kinases) that phosphorylate key regulatory serine residues on the insulin receptor and Insulin Receptor Substrate-1, and thereby down-regulate insulin-stimulated GLUT4 translocation[28].

Increased FFA flux from insulin-resistant adipose tissue, in concert with prolonged hyperglycaemia, have also been associated with mitochondrial dysfunction and impaired glucose and fat oxidation capacity[27,29]. The latter may cause incomplete fat oxidation[27], resulting in the production of deleterious fatty acid derivatives (such as diacylglycerols and ceramides) that appear to aggravate insulin-resistance in a similar manner to inflammatory cytokines[27]. It has also been proposed that such mitochondrial defects increase reactive oxygen species (ROS) production, which may cause detrimental oxidative damage to cellular components and further exacerbate insulin-resistance[27,29]. Furthermore, un-regulated lipolysis increases substrate availability for fat accumulation in ectopic (non-

adipose) tissues, including skeletal muscle, the pancreas and the liver[27,30]. Although the liver develops resistance to the insulin-mediated inhibition of glucose output, becomes resistant to the insulin signal to reduce glucose output, the pathway that activates hepatic lipogenesis retains normal function[18,24]. Ectopic fatty acid deposition at the liver manifests as non-alcoholic fatty liver disease (NAFLD)[18,24]. This may be further compounded by *de novo lipogenesis,* whereby excess carbohydrate directed to the liver, is converted to fat and stored as TG[30]. Indeed, NAFLD has been commonly associated with traits of the MetS, which is likely mediated by the mutually-aggravating relationship between NAFLD and insulin-resistance[31]. Overall, insulin-resistance coupled with adipose tissue expansion, seem to initiate widespread metabolic disturbances by fostering hyperinsulinaemia, inflammation, oxidative stress and toxic lipid accumulation in non-adipose tissues. However, the strongest link between obesity, insulin-resistance and cardiovascular risk is the atherogenic dyslipidaemia that stems from abnormal liver function[18].

The dyslipidaemia that characterises the MetS includes elevated plasma FFA and TG, low concentrations of high-density-lipoproteins (HDL) and a predominance of small, dense low-density-lipoprotein (LDL) particles[24]. Circulating lipids are largely influenced by hepatic insulin-resistance and hyperinsulinaemia[18,24]. As mentioned, hyperinsulinaemia up-regulates hepatic lipogenesis, which increases TG synthesis and export via very-low-density-lipoprotein (VLDL) particles (VLDL-TG)[14]. Simultaneously, both hepatic LDL-receptor expression and lipoprotein lipase activity are down-regulated[12,14]. This increases the secretion of apolipoprotein B particles (predominant in LDL and VLDL) and impairs VLDL-TG clearance respectively[12,14]. The resultant 'hypertriglyceridaemia' reduces circulating HDL by increasing the TG-cholesteryl ester exchange with HDL particles, producing small dense HDL-TG that are more readily cleared from circualtion[12]. The predominance of small LDL particles (known as a 'Pattern B' LDL profile) appears to arise from the cleavage of larger VLDL particles, which are subsequently enriched with triglyceride and hydrolysed to form small dense LDL[12,32]. These pose greater cardiovascular risk because they have lower affinity for clearance by the LDL-receptor. They are also more easily able to transit through the basal membrane of the endothelium, adhere to the vasculature walls and foster arterial plaque build-up that is susceptible to oxidative damage[12]. Other remnant particles of VLDL-TG hydrolysis, including intermediate-density lipoproteins (IDL) and chylomicron remnants, have also been associated with the progression of atherogenic risk[32].

Endothelial dysfunction (recognised as hypertension in the MetS) is also closely linked to insulin-resistance and dysregulated adipose tissue[14]. Endothelial cells line the inner surface of blood vessels and respond to external stimuli by releasing vasoactive molecules[14]. For example, insulin is a potent vasodilator and acts on functioning endothelial cells to produce nitric oxide that stimulates blood flow and capillary recruitment[14]. Insulin thereby exerts an an indirect effect on blood pressure and facilitates both glucose delivery to and uptake by peripheral tissues[14,24]. Endothelial expression of cell adhesion molecules also mediates circulatory inflammation and coagulation and protects against the development of atherosclerotic plaques[14]. Endothelial insulin-resistance appears to develop from the associated conditions of hyperglycaemia, increased FFA, ROS and pro-inflammatory cytokines (e.g. TNF-α) and reduced anti-inflammatory adiponectin[14,24]. These collectively impair insulin signalling and reduce nitric oxide production and its normal physiological effects[14]. In the context of other insulin-mediated processes that remain functional (renal sodium reabsorption and sympathetic-nervous-system activation), vasoconstriction and hypertension are further aggravated[12]. Combined with the increased vascular inflammation and oxidative susceptibility, these disturbances foster a significantly pro-atherogenic vasculature[12].

Although not recognised as a component of the MetS, dysregulated leptin signalling appears to be strongly associated with obesity and MetS[33,34]. Leptin (the 'satiety hormone') is a hormone secreted from adipose tissue in proportion to the mass of adipocytes[34,35]. Leptin targets the hypothalamus in the brain to regulate appetite, metabolism and overall energy balance[35,36]. High leptin normally signals that energy stores are adequate, and the body does not require further energy intake but should rather expend energy (by physical activity) at a normal rate[35,36]. Low leptin conversely stimulates appetite and hunger, thereby promoting energy intake and inhibiting physical activity to conserve energy[36]. Therefore, leptin signalling has been implicated for its likely role in preventing humans from starvation and overeating[36,37]. Obese individuals typically present with systemically elevated leptin levels[33,35]. Theoretically this would promote satiety and reduce food consumption[33,35]. However, evidence suggests that the hypothalamus has developed resistance to the leptin signal such that it no longer responds appropriately[35]. Instead, the brain 'thinks' that the body is starving, which triggers overconsumption and reduced

energy expenditure to conserve the energy stores it thinks are threatened[38]. This would further aggravate the obese condition in a vicious cycle[35,38]. Apart from expanding adipose tissue, evidence has implicated the modern diet for disrupting leptin's normal appetite-suppressing effects ('diet-induced leptin-resistance')[37,39,40]. Although the mechanisms remain unclear, excess consumption of highly palatable, high-sugar, high-fat foods may dysregulate satiety mechanisms and foster both insulin-and- leptin-resistance[37,41]. These defects may well be the primary drivers of obesity, and they appear to be both self-aggravating and integral to the development of associated metabolic abnormalities[37].

Much remains to be determined about the pathogeneis of obesity and MetS. However, the available evidence supports an integral role for insulin-(and leptin)-resistance in promoting these conditions. Although genetic predisposition significantly influences individual susceptibility, the recent surge in chronic NCDs implicates modern environmental and lifestyle causes. Specifically, contemporary patterns of unhealthy diet, physical inactivity, sedentary behaviour, sleep deprivation and chronic stress represent the most plausible culprits.

1.4.) Causes of obesity and associated metabolic disturbances

1.4.1.) Non-modifiable factors (Genetics)

Considerable research has been dedicated to identify 'fat genes' that predispose individuals to obesity. This has proven complex since obesity-related phenotypes (except for rare monogenic cases) are influenced by multiple genetic loci variants, single-gene mutations and single-nucleotide-polymorphisms (SNPs)[42]. Indeed, genome-wide association studies (GWAS) have identified over 50 genes associated with obesity, the majority of which impart small individual effects, but when presenting together may significantly influence the observed phenotype[43]. Most of the implicated genes play a role in the central regulation of food intake, eating behaviour and body-weight[43]. For example, SNPs in the anorexigenic genes, *MC4R* (melanocortin-4-receptor) and *POMC* (pro-opiomelanocortin) have been linked to impaired satiety, snacking behaviour and increased total energy intake[43,44]. Variants in the *FTO* (Fat mass and obesity-associated) gene have been routinely associated

with increased food intake, appetite and dysregulated appetite-related hormones (e.g. leptin and ghrelin)[43,44]. The latter has been supported by extensive animal research that has shown increased food intake and obesity in response to the overexpression of *FTO*[43]. In humans, several genetic variants of FTO have been shown to confer risk for obesity[45]. The *FTO* gene has also been associated with individually variable weight-loss in response to lifestyle (diet and exercise) interventions: individuals who carried the homozygous obesity-predisposing allele (A) lost more weight compared to non-carriers[44]. Another study found that the cumulative obesity susceptibility from 12 SNPs was blunted by up to 40% by high levels of physical activity[46]. This implicates genetic variance for mediating individual responses to external stimuli.

Genetic variance may further influence obesity predisposition by modulating resting metabolic rate (RMR) or the rate of energy utilisation required for basal bodily functions to continue at rest. RMR may be modified by multiple factors, including age, physical activity, lean muscle mass, diet-induced weight loss and thyroid function, however, it has been reported to be up to 40% heritable[47]. Not much is known about the influence of human genetic variance on RMR, but recent evidence has shown that variants involved in energy homeostasis and nutrient processing may predispose certain individuals to reduced RMR and weight-gain[48]. The kinase suppressors of Ras protein 2 (KSR2) interacts with the 'Raf-MEK-ERK' enzymatic pathway as part of the regulation of cellular metabolism and substrate utilisation[48]. Specific KSR2 variants associated with obesity in humans were found to disrupt signalling in a manner which impaired fatty acid and glucose oxidation and lowered RMR[48]. Given that RMR normally accounts for the predominant portion of daily energy expenditure (60 - 70%), a reduced RMR may increase the difficulty of maintaining energy balance and a healthy weight[49].

It seems logical that co-occurring features of the MetS, and consequent CVD risk, share common genetic determinants[50]. Although evidence supports this contention, the estimated heritability differs significantly between studies[50]. For example, BMI has been estimated to be between 25% and 60% heritable, fasting glucose between 10% and 75% and hypertension approximately 50%[50]. Insulin-resistance and T2D both appear to have a large genetic component, estimated to be 46% to 90% heritable[40]. In line therewith, a

positive family history of diabetes is strongly predictive of future offspring pathology, and relatives of diabetics exhibit increased glucose intolerance[18,50]. In this regard, evidence has implicated the peroxisome proliferator-activated receptor (PPAR) – ϒ gene that modulates adipogenesis, as well as genetic variants that influence pancreatic beta cell function[50]. Other studies of twin pairs and families have explored pleiotropic genetic variants that influence two or more phenotypic traits and hence may explain the typical clustering of MetS risk factors[50]. For example, the Swedish Adoption/Twin Study of Aging found that BMI and insulin-resistance in particular, but also TG and HDL-C concentrations and systolic blood pressure, were all influenced by a single latent genetic factor[51]. Similarly, in the San Antonio Heart Study, nondiabetic individuals with a parental history of T2D exhibited a significantly more atherogenic lipid profile compared to individuals without such history, and this was largely explained by differences in BMI, waist-to-hip ratio and fasting insulin levels[52]. Furthermore, recent meta-analyses of GWAS indicated relatively strong evidence for pleiotropic genetic influence on the clustering of CVD traits, particularly coronary artery disease with an atherogenic lipid profile[50]. On the other hand, genetic variation also appears to contribute to the disassociation between obesity-related phenotypes. For example, a specific SNP in the *visfatin* gene, which codes for the insulin-mimicking adipocytokine visfatin, was recently found to protect against insulin-resistance and CVD risk in both obese and non-obese sub-groups[53]. Therefore, there has been considerable evidence for the heritability of obesity, insulin-resistance and associated cardio-metabolic outcomes. However, in the majority of cases, genetic predisposition may explain part of the observed phenotype, but it requires interaction with 'modifiable' or environmental factors to trigger the pathology.

1.4.2.) Modifiable factors

1.4.2.1.) At the Global level

The recent rise in obesity prevalence has coincided with significant transitions in global economic structures, living and work environments and nutritional exposures[3]. Globalisation and the economic growth, urbanisation and trade liberalisations that have accompanied it, have improved widespread aspects of life[3]. Particularly in low-to-middle-income countries, more people have access to basic amenities, food security and

opportunities to earn an income[3]. However, such advancements have also fostered an 'obesogenic environment' that is characterised by a built living-environment, office-based work, mechanised transport, screen-oriented leisure activity, year-round produce availability, and aggressive food marketing strategies that promote 'over-nutrition' of nutritionally-poor foods[2,3]. This environment has promoted the two leading ('the Big Two') causes of a positive energy balance and associated diseases, namely excess caloric intake and increased sedentary behaviour with reduced physical activity[54,55]. It follows that mainstream advice to reverse this trend has been to simply reduce energy intake and increase energy expenditure[3,41]. However, more recently it has been proposed that weight regulation has a more complex aetiology: that different types of calories have different metabolic implications, and that these may counteract the benefits of 'moving more' to foster weight-gain[41,56].

1.4.2.2.) Dietary Exposures

According to the Lancet Global Burden of Disease reports, unhealthy diet may account for more disease than physical inactivity, alcohol consumption and smoking combined[57]. Profound changes in food choice, availability, production and guidelines appear to have fostered a dominant dietary pattern that promotes weight-gain and obesity-associated disease[3]. Research on the primary dietary culprits has been contentious, owing largely to conflict between nutrition research and health authorities. In 1977, the US Senate Select Committee on Nutrition and Human Needs published the *Dietary Goals for the United States.* It advised people that if they were to remain healthy and control their body weight, they should reduce their consumption of fat, particularly saturated fat and cholesterol. This was based on the prevailing ideas at the time, which persist to an extent today: i.) that saturated fat intake associates with increased serum cholesterol and deaths from coronary heart disease[39], and ii.) that body weight regulation is as simple as matching energy intake to energy expenditure[41,58]. The notion that saturated fat consumption increases CHD risk appears to have been spurious and recent findings suggest that the underlying 'Diet-Heart Hypothesis' was incorrect[58,59]. Ironically, recovered data from the Minnesota Coronary Experiment, which was designed to prove said hypothesis, showed that although replacing saturated fat with vegetable oils did reduce serum cholesterol, the latter was associated

with increased risk of death[58,59]. Similar studies have found no association between total cholesterol and mortality[58].

The second prevailing idea was that obesity is a quantitative problem of excess caloric intake, which disregards the qualitative notion of caloric quality and its subsequent biological effects[41]. Since fat constitutes more energy per gram (9 kcal.gram^{-1}) compared to protein or carbohydrate (both 4 kcal.gram^{-1}), public health messages have been biased against fat intake, but in favour of less calorie-dense lean meats, whole-grains, fruits, vegetables and low-fat dairy products[41]. Indeed, the 'demonization' of fat and particularly saturated fat intake, has had a profound nutritional impact[56]. Big food industry has capitalised by mass producing foods that contain cheap vegetable oils such as corn, soybean and rapeseed oils (rich in omega 6 polyunsaturated fatty acids), as well as simple carbohydrates in the form of refined grains and sugars[2,40]. There is now compelling evidence that refutes caloric equivalence and suggests that such dietary components have actually contributed to the epidemics of obesity and MetS.

Omega 6 (n-6) polyunsaturated fat and *trans* fat intake have increased considerably in recent decades, while omega 3 (n-3) intake has declined[60]. It has been proposed that the optimal dietary n-6 : n-3 ratio, as consumed during hunter-gatherer periods, is 1:1[61], whereas the modern Western diet, rich in vegetable oils, has been reported to average between 10:1 and 20:1[60]. Furthermore, owing to the integration of dietary fatty acids into cellular membranes, the quantity of n-6 fatty acids in human adipose tissue biopsies has dramatically increased (by 3-fold) since the 1960s[62,63]. Although some polyunsaturated n-6 fatty acids are essential (e.g. linoleic acid) , their higher unsaturated derivatives (e.g. arachidonic acid) give rise to pro-inflammatory 'eicosanoids' via fatty acid desaturation and elongation reactions[60,64]. Consequently, excess dietary n-6 fatty acids can promote inflammation, vasoconstriction, platelet aggregation and lipogenesis, which has been suggested to increase risk for CVD[60]. However, as reviewed by Russo (2009), many studies have failed to show any meaningful relationship between tissue n-6 fatty acid presence and CVD risk[64]. In contrast, the evidence that n-3 fatty acids (particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) are cardio-protective, has been consistently strong[60,64]. Indeed, n-3 fatty acids, have anti-inflammatory, vasodilatory effects and

enhance both lipid oxidation and insulin-sensitivity by activation of the PPAR-alpha(α) transcription factor[60]. This has motivated the viewpoint that reducing n-6 fatty acid consumption is not necessarily as important as increasing n-3 fatty acid consumption[65]. In contrast, there is strong evidence implicating *trans* fatty acids for increasing CVD risk[66]. *Trans* fatty acids are produced during the 'partial hydrogenation' of polyunsaturated vegetable oils that is performed during the production of processed foods to render them more stable and prolong their shelf life. *Trans* fatty acid consumption has been associated with insulin-resistance, dyslipidaemia (low HDL and high TG), inflammation, endothelial dysfunction and thrombosis[67,68]. *Trans* fatty acids are particularly atherogenic owing to their incorporation into endothelial cell membranes, where they foster characteristics of endothelial dysfunction and atherosclerotic plaque formation[68]. Furthermore, they are highly susceptible to oxidative damage (during cooking, processing, storage and inside the body), which produces reactive compounds (e.g. ROS) that are systemically harmful [60]. For example, increased lipid peroxidation causes oxidative stress, platelet aggregation and immune cell adhesion to the vascular endothelium[60]. Therefore, excess consumption of processed foods rich in *trans* fatty acids, possibly exacerbated by excess n—6 and/or too little n-3 fatty acids, appears to foster an atherogenic vasculature.

Global consumption of simple carbohydrates (primarily refined grains and sugars) has also increased in parallel with obesity and MetS prevalence[41,69]. Simple carbohydrates have a high-glycaemic index (GI) since they are rapidly absorbed compared to proteins, fats and complex carbohydrates[70]. Refined grains such as white rice and white flour, have had their bran and germ portions removed, which contain the fibre, antioxidants, vitamins and minerals, leaving an energy dense but nutrient poor food[71]. Fibre has multiple health benefits: it functions as a prebiotic that feeds 'good' bacteria in the intestine, it slows nutrient absorption, promotes greater satiety, and reduces the GI of carbohydrate-containing foods[72]. The removal of fibre alters the physiological and hormonal effects of the consumed grain, such that they become more similar to sugar. Indeed, refined grains and sugar cause a pronounced insulin spike to dispose of the glucose load and to inhibit lipolysis[19,41]. This can cause a rapid decline in blood glucose ('reactive hypoglycaemia'), and a simultaneous reduction in available FFA[41,70], promoting a recurring loop of hunger and overconsumption of simple carbohydrates[41,70]. In predisposed individuals, this may manifest as chronic hyperinsulinaemia, insulin-resistance, and eventually pancreatic beta

cell dysfunction[20,73]. Consequently, refined grain consumption has been consistently associated with increased risk of weight-gain, systemic and gut inflammation, digestive problems, T2D and CVD[71,74].

It is now generally accepted by most researchers and health organisations that sugar in particular, has a detrimental impact on weight gain and metabolic health[40,75]. However, this is not without some dispute[40]. Concomitant with the rise in overweight and obesity, global consumption of sugar, largely in the form of sugar-sweetened beverages, has significantly increased over the past few decades[3,76,77]. In the USA for example, National Health and Nutrition Examination Survey (NHANES) data from 2005 to 2010 showed that men and women consumed an average of 335 kcal/day and 239 kcal/day from added sugars, representing 12.7% and 13.2% of total caloric intake respectively[78]. As comprehensively reviewed elsewhere[79], increased sugar consumption as part of free-living diets has been strongly associated with weight-gain[67]. Sugar intake has further been associated with components of the MetS, as well as T2D and CVD, independent of BMI and total energy intake[40,80]. This suggests that independent of its caloric value, sugar can promote metabolic pathology[40]. Randomised controlled trials of added sugar consumption in humans, both in the context of *ad libitum* diets and energy-controlled, weight-maintenance diets, have strongly suggested that sugar adversely impacts metabolic risk (e.g. increased TG, reduced HDL-C and reduced insulin-sensitivity)[40]. However, the *ad libitum* studies were confounded by variable individual diets and controlled studies were limited by methodological flaws[40]. Consequently, a recent review remarked on the absence of definitive trials concerning sugar's potentially independent role in metabolic disease[40]. The controversy has been compounded by the null findings of sugar-industry-funded studies[40,81]. Despite this uncertainty, robust mechanisms have been proposed[40].

Sugar (sucrose) is a disaccharide composed of glucose and fructose monomers. Whereas hepatic glucose metabolism is regulated by insulin and hepatic energy need such that much of the ingested glucose reaches circulation, dietary fructose is not regulated by hepatic energy status and is predominantly metabolised in the liver[40]. Dietary fructose, therefore, provides excess substrate for the liver, which stimulates *de novo lipogenesis*[40]. This increases hepatic lipid stores, promotes hepatic insulin-resistance, NAFLD, VLDL-TG

production and consequent dyslipidaemia[40]. Fructose metabolism to fructose-1-phosphate also converts significant amounts of adenosine triphosphate (ATP) to adenosine monophosphate, which leads to uric acid production via the purine degradation pathway[82]. Uric acid appears to be a potent mediator of hypertension and T2D, by promoting inflammation, oxidative stress, insulin-resistance and endothelial dysfunction[40,75]. In addition, studies conducted in rat models suggest that fructose induces leptin-resistance, independent of increased adiposity or elevated leptin levels[37]. In the presence of a highly palatable diet (high-sugar, high-fat), impaired satiety would promote over-consumption and weight-gain[37]. Nonhuman animal models have found that sugar consumption stimulates the same reward and hedonic neural pathways as psychostimulant and opiate drugs, by binding to opioid and dopamine receptors and stimulating the release of dopamine and acetylcholine[83,84]. In this manner, sugar consumption induces a reward and craving that is comparable in magnitude to that of cocaine[85], and fosters sugar-addiction[83,84]. Further research is required to confirm these findings in humans. However, human studies have reported that sugar consumption fails to induce normal satiety mechanisms, which may cause excess energy intake (beyond caloric need) at subsequent meals[3,40]. Despite the lack of definitive clinical trials, the epidemiological data and plausible mechanisms suggest that sugar consumption does promote obesity-associated disease, both directly via dysregulated metabolism and indirectly via dysregulated appetite control[40].

Overall, evidence suggests that the modern Western diet, characterised by highly refined high-sugar, high fat foods, has contributed considerably to obesity and MetS. The assumption that any two foods with the same potential energy (calorie) would influence satiety, food intake and body weight similarly, now seems inherently flawed[41]. More recently, alternative dietary practices to the low-calorie, low-fat model have been advocated to better regulate the hormones (e.g. insulin, leptin) involved in food intake and metabolism[41,86,87]. Low-GI carbohydrates (e.g. oatmeal, apples, pearl barley), have been theorised to induce a smaller and more sustained postprandial rise in blood glucose[86,88]. This would induce a lower relative insulinaemia and increase the availability of metabolic fuels, such as hepatic glucose and adipose-derived FFA[86,88]. Low-GI foods have, therefore, been advocated to make one 'feel fuller for longer' than equivalent high-GI foods and reduce subsequent food intake[88]. Studies of short-term satiety differences between low-GI

and high-GI meals, have suggested this may be effective[88]. Sixteen such studies were reviewed by Ludwig *et al.* (2000), and 15 of them found increased satiety, delayed onset of hunger or decreased food intake after a low-GI compared to a high-GI meal[86]. The authors of a 2007 review speculated that this may be partly due to differences in fibre content and palatability[88]. Long-term studies of low-GI diets in relation to weight loss, however, have generally failed to show significant benefits to spontaneous energy intake or body weight regulation[88]. Future studies are needed that are better controlled and compare diets differing only in GI, to make definitive conclusions[88].

Rather than focusing on the GI of individual food components, Zeevi *et al*. (2015) recently brought attention to the postprandial glycaemic response (PPGR) to real-life meals that consisted of arbitrary food combinations[89]. They reported substantial inter-individual variability in the PPGR to identical test foods, and developed an algorithm to predict personalised PPGRs based on variables including meal content, daily activity, blood parameters and microbiome features[89]. Individually-tailored dietary prescriptions significantly improved PPGRs and associated metabolic characteristics[89]. Based on these findings, the authors speculated that dietary advice for optimal body weight regulation and metabolic health should be individualised[89]. Indeed, consumption of foods that induce a low PPGR seems to be important for minimising both postprandial insulinaemic responses and the long-term risk of hyperinsulinaemia and insulin-resistance[86]. A couple of earlier randomised controlled trials have explored varying macronutrient composition or glyceamic load appropriately for individuals with varied insulin-sensitivity and insulin secretory profiles[90,91]. Pittas *et al*. (2005) found that overweight but healthy men and women, who had higher OGTT insulin secretion, lost significantly more weight on a low-GI diet (30% fat, 40% carbohydrate, 30% protein) compared to a high-GI diet (20% fat, 60% carbohydrate, 20% protein)[91]. The latter was particularly adverse for participants with the highest insulin secretion owing to regular postprandial hypoglycaemia, hunger and excess energy intake[91]. Similarly, Cornier *et al.* (2005) randomised nondiabetic obese women, who had been identified as insulin-resistant or insulin-sensitive, to calorie-restricted high-carbohydrate, low-fat (HCLF, 20% fat, 60% carbohydrate, 20% protein) or so-called low carbohydrate, high-fat (LCHF, 40% fat, 40% carbohydrate, 20% protein) diets for 16 weeks[90]. Although all groups (insulin-resistant-HCLF, insulin-resistant-LCHF, insulin-sensitive-HCLF and insulin-sensitive-LCHF) lost significant weight, the extent of weight loss

was determined by insulin-sensitivity status[90]. Specifically, the insulin-resistant group lost most weight on a LCHF diet and the insulin-sensitive group on the HCLF diet[90]. These studies show the importance of tailoring diets to be appropriate for individual physiology, and suggest that appropriate carbohydrate restriction may attenuate the risk of hyperinsulinaemia and weight-gain most successfully.

'Carbohydrate restriction' refers to a dietary pattern that is low in all carbohydrates, including wholegrains, refined grains and sugars. It has recently received significant attention for its purported potential in addressing obesity and metabolic illness[56,87]. Carbohydrate restriction may take many forms, including high-protein, low-carbohydrate (e.g. Atkins), LCHF, very-low carbohydrate, high-fat (VLCHF), ketogenic (VLCHF with elevated blood ketone concentrations) and intermittent fasting. Proponents of carbohydrate restriction have advanced that the human body has adapted evolutionarily from our hunter-gatherer ancestry to the use of fat as its predominant fuel source[92]. The human genome may thus be ill-adapted to the agricultural-based, high-carbohydrate dietary practices of modern society[92]. This has created a gene-environment mismatch with deleterious health consequences[92]. Given that obesity-associated disease appears to be driven by insulin-resistance and compensatory hyperinsulinaemia[18], appropriate carbohydrate restriction that minimises insulin secretion would theoretically benefit body weight regulation and metabolic health[87].

Although the carbohydrate 'threshold' differs on an individual basis, numerous studies that restricted carbohydrate intake to less than 50 grams per day (VLCHF diets) have reported improvements in MetS. For example, a 2013 meta-analysis of randomised controlled trials with at least 12 months follow-up concluded that well formulated *ad libitum* VLCHF diets were more effective than calorie-restricted HCLF diets for reducing body weight and improving MetS risk factors, including TG, HDL-C and blood pressure[93]. Other studies conducted for a range of time periods (3 months up to 2 years) have shown VLCHF diets to be at least as effective as HCLF, Mediterranean or so-called 'Healthy Eating' diets for improving fasting blood insulin, glucose and glycosylated haemoglobin (HbA1C) concentrations, insulin-sensitivity, adiposity and improving lipid profiles by increasing LDL-C particle size and HDL-C concentrations whilst reducing serum TG[94–101]. In light of these findings, a recent critical review of the role of nutrition in metabolic health concluded that

VLCHF diets represent the most effective intervention for reducing all symptoms of the MetS and preventing the development of T2D independent of weight loss[87]. Additionally, *ad libitum* VLCHF diets appear to be most beneficial for promoting weight loss in both diabetic and non-diabetic individuals[87]. The latter may result from increased protein and fat consumption that, in the absence of simple carbohydrates, appears to improve satiety and promote spontaneous caloric reduction despite unlimited access to food[87]. Additionally it has been proposed that the limited selection of foods on a VLCHF diet enforces this caloric restriction[98]. Indeed, avoiding carbohydrate-rich foods may be particularly difficult for many individuals and has been raised as a concern to the long-term adherence to VLCHF diets, on the basis that sustained diet adherence rather than any specific diet seems to determine weight management success[102]. However, evidence from clinical trials suggests that at least up to 12 months, adherence to VLHF diets is comparable to that of other dietary strategies[87,103]. For example, Hu *et al.* (2016) recently reported comparable adherence from 148 adults randomised to low-carbohydrate (< 40g.day^{-1}) or low-fat (<30%) diets, with greater weight-loss experienced by those following the low-carbohydrate diet[103]. Despite the increasing anecdotal evidence, it remains to be clinically determined if free-living individuals would be able to sustain similar adherence to VLCHF diets over the long-term. Regardless, individualised nutrition regimes (carbohydrate restriction for some) that are easily sustained seem integral to improving the global burden of non-communicable disease [87,104].

1.4.2.3) Physical Inactivity and Sedentary Behaviour

It is widely believed that decreased physical activity has contributed to the increased prevalence of overweight and obesity[105], based on the assumption that reduced daily energy expenditure promotes positive caloric balance[41,86]. For this discussion, it is important to distinguish between 'physical inactivity' and 'sedentary'. The former specifically refers to not meeting physical activity guidelines[92], in which health authorities commonly prescribe 30 minutes of moderate-intensity physical activity on at least 5 days every week, 20 minutes of vigorous-intensity physical activity on at least 3 days per week or an equivalent combination thereof[106,107]. 'Sedentary' specifically refers to time spent sitting, lying down or expending low levels of energy[108]. Indeed, self-reported data indicates that globally 31% of adults do not meet physical activity guidelines[107], and 2010 data showed that 52% of South Africans were well below the age-standardised recommendations[109].

Interestingly, data from the USA suggests that leisure-time physical activity (e.g. gym, recreational sport) has remained stable or increased over the past 50 years, however, activity associated with employment, transportation (e.g. walking or cycling) and household chores has declined substantially[3]. Instead, American adults for example spend an average of 55% of their waking time sedentary, and various population-based studies report adults sitting for more than 5 hours.day^{-1} [1110]. In this regard, health authorities have recommended the public limits sedentary behaviour as far as possible, using strategies such as active transport, standing desks or prompts to break up sitting time, in an attempt to improve body-weight regulation[86,110]. However, whether or not reduced energy expenditure has contributed meaningfully to overweight and obesity, and conversely whether or not exercise reduced sedentariness would protect against weight-gain, remains controversial[56,105].

Observational studies of physical activity and future weight-gain have reported conflicting findings, with positive associations having been weak[41,105]. This may have been due to inherent confounding, difficulty in quantifying physical activity and sedentary behaviour accurately, inconsistent measurement methods, and potential reverse causality[41,105]. Previous systematic reviews and meta-analyses have reported that exercise interventions without caloric restriction have achieved minimal weight-loss[111,112]. Further, some studies have found no change, or even weight-gain, in response to exercise interventions with *ad libitum* dietary intake[113]. This evidence would suggest that exercise needs to be combined with conscious caloric restriction to achieve meaningful weight-loss[113]. Indeed, multiple systematic reviews have reported significant and clinically meaningful weight-loss from interventions that combined exercise with diet (caloric restriction)[112,114,115]. Furthermore, the significant improvements from 'diet only' arms motivated the interpretation that diet is the primary determinant of body composition[112,114]. In support of these concepts, for example, Pontzer *et al*. (2012) compared the energy expenditure of Hadza hunter-gatherers in Tanzania to those of comparable American adults living sedentary 'Westernised' lifestyles[116]. There was no difference in total energy expenditure between populations despite the Hadza being more physically active and significantly leaner[116]. The two populations were, however, differentiated by dietary intake: the Hadza consumed natural, raw foods, while the American adults consumed a highly refined high-sugar, high-fat diet[116]. Prospective associational studies between sedentary behaviour and weigh- gain

have proven unclear[110]. Specifically, studies have found no association, weak positive associations or even reverse causality, in which baseline adiposity predicted future sedentary behaviour[110]. Therefore, the notion that physical inactivity and a sedentary lifestyle have caused obesity does not appear to be as convincing as has been conveyed. This may be due to the overwhelming influence of some of the aforementioned dietary factors on the hormones that regulate food intake[41,56].

Energy intake and energy expenditure are tightly coupled by hunger-satiety signalling pathways between the brain, adipose tissue and gut[38]. This neuro-hormonal regulation of food intake (a functional 'appestat') is integral to body-weight regulation[41,117]. Increased physical activity (energy expenditure) has been shown to trigger signals to the brain to consume more calories in order to restore energy balance[41,117]. For example, hormonal tracking of professional cyclists during the gruelling Giro d'Italia cycling stage race, showed a steady decrease in leptin and concomitant rise in adiponectin, indicating an increasing need for energy or refuelling in these athletes[118]. Conversely, a 7-day simulation of the Tour de France in 13 highly trained cyclists found that athletes did not replenish their energy stores adequately, suggesting that the appestat may be overwhelmed or does not in such intensive scenarios[119]. However, in the general population, where energy surplus is more of a concern and exercise intensity is relatively low and constrained to an hour or two on any given day, it may be that compensatory mechanisms contribute significantly to the limited success of exercise interventions that do not restrict dietary intake[41,113,120]. Further, the often-cited overestimation of exercise energy expenditure[121], may increase the degree of overconsumption[41]. Particularly in the context of a highly processed diet individuals engaging in exercise may remain susceptible to impaired satiety regulation[41,117]. The brain would be instructed to 'exercise less and eat more', thus promoting a vicious cycle of hunger, overeating of non-satiating food and subsequent inactivity[37,41,117,122]. In this context, the pro-weight-loss effects of exercise may be effectively nullified[38,34], and reduced population-wide physical activity levels may be a consequence rather than a cause of the obesity epidemic[41,117,122].

The evidence relating physical inactivity and sedentary behaviour to metabolic disease is more compelling[109,117,123]. According to the WHO, physical inactivity is the fourth leading

cause of NCD-related mortality[109]. Moreover, individuals who are insufficiently active have been reported to have a 20% to 30% increased risk of all-cause mortality compared to those meeting recommendations[109]. Approximately 3.2 million deaths.year^{-1} have been attributed to physical inactivity[109]. Prospective studies have associated physical inactivity with increased risk of hypertension, stroke, T2D and CVD, depression and certain cancers, often in a dose-response manner[109,124]. Conversely, it has been well-established that regular exercise improves cardio-metabolic outcomes, including blood pressure, insulin-sensitivity and blood lipid profile, independent of weight-loss[124–126]. Furthermore, such benefits have been accrued by lean and overweight individuals alike, and favourable cardiovascular fitness, independent of body weight, appears to be protective against metabolic diseases[127–129]. The mechanisms of 'exercise as medicine' appear to be widespread.

Insulin-sensitivity is improved both acutely and chronically in response to exercise training[108]. The former relates to the contraction-mediated translocation of GLUT4 to the muscle cell membrane, which enhances glucose uptake[124,126]. Exercise training may improve insulin-sensitivity by reduced adiposity, with increased muscle mass as well as increased muscle GLUT4 content, glycogen storage capacity, mitochondrial density and oxidative capacity[124,126]. Exercise training also increases HDL-C concentrations, reduces TG and increases LDL particle size, which may be related to altered insulin-sensitivity[126]. Systolic and diastolic blood pressure are both improved by exercise training, particularly in individuals with existing hypertension[126]. This may result from exercise-induced improvements in both cardiac and endothelial function, as well as enhanced insulin-sensitivity and vasodilation[124,126]. Despite an acute exercise bout inducing an inflammatory response and oxidative stress, these are necessary for adaptation, and over the long-term improved cardiorespiratory fitness reduces systemic inflammation[126]. Exercise has been found to have remarkable benefits for mental health and preventing or delaying the onset of neurodegenerative diseases[130]. Therefore, engaging in regular physical activity is crucial to maintaining metabolic health and reducing cardiovascular risk, independent of body-weight[95] .

Sedentary behaviour, particularly increased sitting time, has been found to increase risk of MetS, CVD and all-cause mortality, independent of leisure time spent in physical activity and other potential confounders[131–133]. Specific sedentary behaviours, particularly time spent watching television, have also been independently linked to MetS[133]. This evidence suggests that frequent engagement in physical activity may not compensate adequately for long periods of sedentary time[131,132]. Although the underlying mechanisms appear unclear, the pathology may relate to prolonged periods of absent muscle contraction[134]. In fact, the effects of sedentary predominance have been likened to those of bed-rest on cardiac function, deep vein thrombosis, impaired glucose tolerance and lipoprotein lipase activity, the latter of which reduces TG clearance and HDL production[131,134]. However, other studies have suggested that performing sufficient moderate-to-vigorous activity and maintaining cardiorespiratory fitness would be protective against illness, regardless of time spent sedentary[135,136]. Indeed, 'sedentary research' is an emerging field and it appears that the potential risks of significant sedentary time have been largely based on association[137]. Definitive studies are required to determine whether or not sedentary behaviour independently promotes metabolic disease[131].

1.4.2.4.) Sleep Curtailment

It is well established that sleep is critical for brain restorative processes and for maintaining overall well-being[138]. Although much about sleep physiology remains unexplained, evidence suggests that sleep is integral to learning, memory processing, brain development, cellular repair, neuroendocrine function, autonomic balance and glucose metabolism[138–140]. Reproducible patterns of pituitary- and adrenal hormone release, which are integral to most bodily functions, are dependent on similarly reproducible patterns of specific stages of sleep[140]. Current consensus is that adults should get at least 7 hours of sleep per night[141]. However, modern society, with its long work and commuting hours, night-based leisure activities and shift-work is experiencing an endemic of behavioural sleep curtailment[138]. Exposure to artificial light after sunset and before sunrise, especially from screen-based entertainment, may delay bed time and sleep onset, disturb sleep quality and reduce overall sleep time[138]. As a result, data from the 2014 Behavioural Risk Factor Surveillance System in the USA found that more than one-third of the 444, 306 adult respondents reported sleeping less than 7 hours per night[142]. Over the long-term this would accumulate

as a significant sleep 'debt', which has been linked to poor subjective and objective measures of general, physical and mental health and impaired quality of life[143–145].

Numerous studies have demonstrated the adverse effects of sleep deprivation on body-weight and adiposity[146]. Cross-sectional studies, using both self-report and polysomnography, typically report a U-shaped relationship between sleep duration and weight or measures of central adiposity[138,146,147]. Specifically, short sleep (recognised as less than 6 hours.day^{-1} for adults) and prolonged sleep (more than 9 hours.day^{-1}) appear to promote weight-gain, whilst 7 to 8 hours of sleep per night (considered optimal) has been associated with reduced weight and adiposity[147,148]. Similarly, prospective cohort studies have consistently found increased risk of weight-gain in individuals with shorter sleep durations, independent of caloric intake and physical activity[146]. These findings may relate to impaired glucose and lipid metabolism or altered neuro-hormonal signalling that disrupts appetite regulation (e.g. decreased leptin and increased ghrelin) and promotes unhealthy eating habits[146]. Further physiological studies are required to better elucidate the pathways at play[146].

Partial sleep deprivation also appears to promote cardio-metabolic abnormalities[139]. Healthy individuals exhibit a daily variation in glucose tolerance and adequate sleep is crucial to regulating this natural rhythym[149]. Recurrent partial sleep deprivation has been shown to alter glucose-regulation, and six consecutive nights of curtailed sleep was found to impair glucose tolerance by 40% in healthy young men[143,149]. Both objectively-measured poor sleep quality and perceived sleep debt have been associated with poor glycaemic control, and epidemiological evidence has independently associated short sleep duration with increased risk of T2D[149]. Similar effects have been seen in studies that induced circadian misalignment to mimic shift-work and air-travel across time zones[150]. Multiple mechanisms have been proposed to link impaired sleep to compromised glucose metabolism; these include reduced cerebral glucose metabolism as well as increased sympathetic nervous system activity that reduces insulin secretion and increases the counter-regulatory hormones, glucagon and cortisol[140,149]. Partial sleep deprivation dampens the immune system, foster low-grade inflammation and insulin-resistance, increases risk of hypertension, dyslipidaemia, MetS and CVD, as well as depression and

mortality from all causes[141,151]. Indeed, good sleep habits appear to be crucial to maintaining cardio-metabolic health and will likely receive increased attention in forthcoming years.

1.4.2.5) Chronic Stress

Stress refers to any challenge to an organism's natural homeostasis, including infection, pollution, and temperature variations[152]. Under normal circumstances these induce a stress response (behavioural and physiological) that attempts to restore equilibrium, and is primarily mediated by changes in the autonomic nervous system and the neuroendocrine hypothalamic-pituitary-adrenal (HPA) axis[152]. Stress responses normally facilitate beneficial adaptation to acute stressors, however, they are susceptible to becoming maladaptive in the context of chronic stress[153]. This is exemplified by the stress caused by traits of modern society, such as frenetic work schedules, ultra-competitive capitalist job markets, economic strain, congested commutes, and sleep deprivation[153]. Such relentless demands foster a chronic stress response within the body, which may promote pathology[153]. Cross-sectional studies attempting to link stress in the work environment with components of the MetS have been inconsistent, possibly because of the restricted snapshot of individual stress levels[154]. The Whitehall II case-control study found elevated cortisol (the 'stress hormone'), impaired autonomic nervous system regulation with greater sympathetic activation, and increased systemic inflammation, in working men with the MetS compared to healthy controls[155]. In a 14-year prospective study, Chandola *et al*. (2006) found a dose-response relationship between job-related stress and increased risk of MetS, independent of occupation and confounding health behaviours[154]. Therefore, it appears that chronic psychosocial stress and metabolic disease may be closely connected.

Multiple studies have shown altered HPA axis function in obesity-related conditions. For example, elevated 24 hour and postprandial urinary or salivary cortisol have been associated with abdominal obesity and indices of glucose intolerance, high blood pressure and insulin-resistance[152]. Cortisol also appears to interact with other causes of obesity, including altered appetite regulation and sleep deprivation[152]. For example, whilst leptin levels normally increase during sleep to inhibit food intake, sleep deprivation has been

found to suppress leptin levels, concurrent with elevated cortisol and increased food intake[152]. This would suggest that the stress of chronic sleep deprivation promotes obesity partly through HPA axis dysregulation[152]. Chronic stress appears to promote unhealthy eating habits and obesity via similar pathways[156]. Individuals with greater cumulative stress (associated with higher glucocorticoid secretion) have been found to be more likely to exhibit addictive eating habits, binge eating and snacking, and increased desire for highly-palatable Western foods[152,156,157]. Elevated cortisol secretion in this context would compromise glucose metabolism and appetite-regulation in a manner that promotes insulin-resistance and abdominal obesity[156]. Therefore, it is plausible that chronic stressors increase metabolic disease risk, yet further research is required to better elucidate the mechanisms involved.

1.4.2.6) Other potential contributors

Contemporary society has developed other traits that, although lesser acknowledged, appear to have aggravated the epidemics of obesity and metabolic illness. These will be briefly described here, since they have been comprehensive reviewed by McAllister *et al*. (2009)[54]. Firstly, there has been a wealth of recent research into the adverse effects of mismatched intra-uterine exposures with early-life environments[54,55]. Early developmental exposures influence the functional and organ development of new-borns to adapt them to their forthcoming environment, referred to as 'developmental plasticity'[54]. When there is a mismatch between the resulting phenotype and the early childhood environment (e.g. malnutrition *in utero* compared to abundance in childhood), individuals are more susceptible to insulin-resistance and pathogenic adiposity[54]. Maternal over-nutrition, and the resultant hyperinsulinemia, promote adipogenesis and altered appetite regulation during late foetal life, such that offspring are predisposed to adulthood obesity[54]. Further, maternal obesity may cause epigenetic modifications (e.g. DNA methylation, histone modifications), that alter gene and protein expression and influence individual susceptibility to obesity-associated illness[54]. Consequently, many overweight adults may have been pre-determined by their *in utero* and early childhood exposures to become obese given a conducive environment.

Recent changes in smoking prevalence, pharmaceutical administration, industrial production of environmentally harmful substances, and reproductive habits also warrant examination[54]. Whilst smoking is a well-established CVD risk factor, the appetite-suppressant effect of nicotine has been suggested to reduce the risk of weight-gain in smokers[158]. Given the increased public advocacy and health warnings against smoking, its prevalence has declined significantly over recent decades[54]. This may have caused ex-smokers to gain weight[54]. On another note, the exponential growth of industry has increased the infiltration of harmful substances (e.g. heavy metals, solvents, organophosphates) into the environment and human food chain[54]. When ingested these may disrupt endocrine functions (e.g. agonists/antagonists for receptors) that adversely influence fat metabolism and nutrient partitioning[159]. Many of the contemporary pharmaceuticals that have been introduced to treat chronic conditions such as psychosis, depression, diabetes and hypertension, appear to have had adverse side-effects on adiposity[160]. Furthermore, a high BMI has been associated with increased reproductive fitness, which implicates the increasing prevalence of obesity itself for imparting selective pressure for obesity-associated phenotypes[54,161]. In combination, these and other societal factors, although seemingly unrelated, may have contributed to the complex phenomenon of obesity.

In summary, the aetiology of obesity and MetS is clearly multifactorial. This has made it difficult to generate a unifying and generalisable theory to explain their increasing prevalence. However, the most plausible primary pathophysiology appears to have been insulin-(and-leptin)-resistance and the compensatory hyperinsulinaemia. These conditions have probably been promoted and aggravated by various modern environmental exposures (e.g. poor diet quality, physical inactivity, sleep deprivation, stress, and developmental conditions) to foster pathological glucose and lipid metabolism, increased risk for T2D and CVD, accompanied by weight gain in predisposed individuals[18,87,104]. In order to mitigate the negative health effects of insulin-(and leptin)-resistance, at-risk individuals would benefit mostly from minimising systemic exposure to insulin and foods that impair satiety mechanisms. This may be achieved primarily by reducing carbohydrate consumption below the threshold that causes chronic hyperinsulinaemia[13,20,87], and avoiding highly processed high-sugar, high-fat foods[37]. Ensuring adequate sleep, regularly

engaging in moderate-to-vigorous physical activity and minimising exposure to stressors are also integral strategies to improving overall well-being.

At the same time, it is important to recognise the existence of individuals who may not exhibit the classic clustering of obesity with metabolic pathology, but rather exhibit obesity or metabolic illness independently. Indeed, recent attention has been drawn to the Metabolically Healthy Obese / Overweight and Metabolically Unhealthy Normal Weight phenotypes. These individuals are attractive prospects for learning more of the complex interplay between genetic predisposition, adiposity, and physical fitness in determining cardio-metabolic health.

1.5.) Alternative metabolic phenotypes

1.5.1.) 'Metabolically Unhealthy Normal Weight' phenotype

In contrast to conventional thought, apparently normal weight persons are not always 'thin inside'. The metabolically unhealthy normal-weight phenotype was initially recognised in 1981 as individuals presenting with a BMI below 25 $kg.m^{-2}$, but with metabolic abnormalities more characteristic of MetS, including hyperinsulinaemia, hypertension, hypertriglyceridaemia, greater HbA1c, reduced HDL-C and/or a pro-inflammatory state [162–164]. This sub-group is of particular concern as their elevated risk of disease may easily go unnoticed[163]. Due to variations in MetS diagnostic criteria and cohort (ethnicity, sex and age) differences, estimates of the prevalence of this phenotype have been unclear[163]; however, Wildman *et al*. (2008) found that in the 1999-2004 NHANES cohort, 23.5% of normal-weight adults exhibited at least two cardio-metabolic abnormalities and were deemed metabolically at-risk[165].

A number of cohort studies have indicated greater T2D risk in metabolically unhealthy normal weight persons relative to their metabolically healthy counterparts[164]. Unfortunately, studies have utilised inconsistent criteria for identifying metabolically unhealthy normal weight persons[163], with some using indices of insulin-resistance[166], others

visceral fat area[167], or the presence of at least three metabolic abnormalities analogous to the MetS described above[168]. Meigs *et al*. (2006) assessed CVD and T2D risk over a 7 to 11 year follow-up, and found that 7% and 21% of metabolically unhealthy normal weight participants, who fulfilled the ATP III MetS criteria, developed T2D or experienced CVD events respectively[168]. Both of these were considerably higher than the observed risk in healthy normal-weight or healthy obese participants[168]. Interestingly, it has been estimated that 15% to 20% of incident T2D cases in prospective studies have arisen from normal-weight persons. In a case-cohort study, Eckel *et al*. (2015) identified metabolically unhealthy normal weight persons as those who developed T2D over a mean 7 year follow-up[164]. Many of those who developed T2D had not been identified as insulin-resistant nor did they fulfil MetS criteria at baseline[164]. Furthermore, despite their greater metabolic risk compared to normal-weight controls, their mean waist-circumference in particular (associated with BMI), as well as TG and HDL-C levels, were within the upper range of 'normal' but below MetS thresholds[163,164]. This evidence has led some to suggest that existing MetS criteria and parameter cut-offs are insensitive for identifying risk in normal-weight persons[163,164]. However, given that metabolically unhealthy normal weight persons appear to be at a lower risk than metabolically unhealthy overweight or obese persons[163,164], it may suffice to incorporate other metabolic markers that associate with the metabolically unhealthy normal weight phenotype, including HbA1c or CRP, when determining risk in normal-weight individuals[164]. Regardless, a clear definition of metabolically unhealthy normal weight is required to allow for more consistent criteria to be applied in determining risk across clinical and research settings.

The pathophysiology of the metabolically unhealthy normal weight phenotype is unclear; however, increased abdominal fat and reduced physical activity appear to be integral factors. Firstly, metabolically unhealthy normal weight persons exhibit elevated abdominal and visceral adiposity and reduced fat-free-mass compared to BMI-matched controls, and this has been associated with impaired insulin-sensitivity[163]. Eckel *et al*. (2015) recently reported waist circumference was particularly sensitive to increased risk of incident T2D in normal-weight individuals[164]. This may reflect the functional heterogeneity of different adipose tissue compartments[169]. TG storage in subcutaneous adipose tissue (SAT), located around the hips and thighs, as opposed to visceral adipose tissue (VAT) located in the abdominal area, has been shown to be protective against metabolic abnormalities in

predominantly Caucasian populations[169,170]. Conversely, studies on South African and American black women have shown that at the same BMI or waist circumference, black women present with worse insulin-resistance despite lower VAT[171]. Furthermore, whilst insulin-sensitivity associates most strongly with VAT in white women, it associates more closely with abdominal SAT (ASAT) in black women (particularly deep ASAT rather than superficial ASAT)[171]. This evidence suggests ethnic differences in the relationship between regional fat distribution and metabolic health, for which potential mechanisms include differences in lipolytic activity, sex hormones, and glucocorticoid exposure, and have been reviewed elsewhere[171]. Regardless, it is clear that more research is needed to better understand the functional heterogeneity of different adipose tissue depots[172].

A further consistent finding is that metabolically unhealthy normal weight persons spend less time physically active, more time sedentary and may exhibit reduced aerobic capacity, in comparison to their healthy lean counterparts[163,164]. This agrees with the aforementioned benefits of regular exercise for metabolic health. There is limited literature on dietary components contributing to the metabolically unhealthy normal weight phenotype. However recently, Suliga *et al,* (2015) associated distinct dietary patterns in normal-weight individuals with the prevalence of MetS[173]. After controlling for confounders, the authors reported significantly lower risk of metabolically unhealthy normal weight in individuals consuming the "prudent" dietary pattern, rich in whole-grains and fish and low in refined flour products, sugar and sweets[173]. This seems to reinforce the role of the modern Western diet in driving pathology even in normal-weight persons. Prior history of smoking may further contribute to increased risk of T2D in normal-weight persons[164], and a certain genetic predisposition has been acknowledged in those with a positive family history of T2D or hypertension[163]. Specialised treatment strategies do not currently exist for at-risk normal-weight persons. However, lifestyle modifications that are typically prescribed for the unhealthy-obese, particularly increased physical activity, seem to have been as effective at improving metabolic outcomes[174].

1.5.2.) 'Metabolically Healthy Obese' phenotype

MHO generally refers to those individuals who have a BMI greater than or equal to 30 $kg.m^{-2}$ but two or less accompanying MetS conditions[129,175]. Owing to inconsistent MHO definitions, prevalence estimates have been highly variable[174]. A recent systematic review found that from 27 prospective studies, MHO prevalence ranged from 6% to 75%, depending upon the definition of obesity (e.g. BMI or body fat percentage) and MetS criteria used[176]. Despite similar total body fat to unhealthy obese persons, MHO individuals normally present with less visceral adiposity, normal insulin-sensitivity and glucose control, more favourable lipid and hepatic enzyme profiles, reduced systemic inflammation, and up to 54% less liver fat[170,174,175]. They also exhibit significantly lower VAT and ectopic fat deposition, which may account for their healthier inflammatory and lipid profiles[170]. Interestingly, MHO individuals had smaller fat cells in omental and subcutaneous adipose tissue biopsies compared to unhealthy obese persons, and this was associated with lower levels of the inhibitor of preadipocyte differentiation, preadipocyte factor-1, as well as reduced macrophage infiltration and inflammatory signals[177]. In agreement, genetic studies of the MHO phenotype have implicated genes involved in transcriptional regulation of adipogenesis[174]. Collectively, this evidence suggests that healthy obese persons benefit from greater adipogenic capacity and a more favourable fat distribution.

There have been conflicting opinions as to whether obesity independently increases future risk of disease or if these differences in adipose tissue mitigate risk in MHO persons[127,129,168,178]. Importantly, MHO is often a transient phenotype as many individuals progress from MHO to metabolically unhealthy obese with increasing age[129,178]. Soriguer *et al.* (2013) found that after a 6 year follow-up, 37% of MHO were re-classified as their metabolic health had deteriorated[179]. Schroder *et al*. (2014) similarly found that after 10 years, 50% of MHO participants regressed to metabolically unhealthy[180]. Thus, despite their apparently 'healthy' metabolic profiles at the time of assessment, MHO individuals may be at long-term risk[174]. Indeed, Ärnlov *et al*. (2010) and Ärnlov *et al*. (2011) found that overweight and obese men, regardless of metabolic health status, had greater risk than normal-weight controls of developing CVD and T2D over a 30 year and 20 year follow-up respectively [181,182].

However, Ortega *et al*. (2013) recently suggested that such findings resulted from not having accounted for cardiorespiratory fitness[127]. These authors compared disease risk (co-varying for cardiorespiratory fitness) between MHO, metabolically unhealthy obese and metabolically healthy normal-weight individuals, in a sample of 43 265 adults over a median follow-up of 7 to 15 years[127]. They concluded that when adjusted for fitness, MHO was a benign condition, with reduced risk for all-cause mortality, non-fatal and fatal CVD and cancer mortality, compared to metabolically unhealthy obese persons and they were at no higher risk than metabolically healthy normal-weight participants[127]. Similarly, Meigs *et al*. reported that, after a 7 to 11 year follow-up, MHO persons had reduced risk of T2D and CVD compared to unhealthy obese persons, and were at no greater risk compared to healthy normal-weight individuals[168]. Multiple studies have reported higher levels of physical activity[129] and higher proportions of MHO cohorts meeting physical activity guidelines compared to unhealthy obese controls[178]. Camhi *et al.* (2015) found MHO persons spent significantly less time in sedentary behaviour, but greater time in light physical activity compared to matched unhealthy-obese persons[175]. Collectively, this evidence strongly supports the notion that regular physical activity and consequent fitness is profoundly protective against metabolic disease, independent of adiposity, and largely explains the spectrum of cardio-metabolic risk found in the obese population[178]. The extreme end of this rationale would suggest that athletic individuals, regardless of body weight, should be amongst the healthiest of individuals. While this may often be the case, there is an emerging subset of both professionally and recreationally athletic individuals who are metabolically unhealthy[56].

1.5.3.) Overweight athletes: MHO or Metabolically Unhealthy Overweight?

There is limited research both on the prevalence of overweight or obesity in the athletic population and on the metabolic health of normal-weight or overweight athletes. This may be due to the assumption that regular exercise protects against ill cardio-metabolic health. Studies have reported impaired insulin-sensitivity[183], and increased risk of MetS[184] in heavy field-throwing athletes and weightlifters respectively, compared to physically active controls. Existing American Football linemen, players that typically exhibit significant stature, have also been found to have a high prevalence (up to 50%) of the MetS[185]. An assessment of 302 athletes of varying BMI at the dental clinic of the London 2012 Olympic Games, reported poor dental health, including dental caries, erosion and periodontal

disease[186], which has been positively linked to systemic inflammation and the MetS[187]. Most pertinently, a high prevalence of overweight athletes was reported at the Beijing 2008 Olympic Games[188]. In response, Berglund *et al.* (2011) introduced the concept of '*Adipositas athletica'* to describe a higher than "athletic normal" fat mass in elite athletes[188]. They separated the phenotype into '*adaptiva*' (intentional increase in body fat to gain a competitive advantage), '*secondaria*' (increased body fat secondary to increased strength and body size required for competition), and '*accidentalis*' (unintentional increase in body fat that is disadvantageous during competition, found particularly in low-intensity sports including golf, darts, archery)[188]. Berglund and colleagues implicated unhealthy eating of high-sugar, high-fat foods as the primary driver of fat gain in the latter sub-group[188]. Experienced marathon runners have also been reported to have unexpectedly high sub-clinical atherosclerotic burden and coronary events, comparable to sedentary individuals matched for age and coronary risk factors[189]. Noakes *et al.* (2014) speculated that this too may have had a dietary component[190]. On the other hand, overweight athletes have been found to have impressive cardiorespiratory fitness as well as capable of sustaining vigorous exercise without expected ventilatory constraints[191]. Further, as mentioned above, increased fitness in obese individuals has been found to reduce cardio-metabolic risk to levels comparable with normal-weight individuals[127]. Therefore, the question remains as to whether high fitness, independent of adiposity, protects against cardio-metabolic disease, or if the benefits of regular exercise may become overwhelmed with time by unhealthy lifestyle (especially dietary) habits. The increasingly large representation of overweight recreational runners presents a convenient opportunity to explore these questions.

Over the past couple of decades, South Africa, in line with trends in the secular world[192], has experienced a surge in the popularity of recreational running by members of the general population. Interestingly, anecdotal reports have suggested that many participating runners have been overtly overweight or obese, yet capable of completing the races. For example, unpublished data from the 2014 Two Oceans Ultra and Half-marathons in Cape Town, indicated that, of approximately 17 000 runners between the ages of 30 and 45, more than 50% of men and 25% of women had a BMI (from self-reported weight and height) greater than 25 $kg.m^{-2}$. These proportions were approximately double that of the overweight and obese runners under the age of 30. This seems contradictory to conventional wisdom, given that regular exercise has been advised for promoting weight-

loss or, at least, maintaining a healthy weight[105]. It may be that many of the observed overweight runners had only recently started running (as a means to lose weight); however, it could also be that they had gained weight over the years despite running consistently. Perhaps these recreational runners, who one may speculate typically run at a low intensity, would fall within the realm of the '*accidentalis*' sub-group put forward by Berglund and colleagues[188]. Indeed, they may have gained weight unexpectedly owing to inappropriate dietary habits, or in the words of a recent editorial, maybe "you cannot outrun a bad diet"[56].

Although not specifically investigated in the aforementioned studies, a diet high in carbohydrate, in particular sugary sports drinks, may well have contributed to the negative outcomes in these professionally and recreationally athletic individuals[56,92]. Since the 1960s, exercise science and sporting authorities have advised athletes to obtain a significant proportion of their energy intake in the form of carbohydrate (6 to 10 grams per kg body weight per day)[193,194]. This has been exemplified by the ubiquitous practice of carbohydrate–loading during the days leading up to endurance event, with the intention to saturate the muscle glycogen stores that are rapidly used during high-intensity activity[194]. Furthermore, athletes have been advised to prioritise consumption of easily-digestible (high-GI) carbohydrates immediately before, during and after prolonged exercise to saturate liver glycogen stores, maintain blood glucose levels during exercise and restore muscle glycogen during recovery, respectively[193,194]. Such nutritional strategies have proven advantageous for young, lean insulin-sensitive athletes in various sporting domains, including endurance and aesthetic sports[194,195]. However, for athletes with underlying insulin-resistance, such emphasis on carbohydrate intake may have promoted the hyperinsulinaemia, obesity and associated cardio-metabolic disturbances described above[92]. Furthermore, it may be that young, lean athletic individuals develop metabolic health concerns and performance decrements as they age, since the health returns of regular exercise may become overwhelmed by the detrimental effects of excess dietary carbohydrate (particularly processed carbohydrates) in predisposed individuals[92]. It seems reasonable to speculate further that an inappropriate diet would have had more pronounced adverse effects in insulin-resistant recreational athletes. However, as far as the author of this thesis is aware, the relationship between dietary intake, weight-gain and metabolic health has not been studied in an athletic population.

Therefore, the overweight and obese runners that have been reported to frequent recreational endurance events represent a unique and important sub-group of overweight individuals to study. In contrast to what public health and sporting authorities have advised, regular exercise has not been successful in maintaining a healthy weight. A number of questions remain unexplored, including

i.) are overweight/obese runners metabolically healthy despite being overweight, probably because they engage in physical activity, or

ii.) are they overweight as a result of being metabolically unhealthy despite regular exercise, and

iii.) what inherent factors, such as resting metabolic rate, and lifestyle or environmental factors, including dietary intake, sedentary behaviour, sleep quality and stress levels, may have contributed to their elevated adiposity and metabolic health?

This study sought to answer these questions through a comprehensive metabolic and lifestyle profiling of the emerging overweight or obese female runner, and contribute to the design of future clinical trials in athletic individuals struggling with unwanted weight-gain.

1.6.) Aims

This thesis was a pilot study to characterise the 'Overweight runner' phenotype. It will inform the feasibility and design of a subsequent randomised controlled dietary intervention trial that will aim to reduce weight in these runners.

The primary aim of this study was to determine the degree of insulin-resistance and other risk factors for MetS, including dyslipidaemia, hypertension, impaired glucose tolerance and inflammation, in a cohort of overweight or obese female endurance runners in comparison to lean runners that were matched for age and running experience.

The secondary aim was to investigate resting metabolic rate, habitual diet, competition-specific dietary habits, physical activity, sedentary behaviour, sleep and stress in these

cohorts. We sought to determine whether these would be associated with elevated body weight and / or negative metabolic outcomes in these runners.

1.7.) Hypotheses

The primary hypothesis of this study was that overweight or obese female runners would present with underlying metabolic pathology (primarily a higher degree of insulin-resistance) compared to their lean counterparts.

Secondly, we hypothesised that overweight or obese runners would have been consuming a diet too high in carbohydrate for their level of insulin-resistance, contributing to hyperinsulinaemia, weight-gain and potentially other negative outcomes. The other lifestyle factors that were investigated were largely exploratory, and no hypotheses were made in this regard.

2.) METHODOLOGY

2.1.) Overview of Study Design

This was an analytical, observational, cross-sectional study of overweight female endurance runners compared to lean female runners, who were matched for age, running experience and running calibre.

2.2.) Ethical considerations

This study was approved by the Human Research Ethics Committee, Faculty of Health Sciences, University of Cape Town (UCT) (REF: 816/2014, approved 22 December 2014). All participants provided a written informed consent prior to enrolment in the study. This indicated that they understood and were satisfied with the nature of the study procedures.

2.3.) Participants and Inclusion / Exclusion Criteria

Twenty female recreational endurance runners (10 'Overweight' and 10 'Lean') participated in the study. Participants were eligible if they satisfied the following criteria:

i.) between 30 and 45 years of age,

ii.) adequate endurance running experience by firstly, having completed annual running events of 21.1 km or longer for at least the past 5 years and secondly, having run consistently (on average 3 times per week) during the preceding 6 months,

iii.) deemed 'safe to exercise' in accordance with the Physical Activity Readiness Questionnaire (PAR-Q, *Appendix A*),

iv.) weight-stable (experienced weight fluctuations of less than 5% of their body weight) during the preceding 3 months, and

v.) not made any significant changes to their diet during the preceding 6 months.

Finally, in order to group participants as 'Overweight' and 'Lean', they had to exhibit suitable BMI, Waist Circumference and Body Fat Percentage (BF%, with a degree of leniency) according to the table below. The latter was estimated during eligibility screening using skinfold thicknesses (*section 2.5.2*).

Table 1. Eligibility screening criteria of body composition for inclusion in Overweight and Lean groups.

Measure	Overweight	Lean
Body Mass Index ($kg.m^{-2}$)[196]	≥ 25.0	< 23.0
Waist Circumference (cm)[196]	≥ 80.0	< 75.0
Body Fat Percentage (%)[197]	≥ 28.0	< 25.0

Body measurements for the 'Lean' group were chosen to be slightly stricter than convention in an effort to enhance the degree of comparison between Overweight and Lean runners. The BF% criterion for the Overweight group was less strict than normal for initial screening purposes. This was aimed at avoiding the exclusion of respondents who were on the overweight threshold from screening measures but may have been eligible according to BF% from Dual Energy X-ray Absorptiometry (DXA, *section 2.5.3*).

2.3.) Recruitment and Screening

Female recreational runners based around Cape Town (Western Cape) were recruited. Advertising relied primarily on electronic media. Frequent posts were made on the social media accounts of Western Cape running clubs, upcoming running events, the Sport Science Institute of South Africa (SSISA) and the Exercise Science and Sports Medicine (ESSM) Division of UCT. Electronic adverts were regularly distributed to running club mailing lists. In addition, poster adverts were distributed around the SSISA building, at running club venues, and directly to runners at the finish of select popular events. Initially, advertisements were aimed at recruiting the Overweight participants. Once ten of them had been tested, advertisements were released for Lean controls. These were designed with the intention to match Overweight and Lean groups closely in terms of age, years of

running experience, recent running volume and running calibre. The latter was expressed as energy expenditure per kg bodyweight for their fastest 21.1 km race within the past year (section *2.5.4.*). Interested respondents were initially screened in terms of the aforementioned eligibility criteria using the Eligibility Questionnaire (*Appendix B*). Respondents who were potentially eligible were scheduled for Visit 1 of the study.

2.4.) Testing Protocol

Participants were required to visit SSISA, on Boundary Road, Newlands on three occasions.

2.4.1) Visit 1

Participants arrived at the laboratory at any time that suited them, provided that they were at least 2 hours fasted and had not performed any exercise during that day or the day prior to testing. We explained the study procedures, after which participants were asked to sign their informed consent and confirm their readiness to exercise. They then completed the Detailed Participant Questionnaire (*Appendix C*) to assess their current and past health and nutritional practices, followed by questionnaires concerning their perceived eating habits (*Appendix D*), perceived recent sleep quality (*Appendix E*), perceived recent stress levels (*Appendix F*) and recent experience of gastrointestinal complaints (*Appendix G*). Anthropometric measurements were subsequently recorded to estimate body composition and determine participants' eligibility (BMI, waist-circumference and BF%). Participants were then asked to perform a Peak Treadmill Running Speed (PTRS) Test to assess their fastest running speed. Lastly, participants were asked to recall their prior day's dietary intake (24-hour diet recall, *section 2.5.5.1)* , which served primarily as familiarisation for recording their 3-day diet record (*section 2.5.5.2*).

2.4.2.) Between Visits 1 and 2

Participants were allowed 7 to 8 days between visits 1 and 2. During this period, participants were provided with an accelerometer to measure their physical activity, a

sleep monitor to measure their sleep duration and quality, an exercise logbook to indicate their exercise training schedule, and a logbook to complete their 3-day diet record. Participants were asked to continue with their normal daily activities as much as possible, in order to obtain representative data of their habitual lifestyles.

2.4.3.) Visit 2

Participants were required to arrive at the thermo-neutral laboratory (23°C) in the morning between 06h00 and 08h00. They had to be at least overnight (approximately 12 hours) fasted and were asked to have abstained from both exercise and alcohol consumption during the day prior to testing and on the morning of testing. Participants rested in a supine position on a bed for 10 minutes before measuring resting blood pressure. They were asked to remain relaxed on the bed and refrain from movement or speech for the subsequent measurement of resting metabolic rate. Thereafter, a cannula was inserted into a forearm vein, obtained fasting blood samples to assess health parameters, and conducted an OGTT to measure insulin-sensitivity. Participants were required to remain in a supine position throughout the OGTT. During this period, the accuracy of the participants' 3-day diet records was confirmed. This entailed theassisted recall of commonly forgotten food items, and the use of photographic food manuals and common utensils to assist in portion size estimations.

2.4.4.) Visit 3

Visit 3 was scheduled on any available day in between Visits 1 and 2. Participants were required to arrive at any time that suited them, but at least 2 hours fasted, well hydrated and having performed no exercise on the morning of the testing day. Participants were required to rest supine on a bed and undergo a DXA scan for a detailed determination of their body composition and bone mineral density.

2.5.) Testing and Analytical Procedures

2.5.1.) Personal and family health history (visit 1)

Participants completed the Detailed Participant Questionnaire (*Appendix C*) to assess their family and personal history of disease, their current health status, perceived quality of life, habitual and running-specific nutritional practices, smoking status and alcohol intake. This questionnaire was newly drafted for the purposes of this study.

2.5.2.) Anthropometry (visit 1)

Participants wore undergarments and light running shorts during anthropometric measurements. Height (to the nearest 0.5 cm) and weight (to the nearest 0.1 kg) were measured using a standard stadiometer and calibrated electronic scale (UWE BW-150 Personal scale). Waist (at the level of the umbilicus), hip (over the area of largest girth), thigh, calf, chest and upper arm circumferences were each measured twice (to within 1 mm) with an anthropometric tape as described by Lean *et al*. 1995[196]. Skinfold thicknesses were measured using calibrated skinfold callipers (Holtain, Crosswell, Wales), and in accordance with the International Standards for Anthropometric Assessment (2006). Measures were repeated three times, and the average of the nearest two was accepted. Eight skinfold sites were used: bicep, tricep, subscapular, supraspinale, iliac crest, abdominal, thigh and calf. The 'sum of seven skinfolds' was calculated as the sum of these thicknesses, excluding the iliac crest[198].

BF% was estimated from skinfold thickness measures to assess eligibility. This was performed using the Durnin and Womersley method to estimate body density (BD)[199], which was subsequently converted into BF% using the Siri Equation[200], as detailed below:

$$L = \log (\text{triceps} + \text{subscapular} + \text{biceps} + \text{iliac crest})^{199}$$

$$BD = 1.1423 – (0.0632 * L) \text{ for ages } 30 – 39^{199}$$

$$BD = 1.1333 – (0.0612 * L) \text{ for ages } 40\text{-}45^{199}$$

$$BF\% = 495 / (BD) – 450^{200}$$

2.5.3.) Body Composition and Bone Mineral Density (visit 3)

The participants arrived at the UCT DXA scanning facility at least 2 hours fasted, well hydrated and having performed no exercise on the morning of testing. The participants removed all clothing and jewellery, dressed in a light gown and lay supine on a padded table for approximately 20 minutes. Scans were performed by a registered radiographer using a Hologic QDR Series Bone Densitometer (Discovery W, Serial Number 80191, Hologic, Inc., Bedford, US). Body composition (fat and lean tissue distribution) was measured using a whole body scan and bone mineral density was assessed at the lumbar spine and both proximal hips. Images were recorded and bone mineral density, lean mass, fat mass, and BF% were computed using Apex System Software v 4.0.1. (Hologic Inc., Bedford, US).

2.5.4.) Peak Treadmill Running Speed (PTRS) test and running calibre (visit 1)

The PTRS test was performed on a treadmill (HP Cosmos, VIASYS LE 500CE, Germany) using an incremental (VAMEVAL) ramp protocol at a constant gradient of 1%[201,202]. After a self-paced 10 minute warm up and 5 minute rest the test was started at 8 km.hour^{-1}. The speed was increased by 0.5 km.hour^{-1} at the end of each minute. The participants were verbally encouraged to run for as long as possible, and the test was terminated when they indicated they could not run any longer. The PTRS test was considered valid if it fulfilled the following 2 criteria[203]: a maximum heart-rate (HRmax) within 10 beats per minute (bpm) of age-predicted maximum (220 – age), and a final RPE ≥ 18 on the Borg 6 – 20 RPE scale[204]. PTRS was calculated as the sum of the speed of the final completed stage and the fraction of the stage at which the test was terminated, as follows:

$$\text{PTRS} = \text{Completed full intensity } (\text{km/h}) + \left(\frac{\text{Seconds at final speed}}{60 \text{ seconds}}\right) \times 0.5 \text{ km/h}$$

Heart rate data were recorded during the test, at 1-sec intervals, using a Suunto Ambit 2 heart rate monitor (Suunto Oy, Vantaa, Finland). HRmax was taken as the highest HR reached during the test.

Overweight and Lean runners were matched in terms of running calibre by calculating their energy expenditure (kcal.minute^{-1}) during their fastest 21.1 km race within the previous 12 months. This was performed by adapting the equation of Loftin *et al.* (2010), which was developed for estimating energy expenditure per mile in runners of varying bodyweight[205]. This equation was based on the assumption that it is more difficult for a heavier runner to run a certain distance in a specific amount of time, compared to a lean runner completing this distance in the same time. The formula as per Loftin *et al.* (2010) was[205]:

Kcal.mile^{-1} = [mass (kg) × 0.789] – [gender (men = 1, women = 2) × 7.634] + 51.109

This was converted into kcal.minute^{-1} as per the following:

Kcal.km^{-1} = (kcal.mile^{-1}) / 1.60934

Kcal.minute^{-1} = (kcal.km^{-1}) x 21.1 km / (minutes to complete 21.1km)

2.5.5.) Diet Records and Analysis

Three different dietary assessment tools were used to characterise the participant's habitual dietary intake. Given the inherent limitations in each method, it has been recommended to combine assessment tools to obtain the most accurate estimate of dietary intake[206].

2.5.5.1) 24-Hour Diet Recall (24HR, visit 1)

At the end of Visit 1 and without prior warning, participants were asked to recall, in as much detail, and as accurately as possible, what they had consumed during the previous day. Participants were lead through the process of recalling their previous day's diet from the time of waking until going to sleep. They were prompted to remember any easily-forgotten foods and drinks (e.g. added sugar, cooking oil, snacks). Detailed information was obtained regarding food preparation methods, ingredients in mixed dishes, brand names of commercial products and portion size estimates relative to common household measures (e.g. bowls, spoons, cups) and visual aids (Dietary Assessment and Education Kit, DAEK, South African Medical Research Council, MRC). The 24HR served to familiarise and counsel participants as to the level of detail required in the subsequent 3-day diet record.

2.5.5.2.) 3-Day Diet Record (3DR, between visits 1 and 2)

During the period between visits 1 and 2, participants were provided with a logbook in which to record their dietary intake, in as much detail as possible, for 3 consecutive days. This included one weekend day and two non-weekend days, and at least one exercising day. The 3DR served as the primary tool to estimate habitual dietary intake at the time of testing.

2.5.5.3.) Food Frequency Questionnaire (between visits 1 and 2)

Participants completed a 123-item Food Frequency Questionnaire *(*FFQ, *Appendix H)*. The FFQ was based on that developed by the South African Medical Research Council (MRC) in 2009, which was used to quantify dietary intake amongst South African Marathon Runners during the month leading up to an Ultra Marathon. Although the original questionnaire remained unchanged, participants in the current study were asked to report on their average consumption over the previous 6 months. This was used to obtain a perspective of their longer term dietary practices compared to 3DR. The FFQ asked participants to answer firstly, how often, on average over the past 6 months, they had consumed each item, and secondly, the average amount they had consumed on each occasion. Answers were

computed to provide average daily intakes so as to be comparable with results from the other diet assessment tools used. One Overweight participant did not submit a completed FFQ; therefore, her dietary data could not be compared between assessment tools (*Results section*).

2.5.5.4.) Diet Analysis (after testing)

Quantitative dietary data (from the 24HR, 3DR and FFQ) were analysed in accordance with the South African Food Data System (SAFOODS) of the MRC. Briefly, food items listed by participants were translated into their corresponding codes on the SAFOODS database. Where a specific item was not present on the database, the participant helped select the nearest surrogate. Participants estimated portion sizes in terms of common household utensils with the assistance of the Food Quantities Manual of the MRC and appropriate food models. These were converted into gram amounts of each individual item or ingredient using the Food Quantities Manual. The resulting spreadsheet, containing a list of food codes and corresponding gram values per day, was analysed by Ms Ria Laubscher at the Biostatistics Unit of the MRC. The composition of each participant's diet, in regards to energy, macronutrients, vitamins and minerals, was computed in accordance with the updated South African Food Composition Database (2015). This has been specifically developed to reflect foods found in South Africa. The 3DR and FFQ were compared to assess the level of agreement in energy intake and macronutrient composition between tools. Qualitative data of nutritional practices during normal daily living and in relation to running races (*Appendix C*), were assessed using normal statistical procedures.

2.5.5.5.) Psychological eating traits (visit 1)

We assessed subjective food-related traits using the Three-Factor-Eating Questionnaire (TFEQ-21, *Appendix D*). The TFEQ is a self-rating questionnaire that has been validated in diverse populations to assess the extent of food-related disinhibition (regular episodes of overeating that result from excessive dietary restraint), with obesity[207,208]. Participants were asked to respond to 21 questions: items 1 - 20 on a four-point Likert scale and item 21 on an eight-point numerical scale. Each response was given a score between 1 and 4; items

1 - 16 were reverse coded and item 21 was coded as follows: 1 to 2 scored as 1; 3 to 4 as 2; 5 to 6 as 3 and 7 to 8 as 4. Three separate domain scores were calculated as the mean of the items allocated to each domain: Cognitive Restraint (the conscious restriction of food intake to control body weight), Uncontrolled Eating (the tendency to eat more than usual owing to loss of control over intake) and Emotional Eating (overeating during despondent mood states). Domain scores also ranged from 1 to 4, with higher scores indicating greater propensity for that trait.

2.5.5.6.) Gastrointestinal Complaints (visit 1)

Participants were asked to recall their recent experience of gastrointestinal symptoms in relation to food consumption. The Gastrointestinal Symptoms Questionnaire (*Appendix G*) was based on a previously published questionnaire[209]. In its original form, the questionnaire was used to assess the frequency of abnormal gastrointestinal symptoms experienced by respondents during normal daily activities. It was modified for this study to evaluate symptoms firstly, during normal daily activities and secondly, during exercise sessions. Participants were also asked to recall specific foods that had tended to elicit these symptoms.

2.5.6.) Sleep Assessment and Analysis

2.5.6.1.) Subjective (perceived) sleep assessment (visit 1)

The Pittsburgh Sleep Quality Index (PSQI, *Appendix E*) Questionnaire was used to assess participants' self-perception of their recent sleep quality. The PSQI has been validated to assess sleep quality and disturbance in recent months[210]. The global PSQI score of sleep pathology ranges from 0 to 21. Higher numbers indicate worse sleep quality and values > 5 indicate possible sleep pathology. Participants were scored from 0 to 21 and were categorised as either good sleepers (PSQI score ≤ 5) or poor sleepers (PSQI score of > 5)[211].

2.5.6.2.) Objective sleep assessment (between visits 1 and 2)

Sleep was objectively assessed using Actigraphy, which has been validated as a low-cost alternative to polysomnography for measuring sleep time and quality in free-living settings[212,213]. Participants wore a small, lightweight (± 16 grams) and unobtrusive Actiwatch 2 (Philips / Respironics, Pittsburgh, US) for the seven consecutive days in between Visit 1 and Visit 2. This was worn around their wrist at all times, excluding swimming. The Actiwatch 2 uses a piezoelectric accelerometer to generate an electric charge or voltage proportional to accelerations of the wrist (frequency response of 0.35 – 7.50 Hz), from which it generates activity counts for a given epoch. Epochs are allocated activity counts as a weighted average of activity for the current epoch and that of surrounding epochs. These are measured against activity thresholds for determination of sleep and wake periods. The Actiwatch 2 was configured to record 'Activity Only' in 15 second epochs. Participants were asked to mark the times they went to sleep and woke up every day, using both the Actiwatch 2 event-marker and written on a logbook provided. The data was downloaded and the recorded epochs were identified as sleep or wake by algorithms in the Actiware 5.0 software (Philips / Respironics). The latter was confirmed with reference to the sleep and wake times that had been indicated by the participants[214]. This enabled the software to compute the sleep parameters of interest, including Total Sleep Time, Sleep Efficiency (percentage of time in bed that was spent asleep), Sleep Onset Latency (time lapse between going to bed and falling asleep), and Wake After Sleep Onset (WASO, time spent awake after falling asleep). Data from one Overweight participant was excluded from the analysis, because she performed shift-work at nights and slept during most days, which skewed the data.

2.5.7.) Physical Activity and sedentary behaviour (between visits 1 and 2)

Participants were provided with an accelerometer (GTX3+, ActiGraph LCC, Florida, US) to wear around the hip for the seven consecutive days in between Visit 1 and Visit 2[215,216]. This included all waking hours but excluded water-based activities (e.g. bathing, swimming). It has been acknowledged that the inability of accelerometers to capture non-ambulatory activities, including swimming and cycling, obscures the accuracy of step-count measurements[217]. As has been previously recommended, 200 'bonus steps' per minute of

activity were allocated to participants on the days they performed either cycling or swimming[217]. Four participants reported cycling and three participants reported swimming in their exercise logbooks. Actigraph accelerometers have been extensively used and validated for measuring physical activity and sedentary behaviour [218–220]. The GTX3+ is a tri-axial capacitative accelerometer with a Micro-Electro-Mechanical-System sensor, which is capable of detecting both static and dynamic accelerations in three axes, based on changes in the capacitance of the sensor[221]. Briefly, changes in capacitance cause changes in electric flow, proportional to the detected acceleration. These are subsequently amplified, digitised and the direction of the acceleration is determined, before final phase frequency-filtering ensures that all measurements fall within realistic human movement frequencies[221]. The resultant activity 'counts per minute' (cpm) were compared to pre-set activity thresholds to identify when the wearer was performing different levels of activity (*below*).

The accelerometer was initialised and the recorded data was downloaded and analysed, using Actilife v.6.10.1 Data Analysis Software (Actigraph, Florida, US). Data sampling rate was set at 80Hz and extracted in 15 second epochs for analysis. Uniaxial (axis 1) analysis was performed for comparison with previous studies[222].

Non-wear-time was defined as any period of at least 60 consecutive minutes containing zero activity counts[223]. Allowance was made for a maximum of 2 minutes within the non-wear period with non-zero activity counts[223]. Non-wear-time was excluded from the present analysis. A minimum of 600 minutes of daily wear-time for four days (three weekdays and one weekend day) was required for data inclusion[212]. All Overweight and all except two Lean participants (n=18) provided adequate data for inclusion in the analysis. Of these, seven participants had seven valid days of data, ten provided six valid days and one provided five valid days of data.

In accordance with the *2008 ACSM Physical Activity Guidelines for Americans*, activity was only designated 'Light', 'Moderate' and/or 'Vigorous' if performed in bouts of at least 10 minutes. Various cut-point recommendations exist for classifying accelerometer count values as the following discrete classes of physical activity: sedentary behaviour (SED), light (LIPA), moderate (MPA) and vigorous (VPA) physical activity. 'Matthews' cut-points were

developed with the aim of reflecting both structured (treadmill) walking and running, as well as free-living, lifestyle activities[219]. These cut-points were preferred for analysing the Actigraph data since they purportedly reflect free-living activity most accurately[215,222]. In accordance therewith, SED was considered as any activity < 100 cpm, LIPA was acknowledged as 100 - 759 cpm, 760 – 5998 cpm was deemed MPA, and ≥ 5999 cpm was VPA (MVPA or moderate-to-vigorous physical activity was ≥ 760 cpm). Raw data was analysed according to these cut-points for time spent in different activity levels and this was used to calculate the proportion of wear-time spent in each activity domain.

Based on prior associations with clinical changes in cardio-metabolic biomarkers, a 'sedentary bout' was identified as ≥ 20 consecutive minutes below 100 cpm[224,225]. A 'sedentary break' was any interruption in sedentary time from one minute < 100 cpm to ≥100 cpm the following minute.

Daily physical activity was expressed as

i.) the average percentage of wear-time spent in SED, LIPA, MPA and VPA,
ii.) total volume of activity (average number of steps taken per day),
iii.) number of and time spent in sedentary bouts and
iv.) profile of activity fluctuations throughout an average day (counts in the vertical Axis 1).

2.5.8.) Perceived Stress (visit 1)

The Perceived Stress Questionnaire (PSQ, *Appendix F*), a validated psychosomatic tool, was used to evaluate recent levels of stress (past month)[226]. Participants were asked to respond to 30 questions on a four-point Likert scale. Negative statements were scored 1 to 4 as per the questionnaire; positive statements were scored as (5 – answer). The PSQ score ranges from 30 to 120 with higher values indicating higher levels of stress. Participants were categorised as having experienced a 'Reduced' (score of 30 – 60), 'Average' (score of 60 – 90) or 'High' (score of 90 – 120) level of stress.

2.5.9.) Resting Blood Pressure (visit 2)

Overnight-fasted participants rested in a supine position on a bed for 10 minutes. Resting blood pressure was measured by auscultation using a blood pressure cuff (Aneroid sphygmomanometer, Hi-Care, Seoul, Korea). This was performed on three occasions that were separated by at least one minute and taken on alternating arms. Outlier measures were repeated and the nearest three measures were averaged to calculate individual blood pressure results.

2.5.10.) Resting Metabolic Rate (RMR) and Total Energy Expenditure (visit 2)

RMR was determined with indirect calorimetry using the ventilated hood technique (Quark RMR, COSMED, Rome, Italy). Immediately prior to each test, the Quark was calibrated using a certified calibration gas of known 16% O_2 and 5% CO_2, and a 3 litre syringe cylinder. Following 10 minutes of rest on the laboratory bed and the measurement of resting blood pressure, overnight-fasted participants remained in this supine position, and were asked to relax, keep their eyes open, breathe normally and refrain from any movement or speech during the subsequent 20 minute RMR measurement. A clear ventilated hood was placed over their head. During the initial 5 minutes, the flow rate of the Quark blower was manipulated until the $FeCO_2$ (fractional amount of carbon dioxide expired) was stable between 0.90% and 1.10%. This was taken as an indication that the participants had reached metabolic steady-state. Data recording was started and oxygen consumption and carbon-dioxide production were measured continuously for the subsequent 15 minutes. At the end of the measurement, the data were averaged over 15 second intervals and RMR was calculated as the average of the final 10 minutes. Correct calibration of the Quark was checked periodically by performing a calibration 'burn' of pure ethanol (5 ml) and comparing the recorded data (total CO_2 collected and average respiratory exchange ratio, RER) to calibration standards. The percentage error at each burn was below 3% (*Appendix I*).

The energy requirements of the participants were estimated using the formula for Total Energy Expenditure (TEE)[227].

TEE = Resting Metabolic Rate (RMR) x Physical Activity Level (PAL)

PAL is determined by the level of daily activity, and in the general population has been found to range from 1.4 (sedentary, minimal daily activity) to 2.5 (vigorous activity). For the purpose of this study, participants were allocated an individual PAL based on their level of activity during the days of their 3DR. All participants were allocated a PAL of either 1.8 or 2.0, which represented 'active' or 'very active' lifestyles respectively [227,228].

2.5.11.) Fasting Blood Sampling (visit 2)

Resting blood samples were taken after the RMR measurement and immediately prior to starting the OGTT. A cannula was inserted into the antecubital vein and attached to a three-way stopcock. Blood samples for determination of serum insulin, FFA, lipids (total cholesterol, TG, HDL-C, LDL-C), HDL and LDL particle size distributions, uric acid and alanine aminotransferase (ALT) were collected in collection tubes containing a clot activator and a serum-separating gel. Blood samples were collected in tubes spray-coated with the anticoagulant K_2EDTA for determination of whole-blood HbA1C. Blood was collected in a tube containing the anticoagulant heparin for determination of plasma C-reactive protein and in a tube containing oxalate and fluoride for determination of plasma glucose. All tubes were inverted a minimum of five times to ensure the blood was thoroughly mixed with the tube additives and were processed as described below (*section 2.5.13.*).

2.5.12.) Insulin Sensitivity and Glucose Tolerance (visit 2)

After fasting blood draws, insulin-sensitivity was assessed by performing an OGTT. Participants were fasted overnight and had abstained from exercise and alcohol consumption the day before testing and the morning of testing. Venous blood samples (5 ml) were collected at -10 and -5 minutes, relative to glucose ingestion, for determination of plasma glucose and serum insulin. These were averaged to calculate the baseline (fasting) plasma glucose and serum insulin of the OGTT. The participants ingested 75 grams of

glucose (Re Fuel Products, Cape Town) that had been dissolved in 250 ml of water. Blood samples were taken at 15, 30, 45, 60, 90 and 120 minutes after glucose ingestion for determination of plasma glucose and serum insulin responses. Fasting and OGTT glucose and insulin concentrations were used to calculate various indices of insulin-sensitivity. Where applicable, total area-under-the-curve (AUC) of plasma glucose (GAUC) and serum insulin (IAUC) were calculated using the trapezoid rule.

Hepatic insulin-sensitivity was initially calculated using the validated Homeostatic Model Assessment of Insulin-Resistance, HOMA-IR[229], as follows:

$$\text{HOMA-IR} = (\text{FPG} \times \text{FI}) / 22.5$$

Where FPG is fasting plasma glucose and FI is fasting serum insulin (average of - 10 and - 5 minute measures). A higher value represented greater hepatic insulin-resistance and a threshold value of 2.29 was used to identify insulin-resistance[230].

Since HOMA-IR only incorporates basal glucose and insulin concentrations, it is unable to indicate the sensitivity of the liver to the suppressive effects of insulin on hepatic glucose output[231]. Therefore, we calculated the hepatic insulin-resistance index recommended by Abdul-Ghani *et al*. (2007), since it takes into consideration both the basal insulin and glucose concentrations and the suppression of hepatic glucose output by insulin during the first 30 minutes of the OGTT[231], as follows:

$$\text{Hepatic insulin-resistance index} = GAUC_{30} \times IAUC_{30}$$

Where $GAUC_{30}$ represents the total area under the glucose curve during the first 30 minutes of the OGTT and $IAUC_{30}$ represents the total area under the insulin curve during the same period. A higher value represented greater hepatic insulin-resistance, and this has been validated against the hepatic insulin-resistance index from the gold-standard hyperinsulinaemic-euglyceamic clamp[231].

A specific index of skeletal muscle insulin-sensitivity was also calculated. This takes into account the rate of glucose disposal from its highest value during the OGTT to its lowest value, in relation to the mean concentration of insulin during the OGTT[231]. Since this

normally occurs after 60 minutes, it is assumed that there is no significant change in hepatic glucose output. Thus, the decline in glucose concentration primarily reflects glucose uptake by the peripheral tissues, especially skeletal muscle. The calculation was as follows:

$$\text{Muscle insulin-sensitivity index} = (dG / dT) \times (1 / \bar{I})$$

Where dG / dT is the rate of decline in plasma glucose concentration from its peak to its nadir (where glucose rebounded after its nadir, the rebound was not taken into consideration), and $\bar{I}$ represents the mean insulin concentration during the OGTT. A lower value, indicative of a slower rate of decline in plasma glucose and / or a higher insulin concentration, represented greater skeletal muscle insulin-resistance. This has been validated against insulin-stimulated glucose disposal during the gold-standard hyperinsulinaemic-euglycaemic clamp[231].

The Matsuda Index (Matsuda) has been validated as a surrogate for measuring whole-body (hepatic and skeletal muscle) insulin-sensitivity[232]. Matsuda incorporates basal glucose and insulin as well as the mean concentrations during the OGTT. A Matsuda Index score was calculated as follows:

$$\frac{10\,000}{\sqrt{(FPG\ X\ FI) * (\bar{G}\ X\ \bar{I})}}$$

Where FPG is Fasting Plasma Glucose and FI is Fasting Insulin; $\bar{G}$ is the mean plasma glucose during the OGTT and $\bar{I}$ is the mean serum insulin during the OGTT. A lower value represented greater whole-body insulin-resistance, and a threshold value of 5.0 was used to diagnose insulin-resistance[230].

Total area-under-the-curve of plasma glucose and serum insulin during the 120 minute OGTT ($GAUC_{120}$ and $IAUC_{120}$ respectively) were used to calculate an index of insulin secretion during the OGTT as follows[233]:

$$(IAUC_{120}) / (GAUC_{120})$$

2.5.13.) Blood Processing and Storage (after testing)

Blood tubes used for determination of total cholesterol, TG, HDL-C, LDL-C, Uric Acid, ALT, C-reactive protein and HbA1c were stored on ice, and sent immediately after the OGTT to Metropolis Pathology Laboratory (Taljaard Incorporated, Century City, Cape Town) for on-the-day analysis. The remainder of the resting and OGTT blood samples were kept on ice until the end of the OGTT. They were then centrifuged for 10 minutes at 4°C and 3000 rpm. Samples were aliquoted into separate tubes for storage at -80°C (except for glucose samples which were stored at -20°C) until they were analysed at the end of data collection.

2.5.14.) Substrate Analyses

Unless otherwise specified, all substrate analyses were performed by the author of this thesis. Serum insulin was determined using an Automated Chemiluminescence System (Centaur CP, Siemens Healthcare Diagnostics Inc., NY, US). Plasma glucose was measured using the glucose oxidase method (YSI 2300 STAT Plus Analyser, Ohio, US). Both insulin and glucose analyses were conducted by the analyst at the laboratory of the ESSM Division of UCT. Concentrations of plasma lipid species (including total cholesterol, TG, HDL-C and LDL-C), as well as C-reactive protein, HbA1C, Uric Acid and ALT were measured by Metropolis Pathology Laboratory (Century City, Cape Town).

Serum FFA was determined spectrophotometrically using a commercial kit (FFA half-micro test; Roche Applied Science, Mannheim, Germany). Briefly, 10 µl of serum sample was added to a 200 µl solution containing acyl-CoA synthetase, ATP and coenzyme A (CoA) and incubated for 10 minutes at 25°C. This activated the FFA to form acyl-CoA. Surplus CoA was removed by adding N-ethyl-maleinimide. Acyl-CoA was oxidised to enoyl-CoA and H_2O_2 by adding acyl-CoA oxidase. In the presence of added peroxidise enzyme, 2,4,6-tribromo-3-hydroxy-benzoic acid and 4-aminoantipryne were converted to a red dye. The intensity of the red dye was measured in the visible wavelength range at 546 nm (Bio Tek Synergy HT Multi-Mode Microplate Reader, Bio Tek Instruments Inc., Vermont, US) and used to calculate FFA concentration.

Lipoprotein fraction and sub-fraction analysis was performed in collaboration the UCT Hatter Institute for Cardiovascular Research in Africa (MRC, Cape Town). HDL sub-fractions were analysed using the Lipoprint ® HDL Sub-fractions test (Quantimetrix, Redondo Beach, CA); VLDL, IDL and LDL fraction and sub-fraction analysis was performed using the Lipoprint ® LDL Sub-fractions test (Quantimetrix). Both systems used polyacrylamide-gel-electrophoresis to separate lipoprotein fractions according to size (the procedures were similar). Briefly, 25 µl of serum sample was mixed with a 300 µl Lipoprint loading gel, containing Sudan black dye, which binds proportionately to the cholesterol present in the sample. The mixture was placed onto the upper part of a high resolution 3% polyacrylamide gel. Following 30 minutes of photopolymerisation at room temperature, electrophoresis was performed in an electrolyte (Tris) buffer for 50 minutes at a current of 3 mA per gel tube. After 30 minutes rest, gel tubes were scanned and analysed for the level of cholesterol in each sub-fraction using the Lipoware software. In the resulting HDL profile, the VLDL remained at the origin [Retention Factor (Rf) = 0.0] while albumin migrated as the leading front (Rf = 1.0). IDL and LDL were also located at the origin, followed by 10 distinguishable HDL bands: HDL-1, -2 and -3 were defined as 'Large HDL'; HDL-4, -5, -6 and -7 were defined as 'Intermediate HDL' and HDL-8, -9 and -10 were defined as 'Small HDL'. Each subclass was quantified and expressed as a percentage of total HDL concentration. In the LDL profile, VLDL remained at the origin but HDL was visualised as one band near the end of the gel. Between these, Mid-bands – C, - B and –A (constituting IDL) were seen below the VLDL band, followed by the separate LDL sub-fractions that were present in the samples. LDL – 1 and – 2 were defined as 'Large buoyant' LDL, whilst LDL - 3, - 4, - 5, - 6 and - 7 were defined as 'Small dense' LDL. Each subclass was quantified and expressed as a percentage of total LDL concentration. A predominance of Large buoyant LDL particles was indicative of LDL 'Pattern A', whilst a predominance of small dense LDL was indicative of LDL 'Pattern B'[32,234].

2.5.15.) Metabolic Syndrome Diagnosis

Three different sets of established criteria were used to assess whether or not individual participants exhibited the MetS, namely the World Health Organisation (WHO, 1998)[15], the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) as updated by the American Heart Association (2005)[16], and the International Diabetes Federation (2005)[17]. They all encompass the four features of the MetS, central obesity,

insulin-resistance, dyslipidaemia, and endothelial dysfunction, but vary slightly in the relative emphasis placed on them and the cut-off values used for the relevant parameters. The specific criteria for women, and which were applied in the present study, are summarised in Table 2 below[14].

Table 2. Summary of the three sets of criteria used to investigate the presence of Metabolic Syndrome in participants.

	WHO	**NCEP ATP III**	**IDF**
Absolutely required	Insulin-resistance (e.g. IFG, IGT or Matsuda)	None	Central obesity (waist circumference ≥ 80 cm)
Additional criteria	Plus 2 of the following	Any 3 of the following	Plus 2 of the following
Central obesity	Waist : hip ratio ≥ 0.85 or BMI ≥ 30 $kg.m^{-2}$	Waist circumference ≥ 88 cm	Central obesity required above
Insulin-resistance / Glucose Intolerance	Insulin-resistance required above	Fasting glucose ≥ 6.1 mM	Fasting glucose ≥ 6.1 mM
Dyslipidaemia	Triglycerides ≥ 1.70 mM or HDL-C < 1.00 mM	Triglycerides ≥ 1.70 mM or HDL-C < 1.30 mM	Triglycerides ≥ 1.70 mM or HDL-C < 1.30 mM
Hypertension	≥ 140 / 90 mmHg	> 130 mmHg systolic or > 85 mmHg diastolic	> 130 mmHg systolic or > 85 mmHg diastolic

WHO, World Health Organisation; NCEP ATPIII, National Cholesterol Education Program Adult Treatment Panel III (as updated by the American Heart Association); IDF, International Diabetes Federation; IFG, Impaired Fasting Glucose; IGT, Impaired Glucose Tolerance

2.5.16.) Statistical Analyses

Statistical analyses were performed using Statistica software (version 13, Statsoft, Tulsa, US) and graphical representations were performed using GraphPad Prism (version 5, GraphPad Software Inc., La Jolla, CA, US). Before performing statistical tests, data were checked for normality using the Shapiro-Wilks test, and checked for homogeneity of variance using Levene's test. Normally distributed data were reported as mean ± standard deviation (SD) and non-parametric data were reported as median (interquartile range). Differences between Overweight and Lean groups were determined using an independent t-test if normally distributed, and a Mann-Whitney-U test if not normally distributed. Paired t-tests were used to explore within-group differences between diet assessment tools, between individual energy intake (3DR) and estimated TEE, as well as physical activity differences between weekdays and weekend days. Between-group differences in dietary macronutrient composition, the proportion of accelerometer wear-time spent in different activity levels, and the proportion of groups following select nutritional practices, were all analysed using chi-square tests. Fisher's exact test was used to explore differences in categorical variables in 2 x 2 contingency tables. Between-group differences in lipoprotein composition, as well as within-group macronutrient differences between diet assessment tools, were explored using independent t-tests and appropriate Bonferroni correction factors for multiple comparisons. Correlations between variables were investigated using the Pearson (parametric) or the Spearman (non-parametric) correlation coefficients. Multiple regression analysis was explored to adjust for potential confounders that may have contributed to significant between-group differences. Given the small sample size, 'group' was included as the primary predictor variable and potential confounders were included one after another (including a maximum of two predictor variables). OGTT glucose and insulin curves, as well as daily physical activity profiles, were both analysed for between-group differences and group * time interactions using a repeated-measure two-way ANOVA. Significance was accepted at $p < 0.05$ for all tests except where a Bonferroni correction factor was used.

3.) RESULTS

3.1.) Participant Characteristics

As intended, the Overweight and Lean groups were well-matched for age, but differed considerably in body composition (Table 3). BMI and waist circumference (which were used to group participants) were significantly higher in the Overweight group ($p < 0.0001$) with no overlap. Hip girth was also larger in the Overweight group ($p < 0.001$), but the waist-to-hip ratio was the same. The sum of seven skinfolds ($p < 0.001$), which was used to estimate BF% during screening, was significantly higher in the Overweight group (Table 3). However, there was considerable intra-group variation (Table 3). The ratio of android (abdominal) fat to gynoid (hip and thigh) fat ($p < 0.001$), as well as the ratio of abdominal fat mass to limb fat mass ($p < 0.01$), were greater in the Overweight group than the Lean group. Whole-body bone density was similar between groups ($p = 0.44$) and there were no regional differences in bone density (not shown).

Mean BF% from the gold-standard measure of body composition (DXA, Figure 1), was also significantly higher in the Overweight group (32.1 ± 3.9 vs. 21.8 ± 3.9, $p < 0.0001$). However, there was a considerable range of approximately 11% in both groups. According to DXA, three of the participants that had been recruited as 'Overweight' had BF% values below 30% (Figure 1). This has been regarded as the lower threshold for excess body fat in the general population[197]. No Lean participants exhibited an 'Athletic' BF% of between 8% and 15%[197]. Conversely, four of them had BF% above 25% and one participant overlapped with the Overweight group (27.6%). This participant was an interesting anomaly given that her BMI (19.7 $kg.m^{-2}$) and waist circumference (67.0 cm) were amongst the lowest recorded in this study. Interestingly, she was the only participant to exhibit bone mineral density lower than age-based expectations (Z score < -2.0). Her estimated BF% from skinfolds had also predicted a lower BF% compared to DXA. This was likely due to the limitation of having used a formula that did not incorporate lower-body skinfolds (*Discussion section*). However, this participant met the original criteria of being overtly lean and she had easily maintained a low body weight. Furthermore, neither excluding her from the analysis, nor moving her to the Overweight group based on BF% alone, meaningfully altered the metabolic or lifestyle results. Therefore, it was decided to include her in the

Lean group for the analysis. These BF% results meant that despite distinct BMI and waist circumference, the two groups were less distinct in terms of actual adiposity than expected. The resultant spectrum of BF%, however, facilitated the correlation analyses with relevant metabolic and lifestyle parameters in the sections that follow.

Table 3. Age, anthropometric measures and DXA indices of adiposity and bone mineral density in Overweight (n=10) and Lean (n=10) groups.

Variable	Overweight	Lean	p value
Age (years)	38.7 ± 4.6 (31 – 45)	37.7 ± 4.3 (31 – 45)	0.62
Height (cm)	166 ± 7 (157 -178)	170 ± 6 (160 – 179)	0.12
Weight (kg)	74.4 ± 6.4 (67.1 – 86.4)	59.4 ± 7.8 (51.6 – 71.4)	**< 0.001**
BMI (kg/m^2)	27.1 ± 1.6 (25.3 – 29.6)	20.4 ± 1.7 (18.0 – 23.1)	**< 0.0001**
Waist (cm)	83.7 ± 4.4 (76.3 – 88.1)	69.7 ± 4.3 (63.2 – 75.5)	**< 0.0001**
Hip (cm)	107.6 ± 5.4 (101.2 – 116.9)	93.0 ± 4.2 (87.0 – 101.5)	**< 0.0001**
Waist : Hip *	0.77 (0.09)	0.74 (0.05)	0.24
Sum of seven skinfolds (mm) *	146 (28)	83 (19)	**< 0.001**
Android / Gynoid Ratio	0.8 ± 0.1 (0.7 – 1.0)	0.6 ± 0.1 (0.5 – 0.8)	**<0.001**
Trunk/Limb Fat Mass Ratio	0.9 ± 0.2 (0.6 – 1.2)	0.6 ± 0.1 (0.5 – 0.9)	**<0.01**
Bone mineral density (g/cm^2)	1.2 ± 0.1 (1.1 – 1.3)	1.1 ± 0.1 (0.9 – 1.3)	0.44

Values are mean ± SD (range) or median (interquartile range). Where normally distributed, p values were determined using independent t-tests; where not normally distributed (*), p values were determined using Mann-Whitney-U tests. Bold p values indicate statistically significant differences between groups ($p < 0.05$).

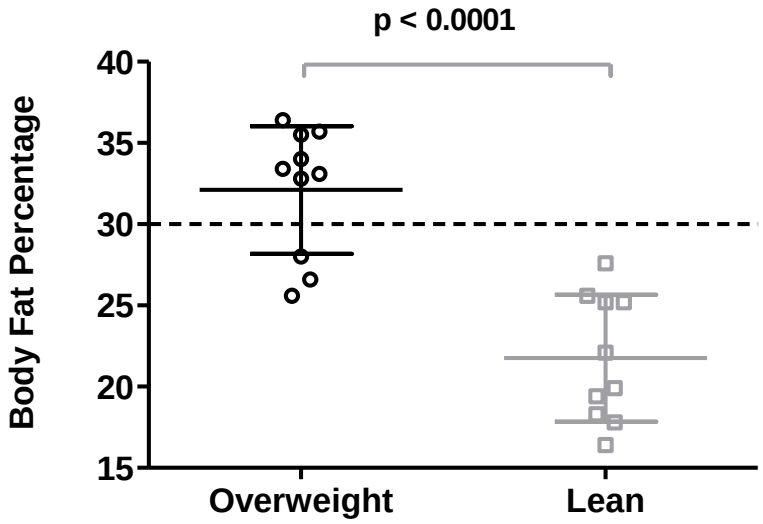


Figure 1. Body fat percentage (DXA) in Overweight (n=10) and Lean (n=10) groups. Data are shown as individual values (markers) and as mean ± SD (solid line ± whiskers). *Dashed line, cited threshold for 'Overweight'* [197]*.* p value was determined using an independent t-test.

3.2.) Running Characteristics

The groups were well-matched for years of running experience, current volume of training (both in terms of distance per week and hours per week), as well as running calibre, which was expressed as energy expenditure ($kcal.min^{-1}$) during their fastest half-marathon of the previous 12 months (Table 4). The latter was calculated by taking into account the different bodyweights of the participants (*Methods section*), which meant that the groups were matched despite the Lean runners having had significantly faster half-marathon times ($p < 0.05$). Consistent therewith, the Lean participants attained a higher peak treadmill running speed (PTRS, $p < 0.05$), and there was a significant negative correlation between BF% and PTRS (Pearson $r = -0.70$, $p < 0.001$). Both groups reported a final RPE of 18 and a similar maximum heart rate ($p = 0.97$ and $p = 0.74$ respectively). This would suggest that they gave a similar degree of effort during the PTRS test.

Table 4. Running-related matching criteria, and results from the PTRS for Overweight (n=10) and Lean (n=10) groups.

	Variable	Overweight	Lean	p value
Running experience	Total years *	6.0 (5.3)	9.0 (8.6)	0.11
	Recent consecutive years *	6.0 (5.3)	6.5 (5.5)	0.40
Current running volume	Distance (km.week^{-1})	42.0 ± 10.9 (30-60)	44.5 ± 12.1 (25 – 65)	0.63
	Time (hours.week^{-1})	4.8 ± 1.1 (3.3 – 7.0)	4.9 ± 1.7 (3.0 – 8.0)	0.91
Fastest 21.1 km during last 12 months	Time (minutes)	136 ± 15 (110 – 159)	123 ± 11 (105 – 140)	**<0.05**
	Energy expenditure (Kcal.min^{-1}) *	8.9 (2.1)	8.6 (1.5)	0.62
Peak treadmill running speed test	Peak speed (km.h^{-1})	12.6 ± 1.0 (11.1 - 14.1)	14.0 ± 1.3 (12.0 – 16.5)	**0.01**
	Time to exhaustion (minutes)	10.3 ± 2.0 (7.0 – 13.2)	13.0 ± 2.6 (8.9 – 18.0)	**0.01**
	HRmax (beats.minute^{-1})	182 ± 12 (156 – 199)	180 ± 6 (172 – 193)	0.74
	Final RPE *	18.0 (0.5)	18.0 (0.5)	0.97

Values are mean ± SD (range) or median (interquartile range)(*). *21.1 km*, *half-marathon distance race*, *HRmax*, *maximum heart rate*; *RPE*, *Rating of perceived exertion*. Where normally distributed, p values were determined using independent t-tests; where not normally distributed (*), p values were determined using Mann-Whitney-U tests. Bold p values indicate statistically significant differences between groups ($p < 0.05$).

3.3.) Resting Blood Pressure

Systolic and diastolic blood pressure were not normally distributed and they are represented as median ± interquartile range in Figure 2. The median systolic blood pressure of the Overweight group was higher than that of the Lean group ($p < 0.05$), however, median diastolic blood pressure was similar. When expressed as the mean blood pressure, both Overweight (118 / 72 mmHg) and Lean (109 / 68 mmHg) groups exhibited 'normal' values (< 120 / 80 mmHg). On an individual level, there were four Overweight participants who exhibited pre-hypertensive systolic blood pressure (≥ 120 mmHg), two of which also exhibited pre-hypertensive diastolic blood pressure (≥ 80 mmHg). One of these participants was also insulin-resistant by the Matsuda Index (3.5), and another participant with pre-hypertensive systolic blood pressure only, was insulin-resistant. In agreement therewith, multiple regression analysis with 'group' and 'IAUC' included as predictor variables, showed that IAUC during the OGTT was more predictive of systolic blood pressure than the distinction of Overweight and Lean groups (Table 5).

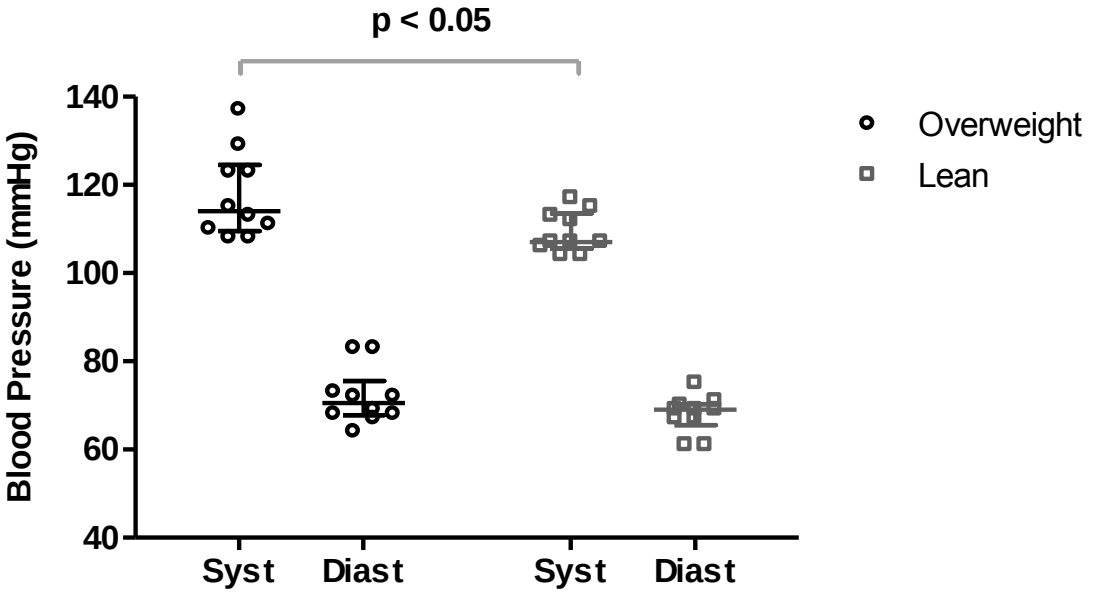


Figure 2. Distribution of Systolic (Syst) and Diastolic (Diast) blood pressure within Overweight (n=10) and Lean (n=10) groups. Data are presented as individual values (markers), and median ± inter-quartile range (solid line ± whiskers). p value was determined by a Mann-Whitney-U test.

Table 5. Regression analysis for systolic blood pressure, when group (Overweight or Lean) and insulin area-under-the-curve (IAUC) during the OGTT were predictor variables (R^2=0.41, $p < 0.012$).

	B	Standard error of b	p value
Intercept	110.832	8.55	<0.0001
Group	-5.336	3.46	0.14
IAUC	0.003	0.001	0.04

p values were determined using multiple regression analysis

3.4.) Cardio-metabolic Blood Parameters

Participants were free of any known illness or injury and had not been diagnosed by a clinician with any component of the MetS, although this was not an eligibility criterion. No participants were taking statins or any blood-pressure-lowering, cholesterol-lowering or sugar-lowering medication. One Overweight participant was on hormone-replacement medication, one Lean participant was on medication for an under-active thyroid gland, and two Lean participants were taking oral contraceptive pills.

Table 6 shows cardio-metabolic health markers that were measured in fasting blood samples. HbA1c, a measure of glucose control during recent months, was similar between groups ($p = 0.76$), as was serum uric acid ($p = 0.73$). All individual and both group mean results of these tests were well within normal ranges. There was considerable intra-group variation in ALT, particularly the Lean group, and two Lean participants had ALT concentrations above the normal range. However, there was no between-group difference in median ALT ($p = 0.24$). Although the Overweight group had a higher level of systemic inflammation, as indicated by higher plasma C-reactive-protein (CRP) concentrations ($p < 0.05$), CRP values for all participants were normal by clinical standards (< 3.0 mg/L)[235]. CRP did exhibit a significantly positive correlation with BF% (Spearman $r = 0.52$, $p < 0.05$). There were no between-group differences in serum TG, total cholesterol (TC) or the TG / HDL-C ratio. However, the TC / HDL-C ratio and LDL-C concentrations were significantly higher in the Overweight group ($p < 0.05$), and HDL-C concentrations tended to be lower in the Overweight group ($p = 0.08$). Further HDL-C concentrations correlated negatively with BF%

(r = -0.49, p < 0.05). FFA concentrations of all participants were within the normal range, however there was considerable intra-group variation and no between-group difference (p = 0.72).

Table 6. Cardio-metabolic health markers from overnight-fasted blood samples in Overweight (n=10) and Lean (n=10) groups.

Blood marker	Normal range	Overweight	Lean	p value
Uric acid (mM) *	0.15 - 0.37	0.24 (0.05)	0.27 (0.12)	0.73
HbA1c (%)	4.5 - 6.3	5.1 ± 0.2	5.1 ± 0.2	0.76
ALT (IU/L) *	10 – 31	13.0 (3.5)	16.0 (13.8)	0.24
CRP (mg/L) *	0.0 - 3.0	0.95 (1.4)	0.50 (0.53)	**<0.05**
Total cholesterol (mM)	< 5.00	5.07 ± 0.72	4.90 ± 0.90	0.65
HDL-C (mM)	> 1.20	1.88 ± 0.22	2.17 ± 0.44	0.08
Total Cholesterol / HDL-C	< 4.0	2.70 ± 0.40	2.30 ± 0.42	**<0.05**
LDL-C (mM) *	< 3.0	2.82 (0.73)	2.24 (0.96)	**<0.05**
Triglycerides (mM)	< 1.70	0.68 ± 0.18	0.65 ± 0.13	0.60
Triglycerides / HDL-C	< 0.90	0.37 ± 0.11	0.31 ± 0.09	0.21
FFA (mM)	0.00 - 0.72	0.29 ± 0.12	0.26 ± 0.16	0.72

Values are mean ± SD or median (interquartile range) (*). *HbA1c, glycosylated haemoglobin; ALT, alanine aminotransferase; CRP, C-reactive protein; C, cholesterol, HDL, high-density-lipoprotein; LDL, low-density-lipoprotein; FFA, Free-Fatty-Acids*. Where normally distributed, p values were determined using independent t-tests; where not normally distributed (*), p values were determined using Mann-Whitney-U tests. Bold p values indicate statistically significant differences between groups (p < 0.05).

Table 7 shows a.) the contribution of VLDL, IDL, LDL and HDL lipoprotein fractions to total cholesterol (TC), and b.) the sub-fraction composition of each lipoprotein fraction. The former was investigated for statistical differences using independent t-tests and a Bonferroni correction factor ($\alpha = 0.05/4$). Only IDL-C contributed significantly more to TC in the Overweight group compared to in the Lean group ($p < 0.0125$). There was a tendency for HDL-C to contribute more to TC in the Lean group, but this did not reach statistical significance after Bonferroni correction ($p = 0.03$). Within the IDL, HDL and LDL fractions, there were no between-group differences in the proportions constituted by the respective sub-fractions. Specifically, in both groups, IDL-C was the dominant sub-fraction of IDL, followed by IDL-A and IDL-B. Large HDL (HDL – 1, - 2, - 3) and intermediate HDL (HDL – 4, - 5, - 6, - 7) each accounted for approximately 45% of the HDL fraction, with small HDL (HDL – 8, - 9, - 10) making up approximately 10%. LDL-1 represented the vast majority of LDL particles in both groups, followed by LDL-2. The majority of participants exhibited only these 'large buoyant' LDL particles. One Overweight and two Lean participants exhibited a very small proportion of 'small dense' LDL-3 particles. However, none of these were near sufficient to suggest a 'Pattern B' LDL profile that has been associated with greater atherogenic risk[234].

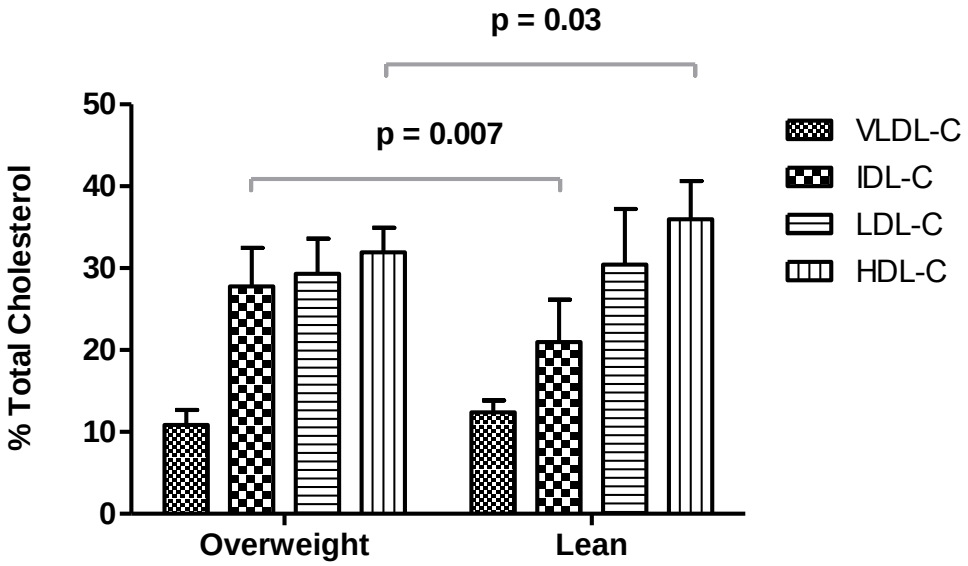


Figure 3. Percentage contributions of the different lipoprotein fractions to Total Cholesterol in Overweight (n=10) and Lean (n=10) groups. C, cholesterol, VLDL, very-low-density-lipoprotein, IDL, intermediate-density-lipoprotein, LDL, low-density-lipo*protein, HDL, high-density-lipoprotein.* Bars represent mean ± SD. p value was determined using an independent t-test and significance ($\alpha = 0.05/4$) was determined by applying a Bonferroni correction factor.

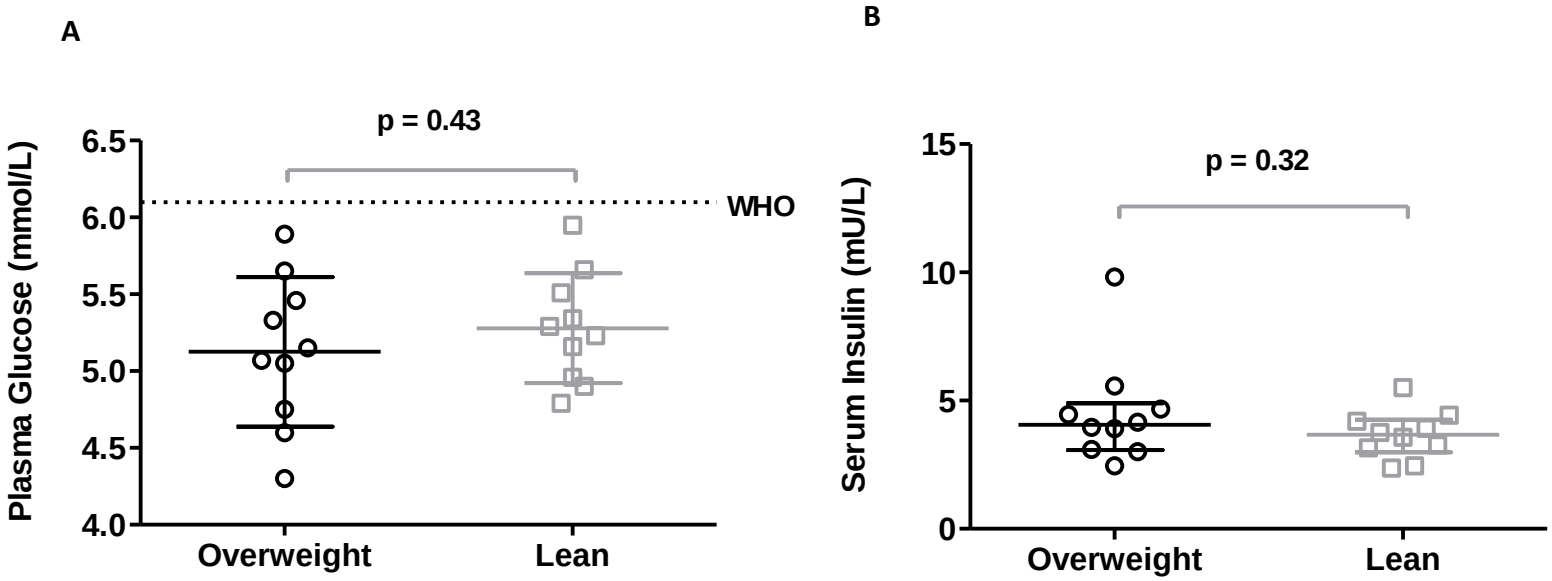
Table 7. Lipoprotein sub-fraction contributions to the respective lipoprotein fractions in Overweight (n=10) and Lean (n=10) groups.

Lipoprotein fraction / *sub-fraction*	**Overweight**	**Lean**	**p value**
VLDL-C (α)	10.84 ± 1.84	12.41 ± 1.43	0.05 (NS)
Total IDL-C (α)	27.77 ± 4.72	20.97 ± 5.19	**< 0.01**
*IDL – C **	33.21 ± 6.56	34.05 ± 8.01	0.80
*IDL – B * (#)*	20.70 (2.97)	20.80 (7.93)	0.97
*IDL – A **	43.80 ± 6.71	43.20 ± 10.11	0.88
Total LDL-C (α)	29.30 ± 4.29	30.44 ± 6.79	0.66
*LDL - 1 (Large) **	80.40 ± 7.07	77.89 ± 11.58	0.57
*LDL - 2 (Large) **	18.98 ± 5.81	20.64 ± 8.79	0.61
*LDL - 3 (Small) * (#)*	0.0 (0.0)	0.0 (1.84)	0.50
*LDL - 4, 5, 6 (Small) **	0.00 ± 0.00	0.00 ± 0.00	N/A
Total HDL-C (α)	31.94 ± 2.99	35.99 ± 4.66	0.03 (NS)
*HDL - 1, 2, 3 (Large) **	44.31 ± 8.55	43.47 ± 3.79	0.81
*HDL – 4, 5, 6, 7 (Intermediate) **	45.61 ± 6.13	45.24 ± 2.91	0.87
*HDL – 8, 9, 10 (Small) * (#)*	10.35 (6.10)	11.30 (1.0)	0.60

Values are percentages expressed as mean ± SD or median (interquartile range) (#). *α, lipoprotein fractions that are percentages of total cholesterol; * , sub-fractions of each lipoprotein fraction, that are percentages of the respective lipoprotein fraction*; *NS, non-significant difference*. p values were determined using independent t-tests, except where not normally distributed (#) they were determined using Mann-Whitney-U tests. Statistical significance (α = 0.05/4) was determined by applying a Bonferroni correction factor. Bold p values indicate significant differences between groups ($p < 0.05$).

3.5.) Insulin-Resistance and Glucose Tolerance

Figure 4 shows the distribution of fasting plasma glucose (FPG), fasting serum insulin (FI) and the HOMA-IR index of hepatic insulin-resistance within Overweight and Lean groups. There were no between-group differences in mean FPG (Overweight, 5.13 ± 0.49 vs. Lean, 5.28 ± 0.36, p = 0.43) or median (inter-quartile range) of FI (Overweight, 4.05 (1.82) vs. Lean, 3.67 (1.27), p = 0.32). No participant exhibited impaired fasting glucose (IFG), as defined by the World Health Organisation[15] (≥ 6.1 mmol.L^{-1}, Figure 4A), and all FI levels were within laboratory norms (< 25 mU.L^{-1}). FI exhibited a strong positive correlation with BF% (Pearson r = 0.53, p < 0.05). Median (inter-quartile range) HOMA-IR values were similar between groups (Overweight, 0.95 (0.54) vs. Lean, 0.83 (0.30), p = 0.34). However, there was considerable intra-group variation, particularly in the Overweight group (Figure 4C) and HOMA-IR also correlated significantly with BF% (Spearman r = 0.46, p < 0.05). The majority of Overweight participants had HOMA-IR values comparable to those of the Lean group, but two runners did exhibit noticeably higher values. However, these were not high enough (≥ 2.29) to identify hepatic insulin-resistance[230].



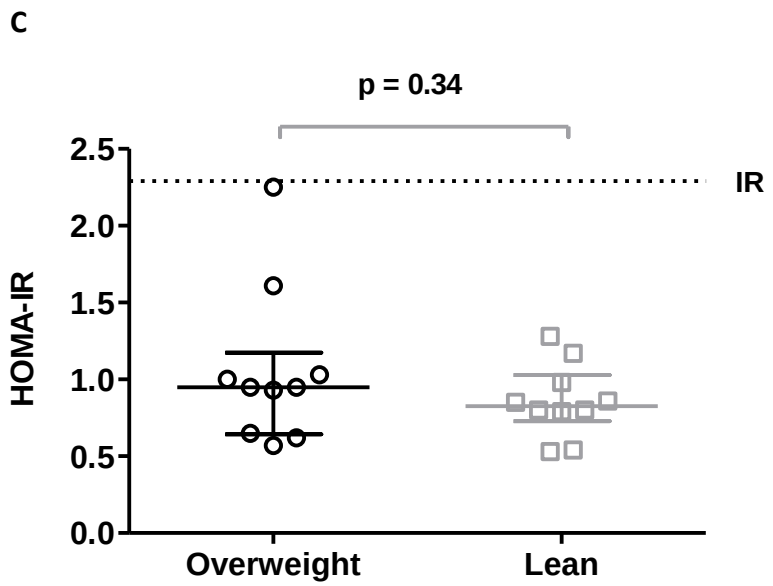


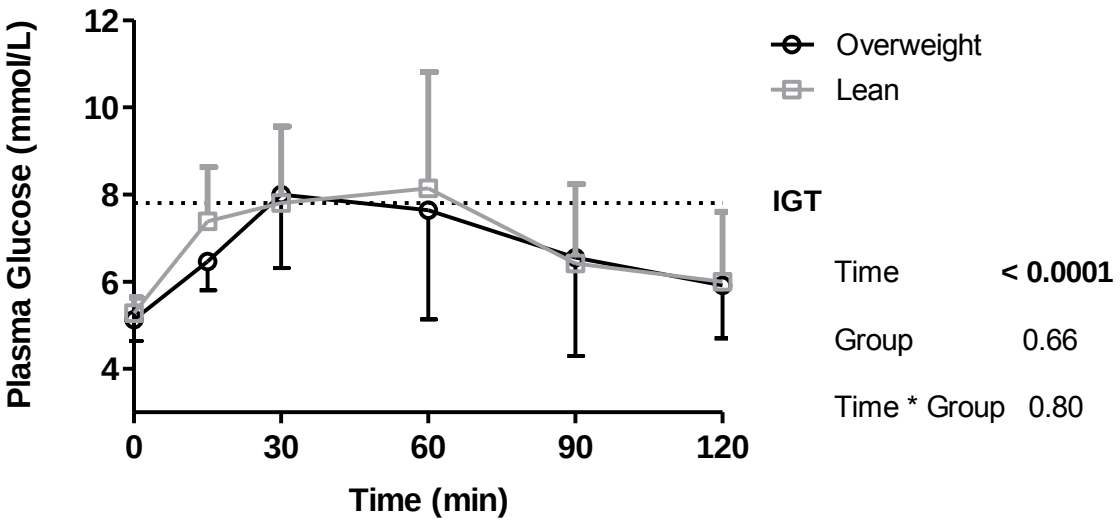
Figure 4. Distribution of individual fasting plasma glucose (FPG), fasting insulin (FI) and HOMA-IR in Overweight (n=10) and Lean (n=10) groups. A. Distribution of FPG. Data are presented as individual FPG (markers), and mean ± SD (solid line ± whiskers). *Dashed line indicates the lower threshold for impaired fasting glucose (WHO)*[15]. p value was determined using an independent t-test. **B**. Distribution of FI. Data are presented as individual FI (markers) and median ± interquartile range (solid line ± whiskers). p value was determined using a Mann-Whitney-U test. **C**. Distribution of HOMA-IR. Data are presented as individual HOMA-IR (markers) and median ± interquartile range (solid line ± whiskers). *Dashed line indicates the lower threshold for insulin-resistance*[230]. p value was determined using a Mann-Whitney-U test.

The plasma glucose responses during the OGTT were largely similar between groups (Figure 5A). Both groups exhibited a significant change in plasma glucose over time (time effect, $p < 0.0001$, $F = 12.28$, partial eta squared = 0.41). However, the two groups did not differ in their mean glucose level at any time-point (group effect, $p = 0.66$, $F = 0.19$, partial eta squared = 0.01). As a result, the shape of the glucose curve was similar between groups (interaction effect, $p = 0.79$, $F = 0.47$, partial eta squared = 0.03), which was indicative of no significant difference in blood glucose response to the glucose load. Despite this, there was substantial individual variation at every time point within both groups (Figure 5C and 5E). Mean plasma glucose at 120 minutes was below 6.0 $mmol.L^{-1}$ in both groups (Overweight, 5.91 ± 1.22 vs. Lean, 5.99 ± 1.61), and there were even Overweight and Lean participants

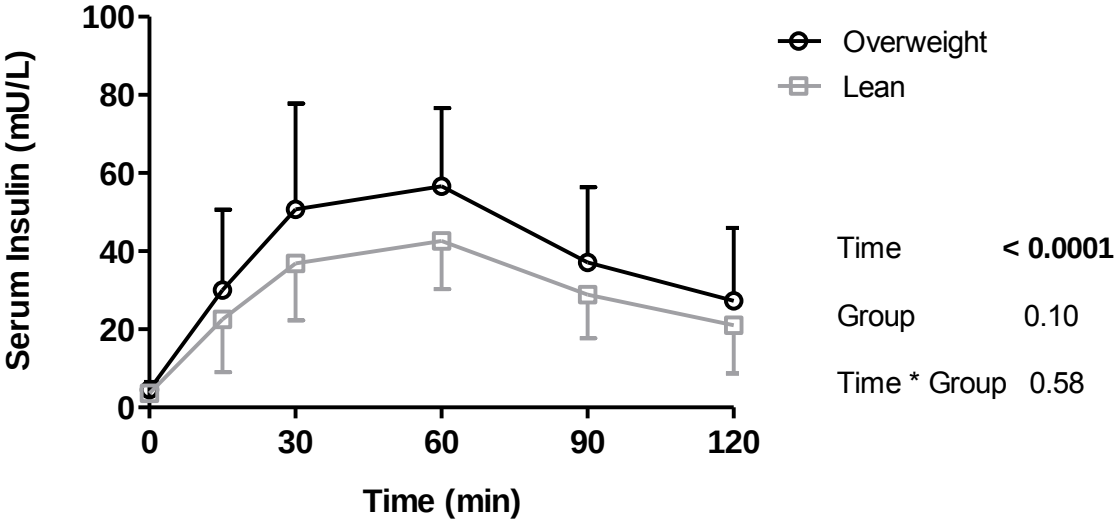
whose glucose levels had returned to baseline by 60 minutes. However, there was one participant in each group who had impaired glucose tolerance (IGT, plasma glucose of between 7.8 and 11.1 mmol.L^{-1} at 120 minutes, World Health Organisation)[15]. Consistent with these findings, the mean ± SD of total area under the glucose curve (GAUC) was similar between groups (Overweight, 830 ± 169 vs. Lean, 853 ± 173, $p = 0.76$) but varied considerably: 575 to 1110 in the Overweight group and 667 to 1147 in the Lean group.

The mean serum insulin response during the OGTT was also similar between groups (Figure 5B). Both exhibited a significant change in insulin over time (time effect, $p < 0.0001$, $F = 31.83$, partial eta squared = 0.64) and the shape of the curves was similar (interaction effect, $p = 0.58$, $F = 0.76$, partial eta squared = 0.04), both peaking at 60 minutes before returning to below 30 mU.L^{-1} at 120 minutes. There was a trend for a greater insulin response in the Overweight group (group effect, $p = 0.10$, $F = 2.93$, partial eta squared = 0.14), but this did not reach statistical significance. This was likely due to the considerable intra-group variation (Figures 5D and 5F). Some participants within each group had early, sharp insulin peaks; others had wide, gradual increases, whilst a few showed very little change. This was reflected in the comparison of the groups' total area under the insulin curve (IAUC). Both mean IAUC (Overweight, 4847 ± 1850 vs. Lean, 3653 ± 922) and the ratio of IAUC / GAUC (Overweight, 5.94 ± 2.22 vs. Lean, 4.48 ± 1.60) were highly variable and tended to be higher in the Overweight group ($p = 0.08$ and $p = 0.11$ respectively), but neither were statistically significant. However, IAUC exhibited a significant positive correlation with BF% (Spearman $r = 0.46$, $p < 0.05$).

A


Plasma Glucose (mmol/L)
12
10
8
6
4
0
30
60
90
120
Time (min)
Overweight
Lean
IGT
Time < 0.0001
Group 0.66
Time * Group 0.80

B


Serum Insulin (mU/L)
100
80
60
40
20
0
0
30
60
90
120
Time (min)
Overweight
Lean
Time < 0.0001
Group 0.10
Time * Group 0.58

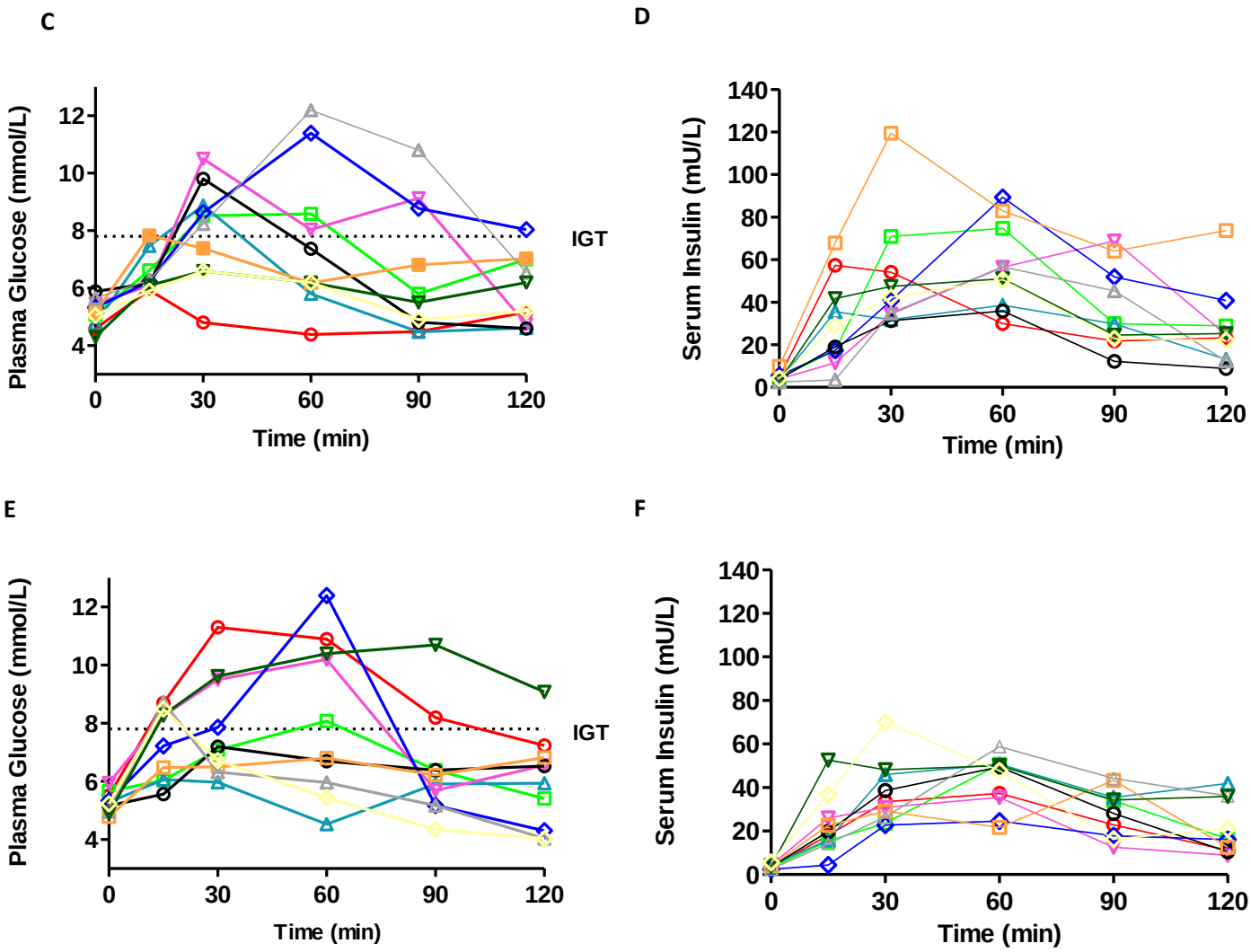


Figure 5. Plasma glucose and serum insulin curves in Overweight (n=10) and Lean (n=10) groups during the 120 minute 75 gram Oral Glucose Tolerance Test (OGTT). A. Mean plasma glucose response during the OGTT. *IGT, Impaired glucose tolerance (WHO)*[15]. Data are displayed as mean ± SD at each time-point. Statistical significance was investigated using a repeated measures two-way ANOVA. **B.** Mean serum insulin response during the OGTT. Data are displayed as mean ± SD at each time point. Statistical significance was investigated using a repeated measures two-way ANOVA. **C.** Individual plasma glucose responses during the OGTT in the Overweight group. **D.** Individual serum insulin responses during the OGTT in the Overweight group. **E.** Individual plasma glucose responses during the OGTT in the Lean group. **F.** Individual serum insulin responses during the OGTT in the Lean group.

Indices of hepatic, skeletal muscle and whole-body insulin-sensitivity were calculated from the OGTT glucose and insulin responses (*Methods section*). There was no between-group difference in the median (interquartile range) of either the hepatic insulin-resistance index (Overweight, 15.5 (6.5) vs. Lean, 13.0 (7.8), p = 0.45) or skeletal muscle insulin-sensitivity index (Overweight, 14.0 (18.3) vs. Lean, 15.5 (16.3), p = 0.85). There was again significant intra-group variation but neither index correlated with BF% or other metabolic markers. Figure 6 shows the distribution of the Matsuda index, which was calculated as a marker of whole-body insulin-sensitivity. There was considerable intra-group variation and no between-group difference in the median (interquartile range) of Matsuda scores (Overweight, 8.8 (3.8) vs. Lean, 8.7 (1.8), p = 0.85). However, Matsuda exhibited a significant, negative correlation with BF% (Pearson r = -0.47, p < 0.05). Two Overweight participants were classified as 'insulin-resistant' as they were below the threshold of 5.0 used to identify insulin-resistance[230]. It was these participants who had exhibited the greatest and most sustained insulin response during the OGTT (Orange and Blue curves in Figure 5D). No Lean participants were found to be insulin-resistant by Matsuda.

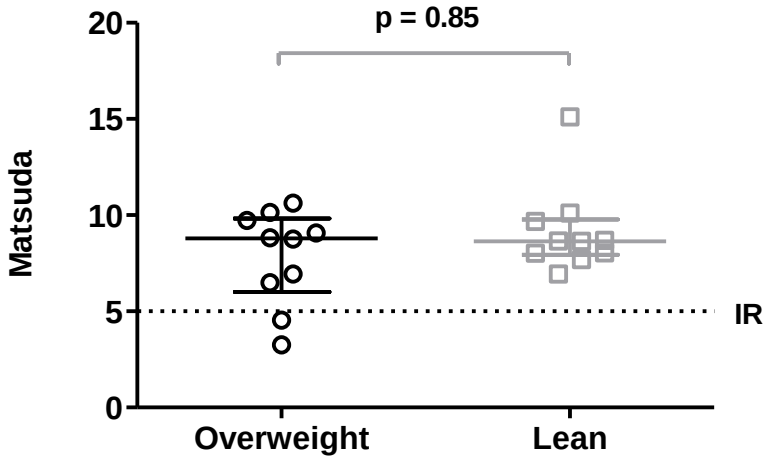


Figure 6. Distribution of the Matsuda Index of insulin-sensitivity in Overweight (n=10) and Lean (n=10) groups. Lower values indicate worse insulin-sensitivity. *IR, cut-point (< 5.0) for insulin-resistance*[230]. Data are shown as individual Matsuda indices (markers), and as median ± interquartile range (solid lines ± whiskers). p value was determined using a Mann-Whitney-U test.

3.6.) Metabolic Syndrome

Individual participants were assessed for the presence of MetS according to the WHO, NCEP ATP III and IDF criteria (*Methods section*). Overall, despite a few participants exhibiting certain abnormalities in isolation or in pairs (e.g. central obesity, systolic and/or diastolic blood pressure and/or impaired glucose tolerance or insulin-resistance), no participant had a metabolic profile that satisfied any of the three sets of criteria. It was largely the absence of dyslipidaemia (abnormal TG or HDL-C concentrations) that precluded a few participants from a MetS diagnosis. One participant in particular had considerable abdominal adiposity (waist-to-hip ratio of 0.86), insulin-resistance by Matsuda (3.26), and pre-hypertensive systolic blood pressure (129 mmHg); however, the latter fell short of the WHO requirement for hypertension, and her lipid profile was healthy. Another participant had pre-hypertensive systolic and diastolic blood pressure (137 / 83 mmHg), impaired glucose tolerance (plasma glucose concentration of 8.04 $mmol.L^{-1}$ at 120 minutes during the OGTT) and central obesity by DXA (35.7% body fat). However, her other central adiposity measures were normal according to MetS criteria and her lipid profile was favourable.

3.7.) Genetic Influence

We did not explore genetic factors specifically in this study. However, participants were asked in the Detailed Participant Questionnaire about their family history of obesity and metabolic disease. Interestingly, six overweight participants had a positive family history of obesity (five participants with obese parents and one participant with an obese sibling), whereas only one Lean participant reported an obese parent. This tended towards statistically significance (Fisher's exact, $p = 0.06$). Furthermore, four Overweight participants had parents with diagnosed T2D in contrast to no Lean participants, but this was also not statistically significant ($p = 0.09$).

3.8.) Dietary Intake

3.8.1.) Between-group comparison

Table 8 summarises the mean energy intake and macronutrient gram intake as derived from the analysis of the 3DR. Total energy intake was approximately 2000 kcal.day^{-1} and was similar between groups ($p = 0.23$), as were the absolute gram intakes of protein ($p = 0.62$), fat ($p = 0.47$) and carbohydrate ($p = 0.23$). The proportions of energy derived from protein (Overweight, 19.9 ± 4.4% vs. Lean, 16.4 ± 4.1%), fat (Overweight, 43.9 ± 9.9% vs. Lean, 43.0 ± 8.4%), carbohydrate (Overweight, 32.8 ± 11.3% vs. Lean, 35.8 ± 11.3%) and alcohol (Overweight, 3.3 ± 3.4% vs. Lean, 4.8 ± 4.0%) were almost identical between groups (chi-square test, $p = 0.78$). However, there was considerable intra-group variation.

The 3DR was also analysed for total sugar and fibre intake, and vitamins and minerals (Table 8). Interestingly, the Lean group consumed a greater amount of both total sugar and fibre ($p < 0.05$). There was considerable intra-group variation in vitamin and mineral intake. For example, vitamin C intakes ranged from 9.6 to 173.2 mg.day^{-1} in the Overweight group and from 16.4 to 177.6 mg.day^{-1} in the Lean group. Therefore, the mean intakes of most micronutrients were similar between groups ($p > 0.05$), although the Lean group had a statistically higher intake of copper, folic acid and vitamin E ($p < 0.05$). Overall, these and the aforementioned dietary findings should be interpreted with a degree of caution, given the inherent error associated with self-report dietary intake (*Discussion section*). No dietary variables were associated with BF% or other metabolic parameters across the two groups.

Table 8. Daily dietary intake, in terms of total energy, macronutrients, vitamins and minerals in Overweight (n=10) and Lean (n=10) groups from the analysis of the 3-day diet records (3DR).

Variable	Overweight	Lean	p value
Energy intake (kcal)	1928 ± 354 (1351 - 2494)	2166 ± 489 (1649 - 2968)	0.23
	Macronutrients		
Protein (g)	93.9 ± 16.4 (63.7 - 125.7)	88.5 ± 29.3 (45.8 - 151.4)	0.62
Total Fat (g)	94.6 ± 25.8 (40.7 - 123.9)	105.7 ± 39.5 (64.9 - 168.5)	0.47
*- Saturated (g) **	37.7 (15.7)	26.2 (25.3)	0.74
- Mono-unsaturated (g)	34.5 ± 12.5 (14.1 - 52.8)	40.5 ± 18.1 (18.9 - 75.4)	0.40
- Polyunsaturated (g)	15.5 ± 6.7 (8.7 - 28.1)	18.8 ± 7.4 (9.3 - 33.3)	0.32
*Cholesterol (mg) **	410 (208)	376 (130)	0.48
Carbohydrate (g)	157.4 ± 56.0 (49.0 - 255.7)	188.0 ± 54.4 (94.4 - 246.5)	0.23
Total sugar (g)	47.0 ± 18.6 (25.4 - 86.9)	72.6 ± 32.5 (28.0 - 132.3)	**0.04**
Total fibre (g)	14.3 ± 5.2 (1.9 - 19.9)	20.8 ± 5.1 (14.4 - 31.6)	**0.01**
Alcohol (g)	10.1 ± 10.7 (0.0 - 33.4)	15.5 ± 14.7 (0.0 - 44.5)	0.36

Table 7 continued

Variable	Overweight	Lean	p value
	Vitamins and Minerals		
Calcium (mg)	807 ± 249 (497 - 1178)	1094 ± 602 (385 - 2506)	0.18
Iron (mg)*	11.5 (4.0)	13.0 (10.2)	0.28
Copper (mg)	1.2 ± 0.4 (0.6 - 2.0)	1.9 ± 0.8 (0.8 - 3.4)	**0.02**
Sodium (mg)	2700 ± 636 (976 - 3110)	2818 ± 667 (1305 - 3405)	0.69
Potassium (mg)	2368 ± 673 (2088 - 4061)	2103 ± 752 (1446 - 3630)	0.42
Vitamin B6 (mg)	2.1 ± 0.7 (0.6 -3.4)	2.0 ± 0.8 (0.9 - 3.6)	0.82
Vitamin B12 (µg)*	5.1 (3.5)	4.6 (3.4)	0.68
Folic Acid (µg)*	9.3 (19.2)	29.3 (37.2)	**0.05**
Vitamin C (mg)	89.9 ± 56.0 (9.6 - 173.2)	93.7 ± 47.2 (16.4 - 177.6)	0.87
Vitamin D (µg)*	5.9 (5.2)	5.4 (4.9)	1.00
Vitamin E (mg)*	7.3 (5.4)	10.9 (2.6)	**0.04**

Values are mean ± SD (range) or median (interquartile range)(*). Where normally distributed, p values were determined using independent t-tests; where not normally distributed (*), p values were determined using Mann-Whitney-U tests. Bold p values indicate statistically significant differences between groups ($p < 0.05$).

3.8.2.) Dietary assessment tool comparison

The 24HR served as familiarisation for completing the 3DR. The data obtained from the 24HR is not shown, but it did not differ statistically from that of the 3DR. The 3DR and FFQ data were compared to assess the level of agreement between tools that represented habitual dietary intake at the time of testing and during the prior 6 months, respectively. On average, estimated energy intake from the 3DR and FFQ were similar within both groups (Overweight, 1916 ± 374 kcal.day^{-1} vs. 1821 ± 658 kcal.day^{-1} respectively; Lean, 2166 ± 489 kcal.day^{-1} vs. 2023 ± 837 kcal.day^{-1} respectively). The within-group variation of the FFQ was more pronounced than that of the 3DR. The Bland-Altman comparison plot of energy intake from the 3DR and FFQ (Figure 7), however, illustrates the large within-individual differences between methods. The mean bias was for the 3DR to report 120.4 kcal.day^{-1} higher than the FFQ, and the 95% limits of agreement were substantial (-1282 to + 1523 kcal.day^{-1}). Estimated energy intake appeared to differ systematically between methods. Specifically, the FFQ tended to under-report at lower intakes, but over-report at the higher intakes.

The estimated macronutrient compositions from the 3DR and FFQ were analysed for statistical differences using paired t-tests and an appropriate Bonferroni correction factor ($\alpha = 0.05/4$). In the Overweight group only, the percentage of energy derived from fat was significantly lower ($p < 0.01$) as per the FFQ compared to the 3DR (33.28 ± 6.23% vs. 42.69 ± 9.72% respectively). This also tended to be significant in the Lean group (FFQ, 36.11 ± 7.19% vs. 42.99 ± 8.37%) but did not reach statistical significance ($p = 0.02$). Conversely, the proportion of energy derived from alcohol was significantly higher ($p < 0.001$) according to the FFQ compared to the 3DR in the Overweight group (8.46 ± 4.25% vs. 3.35 ± 3.63% respectively). Again, this tended to be the case in the Lean group (7.89 ± 2.61% vs. 4.80 ± 3.98%) but was not statistically significant ($p = 0.017$). Within both groups, proportional carbohydrate intake was slightly higher according to the FFQ compared to the 3DR (Overweight, 38.40 ± 9.10% vs. 34.00 ± 11.26%, respectively; Lean, 38.90 ± 9.83% vs. 35.75 ± 11.29%, respectively), but these were not statistically significant (Overweight, $p = 0.06$ and Lean $p = 0.38$). Proportional protein intake was relatively consistent between the 3DR and FFQ estimates.

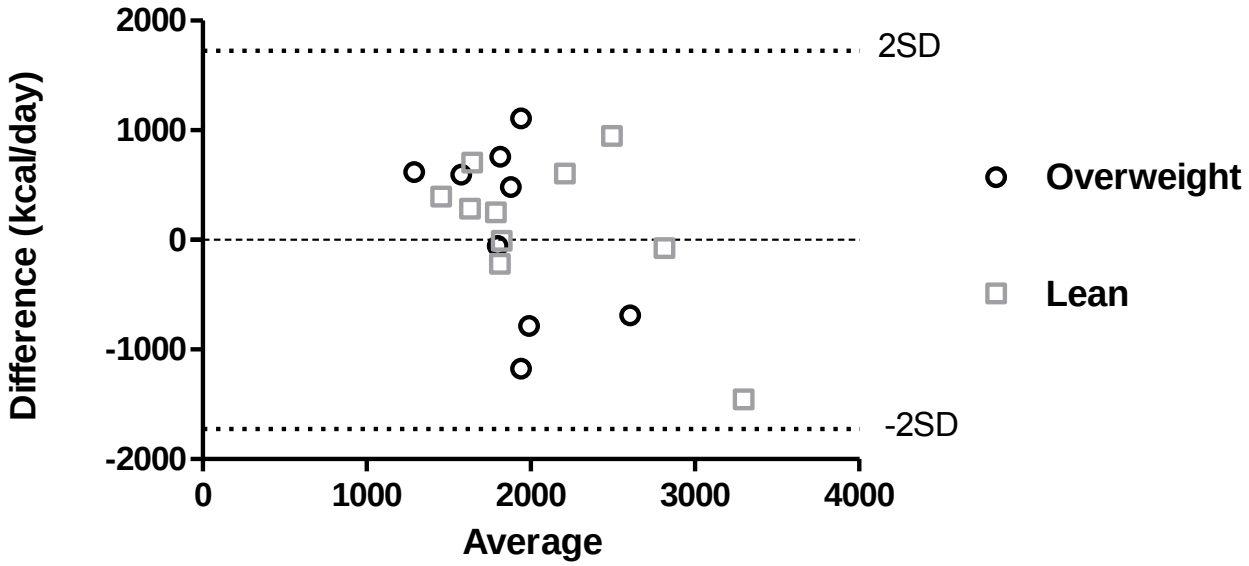


Figure 7. Bland-Altman representation of individual energy intake (kcal.day^{-1}) as estimated from the 3DR compared to the FFQ in Overweight (n=9) and Lean (n=10) groups. *Difference, calculated as the 3DR energy intake minus the FFQ energy intake. Average, calculated as the mean energy intake from the 3DR and FFQ. 2SD, two standard deviations of the mean difference between 3DR and FFQ. Dashed line indicates where there is no difference in energy intake between assessment tools.*

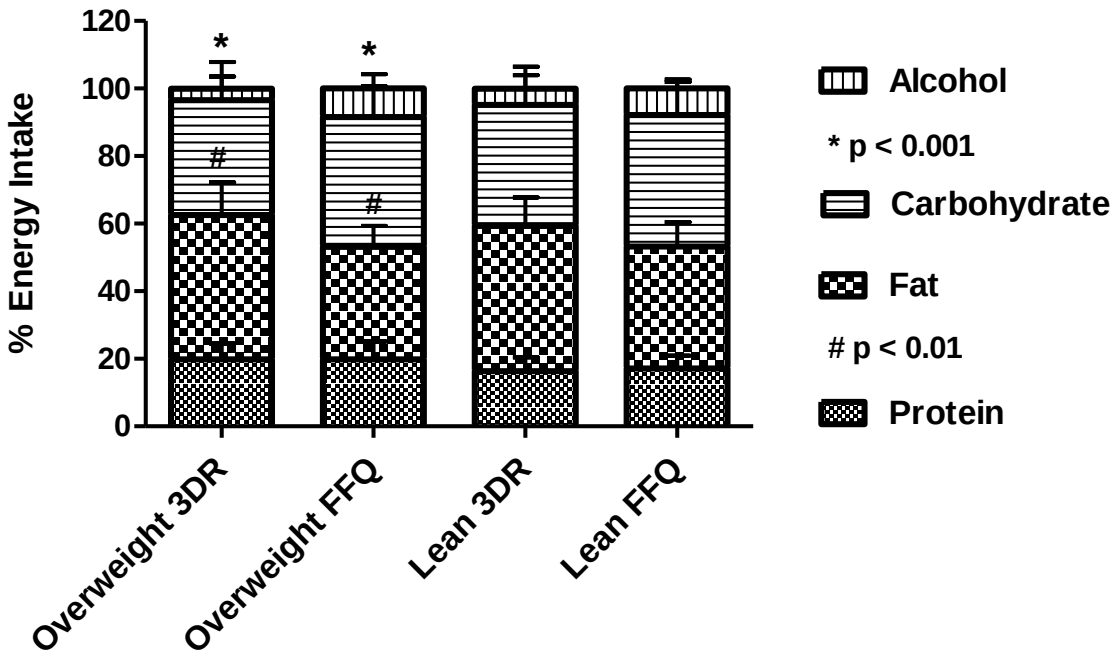


Figure 8. Comparison of the proportional contributions of protein, carbohydrate, fat and alcohol to total energy intake, in Overweight (n=9) and Lean (n=10) groups, according to the 3DR compared to the FFQ. Data are presented as mean ± SD (bar ± whiskers). p values were determined using paired t-tests and significance (α = 0.05/4) was determined by applying a Bonferroni correction factor.

3.9.) Resting Metabolic Rate (RMR)

Mean absolute RMR, as determined by indirect calorimetry, was similar between groups ($p = 0.74$). However, when it was expressed relative to both body mass ($p < 0.0001$) and fat-free-mass (FFM, $p < 0.05$), it was significantly higher in the Lean group. The latter shows that the specific metabolic rate of FFM (the main determinant of RMR) was higher in the Lean group. However, the intra-group variation was considerable (Figure 9). Specifically, half of the Lean group had appreciably higher $RMR.FFM^{-1}$ compared to the Overweight group, whilst the other five Lean participants were comparable to the Overweight group. Neither absolute nor relative RMR were meaningfully correlated with or predictive of BF%.

RMR was used to estimate the total energy expenditure (TEE) of the participants by taking into account each participant's level of activity during the days of diet recall (*Methods section*). TEE effectively represents the participants' daily energy requirements to maintain energy balance. Both Overweight and Lean groups had an estimated TEE of approximately 2750 kcal.day^{-1} (Table 9). This was appreciably higher than their respective energy intakes, which were both approximately 2000 kcal.day^{-1} (paired t-tests, Overweight $p < 0.01$ and Lean $p < 0.05$, Figure 9). Further, the mean proportion of estimated TEE that was provided for by caloric intake was only 72% and 80% in Overweight and Lean groups respectively (Table 9). Assuming that the estimates of energy intake and TEE were accurate (*Discussion section*), this would imply that participants were in caloric deficit during testing. Indeed, two Overweight participants were found to have consumed less than half of their estimated energy requirements (Figure 9), whereas one participant from each group did match their estimated energy intake and expenditure.

Table 9. Parameters pertaining to the resting metabolic rate (RMR) and total energy expenditure (TEE) of Overweight (n=10) and Lean (n=10) groups.

Variable	Overweight	Lean	p value
RMR (kcal.day^{-1})	1452 ± 173 (1192 – 1802)	1427 ± 154 (1232 – 1664)	0.74
RMR (kcal.kg bodyweight^{-1}. day^{-1})	19.5 ± 1.7 (17.2 – 22.2)	24.2 ± 2.0 (21.0 – 27.8)	**< 0.0001**
RMR (kcal.kg FFM^{-1}.day^{-1})	29.5 ± 2.1 (26.5 – 33.3)	31.6 ± 2.3 (28.7 – 35.7)	**< 0.05**
TEE (kcal.day^{-1})	2728 ± 340 (2353 – 3320)	2742 ± 346 (2218 – 3328)	0.93
Energy Intake / TEE	0.72 ± 0.18 (0.48 – 1.05)	0.80 ± 0.20 (0.57 – 1.19)	0.35

Values are mean ± SD (range). *FFM, Fat Free Mass; TEE, Total Energy Expenditure.* All variables were normally distributed and p values were determined using independent t-tests. Bold p values indicate statistically significant differences between groups ($p < 0.05$)

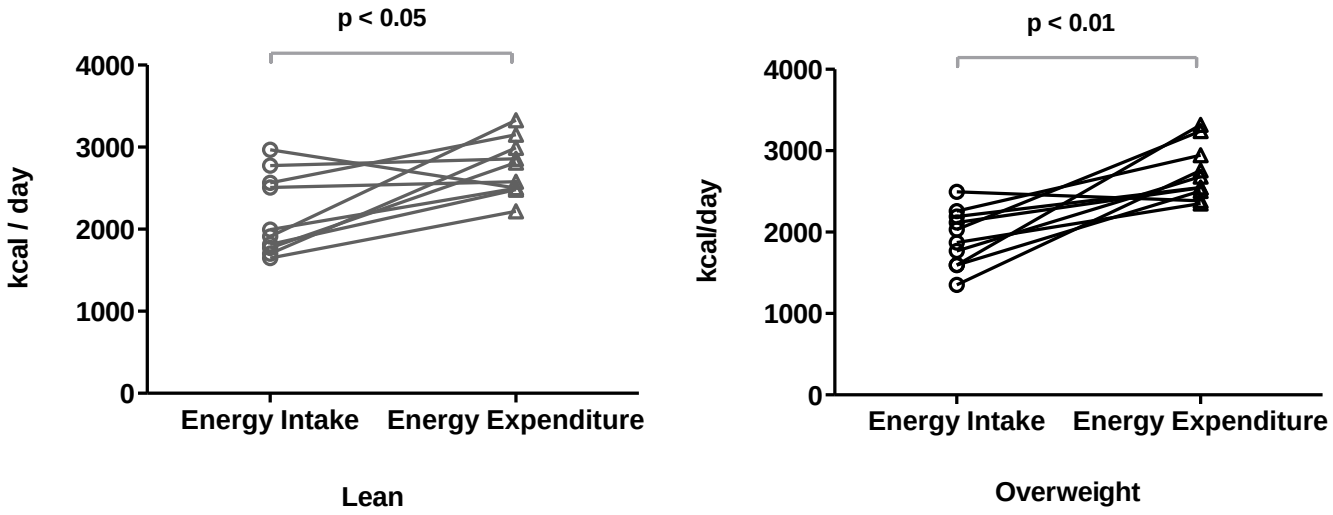


Figure 9. Relationship between mean daily Energy Intake and estimated Total Energy Expenditure (kcal.day^{-1}) in Overweight (n=10) and Lean (n=10) groups. Energy intake was determined from the 3DR, and TEE was estimated using Resting Metabolic Rate from indirect calorimetry and individual PAL factors of 1.8 or 2.0. p values were determined using paired t-tests for within-group differences.

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3.10.) Physical Activity and Sedentary Behaviour

Mean daily GTX3+ accelerometer wear-time (Overweight, 919.5 ± 106.3 vs. Lean, 884.8 ± 76.2 minutes.day^{-1}) was similar between groups (p = 0.45). Figure 10 depicts the proportion of absolute wear-time that Overweight and Lean groups spent in different activity levels as determined by the accelerometers. There were no between-group differences (chi-square test, p = 0.97). Overweight and Lean groups spent the majority of wear-time sedentary (74 ± 6% vs. 72 ± 6% respectively), and comparable proportions of wear-time in light (14 ± 4% vs. 15 ± 5% respectively) and moderate-to-vigorous physical activity (MVPA, 11 ± 3% vs. 13 ± 4% respectively). Overall activity was inferred from the number of steps taken per day (Table 10). On average, both groups exceeded 10 000 steps.day^{-1}. Although the Lean group appeared to average more steps.day^{-1}, there was no difference between groups (p = 0.43). This was owing to the considerable intra-group variation, particularly within the Overweight group (range: 6861 – 18 263 steps.day^{-1}). The findings were similar when steps were

expressed relative to minutes of daily wear time (Table 10). No measures of physical activity correlated with BF% or metabolic parameters.

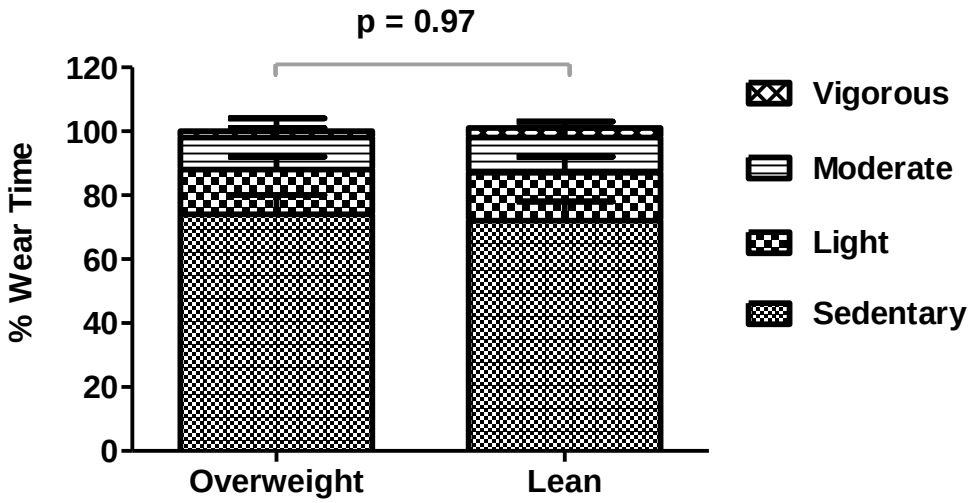

Figure 10. Proportion of wear-time spent in different activity levels by Overweight (n=10) and Lean (n=8) participants, collected using Actigraph GTX3+ accelerometers. Activity levels were determined by Matthews cut-points for counts per minute (cpm) in the vertical axis: sedentary (< 100 cpm), light (100 – 759 cpm), moderate (760 – 5998) and vigorous (≥ 5999 cpm). p value was determined using a chi-square test.

Sedentary behaviour was further analysed in regards to the time that Overweight and Lean groups spent specifically in 'bouts' of sedentary behaviour (≥ 20 consecutive minutes of zero activity). Table 10 suggests that there was a trend approaching statistical significance for Overweight participants to exhibit more sedentary bouts per day ($p = 0.06$) and spend more total time in sedentary bouts ($p = 0.07$). Interestingly, the former also exhibited a significantly positive correlation with BF% (Pearson $r = 0.54$, $p < 0.05$). The average length of a sedentary bout was similar between groups ($p = 0.86$).

Table 10. Overall daily activity in step counts and average sedentary behaviour, as measured using GTX3+ accelerometers in Overweight (n=10) and Lean (n=8) groups.

Variable	Overweight	Lean	p value
Number of SED bouts per day	2.76 ± 1.26 (1.00 - 5.30)	1.71 ± 0.83 (0.60 - 2.80)	0.06
Total time in SED bouts (minutes.day^{-1})	89.2 ± 34.0 (32.9 - 149.5)	60.0 ± 27.4 (18.3 - 89.7)	0.07
Average length of SED bouts (minutes)*	32.4 (7.9)	32.1 (6.5)	0.86
Steps per day	10 742 ± 3552 (6861 – 18 263)	12 073 ± 3273 (7256 – 16 726)	0.43
Steps per minute wear time	11.1 ± 5.3 (3.9 – 21.8)	12.3 ± 3.3 (8.4 – 18.5)	0.58

Values are mean ± SD (range) or median (interquartile range). *SED, sedentary*. Where normally distributed, p values were determined using independent t-tests; where not normally distributed (*), p values were determined using Mann-Whitney-U tests.

Activity patterns were also explored for differences between weekdays (Monday to Friday), and weekend days (Saturday and Sunday). Chi-square analysis showed that the proportion of wear-time spent in different activity levels did not change between weekdays and weekend days (data not shown). When overall activity (steps.day^{-1}) was compared between weekend days and weekdays (Figure 11), only the Overweight group (weekend, 15 907 ± 7606 steps.day^{-1} vs. week, 8114 ± 2579 steps.day^{-1}) was found to take significantly more steps on weekend days relative to their weekdays (paired t-test, $p < 0.01$). In fact, only two Overweight participants exceeded 10 000 steps on an average weekday compared to seven on an average weekend day. Lean participants also tended to be more active on weekends but the difference relative to their weekday activity (15 498 ± 7491 steps.day^{-1} vs. 10 217 ± 3821 steps.day^{-1} respectively) was not statistically significant (paired t-test, $p = 0.15$). Three

of the eight Lean participants averaged more than 10 000 steps on weekdays compared to six on the weekend.

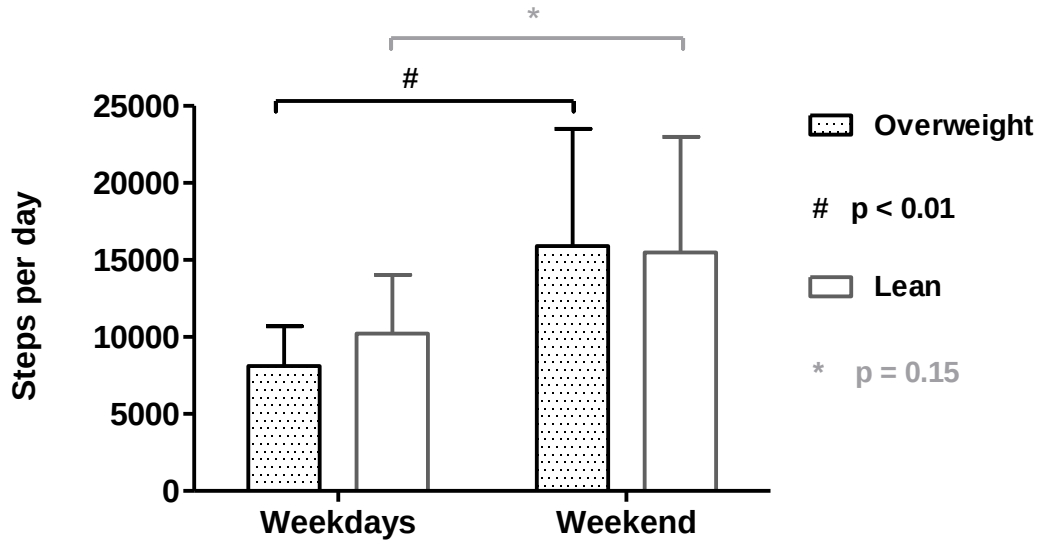

Figure 11. Differences in overall activity (mean steps.day^{-1}) taken on weekdays compared to weekend days, in Overweight (n=10) and Lean (n=8) groups. p values were determined using paired t-tests to compare average weekdays to weekend days within each group.

The hourly activity (counts per minute in the vertical axis) of Overweight (n=10) and Lean (n=8) groups was averaged from valid days in order to construct a profile of activity fluctuations during an average wear day (Figure 12). The profiles were analysed using a repeated measures ANOVA with Bonferroni post hoc tests for possible between-group differences. Both groups exhibited clear changes in activity during the day (time effect, $p < 0.0001$, $F = 8.96$, partial eta squared = 0.36). However, the profiles were similar in shape and there was no group effect ($p = 0.28$, $F = 1.28$, partial eta squared = 0.07) or interaction effect ($p = 0.91$, $F = 0.62$, partial eta squared = 0.04). Both groups tended to be very active in the early morning (06h00 – 08h00) and early evening (16h00 – 18h30), but were relatively inactive during the period between 10h00 – 15h30 and late evening (20h00-24h00) (Figure 12).

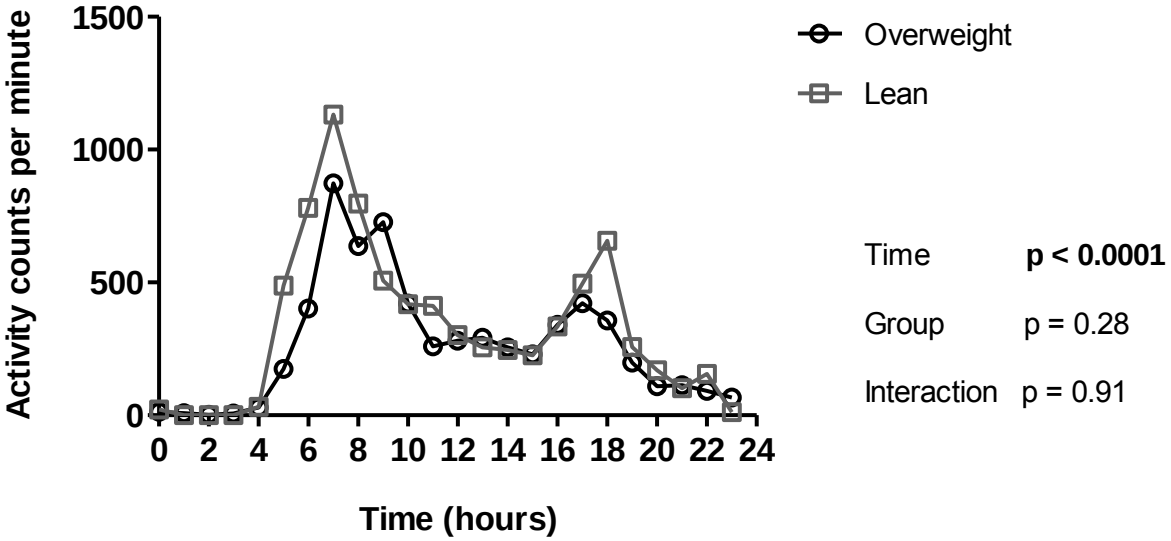

Figure 12. Profile of the average activity fluctuations throughout a typical day in Overweight (n=10) and Lean (n=8) groups. p values were determined using a repeated measures two-way ANOVA.

3.11.) Sleep and Stress

Table 11 shows the average bed time and wake time, time spent asleep, and indices related to sleep efficiency, as determined from 7-day actigraphy. There were no between-group differences in any of the indices assessed ($p > 0.05$). Both groups attained approximately 7 hours of sleep per night, which was on the lower side of adult recommendations[141]. There was, however, wide variation: only three of the nine Overweight and three of the 10 Lean participants averaged at least 7 hours of sleep per night, whereas the remaining 13 participants slept less than 7 hours per night (Figure 13). Despite this, no participants fell below the 'short sleep' threshold (< 6 hours.night^{-1}) that has been linked with ill cardio-metabolic health[149,236]. Furthermore, all except for one Overweight participant, who woke up frequently during the night, were found to have good sleep onset latency (< 20 minutes) and good sleep efficiency (≥ 85%) (Table 11). This would suggest that they fell asleep quickly and spent most of their time in bed asleep. Objective sleep parameters did not exhibit meaningful associations with BF% or metabolic parameters.

Table 11. Average sleep duration and quality in Overweight (n=9) and Lean (n=10) groups, as assessed using 7-day actigraphy.

Variable	Overweight	Lean	p value
Time gone to bed (hh:mm)	22:45 ± 0:39	22:28 ± 0:41	0.36
Wake-Up Time (hh:mm)	06:24 ± 0:31	06:01 ± 0:43	0.21
Time in Bed (hh:mm)	07:39 ± 0:42	07:34 ± 0:35	0.75
Total Sleep (hh:mm)	07:01 ± 0:48	06:52 ± 0:34	0.64
Onset Latency (minutes)	7.3 ± 5.8	5.8 ± 3.5	0.49
Sleep Efficiency (%)	91.55 ± 4.38	90.72 ± 2.79	0.62
Time awake (minutes)	21.3 ± 7.7	25.9 ± 6.9	0.19
Number of awakenings	36.0 ± 6.9	40.3 ± 9.8	0.30

Values are mean ± SD. All parameters were normally distributed and p values were determined using independent t-tests.

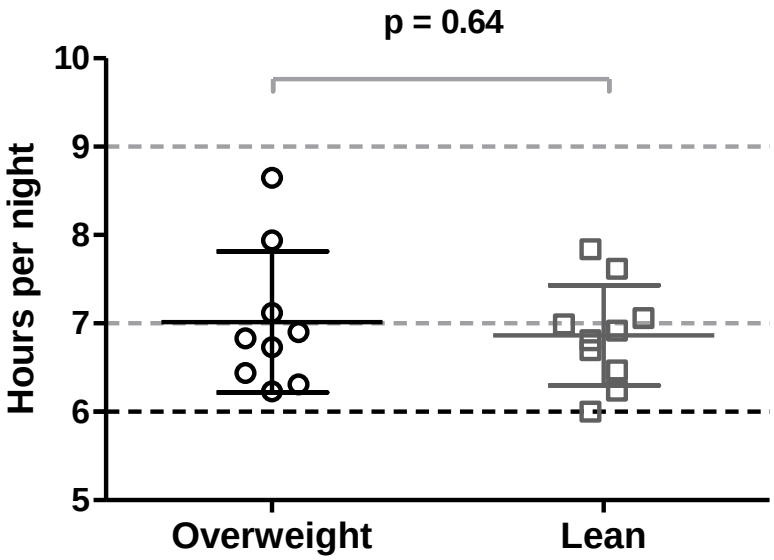

Figure 12. Distribution of average sleep duration (hours per night) during the 7-day Actiwatch recording period. Data are presented as individual 7 day means (markers) and as the group 7-day mean ± SD (lines ± whiskers). Grey lines represent the range for recommended hours of sleep per night for the adult population; the black line represents the threshold for 'short-sleep' associated with ill metabolic health[236].

The Actiwatch results were in slight contrast to the results of the Pittsburgh Sleep Quality Index (PSQI) Questionnaire. Overweight (n=10) and Lean (n=10) groups exhibited average scores of 5.7 ± 3.1 and 5.1 ± 2.6 respectively and there was no between-group difference in median (interquartile range) (5.0 (6.3) vs. 4.5 (3.8), $p = 0.85$). This would suggest that on average both groups perceived that they were 'bad' sleepers (PSQI > 5)[211]. Notably, four of the participants who perceived their sleep the worst, qualitatively reported using medication to help them fall asleep. When compared with the objective measures of sleep duration and sleep quality, PSQI score exhibited no association with objective sleep duration but correlated significantly and negatively with objective sleep efficiency (Pearson $r = -0.63$, $p < 0.01$). PSQI exhibited no association with BF% or metabolic health parameters.

Participants completed the Perceived Stress Questionnaire (PSQ), to evaluate their recent levels of stress. There was no between-group difference in mean PSQ score (Overweight, 61.40 ± 16.67 vs. Lean, 61.90 ± 15.34, p = 0.95), likely owing to the considerable intra-group variation. Of a possible score between 30 and 120, Overweight participants scored between 34 and 89, whilst Lean participants scored between 47 and 87. These scores would be interpreted as 'reduced' to 'average' levels of stress[226]. Figure 14 shows that a higher level of perceived stress was associated with poorer perceived sleep quality. Specifically, there was a strong positive correlation between the PSQ score and the PSQI score of sleep pathology, both when groups were combined (Spearman r = 0.78, p < 0.0001) and within individual groups (Overweight, r = 0.83, p < 0.01 and Lean r = 0.66, p < 0.05). PSQ also had a significantly positive correlation with CRP (inflammation) across both groups (Pearson r = 0.55, p = 0.01).

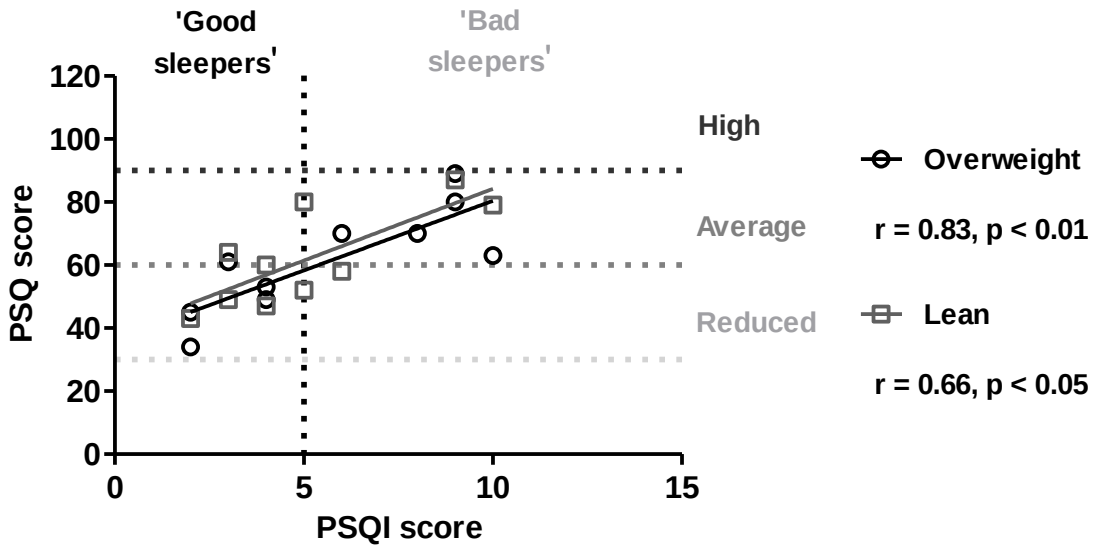

Figure 13. Relationship between individual scores of Overweight (n=10) and Lean (n=10) groups on the Perceived Stress Questionnaire (PSQ) and Pittsburgh Sleep Quality Index (PSQI) Questionnaire. *Dotted vertical line, PSQI score > 5 was used to identify 'bad sleepers'*[211]*. Dotted horizontal lines, used to separate levels of stress according to PSQ score: 30 to 60 represents 'Reduced' stress, 60 to 90 represents 'Average' stress, and 90 to 120 represents 'High' stress*[226]. Correlations were investigated using the Spearman r, and significance was set at p < 0.05.

3.12.) Eating habits and attitudes

Questionnaires were used to explore participants' eating habits and food-related psychology. Figure 15 illustrates the similar mind-sets that were exhibited by Overweight (n=10) and Lean (n=10) groups, as per the Three-Factor Eating Questionnaire (TFEQ). There was no between-group difference (p = 0.68) in median (interquartile range) of total TFEQ score (Overweight, 6.95 (1.52) vs. Lean, 5.95 (2.80)). Group medians were also similar in the three separate domains of the TFEQ: emotional eating (Overweight, 2.2 (1.4) vs. Lean, 2.0 (1.8), p =0.57) uncontrolled eating (Overweight, 2.0 (0.4) vs. Lean, 1.8 (0.8), p=0.70) and cognitive restraint (Overweight, 2.7 (0.4) vs. Lean, 2.6 (0.9), p = 0.70)). Both groups on average exhibited 'cognitive restraint' most strongly of the three domains.

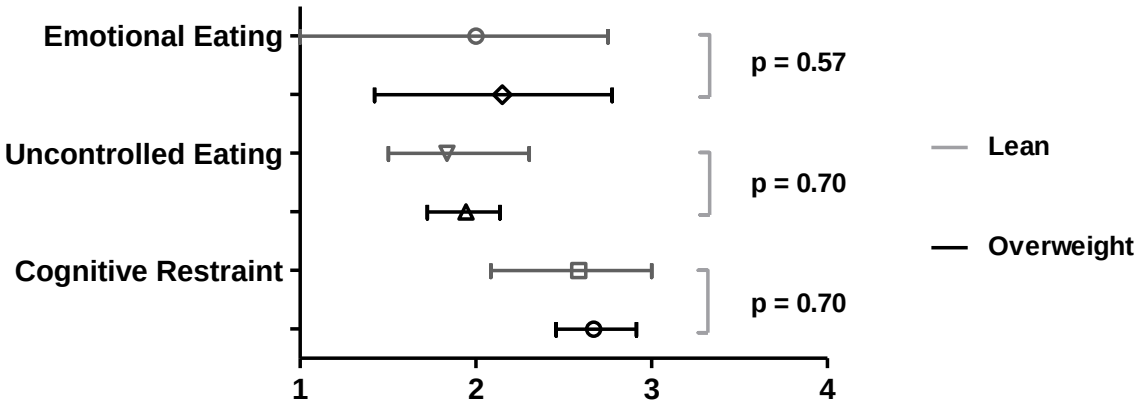

Figure 14. Three Factor Eating Questionnaire (TFEQ) domain scores in Overweight (n=10) and Lean (n=10) groups. Data are presented as median ± interquartile range (marker ± whiskers). Minimum – maximum for each domain is 1 – 4, and higher scores indicate greater propensity for that trait. p values were determined using Mann-Whitney-U tests.

In response to the Gastrointestinal Symptoms Questionnaire (Table 12), Overweight and Lean participants did not report frequent or severe gastrointestinal distress (neither during normal daily activities nor during running). The Overweight group tended to report more gastrointestinal problems during normal daily activities compared to the Lean group (Mann-Whitney-U, p =0.06 for Total Score), however, there was considerable inter-individual variation. Participants typically reported that they had experienced problems

with one gastrointestinal symptom, therefore, the between-group difference was not statistically significant. Further, the symptoms had typically not been experienced recently since most participants reported having foregone the foods that had normally elicited the symptoms. The most frequent gastrointestinal complaint was bloating, which was associated with consumption of wheat-containing foods such as bread, pasta and pizza. These foods were also commonly linked with symptoms of abdominal pain and constipation, while sugar-sweetened beverages seemed to have elicited heartburn in a few participants. Gastrointestinal complaints specific to running were also rare, and when reported, had only been experienced rarely. Sugar-sweetened beverages, such as carbonated and energy drinks, as well as the energy sweets and gels that are typically consumed during running events, were the primary causes of discomfort from bloating and nausea (Table 12).

Table 12. Gastrointestinal complaints reported during normal daily activities and independently during running in Overweight (n=10) and Lean (n=10) groups.

Gastrointestinal Complaints	Possible Score	Overweight	Lean	p value	Associated Foods
During Daily Activities					
Abdominal Pain	5	1.0 (1.25)	1.0 (1.0)	0.86	White bread, pizza
Esophageal symptoms (e.g. heartburn/dysphagia)	10	2.0 (3.0)	2.0 (0.25)	0.46	Sugar-sweetened drinks
Upper dysmotility symptoms (e.g. early satiety, bloating, nausea, vomiting)	25	7.0 (5.3)	6.5 (2.3)	0.44	Pasta, bread, pizza, Beer or cider
Bowel symptoms (Diarrhoea or Constipation symptoms)	30	12.0 (3.8)	11.0 (3.0)	0.17	
Diarrhoea symptoms (≥ 3 Bowel movements / day, loose stools, urgency)	*15*	4.5 (3.0)	4.0 (2.3)	0.61	Coffee, nuts
Constipation symptoms (< 3 bowel movements / week, hard stools, anal blockage)	*15*	3.5 (2.3)	3.0 (1.0)	0.23	Wheat, bread, pasta
Total during daily activities	**70**	**33.0 (7.7)**	**28.5 (5.8)**	0.06	
During Running	**27**	**2.0 (3.5)**	**3.5 (2.2)**	0.32	Energy drinks, sweets, gels, bread Eating too close to a run

Values are expressed as median (interquartile range). p values were determined using Mann-Whitney-U tests.

The Detailed Participant Questionnaire (*Appendix C*) asked participants the extent to which certain food selections and/or omissions applied to them. Figure 16 indicates the percentage of participants that reported to have been making an effort to limit their caloric intake, fat intake and refined carbohydrate intake. There were no between-group differences in any of the three practices (chi-square tests, $p = 0.66$, $p = 1.0$ and $p = 0.47$ respectively). The majority of participants were making an effort to reduce their intake of all three, in particular refined carbohydrates (reportedly all ten Overweight participants and eight of the Lean participants).

Participants were also asked to describe their typical nutritional practices leading up to and on the day of long training runs and races (Figure 16). Of the commonly-adopted practices, only carbohydrate - loading was somewhat recognised and practised by participants: by 50% of both groups (chi-square test, $p = 1.0$). These participants used pasta and sugar-sweetened energy drinks as their primary carbohydrate sources. A couple of participants from both groups also reported consciously increasing their energy intake on the day before a long run or race ('calorie-loading', $p = 0.67$). This typically coincided with carbohydrate-loading since they would consume larger portions of carbohydrate sources such as pasta and potatoes. A few Overweight participants indicated that they had recently started fat-adapting prior to long runs. However, when asked to elaborate, they indicated that they would consume slightly more fat in addition to their normal diet during the day before a long run, and ensure a large portion of protein the night before. Since this was not in line with the scientific notion of 'fat-adapting'[237], these responses were deemed invalid. There were no statistically significant differences between groups.

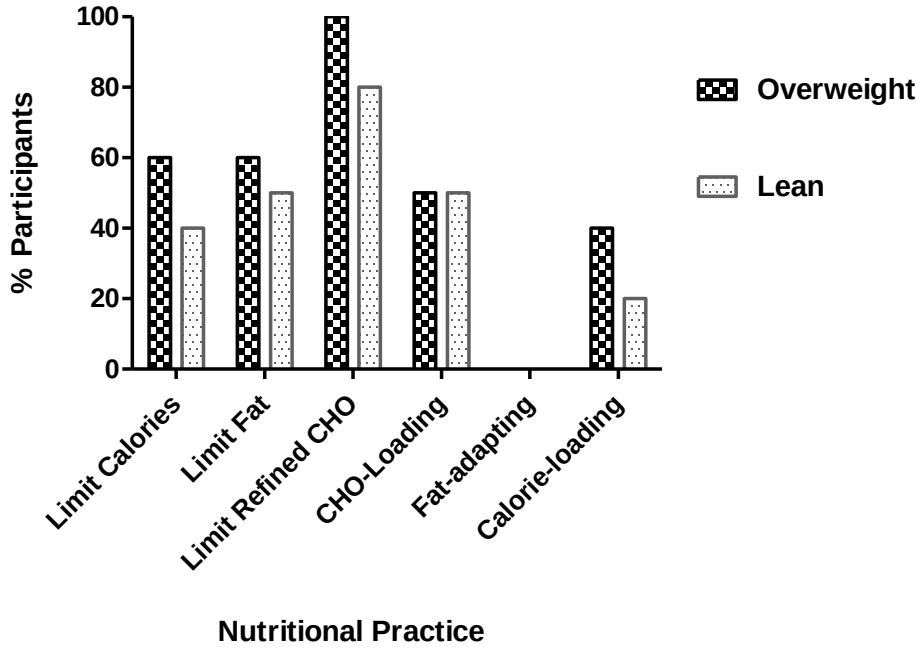

Figure 15. Percentages of participants within Overweight (n=10) and Lean (n=10) groups that reported to follow select nutritional practices, during daily living or specifically leading up to a long run or race. Values are percentages of participants within each group that reported following the respective practice.

There were notable food items reportedly consumed by participants in the period surrounding a long run or race. Table 13 provides a snap-shot of the most common foods participants consumed before, during and after a run, in order of how often they were reported in each group. The majority of items were common amongst Overweight and Lean runners, for example, both groups had pasta and larger portions than normal on the days leading up to a run, both prioritised energy and/or carbonated drinks as well as energy sweets or energy bars during the run, and their most common food consumed post-run was a chocolate milkshake. However, only the Overweight participants indicated that they consumed energy drinks on the days leading up to, and including the morning of the run. Furthermore, whereas most Overweight participants had peanut butter on toast, and an energy drink immediately before a run, Lean participants tended to eat oatmeal or an energy cereal (e.g. Futurelife or ProNutro). Finally, only the Overweight participants indicated that they consumed carbonated and/or recovery drinks post-run. In contrast, the

Lean group did not report any processed foods post-run other than the chocolate milkshake.

Table 13. Foods and drinks commonly consumed by Overweight (n=10) and Lean (n=10) participants during the days leading up to a long training run or race, immediately before, during and after the run.

Overweight				**Lean**			
Days before	**Pre-run**	**During**	**Post-run**	**Days before**	**Pre-run**	**During**	**Post-run**
Pasta	Peanut butter on toast	*Carbonated drinks*	*Steri-stumpie (milkshake)*	*Pasta*	*Oatmeal /* porridge	*Energy sweets (Jelly babies)*	*Steri-stumpie (milkshake)*
Energy drinks (32GI, Energade, Lucozade)	*Banana*	*Jelly babies*	Recovery drink (USN, Energade)	Potatoes	Futurelife ® or ProNutro	*Energy bars (Racefood, Trek, GU, 32GI)*	*Eggs and bacon on toast*
Larger portions	Energy drinks	*Energy drinks or sweets (GU, 32GI)*	Carbonated drinks	*Larger portions*	Coffee and milk	*Carbonated drinks*	Yoghurt with fruit
	Oatmeal	*Sandwiches*	*Eggs and bacon on toast*		*Banana*	*Sandwiches*	
						Banana	

Items in italics were common between groups, and those not in italics were only reported in one group

Finally, the Detailed Participant Questionnaire asked participants to indicate their perception of their individual weight, appearance and dietary habits (Table 14). Participants from the Lean group tended to be more satisfied with their current appearance (Mann-Whitney-U, $p = 0.16$), they reported significantly greater satisfaction with their current weight (Mann-Whitney-U, $p < 0.05$) and the majority of them did not intend to lose weight. In contrast, the Overweight participants wanted to lose an average of 7.8 kg body weight (Mann-Whitney-U, $p < 0.001$). Further, the Lean participants reported having found it easier (Mann-Whitney-U, $p < 0.001$) to maintain their weight (on average 'Easy') compared to the Overweight participants (on average 'Difficult'). This was reflected in the larger weight fluctuations that had been experienced by the Overweight participants during the prior 10 years (independent t-test, $p = 0.01$). Both groups appeared uncertain as to how they perceive their current dietary practices ('Fairly Healthy'), however, the Overweight group was less satisfied with their current diet (Mann-Whitney-U, $p < 0.05$) and tended to have a greater desire to alter their diet (Mann-Whitney-U , $p=0.13$).

Table 14. Self-perceptions of current weight, physical appearance and dietary habits in Overweight (n=10) and Lean (n=10) groups.

Variable	Range	Overweight	Lean	p value
Satisfaction with appearance *	1 = Very dissatisfied 5 = Very satisfied	2.5 (1.5)	4.0 (1.5)	0.16
Satisfaction with weight *		2.0 (0.3)	4.0 (3.0)	**0.02**
Ease of maintaining weight *	1 = Very difficult 5 = No problem	2.0 (0.3)	3.0 (2.0)	**< 0.001**
Weight change during last 10 years kg? (#)		12.9 ± 3.8	8.6 ± 2.9	**0.01**
Target weight loss (kg) *		9.5 (5.2)	1.8 (2.3)	**< 0.001**
Health of current diet *	1 = Very unhealthy 5 = Very healthy	3.0 (0.0)	3.0 (0.3)	0.36
Satisfaction with Diet *	1 = Very dissatisfied 5 = Very satisfied	2.5 (2.0)	4.0 (1.3)	**0.02**
Desire to change diet *	1 = Not at all 5 = Definitely	3.5 (1.3)	3.0 (2.0)	0.13

Values are expressed as median (interquartile range)(*), except (#) which is normally distributed and expressed as mean ± SD. p values were determine using Mann-Whitney-U tests (*), except (#) where an independent t-test was used. Bold p values indicate statistically significant differences between groups ($p < 0.05$).

4.) DISCUSSION

4.1.) Adiposity and metabolic health

This thesis was intended to be a pilot study that would inform a subsequent weight-loss intervention in the emerging 'overweight runner' phenotype. Specifically, it was designed to better characterise the phenotype, assess the feasibility of recruiting overweight runners, estimate the necessary sample size and inform the overall design of the intervention. Our primary aim was to determine whether or not overweight runners would present with metabolic or alternative abnormalities compared to lean runners, and thus whether or not it would be worthwhile to implement a dietary intervention. Secondly, we sought to explore potential factors that may have contributed to their weight-gain, and that could be subsequently investigated in the intervention. The main finding of this study was that Overweight female runners did not present with metabolic abnormalities, despite their elevated adiposity. Two Overweight participants were insulin-resistant, but no participants exhibited the MetS. As far as the author of this thesis is aware, this was the first metabolic profiling of overweight recreational runners. This may be an important step towards understanding the causes of weight-gain in athletic individuals.

Overweight and Lean runners were well matched in terms of age, running experience and running calibre. Participants were recruited based on BMI, waist circumference and skinfold-based estimates of BF%, all of which were expectedly higher in the Overweight group and distinct from the Lean participants. According to DXA, the gold-standard measurement of body composition[238], Overweight runners did have a significantly higher BF%, and this exceeded the threshold of 30% that has been commonly used for identifying excess body fat in the general population[197]. Lean runners had a 'good' average BF%[197]. The latter was considerably lower than that reported (mean 28.4%) in recreational half-marathon female runners by Knechtle *et al*. (2014)[239]. This may suggest that the Lean group was representative of reasonably athletic 'recreational' runners. However, they were not as 'lean' as more competitive runners who have been typically reported to exhibit less than 15% body fat[197,240]. This may have been expected, however, since the study population was over 35 years of age, and was probably less perturbed about other lifestyle factors that influence body fat (e.g. sleep, stress, diet) as professionally athletic individuals.

On an individual level, a lower threshold of 35% body fat has been used for identifying obesity in women[241]. Three Overweight participants were, therefore, 'Obese' and four were 'Overweight' according to these criteria. Surprisingly, DXA revealed that the remaining three Overweight runners had less than 30% body fat. Conversely, four Lean runners unexpectedly exhibited greater than 25% body fat. These findings highlight the limitations of having recruited participants according to BMI, waist circumference and skinfold-based estimates of BF%.

Firstly, it has been well-recognised that BMI provides a limited indication of body composition, given that it is highly correlated with both muscle and fat mass and is unable to differentiate between the proportion and distribution of lean and fat tissue[240,241]. This means that, as observed in the present study, individuals with a low BMI may have had significant fat mass and *vica versa*[214]. Waist circumference may also be limited by providing only a regional estimate of body fatness (abdominal)[242]. Skinfold thicknesses have been reported to lose precision when taken from overweight adults[243], and the predictive equations used to estimate BF% from skinfolds have been reported to have a 3% to 7% standard error[240]. Interestingly, in agreement with their greater waist circumference, DXA revealed that the Overweight group had a greater proportion of fat mass in the 'android' (chest and abdominal) region relative to the 'gynoid' (hips, buttocks and thighs) region. Furthermore, 'athletic' lean women have been reported to accumulate fat predominantly in the lower body with very little in the upper body[240]. In this light, regions of body fat of some Lean, athletic runners may not have been captured by waist circumference or the upper-body skinfolds used to estimate BF% by the Durnin and Wormersley equation[199]. Therefore, although BMI, waist circumference and skinfolds were the most convenient and cost-effective measurements to use during recruitment, they were less accurate than anticipated in predicting whole body fatness.

This was exemplified by the anomaly of the Lean participant with the low BMI (19.7 $kg.m^{-2}$) and waist circumference (67.0 cm) but highest BF% (27.6%). Potential explanations for her elevated adiposity may include her past history of smoking[244], her low caloric intake[260,261], particularly low protein intake (mean 45.8 g/day)[245,246], and high consumption of sugar (mean 132 g/day)[40,75]. Interestingly, her other metabolic parameters were well within

healthy ranges, and she was not 'Metabolically Unhealthy Normal Weight'. This was likely attributable to her history of regular exercise[247]. Ultimately, finding her adiposity to overlap with the Overweight participants meant that the two groups were less separated in terms of body composition than originally desired. As detailed in the *Results* section, however, excluding her from the analysis did not alter the findings of the present study. Regardless, finding significantly higher mean adiposity in the Overweight group and predominant adipose tissue accumulation in the trunk relative to the lower body, would have implicated worse insulin-resistance and greater cardio-metabolic risk compared to the Lean runners[169,248].

In contrast to this train of thought, the Overweight group did not present with clinically meaningful metabolic abnormalities relative to the Lean group. The primary outcome measures of metabolic health were those assessing insulin-sensitivity (or resistance) from both fasting plasma glucose and serum insulin and the responses during the OGTT. It is important at this point to discuss the possible limitations of using surrogate OGTT-derived measures of insulin-sensitivity. Firstly, the Matsuda index, hepatic and skeletal muscle insulin-sensitivity indices were derived in attempt to relate the OGTT (a test of glucose tolerance) to insulin-sensitivity. Whilst they have been validated against the gold-standard measure of insulin-sensitivity (insulin clamp)[232,249], these validations were based on correlation analyses. Indeed, this may show that two variables vary together in a given population, but they fail to indicate the two measures are quantitatively equivalent[250]. This may obscure the relationship between the surrogate measure and consequent metabolic risk. Secondly, although the OGTT mimics the physiological response to glucose ingestion well, the target measure of insulin-sensitivity may be obscured by the influence of the rate of gastric emptying, glucose absorption from the gut, incretin effects, recent diet and hormonal / menstrual status[251,252]. This means that the exact effect of insulin, or sensitivity to its signal, is difficult to deduce from an OGTT. Despite these concerns, however, the OGTT indices used in the present study were most feasible and have been shown to correlate strongly with direct measures and have been comparable to the latter in their relation to cardio-metabolic risk factors[253]. Therefore, although not perfect we believe they provided a valid indication of insulin-sensitivity and possible metabolic risk.

Fasting levels of glucose and insulin, prior to glucose ingestion, may provide an indication of hepatic insulin-sensitivity[249]. Both groups exhibited normal fasting glucose and insulin levels in relation to clinical norms[15,229]. In comparison to prior literature, mean fasting insulin in both groups was below that reported in quartile one (4.9 $mU.L^{-1}$) in a study of prediabetes risk in a predominantly white obese female population[254]. Consistent with these findings, both HOMA-IR and the surrogate of hepatic insulin-resistance were comparable between groups and suggestive of normal insulin-sensitivity[230]. The mean hepatic insulin-resistance index of both groups (Overweight, 16.8 and Lean, 13.3) was similar to that reported (14.1) in a Lean, healthy cohort of males and females aged approximately 40 years[231]. Notably however, one Overweight participant had a fasting insulin result (9.8 $mU.L^{-1}$) that was similar to quartile two of the aforementioned study, which was associated with a 2-fold increased risk of prediabetes[254]. The same participant presented with the highest HOMA-IR (2.25) and hepatic insulin-resistance index (42.0), which may suggest impending prediabetes or impaired glucose tolerance for this individual[230–232].

Based on these findings and prior validation of the hepatic insulin-sensitivity index against direct assessment of hepatic function with the insulin clamp (as described in the *Methods* section)[249], it could be inferred that both groups had normal hepatic insulin function. This may seem surprising given the significant between-group difference in abdominal adipose tissue, which has been regularly linked to hepatic insulin-resistance[255]. However, both fasting insulin and HOMA-IR correlated significantly and positively with BF% across groups. Given the spectrum of BF%, this would suggest that relative hepatic insulin-resistance, although within normal levels, may have contributed to increased adiposity of affected participants. More recently, liver fat accumulation, as opposed to abdominal fat, has been suggested to be a better predictor of insulin-resistance and CVD risk[256,257]. Although not assessed in this study, liver fat may have been healthy in both groups. This would be corroborated by the comparable serum levels of ALT (a marker of liver function) that was found in both groups. Our findings were similar to the mean ALT (15 $IU.L^{-1}$) that was reported in a previous study of 723 middle-aged, lean healthy women[258]. However, mean ALT in both groups were significantly lower than that reported in normal-weight (26 ± 14 $IU.L^{-1}$) and overweight (32 ± 18 $IU.L^{-1}$) women who consumed moderate (less than 40 $g.day^{-1}$) alcohol[259]. Interestingly, two Lean participants had higher than normal ALT levels (> 30

IU.L^{-1}). Given that they had less than 20% body fat and had good hepatic insulin-sensitivity, it seems unlikely that they had pathological liver fat depots. Notably they were also the highest and third highest consumers of alcohol according to the 3DR. It may have been that their regular alcohol consumption, which may have included the night two days prior to the fasting blood draws, contributed to this finding[259]. In regards to the remaining participants, especially in the Overweight group, regular exercise would have conferred a protective effect against the accumulation of liver fat and associated hepatic insulin-resistance[260]. As will be discussed later, this has been documented as a trait of the MHO phenotype[129].

Increased adiposity, particularly in the visceral region, has also been associated with peripheral and whole-body insulin-resistance[26]. Consistent with this notion and previous literature[18,248], we found a significantly positive correlation between total IAUC during the OGTT and BF%, and a negative correlation between the latter and whole-body insulin-sensitivity (by Matsuda) [231,232]. This further supported the hypothesis that insulin-resistance may have contributed to fat gain in the participants with greater adiposity[26]. The group-level OGTT results were relatively surprising. Firstly, the mean glucose response was almost identical between groups, but there was considerable within-group variation. The latter is consistent with previous findings of inter-individual disparity in the 'metabotype' (the characteristic response of one's metabolism to a dynamic challenge, as measured using various physiological markers) and corresponding glucose response during an OGTT[261,262]. Similarly, recent evidence showed that different individuals had highly individual post-prandial glucose responses to identical food items[89]. Within-individual responses to identical oral glucose loads (e.g. OGTT or white bread), have also been reported to vary when retested after a few weeks[263,264]. This phenomenon may be attributed to widespread factors, including physiological stress at the time of testing, recent sleep, exercise and dietary factors[263]. Therefore, the variable glucose and insulin responses in the present study were not unusual, and likely reflected both inter- and intra- individual variability.

Interestingly, one participant from each group exhibited impaired glucose tolerance, but they were otherwise distinct. The Overweight participant was obese by BF% (35.7%), pre-hypertensive (blood pressure of 137 / 83 mmHg) and insulin-resistant by the Matsuda index (4.54). In contrast, the Lean participant had the lowest body fat percentage (16.4%),

and otherwise normal metabolic markers, including a normal Matsuda score of 8.64. The latter had self-reported following a low-carbohydrate dietary pattern, which was corroborated by her 3-day diet (15.1% of her total caloric intake was derived from carbohydrate)[151]. This finding was consistent with multiple studies that have shown low-carbohydrate intake to reduce glucose tolerance in healthy individuals[265–267]. Importantly, it appears that this finding merely reflects an altered metabolic state rather than a pathological state of insulin-resistance[268,269]. Specifically, individuals on a low-carbohydrate diet favour fat oxidation over carbohydrate oxidation and will direct ingested glucose preferentially to glycogen storage[268–270]. Collectively, these may slow the rate of glucose disposal compared to individuals on a mixed macronutrient diet[268–270]. Therefore, it is proposed that the Overweight participant with impaired glucose tolerance was at risk of prediabetes, but the Lean participant had adapted to an alternative metabolic state and was metabolically healthy.

The insulin responses during the OGTT were similarly variable within both groups, in particular the Overweight group. There was, however, a tendency for a higher insulin response and IAUC in the Overweight group. Contrary to the past focus of OGTT interpretation on the plasma glucose response, it appears that the insulin response may be more indicative of the metabolic health of the individual[271,272]. This is because a normal glucose response may be achieved by relative hyperinsulinaemia, which has been implicated to be the primary driver of metabolic pathology[18,20,87]. Having performed approximately 15 000 OGTTs, Dr Joseph Kraft proposed the concept of '*Diabetes in situ*', which incorporated four distinct pathological insulin responses to an OGTT[271,272]. Although we did not measure insulin for the length of time used by Dr Kraft (5 hours), it appears from the individual responses that only one Overweight participant exhibited an insulin profile that resembled *Diabetes in situ*[271,272]. Of the remaining nine insulin responses in the Overweight group, the majority were comparable to the Lean participants, and only two were appreciably higher. Therefore, the tendency for higher insulin in the Overweight group may be attributed to the greater insulin secretion that was required to normalise blood glucose in three of the Overweight participants.

Interestingly, the results of whole-body (Matsuda) and skeletal-muscle insulin-sensitivity indices were not necessarily in agreement with those of hepatic insulin function. Some participants with normal hepatic insulin-sensitivity had reduced peripheral insulin-sensitivity and *vice versa*. This finding was consistent with Reaven (2012), who suggested that different tissues of a given individual may have varying levels of insulin-resistance and that the sequence in which tissues develop pathology may vary between inividuals[18,24]. On average, however, both Matsuda and skeletal-muscle insulin-sensitivity were comparable between groups. This finding contrasted our hypothesis that the increased weight of the Overweight participants would be explained by underlying insulin-resistance[18,20,255]. However, lower Matsuda scores (indicating worse insulin-resistance) did correlate significantly with higher BF%. This discrepancy may have resulted from the unexpected spectrum of BF% we obtained across Overweight and Lean groups. Taken together, the results appear to support the notion that increasing insulin-resistance and hyperinsulinaemia promote fat gain[18], but that insulin-resistance *per se* is unable to explain the past weight-gain of all Overweight participants. Indeed, Matsuda indicated that only two Overweight participants were insulin-resistant and of them also had the highest HOMA-IR. Overall, we found that the majority of Overweight participants were not insulin-resistant, however, and this likely contributed to their largely normal metabolic outcomes.

Overweight and obesity typically present with a cluster of related metabolic disturbances that together increase risk for T2D and CVD[12,14]. However, the present study measured multiple cardio-metabolic markers and found that they were both comparable between the Overweight and Lean groups, and well within healthy ranges. This included HbA1c, as a measure of glucose control, uric acid, ALT as mentioned above, as well as serum HDL-C, TG and FFA concentrations. Median systolic blood pressure and CRP were both significantly higher in the Overweight group. However, the former was comparable between most Overweight participants and the Lean group, and mean blood pressure was within the normal range. CRP was also within the clinically normal range for all participants[235]. Similarly, both total- and LDL-cholesterol concentrations were higher in the Overweight group. However, mean LDL-C was normal, and recent evidence and scientific opinion indicate that these parameters are not reliable predictors of cardio-metabolic risk[58,87,273]. In contrast to popular thought, low total cholesterol has in fact been associated with increased mortality[274].

Rather atherogenic risk has been strongly linked to high TG and low HDL-C concentrations (and a consequently low TG / HDL-C ratio)[273]. In addition, the specific distribution of LDL particles rather than LDL-C concentration has become more accepted for identifying atherogenic risk; with smaller dense particles having been considered pro-atherogenic[12,24,275]. All participants had normal TG and HDL-C concentrations and normal TG / HDL-C ratios, and there was no between-group difference in LDL particle size distribution. In fact, all participants had a vast predominance of large buoyant LDL particles, which has been considered to be a healthy LDL profile[276]. Taken together, our results indicate that the groups were largely comparable in terms of the metabolic parameters that have been closely linked to overweight and obesity, particularly glucose control and lipid profile. Again this was consistent with our finding of similar and normal insulin-sensitivity in Overweight and Lean groups[12,20].

Despite the mean metabolic profile of the Overweight group being normal, it seems worth discussing some between-group differences that did exist with regards to potential pathology in this group. Firstly, despite having a 'normal' lipid profile, HDL-C concentrations tended to be lower in the Overweight group. HDL-C also tended to contribute proportionally less to total cholesterol, as compared to the Lean group. HDL-C has been described as the 'good' cholesterol and high levels have been associated with reduced atherogenic risk[32]. Therefore, one may speculate that reduced HDL-C in the Overweight group, particularly in participants with higher BF%, was suggestive of early dyslipidaemia, but the actual HDL-C levels were well within the normal range. Furthermore, HDL particles are heterogeneous, in that they exhibit varying structures, apolipoprotein compositions and concentrations of esterified cholesterol [275]. Whilst large HDL particles appear to be potently anti-atherogenic, smaller dense HDL particles may be pro-atherogenic[275]. The present study found a predominance of large and intermediate HDL particles in both groups, with minor contributions from small HDL. This would suggest that the HDL-C profiles were healthy in both groups, and this was corroborated by the impressively low TG levels in all participants[276].

In contrast, IDL-C was found to contribute proportionally more to total cholesterol in the Overweight group, as compared to the Lean group. There is limited research on IDL-C, but

it appears that IDL particles are primarily produced in the vasculature as remnant particles from partial lipolysation of VLDL particles and postprandial chylomicrons[32]. In the context of dyslipidaemia, where VLDL-TG production increases and both VLDL and chylomicron clearance are reduced, the presence of IDL particles increases[32]. Earlier investigations associated IDL particle mass with atherogenic progression[32]. More recently, in a cohort of Japanese men, IDL was suggested to be a useful clinical marker of atherogenic risk in the context of increased non-HDL-C (comprised of LDL-C and TG-rich lipoprotein-cholesterol, including VLDL, IDL and chylomicrons) [277]. The purported risk relates to the relative small size of IDL (similar to VLDL) that enables penetration of and retention by the arterial wall[277]. However, the latter finding was in the context of reduced HDL-C and high TG, both of which were absent in the present study[32]. Further, Maki *et al.* (2012) reported atherogenic progression to be associated with increased small dense LDL particles, reduced HDL-C, and increased TG-rich lipoprotein-cholesterol, with the latter having been explained by increased VLDL rather than IDL particles[278]. Therefore, given such uncertainty concerning IDL-C in atherogenic progression, and the otherwise healthy lipid profiles of the Overweight participants, their increased proportion of IDL-C to total cholesterol was unlikely to be of clinical concern.

As alluded to earlier, the Overweight group had higher levels of inflammation and higher systolic, but not diastolic, blood pressure. Although not a criterion for MetS, elevated inflammation has been recognised as predisposing to increased risk of hypertension, MetS and CVD[16] . Both increased adiposity and insulin-resistance, independently, have been associated with a low-grade pro-inflammatory state[279]. In the present study, the increased CRP of the Overweight group may have been due to their greater fat mass, since insulin-resistance was not identified. Indeed, CRP correlated significantly with increasing BF%. The fact that CRP remained within the acceptable range, however, indicated that participants did not have a pathological inflammatory state. Median systolic blood pressure was also slightly higher in the Overweight group but within the normal range. This finding was consistent with the reportedly close link between inflammation and endothelial function[23]. Similarly, finding neither insulin-resistance nor hypertension, was consistent with the close link between insulin-resistance and endothelial dysfunction[14,18]. This was further exhibited by the two Overweight participants who were found to be both insulin-resistant and pre-hypertensive, and the significant degree of variation in blood pressure that was accounted

for by IAUC during the OGTT. Insulin-resistance was, therefore, the most plausible cause of weight-gain and pre-hypertension in these two participants. Although they did not satisfy the MetS criteria at the time of testing, they are likely to be at increased risk of developing MetS in the near future[179,180] .

Overall, the present findings would suggest that the Overweight runners we investigated were comparable to the subgroup of obese individuals that have been referred to as Metabolically Healthy Obese (MHO)[102]. Although none of the Overweight runners were clinically obese by BMI (≥ 30 $kg.m^{-2}$), and only three were obese by BF%, they clearly had increased fat mass relative to the Lean group. However, on average, they did not present with any of the metabolic abnormalities commonly associated with this discrepancy[12,170]. Similar to previous findings in MHO persons[170,174,175], the Overweight runners had normal glucose control, lipid and hepatic enzyme profiles, inflammation and apart from two runners, normal insulin-sensitivity. This may have been related to a favourable adipose tissue distribution in the Overweight runners. Specifically, MHO persons have typically presented with lower visceral fat (particularly intra-abdominal adipose tissue and hepatic fat) compared to unhealthy persons of similar adiposity[129,169,174]. However, these were not measured in the present study.

The most plausible explanation for the generally good metabolic health of the Overweight runners is their history of regular exercise and consequent cardiorespiratory fitness[127,129,174,175]. Overweight and obese persons who have been found to be metabolically healthy reportedly spent significantly more time physically active and less time sedentary compared to metabolically unhealthy obese counterparts[128,174,175]. Greater cardiorespiratory fitness, in fact, may have explained why MHO persons were at no greater disease risk than metabolically-healthy, normal-weight individuals[127]. It is well-known that regular exercise and maintaining a good fitness level confers a multitude of cardio-metabolic health benefits independent of body fatness[110,112]. This includes improved insulin-sensitivity across multiple tissues, reduced blood pressure, beneficial lipid responses, and reduced metabolic disease risk[126,128,280]. The underlying mechanisms appear to be widespread, but likely include increased lean muscle mass, improved muscle insulin-signalling, oxidative and glycogen storage capacity[124,126,128], enhanced endothelial

function[125,281] as well as a systemic reduction in oxidative stress and inflammation[128,282]. In particular, an acute exercise bout and exercise training have been shown to have potent insulin-sensitising effects[283]. For example, Heath *et al.* (1983) showed that 10 days without exercise in trained, lean individuals caused a 100% increase in the insulin response during a 100 g OGTT[283]. Therefore, despite the increased body weight and fatness of the Overweight participants, their consistent exercise routine, which at the time of testing qualitatively involved running, cycling, swimming, weight training, squash and hockey, would have probably been profoundly protective against the development of pathogenic insulin-resistance[126–128,280].

The characteristics of our active, yet Overweight participants were inconsistent with conventional thought that exercise and increased energy expenditure promote weight-loss[105]. On the other hand, exercise intervention trials with *ad libitum* food have rarely reported meaningful weight loss[113,284]. Excess dietary intake, in these instances, probably offset the potential weight-loss effects of exercise. Furthermore, recent scientific opinion has expressed that physical activity, despite its numerous health benefits, plays a limited role in weight-loss[41,56,117]. This has been supported by evidence that energy balance and body weight may be centrally regulated by hormonal hunger-satiety mechanisms that act at the hypothalamus, including gut-brain signals (such as ghrelin) and adipose tissue-brain signals (such as leptin)[37,41,117]. In this regard, increased physical activity (energy expenditure) would promote compensatory increases in caloric intake to maintain energy balance[117]. Factors that disrupt such regulation (such as highly palatable high-sugar, high-fat foods), however, may have promote excess caloric intake and consequent weight-gain in spite of regular exercise and normal metabolic health in our Overweight participants[35,37,41].

Therefore, different mechanisms may have contributed to the weight-gain experienced by different Overweight participants. For the two insulin-resistant (and pre-hypertensive) participants, it is possible that the insulin-sensitising benefits of exercise had become overwhelmed both by considerable predisposition to insulin-resistance[56,190], and aggravating external factors (such as diet) that were not captured at the time of testing[188]. For example, in the context of their underlying insulin-resistance, the finding that both

individuals consumed approximately 50 grams of added sucrose per day may have been more detrimental, relative to participants who were more insulin-sensitive[285,286]. Recent evidence would suggest that a lower-carbohydrate dietary approach may be highly beneficial in negating the effects of their underlying predisposition[87,270,287]. Finding significant correlations between indices of insulin-resistance or hyperinsulinaemia with increased adiposity, would suggest that relative insulin-resistance had contributed to fat gain in affected individuals (Lean and Overweight). However, it could not explain the prior weight-gain of all Overweight participants. It may be speculated that certain genetic and environmental factors predisposed these participants to disrupted appetite regulation (i.e. leptin-resistance)[33,35]. This may have caused them to consume more calories than they had been expending during exercise and gain weight[41,188,190]. Diet-induced leptin-resistance in particular appears to result primarily from the highly palatable, processed foods that have become common-place in the modern Western diet[37]. As shall be discussed, however, the results of the present study were unable to identify such factors.

4.2.) Potential causes of weight-gain in the Overweight group

4.2.1.) Genetic influence

It is well-established that obesity and associated metabolic diseases have considerable heritable components[42,50]. Although we did not explore specific obesity-associated genetic loci or SNPs, we obtained qualitative reports of familial disease history. There was a tendency for a greater proportion of the Overweight group to have had parents or siblings with obesity or T2D. These were consistent with the elevated adiposity and tendency for elevated IAUC during the OGTT in the Overweight group. This was consistent with the cited literature that reported genetic influence accounting for approximately 40% of the obesity phenotype, and 46 to 90% of the insulin-resistant phenotype[40]. Therefore, one may speculate that the six Overweight participants with a positive family history of obesity were predisposed to weight-gain, increased adiposity and insulin-resistance. It would be intriguing to investigate which genetic loci may influence body-weight regulation in this overweight athlete phenotype.

4.2.2.) Resting Metabolic Rate

RMR represents the metabolic cost of supporting normal physiological processes in the resting, post-absorptive body[288]. This includes maintaining the transmembrane ion gradients, cardio-respiratory activity and recovery processes, and RMR accounts for a significant proportion (approximately 60% to 75%) of total daily energy expenditure[49,288,289]. Therefore, factors that alter RMR (including age, sex, genetics, body composition, temperature, energy balance, hormonal and emotional states) would influence daily energy balance, and individuals with a low RMR may be predisposed to weight-gain[288,290]. In the present study, the mean absolute RMR ($kcal.day^{-1}$) of both groups was comparable to prior findings in both young active females[289] and healthy active premenopausal women aged 35 to 50 years[291]. However, in contrast to the literature[290,292], the RMR of the Overweight group was remarkably comparable to that of the Lean group. This was surprising since Overweight individuals generally present with higher absolute RMR, owing to greater cardio-respiratory work and a larger mass of adipose tissue[289,290]. The latter has been suggested to raise energy expenditure via an increased presence of leptin[292], however this could not be surmised from the results of this study. However, it seems to make more sense when one considers that the two groups were similar in terms of the primary active tissue, fat-free-mass (FFM).

FFM is the primary metabolically active component of the body and has been reported to explain between 53% and 88% of the variance in RMR[292]. Adipose tissue only contributes 15% to 20% of the energy expenditure that is contributed by an equivalent amount of FFM[292]. Normalising for total bodyweight , therefore, would erroneously suggest that overweight people have a lower RMR compared to leaner individuals as a consequence of this higher proportion of adipose tissue[289,292]. Therefore, consistent with the literature, RMR in the present study was normalised for FFM[292]. The Lean group presented with a mean relative RMR ($kcal.kg\ FFM^{-1}.day^{1}$) that was comparable to Lean athletic females in prior studies[288,291]. However, the Overweight group had a significantly lower relative RMR compared to the Lean group. This may be interpreted in a variety of ways.

Firstly, this finding may represent a physiological response to long-term caloric deficit[289,293,294]. Overweight participants had clearly struggled with weight fluctuations and they perceived themselves to be restrictive of food intake. Furthermore, as will be discussed later, their self-reported food intake suggested a daily caloric deficit. Although the literature is conflicting in this regard, studies have suggested that energy restriction and consequent weight-loss cause an adaptive down-regulation of RMR and activity energy expenditure, that is considerably greater than that predicted by actual changes in weight and body composition[289,293]. Further, the adaptation has been found to persist long after the period of dynamic weight-loss[293]. This has been used as evidence of an adaptive compensatory response to return to the body's original weight[289,293]. Hence, an adaptive reduction in RMR has been proposed to underlie the tendency for weight-reduced persons to regain weight[289,293]. In support thereof, enhanced 'energy efficiency', characterised by reduced relative RMR, has been reported in male tri-athletes who maintained stable weights despite daily caloric deficits (self-reported and confirmed in a controlled metabolic chamber)[291,295].

However, other studies have suggested that this altered metabolic state was a function of energy-restriction rather than weight-reduction[294,296]. Specifically, caloric restriction was accompanied by concurrent reductions in thyroid hormone and leptin levels as well as RMR, which returned to initial levels after restoration of energy balance[294,296]. Caloric deprivation with reduced adiposity has been found to increase hunger and reduce energy expenditure, apparently to conserve energy and promote weight-regain[296]. When leptin has been administered in this weight-reduced state, however, RMR and thyroid hormones were maintained at pre-weight-loss levels, and this prevented weight-regain[296]. These findings would suggest that measuring RMR in individuals who are not in energy balance, as may have been the case for a number of the participants in the present study, could have provided a misleading impression that they were 'hypometabolic' and prone to weight regain[294,296]. Unfortunately neither leptin nor thyroid hormones were measured in this study, and as will be discussed later, the accuracy of the self-report diet intake was uncertain. Furthermore, the Lean group similarly reported a caloric deficit, and relative RMR did not exhibit any meaningful relationship with BF%. This evidence would oppose the existence of an adaptive down-regulation of RMR. Regardless, it would be speculative to assume either of the aforementioned scenarios applied in the present study.

It is also possible that the lower relative RMR found in the Overweight group was an artefact of potential sources of error in the RMR measurements. Firstly, periodic ethanol calibration burns suggested that the Quark RMR equipment was operating properly and reproducibly, since the calculated error (coefficient of variation) of total CO_2 emitted and RER were consistently below 3% throughout the testing period (*Appendix I*). Despite this, the RER values were admittedly low (< 0.70) during a few of the RMR measurements. Under typical metabolic conditions with stable respiration, RER typically exists between 0.70 and 1.00[297]. These values have conventionally been used to indicate predominant fat oxidation and carbohydrate oxidation respectively[297]. Values below 0.70 may be unusual and have been reported to result from inaccurate gas analysers[298], but have also been found after prolonged fasting[298], and in individuals consuming a VCLHF ketogenic diet[92]. Therefore, finding such values in the present study, although not physiologically impossible, were surprising given that none of the participants were following a ketogenic diet, and they had not fasted for more than 14 hours. Despite the positive calibration results, therefore, this raised concern about the precision of the RMR results themselves.

Independently, measuring RMR in itself is prone to measurement error[288,291]. Despite the good reproducibility from the calibration burns[288], reliability measurements were not performed on actual participants in this study. Therefore, one may speculate that the small between-group difference we identified was smaller than the actual degree of error associated with the measurement[288]. Factors pertaining to the individual participants may have also altered the RMR results, including recent physical activity, energy balance, menstrual status, sleeping arrangements and food intake[288]. In the present study, we did preclude exercise for 36h prior to the RMR measurement , since oxygen consumption may be elevated for up to 24 hours after an exercise bout[288]. However, we did not account for the menstrual status of the participants. RMR is typically lower during the late follicular phase compared to the late luteal phase[288,291]. In hindsight our measurements would have been more accurate had we standardised measurement days to a specific period of the menstrual cycle[288,291]. Furthermore, RMR measurements may have been affected by varying quality of sleep on the night prior to testing, and the possible sense of anxiety on the morning of testing. Prior studies have found RMR to be lower when assessed during a third testing session compared to two earlier habituation trials. Although participants in the

present study had visited the laboratory at least once before the RMR measurement, it is possible that anxiety artificially elevated some RMR outcomes[288]. A final source of error may have been the energy balance of the participants during testing. Particularly in habitually active persons, prior studies have reported a high probability of participants consuming excess calories on the day of relative inactivity that precedes visiting the laboratory[288]. Similarly, knowledge of foregoing breakfast the following day may have altered the food intake of some participants by encouraging them to over-eat the night before testing. This may have had variable effects on different individual RMRs, but would typically inflate RMR above actual values[288]. Therefore, multiple factors may have influenced the RMR measurements and to a more or lesser degree for different participants. Taking these concerns into account, it seems reasonable to merely state the between-group difference that we observed, but make no assumptions about its physiological implications.

4.2.3.) Dietary Intake, Eating Habits and attitudes

Dietary intake as analysed from the 3DR was comparable between Overweight and Lean groups, both in terms of absolute caloric value and macronutrient composition. Interestingly, both groups attained approximately 43% of their calories as fat and only 33% to 36% of calories as carbohydrate. This finding was surprising given that conventional nutrition advice, globally[299,300] and in South Africa[301,302], has advocated a diet higher in carbohydrates (approximately 50% of caloric intake) and lower in total fat (approximately 20% to 35%, or 30% in South Africa) for optimum cardiovascular health and weight maintenance. Reported intakes in both rural and urban South African populations have previously been closer to these guidelines (50% to 65% carbohydrate and 20% to 30% fat)[301,302]. High-carbohydrate intake has been more actively emphasised in the endurance sport context, in light of the general consensus that saturating muscle glycogen stores prior to exercise and maintaining high carbohydrate availability during exercise, are crucial to performance[193,194].

There has been limited research on the dietary practices of recreational runners. However, Butterworth *et al*. (1994) conducted a 3-day food record in a sample of runners who

participated in the Los Angeles Marathon of 1987[303]. They found that of daily caloric intake, 52% was derived from carbohydrate and 31% from fat[303]. Similarly, Nogueira *et al.* (2005) reviewed seven studies that had investigated nutritional strategies in endurance athletes, and concluded that the female athletes had obtained 51% to 60% of their energy intake from carbohydrate and 26% to 33% from fat[304]. Mahoney *et al*. (2016) recently reported that a sample of ultra-marathon runners habitually consumed a diet that was slightly lower in carbohydrate (45% of daily energy) and higher in fat (36% of energy)[305] compared to the earlier studies. Interestingly, Nogueira *et al*. had already stated that the relative carbohydrate intake (4.4 to 7.2 $g.kg^{-1}.day^{-1}$ in females) they found was generally lower than recommendations[304], while Mahoney *et al*. alluded to a high proportion of runners having self-selected a 'low-carbohydrate' diet[305]. The carbohydrate intakes found in the present study, therefore, were not unique in being lower than convention. However, they were both lower than expected, and lower than the cited studies, with a respective intake of approximately 2.2 $g.kg^{-1}.day^{-1}$ and 3.4 $g.kg^{-1}.day^{-1}$ in the Overweight and Lean groups. Conversely, the contribution of fat to energy intake was higher than expected and higher than previously reported[303–305]. Such findings did not support our hypothesis that weight-gain in the Overweight group had resulted from excess carbohydrate intake in the context of insulin-resistance[87,286]. Perhaps, in the context of the relative insulin-sensitivity that we found, their moderate-carbohydrate, moderate-fat ('mixed') diet contributed to their weight gain. Alternatively, similar to Mahoney and colleagues[305], in light of recent media attention that has espoused the benefits of low-carbohydrate diets for health, weight-loss and potentially endurance performance[56,190,305], our results may reflect a recently modified dietary approach. Unfortunately it was not possible to accurately assess their dietary intake during weight-gain, which makes any interpretation speculative.

The three dietary assessment tools used in the present study also showed no evidence of increased caloric intake in the Overweight group compared to the Lean group. Both groups exhibited a surprisingly low caloric intake (approximately 2000 $kcal.day^{-1}$), particularly considering their high level of physical activity. Although the estimated TEE (*Methods section*) would have had a degree of error from both the determination of RMR and the assigned PAL factors, our results suggested that all but two of the twenty participants were in caloric deficit. This may have multiple explanations: the runners may have been actually under-eating[306], they may have (systematically) under-reported their food intake[306–308], or it

may reflect the inherent error associated with dietary assessment tools[206,309,310]. Firstly, considerable evidence has implicated genuine under-eating in the female endurance community relative to respective energy requirements[307,311]. This may relate to both the performance benefits of reduced body weight and the desirability to adhere to the almost expected 'lean' physique of a female runner[306,311]. Recent years have in fact seen an increase in the prevalence of the Female Athlete Triad[279]. This refers to a cluster of health complications, primarily low bone mineral density and menstrual dysfunction, from perpetual under-eating and consequent insufficient energy availability for normal bodily functions and exercise training[312,313]. It has been shown to affect both professional female athletes and recreational athletes alike[312]. It is possible, therefore, that participants, in particular the single Lean participant who presented with lower than age-predicted bone mineral density, were under-eating. This would be consistent with the runners' self-reported restrained eating habits, and consequent attempt to limit caloric intake, fat and refined carbohydrate consumption. However, an accurate determination of energy intake and energy balance would have required a controlled environment and assessments with doubly labelled water.

Self-reported food intake in scientific studies has been consistently, and often considerably, lower than both energy expenditure, and actual energy intake measured using doubly labelled water[306,308,310]. A review by Trabulsi *et al*. (2001), for example, found that studies using 3-day food records had an under-reporting bias of between 10% and 32%. The results of the present study were comparable to the upper end of this range. Certain physiological and psychological traits have been commonly associated with greater propensity to under-report food intake, including increased adiposity[306], the female gender[306], athletic pursuits (especially female endurance athletes)[307], greater eating restraint[306], as well as social desirability and body dissatisfaction[314]. Interestingly, all such traits were present in this study population, particularly the Overweight group. An intriguing precedent in this regard was set by Edwards *et al*. (1993) who assessed dietary intake and energy balance in nine trained female endurance runners[315]. Mean daily energy expenditure (approximately 2990 kcal) and energy intake (approximately 2037 kcal) and the consequent caloric imbalance (32%), were remarkably similar to the findings of the present study. Edwards and colleagues also found lower self-reported energy intake in runners with higher body weight and a negative body image[315]. This was in agreement with the Overweight participants

having reported low satisfaction with their appearance and body weight. One may speculate that such feelings would have in fact been more pronounced in the Overweight runners compared to the lean runners assessed by Edwards' group, and may have increased their tendency to under-report.

Estimating habitual dietary intake accurately was predicted to be difficult since this is a ubiquitous problem in dietary research[206]. The analytical approach of the present study was consistent with the notion that combining multiple assessment tools (3DR, 24HR and FFQ) would improve accuracy[206]. On average, the three tools all indicated that the two groups consumed comparable diets, both in terms of energy intake and proportional macronutrient composition. Although the latter was relatively consistent across tools, total caloric intake varied considerably. For example, the FFQ tended to under-report significantly at lower energy intake, yet over-report at higher intakes, and the agreement between the 3DR and FFQ worsened with increasing energy intake. These findings were in agreement with both reports of significant (20% to 30%) day-to-day variation in individual caloric intake[307], as well as reports of similar systematic bias with increasing energy intake from the FFQ[316]. The latter would suggest that the FFQ functions better at estimating energy intake at a group level rather than on an individual basis[316]. In regards to the macronutrient composition according to the 3DR and FFQ, interestingly, the Overweight group appeared to have consumed a greater proportion of energy from fat and less from alcohol during the 3DR, whereas the Lean group only exhibited a tendency in this regard. Proportional carbohydrate consumption in the Overweight group, in contrast, tended to be lower during the 3DR compared to the FFQ. One may speculate that participants, particularly from the Overweight group, had been influenced by the increasing attention drawn to low-carbohydrate diets, and may have recently attempted to increase their fat intake and reduce their carbohydrate intake. Furthermore, the reduced alcohol consumption may imply a recent attempt by Overweight participants to adopt healthier nutritional practices. However, such interpretations should be made with caution, since our findings may have also been an artefact of the error associated with the respective assessment tools[206].

The 3DR and FFQ have a number of inherent differences and associated limitations. The 3DR should not be affected by recall bias but it may lend itself to under-reporting and deliberate changes to dietary choices[206,309]. This may result because of the burden associated with recording food intake, the anticipation of one's diet being analysed and the consequent desire to be perceived as healthier than normal, or the social stigma attached to over-eating [206,309,317]. Although assessed over multiple days, the 3DR may also be biased by a limited perspective of current as opposed to habitual intake, particularly given the typical degree of within-individual variation from day-to-day[206,309]. This was qualitatively expressed by a few participants in the present study. The FFQ, on the other hand, was used to obtain a better perspective of usual intake over the previous 6 months, but would have been limited by inaccurate recall and the closed-ended nature of the questions and food items[206]. In light of such limitations, Subar *et al*. (2015) recently summated that FFQs "have a finite list of foods or portions with little detail, and [3DRs] are reactive"[317]. The authors went on to conclude that self-reported energy intake should not be used as measure of actual energy intake[317]. Therefore, our dietary findings likely had a degree of validity, which was supported by the similarity of the average results across the three tools. However, it is important to acknowledge that the self-report measures of intake were firstly, estimations of intake, and secondly, likely to have been different from the participants' past dietary habits. This precluded confident conclusions being made about dietary components that may have contributed to prior weight-gain in the Overweight runners.

Aspects of food-related psychology and eating habits were also explored. Interestingly, there were no differences in any of the three domains of the Three-Factor-Eating-Questionnaire (TFEQ). Of the three constructs, cognitive restraint was most strongly reported in both groups. This may initially seem surprising to have found in the Overweight group. However, literature has suggested that 'restrained eating' reflects the tendency to consciously restrict food intake rather than relying on physiological cues of hunger and satiety[318]. Thus, restrained eaters do not necessarily consume less calories than 'unrestrained eaters', merely less than they desire[318]. In fact, restrained eating has been positively correlated with increased weight[318], potentially because it masks underlying appetite dysregulation and predisposes to episodes of overeating[38,207,318]. Previous findings that elevations in adiposity precede changes in cognitive restraint[318], suggest that the mechanisms may involve impaired leptin-signalling with adipose tissue enlargement[38]. The

Overweight participants, therefore, may have adopted a more restrained approach in response to unwanted weight-gain. We found similar restraint in the Lean participants. This may be related to their increased self-awareness and their perception of the 'normal' body image of a female runner[307,312]. Alternatively, they too reported previous weight fluctuations (on average 8.6 kg over the past 10 years) and may have become more restrained in the process of trying to lose said weight. Such speculations are consistent with indications from both groups that they attempted to limit overall calorie intake, as well as fat and refined carbohydrate consumption, both of which have an associated stigma for causing weight-gain[40,319]. It is tempting to speculate that the perception of being restrained in one's eating habits contributed to the Overweight participants qualitatively reporting that they were dissatisfied with their diet and wanted to change it. Although not explored in this thesis, such dissatisfaction may have resulted from feelings of depravity of certain foods that had previously triggered 'reward' or 'pleasure' neural pathways[83,157]. In combination with potentially disrupted appetite regulation, this perceived restraint may have caused them to feel perpetually hungry and understandably dissatisfied[35,38,40]. Overall, the psychological and neural contributions to eating behaviour and body-weight regulation should not be ignored. As alluded to earlier, disruptions in this regard may have contributed to the weight-loss struggles of some of our participants.

Interestingly, participants reported that they had infrequently experienced unpleasant gastrointestinal symptoms. When they had been experienced, most symptoms were associated with the consumption of wheat-containing, carbohydrate-rich foods such as bread, pizza and pasta, as well as sugar-sweetened beverages. This was in agreement with the recent trend for many people to avoid wheat- and gluten- containing foods in light of the associated actual or perceived gastrointestinal distress[320,321]. Although this appears to have had a dietary 'fad' component, and the mechanisms remain unclear, wheat allergy and non-celiac gluten sensitivity have been increasingly related to irritable bowel syndrome, chronic fatigue and auto-immunity[320]. Some have argued that wheat and gluten are harmful to all humans, that the human genome has not had adequate time to adapt to grain consumption and that specific components in modern grains are highly inflammatory[320,322]. Therefore, our finding of rare gastrointestinal distress reflected the participants' attempt to limit their consumption of such foods or they had trained their guts adequately to handle such foods[323].

Participants also reported only few incidences of gastrointestinal distress during running. These were associated with the energy gels, sweets and beverages that have been commonly advised for endurance sport and are provided during most organised events[193,194]. This finding was consistent with reports of gastrointestinal distress, including flatulence, diarrhoea and belching, experienced by endurance athletes consuming carbohydrate-based solutions[323,324]. This phenomenon is multifactorial and may be influenced by mechanical stress to the abdominal organs and gut ischaemia, as well as the osmolality, concentration and type of carbohydrate solution used, and the gut being 'untrained' or ill-adapted to coping with such nutrients during exercise[323]. Although some participants would reportedly avoid carbohydrate-based solutions owing to prior distress (replacing them milk, nuts or biltong) the majority of participants continued to consume them. Thus, they may have 'trained' their guts towards this end.

Both groups in the present study self-reported similar nutritional practices surrounding long runs. Interestingly, these did not necessarily reflect the dietary information we derived from the diet assessment tools. For example, in contrast to the relatively low consumption of carbohydrate during 'normal living', 50% of participants practised carbohydrate-loading during the days leading up to an event. Furthermore, the most commonly consumed food items before, during and after a race were largely carbohydrate-based. Specifically, both groups tended to consume pasta or potatoes the night before a race, energy cereal, toast and/or bananas on the morning of the race, energy sweets and beverages during the race and milkshakes afterwards. Further, many in the Overweight group, but not in the Lean group, reported consuming energy drinks the day before and after an event. These reports were consistent with advice from sporting authorities[193], but contrasted the perceived restraint of refined carbohydrates from the questionnaire in this study. Together this evidence would suggest that the runners were more cognisant of restraining refined carbohydrates during normal daily living compared to during exercise. Potential explanations may include: the perceived importance of regularly consuming rapidly digestible carbohydrates to avoid 'bonking'[325], the exclusive availability of these food sources during race events, or believing they would have been able to 'burn off' any calories by exercising[41,56]. Regardless, the consequent high sugar intake during runs, particularly if performed regularly, may have contributed to weight-gain in some participants (particularly those with insulin-resistance)[18,41,56,188]. Since the participants were

not performing long training runs or events at the time of testing, such dietary choices were probably not captured during testing.

4.2.4.) Physical Activity and Sedentary Behaviour

Reduced physical activity has been commonly cited as a primary cause of the epidemics of obesity and MetS[105]. As discussed earlier, our results were consistent with the latter, since both Overweight and Lean groups exercised regularly and had favourable metabolic profiles independent of adiposity. However, our results were in disagreement with the notion that exercise protects against weight-gain. Firstly, participants were recruited as recreational runners who had taken part in at least half-marathon distance events for the previous five years. Given that both groups averaged over 6 recent consecutive years of running experience, we are satisfied that we had recruited adequately experienced runners. The participants' running mileage at the time of testing was between 30 km and 60 km, with a mean in both groups of approximately 43 km. This was in agreement with previously reported mileage in recreational male runners during an intense training block in preparation for a marathon[326], and exceeded the average mileage (in $km.week^{-1}$ and $hours.week^{-1}$) that was found in female runners preparing for a half-marathon[239]. In addition, Rasmussen *et al.* (2013) regarded 30 to 60 km as an adequate mileage for avoiding injury in preparation for a marathon[327]. The reported half-marathon finishing time in the Lean group was comparable to that of Swiss female runners competing in the Basel half-marathon in 2010 or 2011 (mean 115 minutes), who were similar in terms of age (mean 38.3 years), BMI (mean 21.7 $kg.m^{-2}$) and running experience (mean 6.1 years)[239]. No precedent was found for half-marathon times in Overweight recreational runners, but they were significantly slower compared to the Lean group. This may have been expected, however, since increased BF% has been found to predict slower half-marathon performances[239]. When taking into account their respective bodyweights, however, the two groups were well-matched in terms of running calibre. Therefore, we believe that the participants were highly capable half or full marathon runners despite their considerable difference in body weight and composition. Such a pedigree of exercise training would have benefitted their metabolic health[124,126].

Habitual physical activity was assessed using accelerometry and it exceeded 10 000 steps.day^{-1} in both groups. This met scientifically-based recommendations that "some physical activity is better than none" and that 10 000 steps.day^{-1} is a reasonable target for healthy adults to accrue cardio-metabolic health benefits, reduce morbidity and mortality[328]. Further, the activity of both groups was comparable to the "active" category found by Tudor-Locke *et al*. (2010) in normal-weight, overweight and obese US women (10 000 – 12 499 steps.day^{-1})[329]. In addition, it has been emphasised that a portion (approximately 3000) of the recommended 10 000 steps.day^{-1}, be performed at a moderate-to-vigorous intensity (at least 100 steps.minute^{-1} in bouts of at least 10 minutes)[328]. Physical activity guidelines recommend accumulating 150 minutes of MVPA per week[106]. Given that we investigated active middle-aged women, it was not surprising that both groups exceeded recommendations considerably; specifically participants averaged approximately 90 minutes.day^{-1} of MVPA. Interestingly, MVPA represented a surprisingly insignificant portion of wear-time (Overweight, 11.1% and Lean, 13.5%). This was comparable to the MVPA of US adults in activity 'Class 2' (Class 1 to 6 represented the 'least active' to 'most active' respectively) from the analysis of 2003-2006 NHANES data using the same cut-points as the present study[330]. This finding may have been due to the participants having performed less activity during the testing period compared to their habitual activity levels. Thirteen of the twenty participants indicated this qualitatively in their exercise logbooks. Alternatively, the MVPA we identified was lower than actual MVPA owing to the accelerometer being unable to register non-ambulatory activities[217], including swimming and cycling, which were performed by a significant portion of both groups. Although overall step counts were adjusted in this regard, it would have been imprecise to adjust the times spent in various activity domains. It is further plausible that apart from their designated 'exercise session' on a given day, the participants may have been largely sedentary and performed minimal other MVPA[331].

The proportion of wear-time spent in different physical activity domains (sedentary, light, moderate and vigorous) was highly comparable between groups. A vast majority of time was spent in sedentary behaviour (Overweight, 74.2% and Lean, 72.0%), and very little time was devoted to LIPA (Overweight, 14.0% and Lean, 14.6%). The former was comparable to the sedentary behaviour identified in the women of activity 'Class 2' NHANES (referred to earlier)[330]. This would suggest that the participants in the present study were relatively

inactive. However, it has been acknowledged that accelerometer-based estimates of physical activity may not be entirely accurate[332,333]. Further, Matthews cut-points have tended to over-estimate moderate physical activity and under-estimate both light and vigorous physical activity[215]. Although this would be unable to explain the relatively low proportion of time in MVPA.[330], it may have contributed to the high proportion of sedentary behaviour and little LIPA in the present study[215]. On the other hand, this may have actually reflected the activity pattern of our participants. Whitfield *et al*. (2013) recently described a cohort of 218 recreational half-marathon and marathon runners (approximately 75% female) as being both "highly active and highly sedentary"[331]. Their self-reported training (6.5 hours.week^{-1} including running and cross-training) was comparable to the present study[331]. Interestingly, they spent a considerable portion of waking hours sitting (approximately 10 hours on workdays and 8 hours on non-work days), during which they were working, reading or studying[331]. This was also comparable to the average sedentary times measured in the present study (Overweight, 674 minutes.day^{-1} and Lean, 634 minutes.day^{-1}). The average activity profiles of our groups also indicated relatively low levels of activity other than activity 'spikes' in the early morning and evening, which coincided with their exercise sessions. Together, these findings would suggest that both Overweight and Lean groups had largely sedentary lifestyles independent of their engagement in leisure-time physical activity and meeting physical activity recommendations[108].

Multiple factors may have contributed to this phenomenon. This may have included a predominantly sedentary (desk-based) work environment, motorised transport, screen-based entertainment, or perceived fatigue amongst recreational runners outside of their training sessions[331,334]. Indeed, we observed lower activity during weekdays compared to weekend days. This may have reflected their sedentary employment having limited the available time participants had to be physically active. Furthermore, it seems reasonable to speculate that a morning exercise session would have increased perceived fatigue, while a scheduled training session in the evening may have motivated against other activity during the day. The consequence would be that runners prioritised and largely limited their daily MVPA to these brief training sessions. Ultimately, despite achieving adequate MVPA, it appears that the runners we studied simultaneously engaged in a significant amount of

sedentary behaviour. This would extend the "active couch potato" phenomenon that was described by Owen *et al.* (2010)[134], to a particularly active population.

There has been evidence to suggest that such sedentary behaviour increases cardio-metabolic risk, independent of high MVPA[131,133,134,137]. 'Too much sitting' has been increasingly distinguished from 'too little exercise'[134], and although the mechanisms remain unclear, sedentary behaviour in itself has been found to be an independent risk factor for all-cause mortality[132,335], the MetS[133], T2D[336,337] and CVD[131,337]. In a prospective cohort design, Patel *et al.* (2010) found a dose-response positive association between sitting time and all-cause mortality within five different levels of physical activity, even in individuals performing 2 hours of MVPA per day[131,335]. Although participants in the latter study were aged between 50 and 74 years, this evidence suggests that maintaining regular physical activity may not necessarily offset the detrimental health effects of prolonged sedentary behaviour[131]. On average, we did not find this in the present study. This may have been due to the younger age of our participants, and perhaps metabolic abnormalities underpinned by sedentary behaviour and other factors (e.g. sugar intake) may develop with age[179,180]. Interestingly, when compared to the literature, both the number of sedentary 'bouts' and the accumulated time in sedentary bouts per day, were relatively low in both groups of the present study[338,339]. For example, Ekblom-Bak *et al*. (2015) recently reported an average sedentary time of 9.3 hours.day^{-1} in middle-aged Swedish women, of which 3.2 hours were accumulated in prolonged sedentary bouts[339]. Our participants spent more than 10 hours sedentary, yet Overweight and Lean groups accumulated only 89 and 60 minutes.day^{-1} respectively in prolonged sedentary bouts. However, in support of the cited concern surrounding prolonged sedentary bouts[115,117], mean sedentary bouts per day was significantly correlated with higher BF% in the present study. Overall, this evidence would suggest that our participants interrupted their sedentary behaviour (e.g. sitting) frequently, and were rarely inactive for a prolonged period of time. Although research in is still emerging, breaking up sedentary time in this manner has been associated with more favourable cardio-metabolic health outcomes[131,338,340], and may be one mechanism by which the high sedentariness in the present study was not accompanied by ill metabolic health. Participants who tended to be sedentary for prolonged periods more often, may benefit from consciously initiating frequent sedentary breaks in the future.

On the other hand, there has been evidence down-playing the importance of sedentary behaviour itself, and that has instead implicated MVPA and cardiorespiratory fitness as the primary determinants of cardio-metabolic health[135,136]. For example, van der Velde *et al.* (2015) found significant associations between both MVPA and fitness with more favourable waist circumference, systolic blood pressure, CRP, HDL-C and TG, after adjustment for one another and sedentary behaviour[135]. In contrast sedentary behaviour did not exhibit independent associations when adjusted for MVPA and fitness[135]. This gives further credence to the interpretation that independent of their significant sedentary behaviour, maintaining regular MVPA and fitness would have promoted metabolic health in Overweight and Lean participants alike. Interestingly, a few studies have found that increased adiposity or longitudinal weight-gain predicted future sedentary behaviour and inactivity, independent of baseline activity and weight[122,341]. This would raise the possibility that the Overweight participants, failing meaningful weight-loss, may be at risk of becoming less physically active in the forthcoming years, which would increase their risk of insulin-resistance and illness[283]. Instead of being the result of high levels of physical activity[56,117], therefore, maintaining a healthy body weight, appears to be integral to future activity, health and longevity[122,341].

4.2.5.) Stress and Sleep

This study found no differences between Overweight and Lean groups in either subjective or objective measures of sleep duration and sleep quality. In contrast to prior literature that reported positive associations between short sleep duration and both weight and adiposity gain[146–148,342], it appears that short sleep did not contribute significantly to weight-gain in the Overweight group. The objective Actiwatch recordings indicated that no participants had pathological 'short sleep' (less than 6 hours of sleep per night) as defined in the cited studies[138,146–148,342]. Despite considerable inter-individual variation, both groups averaged approximately 7 hours of sleep per night, which was near to the recommended sleep duration (7 to 8 hours.night^{-1}) for maintaining a healthy weight and metabolism[141]. Short sleep duration has also been associated with impaired glucose control[140,149], increased inflammation and endocrine disruptions[139], that collectively appear to increase risk for MetS[343] and T2D[344]. Therefore, the reasonable sleep duration of both groups would have benefitted their metabolic health.

Indices of sleep quality, in particular onset latency (time taken to fall asleep) and sleep efficiency (proportion of hours slept to total hours in bed) have also been associated with obesity[345] and metabolic disturbances[346]. This may not be surprising given that sleep and specific stages of sleep (i.e. sleep architecture) mediate many restorative processes that are integral to bodily health[138–140]. The objective parameters of sleep quality were highly impressive in both groups: onset latency was below 8 minutes, and sleep efficiency was over 90%. These were both consistent with acceptable research-based standards for sleep onset latency (5 to 20 minutes) and sleep efficiency (≥ 85%)[211]. They were also superior to the results generally reported in population-based cohorts[347], adults[348] and young-adult studies[349]. This would suggest that the Overweight and Lean participants had good sleep quality during testing.

Prior literature would suggest that such sleep hygiene was partly attributable to their high levels of MVPA[350–352]. Although the relationship between exercise and sleep is bi-directional, multiple studies have shown positive associations between greater physical activity and sleep quality[352]. Furthermore, exercise interventions have consistently conferred significant improvements in sleep duration and quality, concomitant with positive psychological changes[350,351,353]. The mechanisms appear to involve physiological changes that benefit homeostatic sleep regulation, enhanced circadian rhythmicity and improved psychological functioning[352]. Regardless, if representative of their prior and habitual sleeping habits, the observed sleep quality would have further benefitted metabolic health[140,149].

In contrast to our objectively-measured findings, our participants perceived their sleep in a less positive manner. According to the results of the Pittsburgh Sleep Quality Index (PSQI) questionnaire, both groups on average perceived themselves as "poor sleepers"[210]. One should be careful, however, not to oversimplify this finding. Both groups were close to the threshold (≥ 5) used to separate 'good' from 'poor' sleepers, and 50% of participants in both groups were classified as 'good' or 'poor' sleepers. Nonetheless, the disagreement between Actiwatch and PSQI data was actually less pronounced than what has been observed previously[354–356]. The cited studies attributed the discrepancy, partly to the

temporal difference between the period of actigraphy and the PSQI reference period (previous month), but primarily to the inherent properties of the objective and subjective measures that effectively assess different aspects of sleep[355]. The PSQI is self-perceptive and appears to associate significantly with subjective mood state, cognitive and psychological functioning at the time of testing[354,355]. This has caused PSQI scores to diverge from objective reality[354,355]. For example, Landry *et al*. (2015) found very weak associations between PSQI scores and objective measures of sleep quality (duration, efficiency and disturbances), and the distinction between 'good' and 'poor' sleepers had no predictive value in determining the actigraph results[355]. Interestingly, although the PSQI was not associated with objective sleep duration in the present study, it did exhibit a significant and negative association with objective sleep efficiency. This would suggest that the self-perceived worse sleepers had lower sleep efficiency. Our objective and subjective findings, therefore, showed greater levels of agreement compared to the cited studies. This may point to a more favourable psychological state in our participants, since the cited research had largely been conducted in elderly populations susceptible to cognitive and memory decline[354–356]. Overall, objective sleep duration and quality were on average impressive and comparable between groups, and this suggests that sleep pathology did not contribute to increased adiposity in the Overweight group.

In a similar manner, our participants generally reported a 'reduced' to 'average' level of recent stress on the Perceived Stress Questionnaire (PSQ), and the results were highly comparable between Overweight and Lean groups. This contrasted the reported association between stress and increased adiposity[152], and may have multiple explanations. Firstly, the PSQ provided a limited view of recent (past month) stress levels[226], as opposed to lifetime stressors or stressful life events that may have influenced body weight and adiposity in years prior to this study[154]. Furthermore, higher levels of physical activity and exercise interventions have been associated with reduced stress levels[357], improved mood and quality of life[358]. This may be partly mediated by the anti-inflammatory effect of regular exercise, particularly MVPA[16,155,358]. In agreement, CRP levels were low in both groups and were significantly positively correlated with the PSQ scores. Together, this evidence gives credence to the reciprocal relationships between exercise, stress and inflammation[359].

We observed a strong correlation between increased perceived stress (PSQ) and worse perceived sleep (PSQI). In other words, participants who self-reported higher levels of recent stress also perceived their recent sleep as less satisfying. This reflected previous literature that highlighted the close relationship between short sleep duration and sleep disturbance with both psychosocial (e.g. PSQ) and physiological (e.g. salivary cortisol, serum norepinephrine) indices of stress[360]. Specifically, heightened physiological stress has been shown to precede shorter sleep duration and sleep disturbance[361], as well as irregular sleep duration from night to night[360]. Subjective stress indices, on the other hand, have tended to reflect perceived sleep indices closely but not necessarily objective sleep[362,363]. The cited studies postulated that this reflects a tendency towards negative emotional responses brought about by enduring distress[363]. This may bias self-reports of personal well-being to be either generally positive or negative[363], which in the present study would have been enhanced by the temporal overlap of the PSQ and PSQI indices. Ultimately, the reduced to average levels of stress, good quality sleep, in combination with regular MVPA, represented healthy lifestyle habits that would have played a significant role in maintaining metabolic health in the Overweight group[174,175].

4.2.6.) Limitations

This study had two primary limitations. Firstly, the sample size was smaller than anticipated because we had difficulty recruiting eligible participants. We had intended to test twenty Overweight and twenty Lean participants, but the difficulty experienced meant that we could only test ten participants per group. We particularly struggled to recruit the more overweight or obese recreational runners that had been reported anecdotally and whom we had previously witnessed at organised events. However, many seemingly overweight / obese runners we did approach had only recently taken up running and were therefore not eligible for this study. This would have contributed to our finding that the Overweight and Lean groups were less distinct in terms of adiposity than we had expected. Further, it is tempting to speculate that runners who are more overweight or obese (for example, BMI ≥ 30 $kg.m^{-2}$) would have been less metabolically healthy than both the Overweight and Lean runners in our study. Alternatively, the obese 'experienced' runners may be a rarer phenotype than we had predicted, or obese runners who became metabolically ill have tended to stop running. Nevertheless, our small sample size may have limited the statistical

power of the between-group comparisons, especially given the significant within-group variation. This would preclude our findings from being generalizable to all overweight athletic individuals.

Owing to the cross-sectional nature of our study, the second limitation was that we were unable to identify causal factors explaining the Overweight participants' weight-gain. Given this was a pilot study, we aimed to identify potential factors associated with greater adiposity, with the intention to identify causal relationships in a subsequent intervention. Although we measured a variety of potentially contributory lifestyle factors (physical activity, diet, sleep and stress), we did not find meaningful associations. However, our findings may not have necessarily reflected the lifestyle habits of the Overweight participants during weight-gain. Specifically, the habits that we observed may have been healthier than what they had previously practised. Although this may not have influenced the between-group comparison of metabolic health parameters, it would have altered the potential explanatory variables that we explored. It was clear during testing that although not currently losing weight, the participants were conscious of their elevated adiposity and had probably altered certain lifestyle habits in an effort to lose weight. Qualitatively, the Overweight participants appeared to be interested and motivated to learn more about their own physiology and possible ways in which to improve their health. There may have, therefore, been a selection bias towards individuals who were more health conscious than other overweight runners.

As have been discussed in more detail above, there were other potential causes of bias or error in this study. Briefly, the self-report dietary intake may have differed from actual intake; the accelerometers were unable to register MVPA from cycling and swimming; physical activity domain cut-points may have under- or over- estimated times spent in different domains; low RERs during a few RMR measurements raised concern about the RMR validity; and we did not strictly control for some potential confounders to RMR and glucose tolerance tests (including menstrual status, previous day's diet and test anxiety). For example, evidence suggests that increased presence of progesterone and estrogen during the luteal phase of the menstrual cycle is concomitant with lower insulin-sensitivity compared to the follicular phase[364]. Further, testing was not standardised in relation to

participants' training and event schedules, which means that the lifestyle data did not necessarily reflect participants at the same stage of training. Finally, the setting of the research laboratory and knowledge of having one's lifestyle analysed, may have influenced the observed lifestyle practices.

4.2.7.) Summary

We investigated the metabolic and lifestyle characteristics of Overweight female runners in comparison to Lean runners who were well-matched for age, running experience and running calibre. As far the author of this thesis is aware, this is the first time that the Overweight recreational runner phenotype has been studied, particularly in comparison to comparable Lean counterparts. We hypothesised that the elevated weight of the Overweight runners would be explained by underlying insulin-resistance and potentially present with related abnormalities, including hypertension, systemic inflammation and dyslipidaemia. Furthermore, we anticipated these conditions would have been promoted or aggravated by certain lifestyle differences between groups, in particular a diet too high in carbohydrate for the expected degree of insulin-resistance.

While the Overweight group did exhibit greater adiposity than the Lean group, there were no clinically meaningful differences in either the metabolic or lifestyle characteristics of the two groups. Although inflammation and systolic blood pressure were higher in the Overweight group, both parameters were within the healthy ranges. However, most outcome measures exhibited considerable inter-individual variation within both groups, and our results suggest that the mechanisms of weight-gain within the Overweight group may have differed between individuals. Two Overweight participants were insulin-resistant and pre-hypertensive, and this have may be indicative of increased metabolic risk. There was also evidence that relative insulin-resistance contributed to increased adiposity across both groups. However, it is uncertain what primarily caused the remaining Overweight participants to gain weight. It is tempting to speculate that dysregulated appetite control played a key role. The normal metabolic health of the Overweight runners probably resulted from healthy lifestyle behaviours, most importantly their history of consistent exercise training and consequent cardiorespiratory fitness, but also apparently adequate

and efficient sleep, infrequent prolonged sedentary bouts and reduced to average levels of stress. We conclude, therefore, that the middle-aged Overweight female runners we investigated were not metabolically at risk compared to matched Lean runners, and that their RMR and lifestyle attributes were not meaningfully dissimilar to their Lean counterparts.

4.2.8.) Future research considerations

Despite not having found the expected differences between Overweight and Lean runners, the 'overweight athlete' remains an intriguing phenotype to explore. Future studies may benefit from a more thorough recruitment process to ideally include overweight runners with greater adiposity and secure a larger between-group distinction. For example, it is recommended that alternative equations be used to estimate BF%. Whereas the Durnin and Womersley formula that was used in the present study only incorporated upper-body skinfolds (bicep, tricep, iliac crest and subscapular)[199], it has been found that including lower-limb skinfolds (thigh and calf) improves accuracy[238,365]. In hindsight, utilising these equations may have provided greater disparity in adiposity between Overweight and Lean groups. Alternatively assuming available funds, researchers would ideally perform DXA scans during the screening process to ensure that respondents fall safely within predetermined BF% criteria.

Recruitment criteria may also need to be modified to secure a larger and more generalisable sample of overweight athletes. It may have been that the more overweight and obese runners that had been reported anecdotally, had not been running for at least 5 years. Future studies may need to relax this criterion to include individuals who adopted running more recently, yet who have continued to struggle with weight-loss. Further, unpublished data from the 2014 Two Oceans Ultra and Half-Marathons suggested that there was a greater prevalence of overweight and obesity in male (52%) as compared to female (26%) runners. Researchers may hence experience less difficulty should they decide to recruit overweight male runners. Additionally, prior evidence would suggest that males tend to exhibit greater insulin-resistance compared to females[366]. Therefore, overweight

male runners may provide greater insight into the role of underlying insulin-resistance in promoting adiposity and metabolic illness in spite of exercise.

Future studies should also address admitted methodological limitations from the present study. Firstly, it is recommended that the testing period be standardised across participants. This is primarily because lifestyle measurements (diet, sleep and physical activity) may be influenced by the timing of testing in relation to the participants' respective training or race schedules, as well as changes in seasons or the weather. Metabolic tests in females should also ideally be standardised to a certain period of the menstrual cycle, since this has been reported to influence both glucose tolerance[364] and RMR[367]. An interesting finding in the present study was that the qualitative eating habits during long runs or races, were rather distinct from (and less healthy than) the results of the diet assessment tools. Future studies may, therefore, benefit from exploring exercise-related dietary intake and its implications for weight-loss.

Additional factors need to be explored more thoroughly for potentially influencing metabolic health in overweight recreational athletes. The Overweight group of the present study shared similarities with the well-documented MHO phenotype[170]. Although our participants shared the high level of cardiorespiratory fitness reported in MHO persons[178], we did not assess other common traits. It would be intriguing to explore adipose tissue distribution and morphology (using magnetic resonance imaging) and liver fat accumulation in Overweight runners compared to matched unhealthy-overweight persons[169]. Furthermore, we experienced difficulty separating the contrasting effects of increased body fatness and regular exercise (MVPA) on metabolic health. One may speculate that having had two additional comparative groups, lean-sedentary and overweight-sedentary, would have provided valuable insight into the complex interplay between these opposing factors. As speculated above, this study did not explore appetite-regulatory systems. This may have been performed, for example, by assessing gut-hormone secretion, leptin-signalling or neurological imaging. Given the cited literature that has implicated impaired appetite-control in promoting over-nutrition[33,36,37,40], it is recommended that future studies explore the physiology and psychology of appetite-regulation in the context of both weight-gain and dietary factors in recreational athletes.

Finally, in line with the original intention of this study, it seems reasonable to recommend randomised intervention trials in overweight endurance athletes. Insulin-resistance and appetite disruptions appear to have been the most plausible explanations for prior weight-gain in the Overweight participants. However, the former only applied to two participants and the latter was speculative. Nonetheless, both abnormalities have been shown to be largely driven and aggravated by dietary factors, particularly refined high-sugar, high fat foods[18,37]. It would be intriguing to explore the weight and metabolic response to dietary interventions that target the mitigation of these disturbances. Indeed, this pilot study was intended to inform a subsequent trial where we would stratify overweight athletes into insulin-resistant and insulin-sensitive groups and randomise them to either a VLCHF diet (aimed at minimising insulin secretion)[87] or a low-calorie, mixed macronutrient diet that would be minimally processed but rather based on natural (whole)-foods[90,173,368]. However, we did not find the level of insulin-resistance or ill metabolic health we expected. Therefore, to perform such a trial would require a slower, more overweight cohort, more likely to have metabolic disturbances, or it should be directed more to weight-loss and performance outcomes. Ultimately, the overweight-athlete paradox represents a unique opportunity to study the interplay between metabolic predisposition and lifestyle factors (especially diet and exercise) in relation to obesity and metabolic disease. The field is wide open for exploration to improve our understanding of these relationships.

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6.) APPENDICES

APPENDIX A

AHA/ACSM Health/Fitness Facility Pre-participation Screening Questionnaire (PAR-Q)

Participant Code: _____ **Date and Time:**_____

Assess your health needs by marking all true statements.

History: You have had:

__ a heart attack

__ heart surgery

__ cardiac catheterization

__ coronary angioplasty (PTCA)

__ pacemaker/implantable cardiac defibrillator/rhythm disturbance

__ heart valve disease

__ heart failure

__ heart transplantation

__ congenital heart disease

Symptoms:

__ You experienced chest discomfort with exertion.

__ You experience unreasonable breathlessness.

__ You experience dizziness, fainting, blackouts.

__ You take heart medications.

Other health issues:

__ You have diabetes.

__ You have lung disease.

__ You have musculoskeletal problems.

__ You have concerns about the safety of exercise.

__ You take prescription medication(s)

If you marked any of the statements in this section, consult your healthcare provider before engaging in exercise.

Cardiovascular Risk Factors:

__ You are a man older than 45 years or a woman older than 55 years

__ You smoke.

__ Your blood pressure is > 140/90.

__ You don't know your blood pressure.

__ You take blood pressure medication.

__ You have high cholesterol.

__ You have a close blood relative who had a heart attack before age 55 (father or brother) or age 65 (mother or sister).

__ You are physically inactive (i.e.: you get < 30 minutes of physical activity on at least 3 days per week).

__ You are > 9kg overweight.

If you marked 2 or more of the statements in this section, consult your healthcare provider before engaging in exercise.

__ None of the above is true.

You should be able to exercise safely without consulting your healthcare provider

APPENDIX B

Eligibility Questionnaire

Participant Code:_____ **Date and Time** _____

SECTION 1: PERSONAL INFORMATION

Age:_____yrs **Height:**_____ cm **Weight:**_____ kg

Are you pregnant? Yes No Maybe

SECTION 2: HEALTH

1. Have you been diagnosed with Type 1 or Type 2 diabetes?

   Yes No

2. Have you been **diagnosed** with other **metabolic conditions** (including thyroid disorders, chronic kidney disease, fatty-liver disease)?

   Yes No

   Please specify: _____

3. Have you suffered a **coronary event** (heart attack) and/or **stroke** (including a Transient Ischaemic Attack or Cerebrovascular event)?

   Yes No

4. Do you currently have a pacemaker?

   Yes No

5. In the **past 3 months** have you taken **chronic medication** for any of the following?

High Blood Cholesterol	Yes	No
Diabetes (insulin therapy)	Yes	No
High Blood Pressure	Yes	No

High Blood Sugar	Yes	No	
Hormonal/Thyroid problems	Yes	No	
Other	Yes	No	Please specify _____

6. Have you had an injury in the past month that prevented you from running?

   Yes No

   If **YES**, please provide details: _____

SECTION 3: DIET

1. Have you **started a new eating plan or significantly changed the type of foods** that you typically eat, within the **past 6 months**?

   Yes No

   If **YES**, please describe what changes you have made: _____

   _____

   If **YES**, what were the reasons that you changed your diet: _____

   _____

SECTION 4: WEIGHT CHANGE

1. Within the **past 3 months**, has your **body weight changed by more than 5%** at any stage? For example, if you weigh ± 80 kg has your weight changed by 4kg (e.g. 78 to 82 kg) within the last 3 months?

   Yes No

   If **YES**, what was your lowest and highest weight that you measured during this time?

   Lowest_____ Highest_____

SECTION 5: RUNNING EXPERIENCE AND OTHER EXERCISE

1. For the **past 6 months**, have you run **at least 3 times per week** for at least 75% of the time?

   Yes No

2. Have you completed **at least 1 marathon and/or 2 half-marathons and/or one 30km trail run per year**, for the **past 5 years**?

   Yes No

   If **YES**, where these: all races /all training runs / mixture of training and racing? (circle)

   If **NO**, but you have been close to completing these runs, please describe your distance running experience over the past 5 years.

   _____

   _____

3. What were your **fastest race times** for a half, full and/or ultra -marathon that you ran **within the past 12 months.** Please indicate whether these were road or trail runs and the distance for other runs.*****

   21.1 km time _____(Road/Trail) Race name/date:_____

   _____

   42.2 km time_____(Road/Trail) Race name/date:_____

   _____

   Other >21.1km_____(Road/Trail) Race dist/name/date_____

4. What were your **fastest race times that you have ever run** for a half, full and/or ultra-marathon**.** Please indicate whether these were road or trail runs and the distance for other runs.*****

   21.1 km time _____(Road/Trail) Race name/date:_____

   42.2 km time_____(Road/Trail) Race name/date:_____

   Other >21.1km_____(Road/Trail) Race dist/name/date_____

5. Have you completed **a half-marathon or longer race/training run within the past 6 months**?

   Yes No If YES, Race or Training run.

6. In **total during your lifetime**, how many **years** have you run for **on a regular basis** (at least 3 times per week for at least 75% of the time)? In other words, add the number of years that you have run regularly, **excluding breaks of more than 3 months**.*

   _____

7. If you **have taken a long break** from running during your lifetime, then working back from present, how many **years** have you run for **regularly** (at least 3 times per week for at least 75% of the time) **since** taking a break for longer than 3 months? *

   _____

8. Over the **past 3 months**, on average, how many **hours** have you run **per week**? *

   _____

9. Over the **past 3 months**, on average, how many **kms** have you run **per week**? *

   _____

10. Is running your primary form of exercise?

    Yes No

11. Is there any **other form of exercise** that makes up **more than one third** of your exercise?

    Yes No

    If **YES**, what are these activities and how many hours do you spend on these activities per week? _____

    _____

Thank you for completing the questionnaire!

APPENDIX C

Detailed participant questionnaire

Participant Code: _____ **Date and Time** _____

Thank you for volunteering for this study. This questionnaire asks about your family health history, your personal health, your perceptions of your health, appearance and quality of life, as well as your dietary intake and running / training routines.

Please answer **all questions honestly**. Where applicable, please **circle the number** which you think applies to you. If you are **unsure** about which response to give, please choose the one that appears **most appropriate**. Your results will be kept strictly confidential and will be stored in coded form. Only the official investigators of this study will be able to identify you.

SECTION 1: PERSONAL DETAILS

1. Ethnicity: (a) Black (b) White (c) Mixed (d) Indian (e) Other _____

2. Usual monthly income (before tax): (a) R0 – R9 999 (b) R10 000 - R24 999 (c) more than R25 000

3. Occupation (or degree/course if studying): _____

4. Highest level of education completed:

(a) primary school (b) high school (b) undergraduate tertiary (c) postgraduate tertiary

5. How many people currently live in your household? _____

6. How many rooms in total are there in your household? (Count an open plan area as the number of functional rooms eg kitchen/dining room open plan = 2 rooms)_____

SECTION 2: FAMILY HEALTH HISTORY

1. Has your **mother or sister** had **heart disease** (e.g. Heart attack / myocardial infarction, angina, heart failure, arrhythmia)?

   before the age of 65? Yes No

   after the age of 65? Yes No

2. Has your **father or brother** had **heart disease** (as above)?

   before the age of 55? Yes No

   after the age of 55? Yes No

3. As far as you are aware, have any of your close **family members** (i.e. brothers, sisters, parents) had any of the following conditions (Yes / No)? If **YES**, please specify the relation.

Condition	**Diagnosis**	**Yes / No**	**Relation**
Obesity	BMI ≥ 30 $kg.m^{-2}$ Waist Circumference ≥ 94 cm		
Pre-diabetes (Impaired Fasting Glucose or Tolerance)	Fasting blood glucose ≥ 5.6 mM 2-hour OGTT* glucose 7.8 – 11.0 mM		
Type 2 diabetes	Fasting blood glucose ≥ 7.0 mM 2-hour OGTT* glucose ≥ 11.1 mM		
High Blood Pressure	Systolic ≥ 130 mmHg or Diastolic ≥ 85 mmHg		
High Blood Cholesterol	Total cholesterol ≥ 5.2 mM		

*OGTT: Oral Glucose Tolerance Test (Blood glucose concentration is measured 2 hours after 75 grams of glucose is consumed)

SECTION 3: PERSONAL HEALTH

1. Do you **have or have you ever had** any of the following conditions?

Glandular Fever	Yes	No
Lung Disease	Yes	No
Gout	Yes	No
Epilepsy	Yes	No
Celiac Disease	Yes	No
Gluten sensitivity	Yes	No

Depression / Anxiety	Yes	No	
Asthma	Yes	No	
Chronic Obstructive Pulmonary Disorder	Yes	No	
Heart murmur	Yes	No	
Arthritis / Osteoporosis	Yes	No	
Chrone's Disease	Yes	No	
Drug or Alcohol Problems	Yes	No	
Prostrate cancer	Yes	No	
Other cancer	Yes	No	Specify_____
Other condition	Yes	No	Specify_____

2. Have you ever been **diagnosed** by a health care professional with any of the following?

High Blood Cholesterol	Yes	No
High Blood Pressure	Yes	No
High Blood Sugar	Yes	No
Abdominal Obesity	Yes	No

3. Are you **currently** seeing a practitioner/doctor for any injuries and /or illnesses?

   Yes No

   If **YES**, please specify**:**_____

SECTION 4: SMOKING

1. Do you **currently smoke** any tobacco products, such as cigarettes, cigars, or pipes?

   Yes No If **YES**, for how long? _____

   If **NO**, have you smoked any tobacco products in the past?

   Yes No If **YES**, for how long? _____

   When did you **stop** smoking? _____

If **Yes** to either of the above, how many cigarettes **do/did** you smoke **per day**?

(1) 1 – 4 / day (2) 5 – 9 / day (3) 10 – 19 / day (4) 20 – 29 / day (5) ≥ 30 / day

SECTION 5: ALCOHOL INTAKE

Note: 1 "alcoholic drink" below is equal to 1 glass of wine OR 1 340ml beer OR 1 tot of spirits.

1. Thinking back over **the past 6 months**, on average how many alcoholic drinks **per week** did you usually drink?

   (1) 0 units (2) 1 – 2 units (3) 3 – 6 units (4) 7 – 10 units (5) 11 – 14 units (6) > 14 units

2. Thinking back over **the past 10 years**, on average how many alcohol drinks **per week** did you usually drink?

   (1) 0 units (2) 1 – 2 units (3) 3 – 6 units (4) 7 – 10 units (5) 11 – 14 units (6) > 14 units

SECTION 6: SUBJECTIVE HEALTH

These questions relate to your experiences and feelings over the **past 3 months**. Please **read each question** and **circle the number on the scale** that gives the best answer for you.

1. In general, would you say your **health** is:

   (1) Very poor (2) Poor (3) Fair (4) Good (5) Very good

2. How often do you **have enough energy** for everyday life?

   (1) None of the time (2) A little of the time (3) A good bit of the time

   (4) Most of the time (5) All of the time

3. Would you say your **sleep quality** is:

   (1) Very poor (2) Poor (3) Fair (4) Good (5) Very Good

4. In total, how many **hours** do you typically **sleep** (excluding lying awake) per day/night?

   (1) ≤ 5 hours (2) 5 - 6 hours (3) 6 - 7 hours (4) 7 - 8 hours (5) > 8 hours

5. How **much stress have you felt** over the **past month**?

   (1) Very little (2) Fair amount (3) Large amount (4) Extreme amount

6. How **satisfied** are you with your **bodily appearance**?

   (1) Very dissatisfied (2) Dissatisfied (3) Neither satisfied nor dissatisfied

   (4) Satisfied (5) Very satisfied

7. How **satisfied** are you with your current **weight**?

   (1) Very dissatisfied (2) Dissatisfied (3) Neither satisfied nor dissatisfied

   (4) Satisfied (5) Very satisfied

8. Would you like to **lose weight**?

   Yes No

   If **Yes**, how many **kg** would you like to lose? _____

   If **Yes**, how **important** is it to you to lose the weight?

   (1) Not at all (2) Kind of important (3) Important (4) It is a priority

   If **Yes**, how **motivated** are you to lose the weight?

   (1) Not at all (2) Kind of motivated (3) Highly (4) Very highly

9. Have you **previously tried** (e.g. by exercise or diet) to lose weight?

   Yes No

   If Yes, **how much weight (kg)** did you manage to lose? _____

   If Yes, to **what extent** were you **successful** in achieving your target weight loss?

   (1) Not at all (2) To some extent (3) About half-way (4) Nearly fully (5) Completely

10. In the past, how **difficult/easy** has it been to **maintain a stable weight that you are satisfied with**?

    (1) Very difficult (2) Difficult (3) Relatively Easy (4) Easy (5) No problem

11. In the **past 10 years**, have you experienced **major weight fluctuations** (frequent 'ups' and 'downs' of more than 5% body weight)? For example, repeatedly jumping between 80 and 84 kg.

    Yes No

12. Please indicate your highest and lowest weight in the past 10 years?

    Lowest weight (kg) _____ Highest weight (kg) _____

SECTION 7: USUAL DIET

This section refers to your **general diet** and **section 8** refers specifically to your running related diet.

1. Are you **currently following**, or **have you followed in the past**, any of these particular **types of diets or eating patterns?** If so, please indicate **when and for how long** you have eaten /ate this way. You may choose more than one.

	Ever?		Current?	How long?	When ended?
Example diet	***Yes***	*No*	*No*	*3 years*	*Dec 2013*
No particular diet but I choose healthy foods	Yes	No			
I don't think much about what I eat	Yes	No			
Mixed or 'balanced' diet	Yes	No			
Low Fat	Yes	No			
Low GI	Yes	No			
Mediterranean	Yes	No			
Low Calorie mixed diet	Yes	No			
DASH (Dietary Approaches to Stop Hypertension)	Yes	No			

Vegetarian	Yes	No	_____	_____
Vegan	Yes	No	_____	_____
Very Low-Carb/ Atkins	Yes	No	_____	_____
Low Carbohydrate	Yes	No	_____	_____
Paleo	Yes	No	_____	_____
Religious (e.g. Halal, Kosher)	Yes	No	_____	_____
Other, please specify……..			_____	_____

2. Do you think you generally **eat too much food**?

   Yes No

3. Do you try to **limit how many calories** (how much food) that you generally eat?

   Yes No

4. Do you try **to limit the amount of fat** in your diet (e.g. by eating low-fat dairy products and lean meats, avoiding fatty foods and cutting the fat off of pieces of meat)?

   Yes No

5. Do you try **to limit the amount of refined carbohydrates** in your diet (e.g. table sugar, white flour, sweets, cakes, biscuits, and sweetened beverages)?

   Yes No

6. How **healthy** would you say your current diet or eating pattern is?

   (1) Very unhealthy (2) Unhealthy (3) Fairly healthy (4) Very healthy

7. How **satisfied** are you with your current diet or eating pattern?

   (1) Very dissatisfied (2) Dissatisfied (3) Neither satisfied nor dissatisfied

   (4) Satisfied (5) Very satisfied

8. Do you think you should **change** your dietary habits?

   (1) Not at all (2) Probably not (3) Unsure (4) Probably (5) Definitely

9. What **mineral / vitamin** supplements (e.g. multivitamin or vitamin C / vitamin D and omega-3), if any, are you currently using?

Name/ Type of supplement	**amount / day**	**Period taken**

SECTION 8: RUNNING NUTRITION

Questions 1 and 2 refer to your nutrition in the **days or weeks leading up to** a long run or race and **Question 3** refers to your nutrition **on the day** of a long run or race.

1. Do you **change your usual eating pattern** in the days or weeks before a long run? In other words, do you change the type and/or amounts of food/drinks you consume to prepare for a race?

   Yes No No, but I have in the past

2. If **YES**, do you **currently** or **have you in the past** practiced any of the following strategies? You can choose more than 1.

   (a) Carbo-loading i.e. you eat a greater-than-normal amount of **carbohydrate** during the days leading up to a long run.

   Currently practicing: Yes No If yes, for how long? _____

   Practiced in the **past**: Yes No If yes, for how long and when did you stop?_____

   If **YES**, please describe how you carbo-load (what types of foods or drinks you choose and when you consume them)? _____

   _____

   _____

   For what reason **do/did** you carbo-load? _____

   _____

(b) Fat adapting i.e. you eat a greater-than-normal amount of fat and a less-than-normal amount of carbohydrate during the days leading up to a long run, **with or without a carbo-loading day**.

Currently practicing: Yes No If yes, for how long? _____

_____

Practiced in the **past**: Yes No If yes, for how long and when did you stop?_____

If **YES**, please describe how you fat-adapt (what types of foods or drinks you choose and when you consume them?): _____

_____

_____

For what reason **do/did** you fat-adapt?_____

_____

(c) Calorie loading i.e. you eat a more than a normal **amount of food** before a long run.

Currently practicing: Yes No If yes, for how long? _____

Practiced in the **past**: Yes No If yes, for how long and when did you stop?_____

If **YES**, please describe how you calorie-load (what types of foods or drinks you choose and when you consume them?): _____

_____

_____

For what reason **do/did** you calorie load?_____

_____

(d) Calorie restricting i.e. you eat less than a normal amount of food in the days/ weeks before a long run.

Currently practicing: Yes No If yes, for how long? _____

Practiced in the **past**: Yes No If yes, for how long and when did you stop?_____

If **YES**, please describe what types of foods or drinks you restrict and when you restrict them:_____

_____

For what reason **do/did** you restrict calories? _____

_____

(e) Other pre race eating pattern

Currently practicing: Yes No If yes, for how long? _____

Practiced in the **past**: Yes No If yes, for how long and when did you stop?_____

If **YES**, please describe _____

_____

_____

For what reason **do/did** you follow this pattern? _____

_____

3. What **foods, drinks and / or nutritional supplements** (e.g. energade, coke, gels, sweets, electrolyte solutions) do you normally consume **before, during and soon after** training runs and / or races?

Before:_____

During:

After:

Thank you for completing the questionnaire!

APPENDIX D

The Three-Factor Eating Questionnaire (TFEQ-R21)

Participant Code:_____ Date and Time_____

Please circle the number which you think applies to you. Please answer honestly.

1. I deliberately take small helpings to control my weight.

(1) Definitely true (2) Mostly true (3) Mostly false (4) Definitely false

2. I start to eat when I feel anxious.

(1) Definitely true (2) Mostly true (3) Mostly false (4) Definitely false

3. Sometimes when I start eating, I just can't seem to stop.

(1) Definitely true (2) Mostly true (3) Mostly false (4) Definitely false

4. When I feel sad, I often eat too much.

(1) Definitely true (2) Mostly true (3) Mostly false (4) Definitely false

5. I don't eat some foods because they make me fat.

(1) Definitely true (2) Mostly true (3) Mostly false (4) Definitely false

6. Being with someone who is eating, often makes me want to also eat.

(1) Definitely true (2) Mostly true (3) Mostly false (4) Definitely false

7. When I feel tense or "wound up", I often feel I need to eat.

(1) Definitely true (2) Mostly true (3) Mostly false (4) Definitely false

8. I often get so hungry that my stomach feels like a bottomless pit.

(1) Definitely true (2) Mostly true (3) Mostly false (4) Definitely false

9. I'm always so hungry that it's hard for me to stop eating before finishing all of the food on my plate.

(1) Definitely true (2) Mostly true (3) Mostly false (4) Definitely false

10. When I feel lonely, I console myself by eating.

(1) Definitely true (2) Mostly true (3) Mostly false (4) Definitely false

11. I consciously hold back on how much I eat at meals to keep from gaining weight.

(1) Definitely true (2) Mostly true (3) Mostly false (4) Definitely false

12. When I smell a sizzling steak or see a juicy piece of meat, I find it very difficult to keep from eating — even if I've just finished a meal

(1) Definitely true (2) Mostly true (3) Mostly false (4) Definitely false

13. I'm always hungry enough to eat at any time.

(1) Definitely true (2) Mostly true (3) Mostly false (4) Definitely false

14. If I feel nervous, I try to calm down by eating.

(1) Definitely true (2) Mostly true (3) Mostly false (4) Definitely false

15. When I see something that looks very delicious, I often get so hungry that I have to eat right away.

(1) Definitely true (2) Mostly true (3) Mostly false (4) Definitely false

16. When I feel depressed, I want to eat.

(1) Definitely true (2) Mostly true (3) Mostly false (4) Definitely false

17. How often do you avoid "stocking up" on tempting foods?

(1) Almost never (2) Seldom (3) Usually (4) Almost always

18. How likely are you to make an effort to eat less than you want?

(1) Unlikely (2) A little likely (3) Somewhat likely (4) Very likely

19. Do you go on eating binges even though you're not hungry?

(1) Never (2) Rarely (3) Sometimes (4) At least once a week

20. How often do you feel hungry?

(1) Only at mealtimes (2) Sometimes between meals (3) Often between meals

(4) Almost always

21. On a scale from 1 to 8, where 1 means no restraint in eating and 8 means total restraint, what number would you give yourself?

Mark the number that best applies to you: 1 2 3 4 5 6 7 8.

Thank you for completing the questionnaire!

APPENDIX E

PITTSBURGH SLEEP QUALITY INDEX QUESTIONNAIRE

Participant Code:_____ **Date and Time** _____

Instructions:

The following questions relate to your usual sleep habits during the **past month only**. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

1. During the past month, when have you usually gone to bed at night?

Usual bed time: _____

2. During the past month, how long, in minutes has it usually taken you to fall asleep each night?

Number of minutes: _____

3. During the past month, when have you usually gotten up in the morning?

Usual getting up time: _____

4. During the past month, how many hours of actual sleep did you get each night? (This may be different than the number of hours you spend in bed)

Hours of sleep per night: _____

For each of the remaining questions, check the one best response.

Please answer *all* questions.

5. During the past month, how often have you had trouble sleeping because you…

(a) Cannot get to sleep within 30 minutes

Not during the past month _____ *Less than once per week*_____

Once or twice a week _____ *Three or more times a week* ___

(b) Wake up in the middle of the night or early morning

Not during the past month _____ *Less than once per week*_____

Once or twice a week _____ *Three or more times a week* ___

(c) Have to get up to use the bathroom

Not during the past month _____ *Less than once per week*_____

Once or twice a week _____ *Three or more times a week* ___

(d) Cannot breathe comfortably

Not during the past month _____ *Less than once per week*_____

Once or twice a week _____ *Three or more times a week* ___

(e) Cough or snore loudly

Not during the past month _____ *Less than once per week*_____

Once or twice a week _____ *Three or more times a week* ___

(f) Feel too cold

Not during the past month _____ *Less than once per week*_____

Once or twice a week _____ *Three or more times a week* ___

(g) Feel too hot

Not during the past month _____ *Less than once per week*_____

Once or twice a week _____ *Three or more times a week* ___

(h) Had bad dreams

Not during the past month _____ *Less than once per week*_____

Once or twice a week _____ *Three or more times a week* ___

(i) Have pain

Not during the past month _____ *Less than once per week*_____

Once or twice a week _____ *Three or more times a week* ___

(j) Other reason(s), please describe _____

_____

6. During the past month, how would you rate your sleep quality overall?

Very good _____ *Fairly good* _____

Fairly bad _____ *Very bad* _____

7. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?

Not during the past month _____ *Less than once per week*_____

Once or twice a week _____ *Three or more times a week* ___

8. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?

Not during the past month _____ *Less than once per week*_____

Once or twice a week _____ *Three or more times a week* ___

9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?

No problem at all _____ *Only a very slight problem* _____

*Somewhat of a problem*_____ *A very big problem* _____

10. Do you have a bed partner or roommate?

No bed partner or roommate _____

Partner/roommate in other room _____

Partner in same room, but not same bed _____

Partner in same bed _____

If you have a roommate or bed partner, ask him/her how often in the past month you have had…

(a) Loud snoring

Not during the past month _____ *Less than once per week*_____

Once or twice a week _____ *Three or more times a week* ___

(b) Long pauses between breaths while asleep

Not during the past month _____ *Less than once per week*_____

Once or twice a week _____ *Three or more times a week* ___

(c) Legs twitching or jerking while you sleep

Not during the past month _____ *Less than once per week*_____

Once or twice a week _____ *Three or more times a week* ___

(d) Episodes of disorientation or confusion during sleep

Not during the past month _____ *Less than once per week*_____

Once or twice a week _____ *Three or more times a week* ___

(e) Other restlessness while you sleep: please describe _____

_____

Not during the past month _____ *Less than once per week*_____

Once or twice a week _____ *Three or more times a week* ___

Thank you for completing the questionnaire!

APPENDIX F

The Perceived Stress Questionnaire

Participant Code:_____ **Date and Time** _____

Instructions: For each sentence, circle the number that describes how often it applies to you **during the last month**. Work quickly, without bothering to check your answers, and be careful to consider **only the last month**.

	Almost never	Sometimes	Often	Usually
1. You feel rested	1	2	3	4
2. You feel that too many demands are being made on you	1	2	3	4
3. You are irritable and grouchy	1	2	3	4
4. You have too many things to do	1	2	3	4
5. You feel lonely or isolated	1	2	3	4
6. You find yourself in situations of conflict	1	2	3	4
7. You feel like you're doing things you really like	1	2	3	4
8. You feel tired	1	2	3	4
9. You fear you may not manage to attain your goals	1	2	3	4
10. You feel calm	1	2	3	4
11. You have too many decisions to make	1	2	3	4
12. You feel frustrated	1	2	3	4
13. You are full of energy	1	2	3	4
14. You feel tense	1	2	3	4
15. Your problems seem to be piling up	1	2	3	4
16. You feel like you're in a hurry	1	2	3	4

17. You feel safe and protected	1	2	3	4
18. You have many worries	1	2	3	4
19. You are under pressure from other people	1	2	3	4
20. You feel discouraged	1	2	3	4
21. You enjoy yourself	1	2	3	4
22. You are afraid for the future	1	2	3	4
23. You feel you're doing things because you have to not because you want to	1	2	3	4
24. You feel criticised or judged	1	2	3	4
25. You are lighthearted	1	2	3	4
26. You feel mentally exhausted	1	2	3	4
27. You have trouble relaxing	1	2	3	4
28. You feel loaded down with responsibility	1	2	3	4
29. You have enough time for yourself	1	2	3	4
30. You feel under pressure from deadlines	1	2	3	4

Thank you for completing the questionnaire!

APPENDIX G

Gastrointestinal Symptoms Questionnaire

Participant Code:_____ **Date and Time** _____

This questionnaire aims to determine whether or not you have typically experienced gastrointestinal (GI) symptoms **associated with eating**. **SECTION A** pertains to eating **during normal meal times or snacks**. **SECTION B** pertains specifically to eating **during running / exercise**. **Please answer honestly** and remember that your information will be kept strictly confidential and only the study investigators will be able to identify you.

SECTION A: GI SYMPTOMS WHILE NOT DOING EXERCISE

Please indicate the **FREQUENCY** of GI symptoms you have experienced within the **past 3 months, excluding the times that you have been exercising by circling the number which applies to you.** Please try to recall the types of foods or drinks which have made you feel the discomfort (list specific foods or general types).

Abdominal Pain or Discomfort

(1) Not at all (2) Rarely (3) Sometimes (4) Often (5) Very often

Food / Drink _____

Early Satiety (Feeling full soon after starting to eat; the person is unable to finish a normal meal)

(1) Not at all (2) Rarely (3) Sometimes (4) Often (5) Very often

Food / Drink _____

Postprandial Fullness (An unpleasant feeling of food staying in the stomach after a normal meal)

(1) Not at all (2) Rarely (3) Sometimes (4) Often (5) Very often

Food / Drink _____

Bloating (A feeling as if the abdomen or stomach were swollen)

(1) Not at all (2) Rarely (3) Sometimes (4) Often (5) Very often

Food / Drink _____

Heartburn/Acid Reflux (A burning pain or discomfort behind the breastline rising up toward the throat)

(1) Not at all (2) Rarely (3) Sometimes (4) Often (5) Very often

Food / Drink _____

Nausea

(1) Not at all (2) Rarely (3) Sometimes (4) Often (5) Very often

Food / Drink _____

Vomiting

(1) Not at all (2) Rarely (3) Sometimes (4) Often (5) Very often

Food / Drink _____

Dysphagia (Difficulty in swallowing, in which solid food or liquids stick on the way down)

(1) Not at all (2) Rarely (3) Sometimes (4) Often (5) Very often

Food / Drink _____

Diarrhoea

(1) Not at all (2) Rarely (3) Sometimes (4) Often (5) Very often

Food / Drink _____

Constipation

(1) Not at all (2) Rarely (3) Sometimes (4) Often (5) Very often

Food / Drink _____

> 3 Bowel movements PER DAY

(1) Not at all (2) Rarely (3) Sometimes (4) Often (5) Very often

Food / Drink _____

< 3 Bowel movements PER WEEK

(1) Not at all (2) Rarely (3) Sometimes (4) Often (5) Very often

Food / Drink _____

Lumpy or hard stools

(1) Not at all (2) Rarely (3) Sometimes (4) Often (5) Very often

Food / Drink _____

Loose or watery stools

(1) Not at all (2) Rarely (3) Sometimes (4) Often (5) Very often

Food / Drink _____

Anal blockage (A feeling of blockage in the anus or back passage that makes it difficult to pass bowel movements)

(1) Not at all (2) Rarely (3) Sometimes (4) Often (5) Very often

Food / Drink _____

Urgency (A need to have a bowel movement that makes the person rush to the toilet)

(1) Not at all (2) Rarely (3) Sometimes (4) Often (5) Very often

Food / Drink _____

Faecal Incontinence (Inability to control bowel movements, leading to an involuntary loss of bowel contents)

(1) Not at all (2) Rarely (3) Sometimes (4) Often (5) Very often

Food / Drink _____

SECTION B: RUNNING ASSOCIATED GI SYMPTOMS

Have you experienced any of the following GI symptoms **during a training run or race** within the **past year**? If **YES**, please indicate **how often** you have experienced the symptom (circle the number which applies to you). Please indicate any **food or drinks** that you think may cause these symptoms during running?

Abdominal pain

Yes No (1) Once (2) A couple of times (3) Regularly

Food / Drink _____

Bloating (A feeling as if the abdomen or stomach were swollen)

Yes No (1) Once (2) A couple of times (3) Regularly

Food / Drink _____

Heartburn / Acid Reflux (A burning pain or discomfort behind the breastline rising up toward the throat)

Yes No (1) Once (2) A couple of times (3) Regularly

Food / Drink _____

Nausea

Yes No (1) Once (2) A couple of times (3) Regularly

Food / Drink _____

Vomiting

Yes No (1) Once (2) A couple of times (3) Regularly

Food / Drink _____

Dysphagia (Difficulty in swallowing, in which solid food or liquids stick on the way down)

Yes No (1) Once (2) A couple of times (3) Regularly

Food / Drink _____

Diarrhoea

Yes No (1) Once (2) A couple of times (3) Regularly

Food / Drink _____

Urgency (A need to have a bowel movement that makes the person rush to the toilet)

Yes No (1) Once (2) A couple of times (3) Regularly

Food / Drink _____

Faecal Incontinence (Inability to control bowel movements, leading to an involuntary loss of bowel contents)

Yes No (1) Once (2) A couple of times (3) Regularly

Food / Drink _____

Thank you for completing the questionnaire!

APPENDIX H

MRC Food Frequency Questionnaire

FOOD FREQUENCY QUESTIONNAIRE **Participant Code:**

For the completion of this questionnaire we would like you to **think carefully** of what you ate and drank, **on average**, within the **LAST 6 MONTHS**.

In the table below you will find a list of foods and drinks (Column 1). We would like you to tick off:

- **How often** you ate or drank the specified item during the **past 6 months**.
- On average, **how much** (or portion size) you ate/drank of the specified item if you did eat it (Column 3).

Guidelines are given in Column 3 to help you with portion size estimation.

The ***standard (Std) portion size*** of each food item/drink is indicated in household measures (e.g. cups, spoons, grams) or a unit (e.g. a hamburger). Where possible the Std portion size is also shown in terms of visual aids, including a matchbox, a tennis ball, and a sketch of half a cup of dished up food.

Sketches of these aids are attached to the questionnaire.

Once you are sure about the size of the Std portion, tick one of the following options in Column 3:

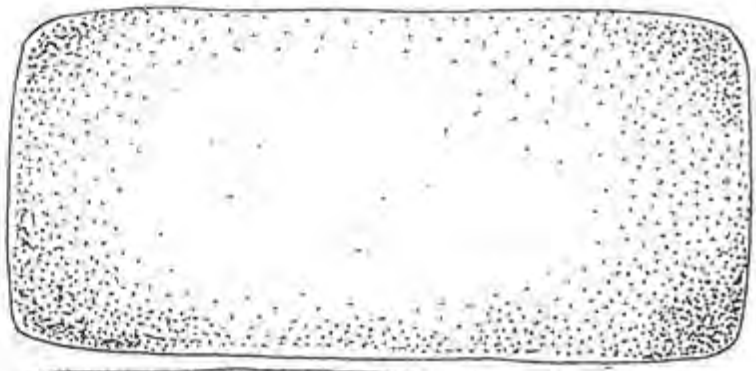
- If the portion you ate/drank of the specified item on average is ±the same as the Std portion size, tick the ***1 x Std*** column; Or
- If the portion you ate/drank of the specified item on average is less than the Std portion size, tick the ***½ x Std*** column; OR
- If the portion you ate/drank of the specified item on average is a little more than the Std portion size, tick the ***1 ½ x Std***; OR
- If the portion you ate/drank of the specified item on average is much more than the Std portion size, tick the ***2 x or 3 x Std*** column.

<u>Explanation of abbreviations/symbols used:</u>

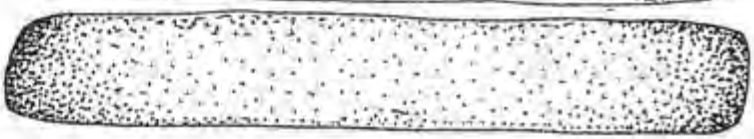
Teaspoon: tsp Table spoon: Tbs Gram: g Milliliters: ml Etc – whatever is necessary	Tennis ball:  ½ cup of dished up food:  Matchbox: 

Please make sure that you **tick every line**. If you did not eat/drink a particular item in the last 6 months, make sure that you tick the "NO" column. Remember that if you ticked the "NO" option in Column 2, you do not have to complete Column 3 (portion size).

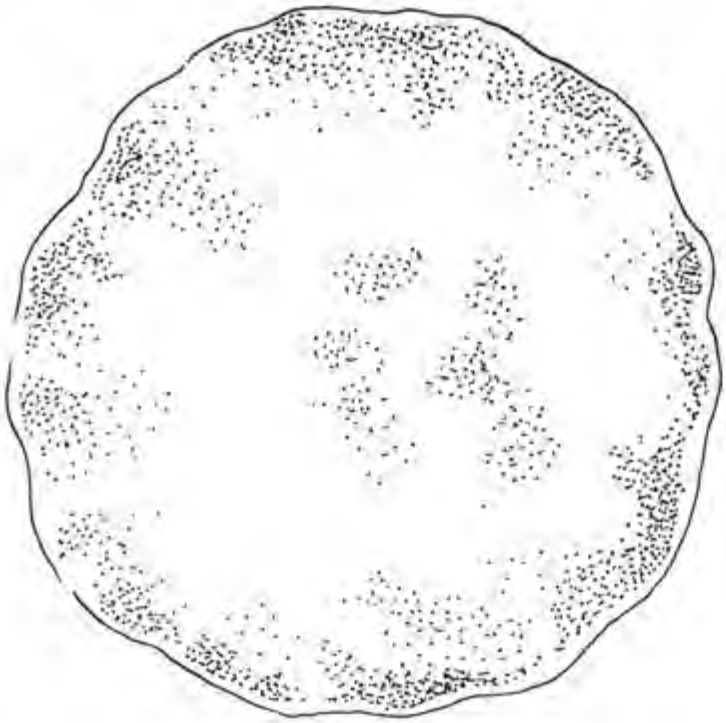
3 x matchboxes: top view



3 x matchboxes: cross section



½ cup: top view



Level table spoon

cup: cross section

evel teaspoon

Heaped teaspoon

EXAMPLE (see first highlighted portion of questionnaire): During Jimmy's training period, one of the foods he eats is brown bread. He eats brown bread every day for breakfast and for lunch. At each meal he eats about 2 slices of bread.

Usual intake during the LAST MONTH OF YOUR TRAINING SEASON before your latest marathon														
Column 1		Column 2							Column 3					
		NO	1-3 per month	1-3 per week	4-6 per week	1 per day	2 per day	3 or more per day	Std portion	1X std	½ X std	1½ X std	2X std	3X std
	EXAMPLE	This person eats 2 slices of brown bread twice a day.												
	BREAD/ROLLS: brown						√		1 roll or 1 med slice				√	
1	RED MEAT: low fat cuts (no visible fat) e.g. Steak, lean minces, roast, ham (beef, veal mutton, pork, venison, ostrich)								90g/ 3X ▱					
2	RED MEAT: high fat cuts e.g. Regular mince, ribs chops with fat (beef, pork, mutton)								90g/ 3X ▱					
3	HAMBURGER								1					
4	SAUSAGE: viennas, russians, frankfurt, boerewors etc.								10cm sausage					
5	MEAT STEW: e.g. chuck, neck (with or without vegetables)								1 cup or 2X ◎					
6	CHICKEN/TURKEY: with skin								90g/ 3X ▱					
7	CHICKEN/TURKEY: without skin								90g/ 3X ▱					
8	FRIED CHICKEN/ SCHNITZEL: Kentucky or homemade								90g/ 3X ▱					
9	FISH: fried in fat/oil								90g/ 3X ▱					
10	FISH: steamed, grilled, braaied (fire)								90g/ 3X ▱					
11	FISH: tinned sardines, pilchards, salmon, tuna								90g/ 3X ▱ or ½ small tin					
12	ORGAN MEATS: liver, kidney, heart, tripe (beef, sheep, chicken)								90g/ 3X ▱					
13	SMOKED/CURED: meat, fish, poultry								90g/ 3X ▱					
14	BACON								1 rasher					
15	PROCESSED MEATS: e.g. Polony, salami								1 thin slice					
16	MEAT PIE/SAUSAGE ROLL								1X ◎ or 1 med					

		NO	1-3 per month	1-3 per week	4-6 per week	1 per day	2 per day	3 or more per day	Std portion	1X std	½ X std	1½ X std	2X std	3X std
17	MARGERINE/OIL/FAT IN PREPARATION (added to chicken/ beef/fish)								1 tsp					
18	EGGS (WITHOUT FAT): boiled/ poached								1 egg					
19	EGGS (WITH FAT): scrambled/ baked/ omelette								1 egg					
20	BREAD/ROLLS: white								1 roll or 1 med slice					
21	BREAD/ROLLS: brown								1 roll or 1 med slice					
22	BREAD/ROLLS: whole wheat/health/low GI								1 roll or 1 med slice					
23	PROVITA/ RYVITA								2 biscuits					
24	PIZZA: commercial/ homemade								4 slices					
25	BREAKFAST CEREAL: high fibre e.g. All bran, highbulk, muesli, weet-bix, Pro-nutro								½ cup or 1 weet-bix or 1X◎					
26	BREAKFAST CEREAL: other e.g. cornflakes, rice crispies, special K								½ cup or 1X ◎					
27	OATS/OAT BRAN: cooked porridge								½ cup or 1X ◎					
28	MAIZE PORRIDGE: stiff/crumbled								½ cup or 1X ◎					
29	RICE: white								½ cup or 1X ◎					
30	RICE: brown								½ cup or 1X ◎					
31	MEALIE RICE/SAMP								½ cup or 1X ◎					
32	CORN: e.g. mealie, sweetcorn								½ cup/1 med/ 1X ◎					
33	POPCORN								1 cup or 2X ◎					
34	LEGUMES (tinned or dry beans) e.g. baked beans, lentils								½ cup or 1X ◎					
35	PASTA: e.g. Macaroni, spaghetti, noodles								1 cup or 2X ◎					
36	PASTA SAUCE: white or cheese sauce								½ cup					
37	POTATO: boiled, mashed, baked								½ cup or 1X ◎ or 1 med					
38	POTATO: roasted/fried in any fat/oil (includes french-fries/slap chips)								½ cup or 1X ◎ or 1 med					

		NO	1-3 per month	1-3 per week	4-6 per week	1 per day	2 per day	3 or more per day	Std portion	1X std	½ X std	1½ X std	2X std	3X std
39	MARGERINE/OIL/FAT IN PREPARATION: (added to pasta, potato, rice)								1 tsp					
40	FULL CREAM (FRESH/ POWDERED): milk, sour milk (Maas) on porridge/cereal or to drink (excluding milk blends)								½ cup or ½ std glass					
41	LOW FAT (FRESH/ POWDERED): milk, sour milk (Maas) on porridge/cereal or to drink (excluding milk blends)								½ cup or ½ std glass					
42	SKIMMED (FRESH/ POWDERED): milk, sour milk (Maas) on porridge/cereal or to drink (excluding milk blends)								½ cup or ½ std glass					
43	MILK IN TEA/COFFEE: full cream/low fat/skimmed								3 Tbs					
44	COFFEE CREAMER IN TEA/COFFEE: e.g. Cremora								1 tsp					
45	YOGHURT: full cream								½ cup or 125ml serving					
46	YOGHURT: low fat								½ cup or 125ml serving					
47	YOGHURT: fat free								½ cup or 125ml serving					
48	DRINKING YOGHURT								½ cup or 125ml serving					
49	FLAVOURED MILK DRINKS: milo, nesquick, horlicks, sterri-stumpie								1 cup					
50	ICE-CREAMS (DAIRY): ice-cream, milkshakes, smoothies, frozen yoghurt								1 cup					
51	ICE-CREAM (NON-DAIRY): sorbet, milkshakes, smoothies								1 cup					
52	SMOOTHIES (WITHOUT ICE-CREAM): fruit and ice blend only								1 cup					
53	CUSTARD, MILK PUDDINGS								½ cup					

		NO	1-3 per month	1-3 per week	4-6 per week	1 per day	2 per day	3 or more per day	Std portion	1X std	½ X std	1½ X std	2X std	3X std
54	CHEESE: on bread or in dishes (excluding cottage/cream cheese)								1 slice or 1X ▱					
55	COTTAGE CHEESE								1 Tbs					
56	CREAM CHEESE								1 Tbs					
57	REGULAR SALAD DRESSING/ MAYONNAISE								1 Tbs					
58	LOW FAT/LIGHT SALAD DRESSING/ MAYONNAISE								1 Tbs					
59	SAUCES: tomato, chutney, mustard, sweet chilli								1 Tbs					
60	PEANUT BUTTER:								1 Tbs					
61	PEANUTS:								30g or 1X ▱					
62	OTHER NUTS:								30g or 1X ▱					
63	RAISINS:								30g or 1X ▱					
64	OTHER DRIED FRUIT:								30g or 1X ▱					
65	CITRUS FRUIT: e.g. Oranges, grapefruit, naartjies, minolas etc.*								1 med or 1X ●					
66	APPLES/PEARS:								1 med or 1X ●					
67	BANANAS:								1 med or 1X ●					
68	PEACH/APRICOT:*								1 med or 1X ●					
69	WATERMELON/ SWEET MELON:*								1 large slice					
70	GUAVAS:*								1 med or 1X ●					
71	MANGO/ PAW-PAW/ PINEAPPLE:*								1 large slice					
72	GRAPES:*								8-10 grapes					
73	STRAWBERRIES:*								½ cup or 1X ◎					
74	AVOCADO:*								½ med					
75	FRUIT SALAD: tinned/ fresh								½ cup or 1X ◎					

*IF A PARTICULAR FRUIT WAS NOT IN SEASON DURING YOUR LAST MONTH OF TRAINING, YOU CAN MARK THE 'NO' COLUMN.

		NO	1-3 per month	1-3 per week	4-6 per week	1 per day	2 per day	3 or more per day	Std portion	1X std	½ X std	1½ X std	2X std	3X std
76	CABBAGE: (raw/ cooked)								½ cup or 1X◎					
77	BROCCOLI, CAULIFLOWER, BRUSSEL SPROUTS: (raw/cooked)								½ cup or 1X◎					
78	SPINACH/MAROG/ IMIFINO: (raw/ cooked)								½ cup or 1X◎					
79	CARROTS (raw/cooked)								½ cup or 1X◎					
80	BEETROOT:								½ cup or 1X◎					
81	TOMATO: (raw/cooked)								½ cup or 1X◎					
82	ONIONS: (raw/cooked)								½ cup or 1X◎					
83	TOMATO AND ONION: stewed								½ cup or 1X◎					
84	GREEN PEAS: (raw/ cooked)								½ cup or 1X◎					
85	GREEN BEANS: (raw/ cooked)								½ cup or 1X◎					
86	MIXED VEGETABLES: carrots, peas, mealies, beans (in any combination)								½ cup or 1X◎					
87	PUMPKIN, HUBBARD, BUTTERNUT								½ cup or 1X◎					
88	GEM SQUASH, MARROWS, PATTY-PANS								1 med/ ½ cup or 1X◎					
89	MIXED SALAD: lettuce, cucumber, tomato, peppers, onions in any combination								½ cup or 1X◎					
90	SWEET POTATOES:								1 med/ 1/2 cup or 1X◎					
91	MARGERINE/OIL/FAT IN PREPARATION: (added to vegetables)								1 tsp					
92	SUGAR: (white and/or brown added to vegetables)								1 tsp (heaped)					
93	MUFFIN/SCONE (bran, whole wheat only)								1 med or 1X●					
94	MUFFIN/SCONE (all other) chocolate/ poppy-seed etc								1 med or 1X●					
95	RUSKS: any								1 med or 2X▭					

		NO	1-3 per month	1-3 per week	4-6 per week	1 per day	2 per day	3 or more per day	Std portion	1X std	½ X std	1½ X std	2X std	3X std
96	VETKOEK, DOUGHNUTS, SAMOOSAS, KOEKSISTER								1 med or 1X◎					
97	SALTY CRISPS: Simba chips, Lays etc.								1 small packet					
98	SALTY BISCUITS: Salty Crax etc.								2 biscuits					
99	ICED CAKES, TARTS, COOKIES with filling								1 med piece or 2 cookies or 1X◎					
100	CAKE: commercial, homemade cakes/ tarts								1 med piece or 2 cookies or 1X◎					
101	JAM/SYRUP/HONEY								1 tsp					
102	SWEETS: boiled sweets, jellies, toffee								4 sweets					
103	SWEETS: super C's, energy sweets								4 sweets					
104	CHOCOLATE								1 small bar/2-3 blocks					
105	ENERGY/HEALTH/ BREAKFAST BARS								1 small bar					
106	TEA NORMAL (excluding rooibos)								1 cup					
107	TEA ROOIBOS								1 cup					
108	COFFEE: instant/ground (excluding decaffeinated)								1 cup					
109	COFFEE: decaffeinated								1 cup					
110	SUGAR: (white & brown in tea/coffee)								1 tsp (heaped)					
111	ORANGE/GUAVA JUICE								1 std glass					
112	OTHER FRUIT JUICE								1 std glass					
113	MIXED JUICES/ CORDIALS : e.g. Oros, powdered mixes etc								1 std glass					
114	ENERGY DRINKS: Energade, Powerade etc								500ml					
115	CAFIENATTED ENERGY DRINKS: Red-Bull, Play etc.								250ml or 1 Tin					
116	WINE: red/white								1 wine glass or ± 200ml					

		NO	1-3 per month	1-3 per week	4-6 per week	1 per day	2 per day	3 or more per day	Std portion	1X std	½ X std	1½ X std	2X std	3X std
117	PORT/SHERRY/ LIQUEUR etc.								1 sherry glass					
118	REGULAR BEER/ CIDERS/COOLERS								340ml (1X can/ bottle)					
119	LIGHT BEER/ CIDERS/COOLERS								340ml (1X can/ bottle)					
120	SPIRITS: brandy, whiskey,rum,vodka,gin								1 tot/ shot					
121	NON-DIET FIZZY SOFT DRINKS: coke, fanta								1 std glass/can					
122	DIET FIZZY DRINKS: e.g. Coke zero, coke light, sprite zero etc.								1 std glass/can					
123	OTHER SOFT DRINKS OR FIZZY FRUIT SQUASH								1 std glass/can					

APPENDIX I

Quark RMR (COSMED) Alcohol Burns

1. 29 July 2015

t	VE	VO2	VCO2	FeO2	FeCO2	CO2 volume	R
hh:mm:ss	l/min	ml/min	ml/min	%	%	ml	---
00:00:01	40.12658	9.2748	0	20.87186	0.069353	0	0
00:00:06	40.17959	8.578101	0	20.87401	0.069261	0	0
00:00:11	40.2326	8.969994	0	20.87286	0.06917	0	0
00:00:16	40.2326	8.969994	0.814266	20.87286	0.072464	0.067853	0.090777
00:00:21	40.12658	10.36339	0.875228	20.86856	0.072655	0.072933	0.084454
00:00:26	42.51191	13.84688	3.857968	20.86035	0.081047	0.321485	0.278616
00:00:31	43.30702	15.37091	3.40076	20.85679	0.079559	0.283385	0.221246
00:00:36	43.25402	13.89043	2.342649	20.86091	0.076593	0.195213	0.168652
00:00:41	43.20101	16.76431	3.461721	20.85276	0.079755	0.288465	0.206494
00:00:46	43.13475	11.92008	3.499822	20.86636	0.079877	0.29164	0.293607
00:00:51	43.13475	11.92008	1.322639	20.86636	0.073733	0.110215	0.110959
00:00:56	43.20101	14.58713	1.284538	20.8589	0.07362	0.107041	0.08806
00:01:00	43.13475	17.36304	3.499822	20.851	0.079877	0.29164	0.201567
00:01:05	43.13475	14.09726	1.322639	20.86022	0.073733	0.110215	0.093822
00:01:10	42.90947	18.96326	4.717956	20.8462	0.083385	0.393147	0.248794
00:01:15	43.01548	34.98733	11.18854	20.80099	0.101664	0.932341	0.319788
00:01:20	42.90947	34.20355	4.717956	20.80296	0.083385	0.393147	0.137938
00:01:25	43.13475	30.42613	6.765597	20.81413	0.089094	0.563777	0.222361
00:01:30	43.13475	23.89458	5.677005	20.83257	0.086022	0.473065	0.237585
00:01:35	43.13475	20.62881	3.499822	20.84178	0.079877	0.29164	0.169657
00:01:40	43.13475	24.98318	6.765597	20.82949	0.089094	0.563777	0.270806
00:01:45	43.20101	23.29586	4.550313	20.83436	0.082822	0.379178	0.195327
00:01:50	43.13475	19.54022	3.499822	20.84485	0.079877	0.29164	0.179109
00:01:55	43.13475	22.80599	6.765597	20.83564	0.089094	0.563777	0.296659
00:02:00	43.13475	41.31205	18.7401	20.78341	0.122888	1.561613	0.453623
00:02:05	43.25402	28.04212	6.697015	20.82108	0.088848	0.558062	0.23882
00:02:10	43.20101	27.65023	7.816087	20.82209	0.092025	0.651315	0.282677
00:02:15	43.13475	20.62881	4.588413	20.84178	0.082949	0.382352	0.222427
00:02:20	41.27949	133.1892	81.85664	20.50722	0.311396	6.821114	0.614589
00:02:25	39.34472	564.119	370.3574	19.1546	1.215898	30.86188	0.656523
00:02:30	39.23871	665.6629	445.5311	18.83485	1.452212	37.12611	0.669304
00:02:35	39.07969	704.7651	473.926	18.70465	1.546287	39.49225	0.672459
00:02:40	39.01343	665.0859	446.7493	18.82473	1.463995	37.22762	0.671717

00:02:45	39.11944	647.3636	433.6252	18.8855	1.419377	36.13399	0.669833
00:02:50	39.06643	613.2254	406.4409	18.98915	1.336499	33.86872	0.662792
00:02:55	39.01343	634.6053	422.8003	18.91984	1.389266	35.23195	0.666241
00:03:00	39.02668	656.4751	435.8557	18.85229	1.429542	36.31986	0.663933
00:03:05	38.96042	686.4658	457.6657	18.7551	1.5	38.13728	0.666698
00:03:10	39.02668	658.6523	435.8557	18.8455	1.429542	36.31986	0.661739
00:03:15	38.8014	667.8727	445.7826	18.80464	1.468579	37.14706	0.667466
00:03:20	39.01343	649.8456	431.509	18.87228	1.41644	35.95764	0.664018
00:03:25	38.96042	703.8833	469.6402	18.70068	1.537415	39.13512	0.667213
00:03:30	38.64238	704.7977	469.8231	18.6797	1.550069	39.15036	0.666607
00:03:35	38.8014	733.1882	489.3263	18.59973	1.605191	40.77556	0.667395
00:03:40	38.8014	660.2526	433.8081	18.82855	1.431011	36.14923	0.657034
00:03:45	38.74839	672.9238	445.8131	18.78591	1.470588	37.1496	0.662502
00:03:50	38.76164	732.8943	487.1719	18.59829	1.6	40.59604	0.664723
00:03:55	38.40384	712.8315	471.0488	18.64044	1.563147	39.2525	0.660814
00:04:00	38.64238	698.2662	461.1143	18.70027	1.522634	38.42466	0.66037
00:04:05	38.70863	724.8822	479.5823	18.62034	1.578227	39.96359	0.6616
00:04:10	38.64238	725.481	480.709	18.61454	1.584362	40.05748	0.662607
00:04:15	38.58937	768.6327	510.1314	18.47527	1.679258	42.50925	0.663687
00:04:20	38.54961	750.9213	498.1798	18.5287	1.643176	41.51332	0.663425
00:04:25	38.4436	741.4288	490.6206	18.55222	1.623578	40.88341	0.661723
00:04:30	38.64238	717.8608	473.0888	18.63855	1.560357	39.42249	0.659026
00:04:35	38.53636	711.6341	469.884	18.65199	1.554333	39.15543	0.660289
00:04:40	38.43035	741.3309	490.6282	18.55172	1.624138	40.88405	0.661821
00:04:45	38.43035	741.3309	489.5396	18.55172	1.62069	40.79334	0.660352
00:04:50	38.37734	771.4195	511.3419	18.45304	1.691989	42.61012	0.662858
00:04:55	38.4436	815.4531	539.6072	18.31782	1.778697	44.96547	0.661727
00:05:00	38.37734	776.8625	512.4305	18.43577	1.695442	42.70084	0.659615
00:05:05	38.28458	773.9995	511.3953	18.43891	1.696089	42.61457	0.660718
00:05:10	38.45685	740.4382	487.3472	18.55617	1.612681	40.61064	0.658188
00:05:15	38.24482	784.5915	519.0383	18.40263	1.722107	43.25146	0.66154
00:05:20	38.19181	788.554	521.2459	18.38654	1.731437	43.43542	0.661015
00:05:25	38.19181	783.111	516.8916	18.40389	1.717557	43.07258	0.660049
00:05:30	38.31108	821.0049	542.9492	18.29125	1.795227	45.24396	0.661323
00:05:35	38.13881	783.8077	515.8335	18.39819	1.71647	42.9844	0.658112
00:05:40	38.33758	756.9739	497.2131	18.49637	1.648807	41.43277	0.656843
00:05:45	38.17856	755.7982	497.3045	18.49011	1.655675	41.44039	0.657986
00:05:50	38.05929	806.0803	533.2967	18.32173	1.775766	44.43961	0.661592
00:05:55	38.27132	741.2438	486.3653	18.54224	1.617036	40.52882	0.656148
00:06:00	37.97978	793.5179	523.545	18.35659	1.748081	43.62701	0.659777
00:06:05	38.23157	817.1513	536.4634	18.29809	1.778163	44.70349	0.656504
00:06:10	38.21832	761.5351	500.5475	18.47434	1.664355	41.71062	0.657287
00:06:15	38.16531	779.6493	514.7296	18.41319	1.711806	42.89242	0.660207
00:06:20	38.07255	784.4064	516.9602	18.39192	1.722938	43.07829	0.659046

00:06:25	38.12555	795.6842	524.5498	18.3594	1.744873	43.71074	0.659244
00:06:30	38.07255	786.5836	516.9602	18.38496	1.722938	43.07829	0.657222
00:06:35	38.07255	756.103	496.2769	18.48242	1.656805	41.35476	0.656361
00:06:40	38.12555	801.1272	527.8156	18.34202	1.755301	43.98287	0.658841
00:06:45	38.0858	774.7071	509.3324	18.4238	1.697982	42.44267	0.657452
00:06:50	37.96653	790.1542	520.2869	18.36649	1.73822	43.35551	0.658462
00:06:55	38.03279	779.7581	511.5401	18.40418	1.707317	42.62663	0.656024
00:07:00	38.01954	756.7997	496.3074	18.47682	1.659115	41.3573	0.655798
00:07:05	37.99303	781.6414	511.5629	18.39554	1.709104	42.62854	0.654473
00:07:10	37.86052	741.4724	485.5129	18.51593	1.631082	40.45779	0.654796
00:07:15	37.83401	764.1368	501.857	18.44133	1.684764	41.81975	0.656763
00:07:20	38.01954	756.7997	496.3074	18.47682	1.659115	41.3573	0.655798
00:07:25	37.87377	789.4684	518.1631	18.36249	1.735479	43.17853	0.656344
00:07:30	37.71475	783.9383	512.8115	18.36964	1.725228	42.73259	0.654148
00:07:35	37.92678	751.7596	489.8292	18.48707	1.642208	40.81747	0.651577
00:07:40	37.83401	777.1999	508.3886	18.3993	1.705779	42.36402	0.654128
00:07:45	37.92678	774.62	508.3352	18.4137	1.701607	42.35958	0.656238
00:07:50	37.92678	757.2025	495.2721	18.4696	1.659679	41.27103	0.654081
00:07:55	37.96653	752.0535	491.9835	18.48866	1.647469	40.99699	0.654187
00:08:00	37.76775	715.749	464.883	18.59298	1.568421	38.7387	0.649506
00:08:05	37.86052	749.0925	488.7787	18.49142	1.641582	40.72993	0.652494
00:08:10	37.83401	746.7194	488.7939	18.49737	1.642732	40.7312	0.654589
00:08:15	37.71475	788.2927	518.2545	18.35559	1.742797	43.18615	0.657439
00:08:20	37.82076	748.7986	489.8902	18.48984	1.646811	40.82255	0.654235
00:08:25	37.91352	752.7502	492.014	18.48305	1.649773	40.99953	0.653622
00:08:30	37.71475	734.9517	479.0652	18.52776	1.616304	39.9205	0.651832
00:08:35	37.76775	701.5973	455.0857	18.6386	1.536842	37.92229	0.648642
00:08:40	37.86052	740.3838	483.3357	18.51943	1.624081	40.27637	0.652818
00:08:45	37.82076	740.0899	484.4472	18.51787	1.629292	40.36898	0.654579
00:08:50	37.96653	698.7125	453.8828	18.65969	1.525305	37.82205	0.649599
00:08:55	37.7545	710.208	461.6249	18.61004	1.558442	38.4672	0.649985
00:09:00	37.7545	705.8537	458.3591	18.62408	1.547912	38.19506	0.649368
00:09:05	37.728	708.9235	459.4629	18.61257	1.552511	38.28705	0.648114
00:09:10	37.82076	723.761	465.9411	18.57043	1.569727	38.82687	0.643778
00:09:15	37.71475	708.8255	456.2048	18.61209	1.542516	38.01554	0.643607
00:09:19	37.7545	702.5879	458.3591	18.63461	1.547912	38.19506	0.652387
00:09:24	37.87377	688.2294	446.316	18.68789	1.504549	37.19151	0.648499
00:09:29	37.70149	706.5504	460.5668	18.61863	1.557118	38.37903	0.651853
00:09:34	37.76775	697.2429	450.7313	18.65263	1.522807	37.55944	0.646448
00:09:39	37.71475	667.459	428.99	18.74561	1.454673	35.74774	0.642721
00:09:44	37.86052	669.6253	429.9948	18.74694	1.452573	35.83146	0.642142
00:09:49	37.7545	641.6268	411.5497	18.83117	1.396981	34.29443	0.641416
00:09:54	37.82076	597.4844	377.7652	18.97687	1.285915	31.47918	0.63226
00:09:59	37.67499	609.4698	387.6464	18.93071	1.322547	32.30257	0.636039

00:10:04	37.86052	590.1581	371.2108	19.00245	1.263563	30.933	0.629002
00:10:09	37.86052	410.5405	247.1114	19.57998	0.864543	20.59179	0.601917
00:10:14	38.33758	85.31292	29.11874	20.6291	0.162461	2.426464	0.341317
00:10:19	38.28458	46.82032	4.11161	20.75112	0.083074	0.34262	0.087817
00:10:24	38.40384	45.5249	2.954437	20.75569	0.079365	0.246193	0.064897
00:10:29	38.33758	42.85785	2.992538	20.76391	0.079502	0.249368	0.069825
00:10:34	38.33758	42.85785	2.992538	20.76391	0.079502	0.249368	0.069825
00:10:39	38.33758	41.76926	2.992538	20.76737	0.079502	0.249368	0.071645
					Totals	**3753.41**	**0.66**
					% Error	**-1.74**	**-1.37**

2. 24 August 2015

t	VE	VO2	VCO2	FeO2	FeCO2	CO2 volume	R
hh:mm:ss	l/min	ml/min	ml/min	%	%	ml	---
00:00:01	41.29013	0.056253	1.454994	20.91983	0.054296	0.121245	25.86538
00:00:06	41.35607	0.553871	0.34617	20.91837	0.05102	0.028846	0.625
00:00:11	41.29013	1.138032	1.454994	20.91664	0.054296	0.121245	1.278517
00:00:16	41.23738	0.739937	1.476629	20.91781	0.054365	0.123048	1.995614
00:00:21	41.21101	2.704449	2.569227	20.912	0.0576	0.214094	0.95
00:00:26	40.68351	3.050619	2.785583	20.91086	0.058347	0.232123	0.913121
00:00:31	40.68351	3.050619	1.703803	20.91086	0.055105	0.141978	0.558511
00:00:36	40.11644	3.098217	1.936386	20.91059	0.055884	0.161359	0.625
00:00:41	40.05051	1.518819	1.96343	20.91538	0.055976	0.163613	1.292735
00:00:46	39.99776	2.202504	3.066846	20.91329	0.059347	0.25556	1.392436
00:00:51	39.99776	2.202504	3.066846	20.91329	0.059347	0.25556	1.392436
00:00:56	39.945	2.886188	3.088481	20.91119	0.059426	0.257363	1.07009
00:01:01	39.99776	2.202504	1.985066	20.91329	0.05605	0.165416	0.901277
00:01:06	39.87907	1.30679	2.033746	20.91601	0.056217	0.169472	1.556291
00:01:11	39.945	2.886188	2.006701	20.91119	0.056124	0.167218	0.695277
00:01:16	39.99776	2.202504	3.066846	20.91329	0.059347	0.25556	1.392436
00:01:21	39.945	2.886188	3.088481	20.91119	0.059426	0.257363	1.07009
00:01:26	39.945	3.967968	3.088481	20.90789	0.059426	0.257363	0.778353
00:01:31	39.945	3.967968	3.088481	20.90789	0.059426	0.257363	0.778353
00:01:36	40.05051	1.518819	1.96343	20.91538	0.055976	0.163613	1.292735
00:01:41	39.945	2.886188	3.088481	20.91119	0.059426	0.257363	1.07009
00:01:46	39.99776	2.202504	3.066846	20.91329	0.059347	0.25556	1.392436
00:01:51	39.87907	1.30679	3.115526	20.91601	0.059524	0.259617	2.384106
00:01:56	39.945	3.967968	3.088481	20.90789	0.059426	0.257363	0.778353

00:02:01	39.99776	3.284283	3.066846	20.90999	0.059347	0.25556	0.933794
00:02:06	41.31651	4.582419	2.525956	20.90648	0.057453	0.210488	0.551228
00:02:11	43.13639	3.171778	2.861307	20.91104	0.058086	0.238433	0.902115
00:02:16	43.13639	5.335338	3.943087	20.90492	0.061143	0.328577	0.739051
00:02:21	43.20232	11.24185	7.161382	20.88828	0.070208	0.596758	0.637028
00:02:26	43.08364	4.937243	3.964723	20.90603	0.061218	0.33038	0.803024
00:02:31	43.03089	3.457368	2.904579	20.91021	0.058229	0.242039	0.840113
00:02:36	43.08364	3.855463	2.882943	20.90909	0.058157	0.240236	0.747755
00:02:41	43.08364	4.937243	3.964723	20.90603	0.061218	0.33038	0.803024
00:02:46	43.13639	17.23491	13.6791	20.87129	0.088658	1.13988	0.793686
00:02:51	43.13639	8.580677	6.106647	20.89575	0.067258	0.508867	0.711674
00:02:56	42.97814	7.386392	4.007994	20.89905	0.061369	0.333986	0.542619
00:03:01	40.05051	5.845938	3.04521	20.90221	0.059269	0.253757	0.52091
00:03:06	40.05051	3.682378	3.04521	20.90879	0.059269	0.253757	0.826968
00:03:11	39.99776	4.366063	3.066846	20.90669	0.059347	0.25556	0.702428
00:03:16	39.99776	4.366063	3.066846	20.90669	0.059347	0.25556	0.702428
00:03:21	40.05051	2.600598	3.04521	20.91208	0.059269	0.253757	1.170965
00:03:26	39.945	5.049748	3.088481	20.90459	0.059426	0.257363	0.611611
00:03:31	39.99776	4.366063	3.066846	20.90669	0.059347	0.25556	0.702428
00:03:36	40.11644	5.261777	3.018165	20.90401	0.059172	0.251504	0.573602
00:03:41	39.99776	3.284283	3.066846	20.90999	0.059347	0.25556	0.933794
00:03:45	40.05051	2.600598	3.04521	20.91208	0.059269	0.253757	1.170965
00:03:50	40.05051	2.600598	3.04521	20.91208	0.059269	0.253757	1.170965
00:03:55	39.99776	3.284283	3.066846	20.90999	0.059347	0.25556	0.933794
00:04:00	40.05051	3.682378	3.04521	20.90879	0.059269	0.253757	0.826968
00:04:05	40.05051	3.682378	3.04521	20.90879	0.059269	0.253757	0.826968
00:04:10	40.05051	4.764158	3.04521	20.9055	0.059269	0.253757	0.639192
00:04:15	40.05051	4.764158	3.04521	20.9055	0.059269	0.253757	0.639192
00:04:20	40.65713	8.26047	4.95996	20.89523	0.064872	0.413313	0.600445
00:04:25	43.25507	7.312831	3.894407	20.89939	0.060976	0.324521	0.532544
00:04:30	43.30782	5.547366	3.872771	20.90438	0.060901	0.322718	0.698128
00:04:35	43.20232	6.914736	3.916043	20.90049	0.06105	0.326324	0.566333
00:04:40	43.25507	6.231051	3.894407	20.90244	0.060976	0.324521	0.625
00:04:45	42.95176	26.65938	17.00017	20.84434	0.09825	1.416624	0.637681
00:04:50	39.45707	475.1869	323.4954	19.45187	1.049465	26.95687	0.680775
00:04:55	39.33838	597.6141	412.25	19.06805	1.327523	34.35279	0.689827
00:05:00	39.15375	662.2093	461.0058	18.8582	1.485349	38.41562	0.696163
00:05:05	39.14057	666.4369	463.1748	18.84434	1.492588	38.59636	0.695002
00:05:10	39.28563	649.1414	450.134	18.90567	1.446794	37.50966	0.69343
00:05:15	39.28563	639.4054	441.4797	18.93588	1.41994	36.7885	0.690454
00:05:20	39.2065	682.0794	473.9656	18.79919	1.523713	39.49555	0.694883
00:05:25	39.16694	659.0635	455.5915	18.86869	1.468013	37.96444	0.691271
00:05:30	39.11419	662.9925	457.7767	18.85367	1.476736	38.14653	0.69047
00:05:35	39.37794	620.63	424.1334	18.99866	1.363027	35.34303	0.683392

00:05:40	39.00869	658.951	452.4111	18.86072	1.463827	37.69942	0.686563
00:05:45	39.101	659.6476	453.455	18.86341	1.463744	37.78641	0.68742
00:05:50	38.96913	718.1503	496.7803	18.67343	1.604061	41.3967	0.69175
00:05:55	39.00869	702.2222	481.6191	18.72549	1.555105	40.13332	0.68585
00:06:00	39.15375	654.6368	446.9427	18.88178	1.441563	37.24374	0.682734
00:06:05	38.86363	755.2164	518.4592	18.55107	1.676281	43.2032	0.686504
00:06:10	39.16694	670.9631	457.7551	18.83165	1.474747	38.14473	0.682236
00:06:15	39.02188	703.4035	482.6955	18.72254	1.557959	40.22302	0.686229
00:06:20	39.00869	692.4861	475.1285	18.75592	1.534821	39.59246	0.68612
00:06:25	38.63944	755.688	518.5511	18.53584	1.686007	43.21086	0.686197
00:06:30	38.82407	739.7729	506.5758	18.59715	1.640625	42.21296	0.684772
00:06:35	38.86363	702.2092	479.5151	18.71734	1.554123	39.95799	0.682866
00:06:40	38.7845	727.5747	496.856	18.63312	1.611697	41.40301	0.682893
00:06:45	38.82407	671.6208	458.9775	18.81114	1.491168	38.2466	0.683388
00:06:50	38.83725	753.9356	516.3064	18.55348	1.670628	43.02381	0.684815
00:06:55	38.679	737.5964	504.4717	18.59529	1.639959	42.03763	0.68394
00:07:00	38.7845	769.7642	524.9823	18.50051	1.700102	43.74677	0.682004
00:07:05	38.63944	787.0597	536.9414	18.43686	1.744027	44.74332	0.682212
00:07:10	38.679	789.5218	538.0069	18.43164	1.745653	44.83212	0.681434
00:07:15	38.69219	776.64	529.3473	18.47307	1.717791	44.11051	0.681586
00:07:20	38.59988	777.0251	529.3851	18.46601	1.7219	44.11366	0.681297
00:07:25	38.69219	745.2683	507.7117	18.57192	1.649625	42.30761	0.681247
00:07:30	38.59988	840.8501	572.6563	18.26443	1.858558	47.71945	0.681045
00:07:35	38.74494	784.6105	533.6528	18.45133	1.729067	44.46928	0.68015
00:07:40	38.61307	807.4144	547.77	18.3709	1.779372	45.64567	0.678425
00:07:45	38.44163	845.0647	573.803	18.24014	1.86964	47.815	0.679005
00:07:50	38.53394	837.1071	570.5198	18.27173	1.854894	47.54142	0.681537
00:07:55	38.50757	804.4547	547.8133	18.37329	1.784247	45.64928	0.680975
00:08:00	38.38888	880.3653	598.7056	18.12436	1.95122	49.89014	0.680065
00:08:05	38.53394	800.3266	542.3935	18.38809	1.765914	45.19765	0.677715
00:08:10	38.34931	807.5875	548.9599	18.35282	1.795048	45.74483	0.679753
00:08:15	38.48119	791.2743	538.0881	18.4133	1.754626	44.83888	0.680027
00:08:20	38.59988	788.9246	536.9576	18.42843	1.745815	44.74468	0.68062
00:08:25	38.56032	791.8714	539.1374	18.41655	1.754446	44.92632	0.68084
00:08:30	38.48119	797.7649	541.3334	18.39273	1.764907	45.10931	0.678563
00:08:35	38.50757	801.2093	543.4861	18.38356	1.770548	45.2887	0.678332
00:08:40	38.40206	817.7216	555.429	18.32418	1.813187	46.2839	0.67924
00:08:45	38.3625	805.5235	546.791	18.36026	1.787556	45.56409	0.678802
00:08:50	38.19106	837.7648	566.3333	18.24586	1.857735	47.19256	0.676005
00:08:55	38.54713	805.835	545.6335	18.37154	1.775573	45.46764	0.677103
00:09:00	38.30975	824.5974	557.6304	18.29604	1.824441	46.46734	0.676246
00:09:05	38.38888	807.8861	546.7802	18.35452	1.786328	45.56319	0.676804
00:09:10	38.40206	818.8034	554.3472	18.32074	1.809753	46.19375	0.677021
00:09:15	38.34931	824.896	559.7777	18.2978	1.829436	46.64628	0.678604

00:09:20	38.34931	815.16	553.2871	18.32875	1.808803	46.10541	0.678747
00:09:25	38.34931	778.3795	527.3243	18.44567	1.726272	43.94194	0.677464
00:09:30	38.52075	758.0377	513.1909	18.52105	1.674084	42.7642	0.676999
00:09:35	38.52075	814.2902	549.9714	18.34303	1.790483	45.82912	0.6754
00:09:40	38.257	801.4819	541.4253	18.36608	1.77525	45.11697	0.67553
00:09:45	38.11194	819.8592	554.4662	18.29758	1.823529	46.20367	0.676294
00:09:50	38.40206	822.0487	556.5108	18.31044	1.816621	46.37404	0.67698
00:09:55	38.45481	810.5473	547.8349	18.35048	1.786694	45.65108	0.675883
00:10:00	38.24381	801.3824	541.4307	18.36552	1.775862	45.11742	0.675621
00:10:05	38.20425	779.4483	525.2203	18.43286	1.725923	43.76661	0.673836
00:10:10	38.24381	790.5646	533.8583	18.4	1.751724	44.48641	0.675287
00:10:15	38.30975	800.7983	541.4037	18.37177	1.772806	45.11517	0.67608
00:10:20	38.257	744.1476	503.5631	18.54878	1.654602	41.96191	0.676698
00:10:25	38.21744	762.2393	515.4789	18.48861	1.694272	42.95485	0.676269
00:10:30	38.257	762.5379	514.3808	18.49018	1.689073	42.86336	0.674564
00:10:35	38.257	758.2108	513.2991	18.50396	1.685626	42.77321	0.676987
00:10:40	38.17788	742.4687	500.3502	18.54922	1.647668	41.69418	0.673901
00:10:45	38.19106	751.2225	507.9172	18.5221	1.671271	42.32474	0.676121
00:10:50	38.05919	766.4539	517.7073	18.465	1.708247	43.14055	0.675458
00:10:55	38.23063	753.6846	507.901	18.51673	1.669541	42.32339	0.673891
00:11:00	38.17788	737.0598	497.1048	18.56649	1.637306	41.42375	0.674443
00:11:05	38.27019	724.7751	488.4127	18.6113	1.605789	40.69943	0.673882
00:11:10	38.27019	742.0836	500.3123	18.55617	1.643694	41.69102	0.674199
00:11:15	38.05919	730.7552	494.9899	18.57935	1.635482	41.24751	0.677368
00:11:20	37.96688	727.895	490.7007	18.58284	1.625564	40.89009	0.674137
00:11:25	38.00644	722.7846	487.4391	18.60167	1.613463	40.6183	0.674391
00:11:30	38.21744	721.1317	485.189	18.61974	1.597654	40.4308	0.672816
00:11:35	37.92731	711.3697	478.8173	18.63352	1.589013	39.89985	0.673092
00:11:40	38.19106	712.2784	477.6274	18.64641	1.574586	39.80069	0.670563
00:11:45	38.08556	684.4377	459.2804	18.72922	1.520083	38.27184	0.671033
00:11:50	38.07238	716.7916	483.085	18.62487	1.596813	40.25547	0.673955
00:11:55	37.98006	698.7864	470.1415	18.67708	1.559028	39.17689	0.672797
00:12:00	37.91413	686.3892	460.4325	18.71304	1.530435	38.36784	0.670804
00:12:04	38.05919	693.9747	464.7001	18.69716	1.538462	38.72346	0.669621
00:12:09	38.17788	653.7628	435.4434	18.83247	1.440415	36.2855	0.666057
00:12:14	38.05919	624.7408	416.02	18.91892	1.382536	34.66695	0.665908
00:12:19	37.92731	665.9349	446.3639	18.77955	1.484701	37.19551	0.670282
00:12:24	38.00644	567.0083	369.5251	19.10132	1.235253	30.79253	0.65171
00:12:29	38.11194	540.76	348.928	19.19031	1.16609	29.07617	0.645255
00:12:34	38.08556	371.8034	234.2702	19.72992	0.799861	19.52174	0.630092
00:12:39	38.70538	86.56401	38.21387	20.64736	0.170358	3.184362	0.441452
00:12:44	38.63944	36.30453	7.951081	20.80546	0.075085	0.662564	0.219011
00:12:49	38.63944	33.05919	6.869301	20.8157	0.071672	0.572419	0.207788
00:12:54	38.75813	35.03668	6.820621	20.8098	0.071453	0.568362	0.194671

00:12:59	38.75813	35.03668	5.738841	20.8098	0.06805	0.478218	0.163795
00:13:04	38.74494	34.93716	5.74425	20.81007	0.068074	0.478668	0.164417
00:13:09	38.70538	35.72037	6.842257	20.8075	0.07155	0.570165	0.191551
00:13:14	38.79769	34.25347	6.804394	20.81237	0.07138	0.56701	0.198648
00:13:19	38.79769	34.25347	6.804394	20.81237	0.07138	0.56701	0.198648
00:13:24	38.85044	34.65157	6.782759	20.81127	0.071283	0.565207	0.195742
00:13:29	38.90319	33.96788	5.679344	20.81356	0.067797	0.47326	0.167197
00:13:34	38.94275	34.26645	5.663117	20.81273	0.067728	0.471908	0.165267
00:13:39	38.94275	34.26645	5.663117	20.81273	0.067728	0.471908	0.165267
00:13:44	38.98232	34.56503	6.72867	20.81191	0.071042	0.5607	0.194667
00:13:49	38.9955	34.66455	6.723261	20.81163	0.071018	0.560249	0.193952
00:13:54	38.98232	33.48325	6.72867	20.81529	0.071042	0.5607	0.200956
00:13:59	39.03507	34.96312	5.625255	20.81081	0.067568	0.468752	0.160891
00:14:04	38.95594	33.2842	5.657708	20.81584	0.067705	0.471457	0.169982
00:14:09	38.9955	34.66455	5.641481	20.81163	0.067636	0.470105	0.162745
00:14:14	39.02188	32.70004	6.712443	20.81784	0.07097	0.559348	0.205273
00:14:19	39.03507	33.88134	6.707034	20.81419	0.070946	0.558897	0.197957
00:14:24	38.9955	33.58277	6.723261	20.81502	0.071018	0.560249	0.2002
					Totals	**3837.052**	**0.67956**
					% Error	**0.45**	**1.88**

3. 23 October 2015

t	VE	VO2	VCO2	FeO2	FeCO2	CO2 volume	R
hh:mm:ss	**l/min**	**ml/min**	**ml/min**	**%**	**%**	**ml**	**---**
00:00:02	40.55947	14.39346	0.586631	20.87674	0.051763	0.048884	0.040757
00:00:07	40.55947	15.46985	1.66302	20.8735	0.054998	0.138579	0.107501
00:00:12	40.50698	16.15013	0.608159	20.8714	0.05183	0.050678	0.037657
00:00:17	40.44137	13.50221	0.635069	20.8793	0.051914	0.05292	0.047034
00:00:22	40.50698	13.99735	0	20.87787	0.048591	0	0
00:00:27	40.50698	15.07374	0	20.87464	0.048591	0	0
00:00:32	40.50698	15.07374	0	20.87464	0.048591	0	0
00:00:37	40.50698	15.07374	0.608159	20.87464	0.05183	0.050678	0.040346
00:00:42	40.55947	29.46289	12.4269	20.83145	0.08735	1.035534	0.421781
00:00:47	40.50698	19.37929	2.760935	20.86168	0.058309	0.230069	0.142468
00:00:52	40.61195	14.78957	0.565104	20.87561	0.051696	0.04709	0.03821
00:00:57	40.50698	18.3029	2.760935	20.86492	0.058309	0.230069	0.150847
00:01:02	40.55947	14.39346	0	20.87674	0.048528	0	0
00:01:07	40.50698	26.91401	8.142876	20.839	0.074506	0.678546	0.302552

00:01:12	40.50698	23.68484	5.990099	20.84872	0.068027	0.499155	0.252909
00:01:17	40.50698	19.37929	2.760935	20.86168	0.058309	0.230069	0.142468
00:01:22	40.50698	32.29595	11.37204	20.82281	0.084224	0.947632	0.35212
00:01:27	40.50698	39.83066	16.75398	20.80013	0.100421	1.396109	0.42063
00:01:32	40.50698	21.53207	3.837323	20.8552	0.061548	0.319764	0.178214
00:01:37	40.55947	34.84483	13.50329	20.81527	0.090586	1.125229	0.387526
00:01:42	40.55947	50.99065	25.34356	20.76674	0.126173	2.111879	0.497024
00:01:47	40.50698	31.21956	10.29565	20.82604	0.080985	0.857937	0.329782
00:01:52	40.55947	19.7754	2.739408	20.86056	0.058234	0.228275	0.138526
00:01:57	40.55947	18.69901	1.66302	20.8638	0.054998	0.138579	0.088936
00:02:02	40.44137	14.5786	0.635069	20.87605	0.051914	0.05292	0.043562
00:02:07	40.55947	23.00457	4.892184	20.85086	0.064704	0.407666	0.212661
00:02:12	40.44137	21.03693	4.940621	20.85659	0.064893	0.411702	0.234855
00:02:17	40.55947	16.54624	0.586631	20.87027	0.051763	0.048884	0.035454
00:02:22	40.50698	34.44872	12.44843	20.81633	0.087464	1.037327	0.361361
00:02:27	40.55947	44.53233	19.96162	20.78615	0.109997	1.663401	0.44825
00:02:32	40.55947	41.30316	18.88523	20.79586	0.106762	1.573706	0.457234
00:02:37	40.50698	66.74036	33.97619	20.71914	0.152251	2.831236	0.50908
00:02:42	40.55947	62.83092	30.7255	20.73115	0.142349	2.560356	0.489019
00:02:47	40.50698	81.8098	44.74007	20.67379	0.184645	3.72819	0.546879
00:02:52	38.44686	221.2623	138.1544	20.21843	0.488055	11.51241	0.624392
00:02:57	36.7279	548.4283	362.7482	19.09968	1.254019	30.2278	0.661432
00:03:02	36.83288	583.665	389.6148	18.98824	1.339508	32.4666	0.667532
00:03:07	36.34737	641.3551	433.9458	18.76895	1.505415	36.16071	0.676608
00:03:12	36.62293	604.6847	405.8467	18.9072	1.400932	33.81921	0.671171
00:03:17	36.57044	609.6705	406.9446	18.88769	1.40653	33.9107	0.667483
00:03:22	36.55732	598.8076	399.4153	18.92319	1.38191	33.28328	0.667018
00:03:27	36.67542	600.7752	400.4433	18.92308	1.381038	33.36894	0.666544
00:03:32	36.55732	599.884	399.4153	18.9196	1.38191	33.28328	0.665821
00:03:37	36.51795	635.1077	424.1884	18.79986	1.466044	35.34762	0.6679
00:03:42	36.62293	621.9069	413.3814	18.84987	1.426012	34.44708	0.6647
00:03:47	36.39986	626.6817	416.7021	18.8212	1.445566	34.72379	0.664934
00:03:52	36.39986	627.7581	416.7021	18.81759	1.445566	34.72379	0.663794
00:03:57	36.51795	624.3438	412.3481	18.83579	1.426518	34.36097	0.66045
00:04:02	36.37361	599.574	394.1087	18.91053	1.370851	32.84108	0.657315
00:04:07	36.34737	656.4245	437.175	18.71841	1.516245	36.42979	0.665994
00:04:12	36.71478	675.3431	448.8646	18.67763	1.540386	37.40389	0.664647
00:04:17	36.81976	685.8228	457.4326	18.64932	1.564505	38.11786	0.666984
00:04:22	37.59394	626.0058	409.754	18.89005	1.378709	34.1448	0.654553
00:04:27	37.62019	698.3218	463.5627	18.65713	1.552145	38.62868	0.663824
00:04:32	37.88262	665.858	439.7745	18.77728	1.465189	36.64641	0.660463
00:04:37	37.52833	680.4064	449.6073	18.70979	1.51049	37.46578	0.660792
00:04:42	37.58082	695.872	461.426	18.66271	1.546788	38.45063	0.66309
00:04:47	37.62019	717.6968	476.4793	18.59435	1.594001	39.70502	0.663901

00:04:52	37.33151	753.1918	501.3546	18.46046	1.68717	41.77788	0.66564
00:04:57	37.58082	733.5455	486.183	18.5405	1.627095	40.51363	0.662785
00:05:02	37.47585	742.4408	493.7607	18.5049	1.656162	41.14508	0.665051
00:05:07	37.38399	747.1296	497.0276	18.48368	1.670762	41.41731	0.66525
00:05:12	37.54145	708.4915	467.9005	18.61936	1.569381	38.99015	0.660418
00:05:17	37.54145	717.1026	474.3588	18.5914	1.590353	39.52832	0.661494
00:05:22	37.17404	804.7465	535.8636	18.28097	1.807271	44.65352	0.665879
00:05:27	37.37087	751.3361	495.9566	18.4691	1.667837	41.32806	0.660099
00:05:32	37.38399	790.1851	525.0137	18.34328	1.762022	43.74939	0.664419
00:05:37	37.10844	769.8069	511.1336	18.39109	1.729137	42.59277	0.663976
00:05:42	37.41024	740.8693	490.5585	18.50579	1.648544	40.87824	0.662139
00:05:47	37.34463	747.9089	494.8909	18.47857	1.665495	41.23926	0.6617
00:05:52	37.38399	747.1296	492.722	18.48368	1.656722	41.05853	0.659487
00:05:57	37.18717	765.0191	505.7194	18.41214	1.707833	42.1416	0.661055
00:06:02	37.4496	770.2288	507.7645	18.41275	1.702873	42.31202	0.659239
00:06:07	37.01658	799.2526	530.5463	18.28784	1.797235	44.21042	0.663803
00:06:12	37.17404	775.684	513.2595	18.37628	1.733145	42.76991	0.661686
00:06:17	37.23965	828.9221	550.9062	18.20648	1.853418	45.90701	0.664605
00:06:22	37.13468	790.4563	521.8867	18.32509	1.763251	43.48882	0.660235
00:06:27	37.05595	792.0149	524.0718	18.31445	1.774079	43.6709	0.661694
00:06:32	37.06907	816.8709	540.2123	18.23363	1.826549	45.01589	0.661319
00:06:37	37.23965	764.3388	502.4687	18.4179	1.694856	41.87072	0.65739
00:06:42	37.18717	816.6858	542.3166	18.24277	1.827805	45.19124	0.664046
00:06:47	36.99034	786.1379	519.7932	18.32919	1.763037	43.31436	0.661198
00:06:52	37.12156	795.7392	527.2741	18.30682	1.781548	43.93775	0.662622
00:06:57	36.92473	828.6982	548.8826	18.18408	1.862118	45.73838	0.662343
00:07:02	37.13468	797.991	527.2687	18.30035	1.780919	43.9373	0.660745
00:07:07	37.08219	802.9769	530.5194	18.28025	1.794055	44.20818	0.660691
00:07:12	37.0297	788.5877	521.9298	18.32388	1.768249	43.49241	0.661854
00:07:17	36.67542	834.3514	552.214	18.14669	1.88551	46.01599	0.661848
00:07:22	37.01658	800.329	528.3935	18.2843	1.790145	44.03103	0.66022
00:07:27	36.85912	800.217	527.3817	18.27341	1.794233	43.94672	0.659048
00:07:32	36.89849	806.9724	533.8239	18.25391	1.813656	44.48354	0.661514
00:07:37	36.9641	780.5579	514.422	18.34576	1.746539	42.86678	0.659044
00:07:41	36.78039	825.4562	544.6362	18.18409	1.855155	45.38454	0.6598
00:07:46	36.80663	815.9667	539.2435	18.21747	1.836007	44.93516	0.660865
00:07:51	36.9641	775.1759	511.1928	18.36351	1.735889	42.5977	0.659454
00:07:56	36.85912	749.6268	494.0137	18.44073	1.683873	41.16616	0.659013
00:08:01	37.0297	759.5252	499.3257	18.41956	1.693834	41.60881	0.657418
00:08:06	36.81976	811.7602	536.009	18.23236	1.824661	44.66563	0.660305
00:08:11	36.87224	794.9341	525.2235	18.29181	1.786477	43.76688	0.660713
00:08:16	36.83288	788.1787	520.9341	18.31136	1.774136	43.40944	0.660934
00:08:21	37.01658	791.7179	521.9352	18.31266	1.768876	43.49286	0.659244
00:08:26	37.48897	758.6857	496.9845	18.45292	1.666083	41.41372	0.65506

00:08:31	37.27902	766.7887	503.5289	18.41253	1.696586	41.95907	0.656672
00:08:36	37.23965	810.6235	534.7603	18.26638	1.800564	44.56158	0.65969
00:08:41	37.22653	771.7745	507.856	18.39267	1.713077	42.31964	0.658037
00:08:46	37.12156	792.5101	521.8921	18.31743	1.763874	43.48927	0.658531
00:08:51	37.23965	766.4916	503.5451	18.41085	1.698379	41.96041	0.656948
00:08:56	37.33151	768.2612	503.5074	18.41125	1.6942	41.95727	0.655386
00:09:01	37.29214	760.4294	498.1416	18.4342	1.678395	41.51014	0.655079
00:09:06	37.18717	751.0261	492.8027	18.45801	1.66549	41.06525	0.656173
00:09:11	37.43648	756.1368	494.8533	18.45776	1.661409	41.23612	0.654449
00:09:16	37.37087	742.725	485.1927	18.49719	1.632725	40.43111	0.65326
00:09:21	37.60706	742.3547	480.7902	18.51361	1.608514	40.06425	0.647656
00:09:26	37.33151	783.3306	509.9657	18.36204	1.71529	42.49545	0.651022
00:09:31	37.48897	722.0885	465.7693	18.57193	1.564578	38.81255	0.645031
00:09:36	37.27902	757.1012	489.5359	18.44421	1.650827	40.79303	0.646592
00:09:41	37.52833	709.4689	457.142	18.61538	1.534965	38.09364	0.644344
00:09:46	37.37087	722.2736	466.8941	18.5639	1.573034	38.90628	0.646423
00:09:51	38.09257	744.9423	480.5911	18.536	1.588012	40.04766	0.645139
00:09:56	38.0532	726.3467	467.6906	18.5931	1.548276	38.97266	0.643894
00:10:01	38.0532	725.2703	467.6906	18.59655	1.548276	38.97266	0.64485
00:10:06	38.15818	720.6805	462.2656	18.61761	1.526823	38.52059	0.641429
00:10:11	38.04008	720.8657	463.3904	18.60987	1.535012	38.61433	0.642825
00:10:16	37.9876	690.3307	441.8842	18.70466	1.468048	36.82221	0.640105
00:10:21	38.27627	634.3844	399.7867	18.89955	1.323277	33.31422	0.630196
00:10:26	38.09257	654.5257	414.9314	18.82535	1.377885	34.57624	0.633942
00:10:31	38.68305	626.6903	389.9323	18.94505	1.278833	32.49306	0.622209
00:10:36	38.69617	585.8866	360.8645	19.07426	1.186843	30.07084	0.615929
00:10:41	38.81427	400.5627	234.8786	19.66193	0.787694	19.57244	0.586372
00:10:46	39.12919	90.78687	28.08296	20.63716	0.137492	2.340153	0.309328
00:10:51	39.28665	51.07246	5.414232	20.76152	0.0668	0.451168	0.106011
00:10:56	39.40475	48.73455	3.213018	20.76923	0.05994	0.267741	0.065929
00:11:01	39.33914	47.16302	3.239928	20.77385	0.06004	0.269983	0.068696
00:11:06	39.28665	47.8433	3.261456	20.77154	0.06012	0.271777	0.06817
00:11:11	39.40475	54.11649	3.213018	20.75258	0.05994	0.267741	0.059372
00:11:16	39.45724	55.58898	3.191491	20.74825	0.05986	0.265947	0.057412
00:11:21	39.33914	53.62135	3.239928	20.75384	0.06004	0.269983	0.060422
00:11:26	39.50972	49.52677	3.169963	20.76719	0.059781	0.264153	0.064005
00:11:31	39.6147	48.16621	3.126907	20.77178	0.059622	0.260565	0.064919
00:11:36	39.68031	54.04329	3.099998	20.75397	0.059524	0.258323	0.057361
00:11:41	39.6147	54.62454	3.126907	20.7519	0.059622	0.260565	0.057244
00:11:46	39.6147	54.62454	3.126907	20.7519	0.059622	0.260565	0.057244
					Total	**3769.41**	**0.66**
					% Error	**-1.32**	**-1.19**

4. 23 November 2015

t	VE	VO2	VCO2	FeO2	FeCO2	CO2 volume	R
hh:mm:ss	l/min	ml/min	ml/min	%	%	ml	---
00:00:00	39.07998	1.559171	0.032303	20.91516	0.0501	0.002692	0.020718
00:00:05	39.13219	0.878649	0.010768	20.91728	0.050033	0.000897	0.012255
00:00:10	39.07998	1.559171	0.032303	20.91516	0.0501	0.002692	0.020718
00:00:15	39.13219	0.878649	0.010768	20.91728	0.050033	0.000897	0.012255
00:00:20	39.07998	1.559171	0.032303	20.91516	0.0501	0.002692	0.020718
00:00:25	39.07998	1.559171	0.032303	20.91516	0.0501	0.002692	0.020718
00:00:30	39.07998	1.559171	0.032303	20.91516	0.0501	0.002692	0.020718
00:00:35	38.97556	2.920215	0.075374	20.91092	0.050234	0.006281	0.025811
00:00:40	39.02777	2.239693	0.053839	20.91304	0.050167	0.004486	0.024038
00:00:45	39.13219	1.955424	0.010768	20.91394	0.050033	0.000897	0.005507
00:00:50	39.13219	1.955424	0.010768	20.91394	0.050033	0.000897	0.005507
00:00:55	39.13219	0.878649	0.010768	20.91728	0.050033	0.000897	0.012255
00:01:00	39.07998	1.559171	0.032303	20.91516	0.0501	0.002692	0.020718
00:01:05	39.13219	1.955424	0.010768	20.91394	0.050033	0.000897	0.005507
00:01:10	39.07998	1.559171	0.032303	20.91516	0.0501	0.002692	0.020718
00:01:15	38.97556	2.920215	0.075374	20.91092	0.050234	0.006281	0.025811
00:01:20	40.93347	2.70486	0.344568	20.91199	0.05102	0.028713	0.127389
00:01:25	41.84717	2.101866	0	20.91391	0.049906	0	0
00:01:30	41.84717	3.178641	0	20.91079	0.049906	0	0
00:01:35	41.89938	3.574895	0	20.90966	0.049844	0	0
00:01:40	40.48968	4.720584	0.52762	20.90587	0.05158	0.043967	0.11177
00:01:45	39.07998	4.789498	1.109079	20.90514	0.05344	0.09242	0.231565
00:01:50	41.56001	11.767	3.316469	20.88568	0.059673	0.276361	0.281845
00:01:55	39.13219	262.5351	173.3716	20.10674	0.587058	14.44706	0.660375
00:02:00	38.53177	518.5579	337.2892	19.28862	1.111111	28.10631	0.650437
00:02:05	38.25766	603.6964	404.1623	19.00716	1.330604	33.67885	0.669479
00:02:10	38.25766	576.777	385.8571	19.09246	1.272603	32.15347	0.668988
00:02:15	38.10102	620.8128	418.225	18.94484	1.38061	34.85069	0.673673
00:02:20	38.30987	614.8604	413.8318	18.97445	1.359455	34.4846	0.67305
00:02:25	37.98355	628.5354	424.7341	18.91409	1.405498	35.39309	0.675752
00:02:30	38.15324	591.0593	394.5144	19.04208	1.303455	32.87489	0.66747
00:02:35	38.20545	637.7569	428.9497	18.89648	1.411001	35.74438	0.672591
00:02:40	38.10102	627.2734	420.3786	18.92429	1.387461	35.03014	0.670168
00:02:45	37.98355	633.9193	426.8877	18.89691	1.412371	35.57255	0.67341
00:02:50	38.15324	642.7445	432.2015	18.87787	1.423195	36.01535	0.672431
00:02:55	37.77471	701.2479	476.5055	18.66966	1.579129	39.7072	0.679511

00:03:00	37.94439	696.0751	469.9748	18.69625	1.551428	39.163	0.675178
00:03:05	37.89218	655.8381	439.8467	18.82191	1.457113	36.65242	0.670663
00:03:10	37.93134	664.7495	446.2912	18.7956	1.476256	37.18944	0.671367
00:03:15	37.77471	713.0924	480.8126	18.63165	1.592951	40.06611	0.674264
00:03:20	37.87913	663.2765	444.1591	18.79738	1.471399	37.01178	0.669644
00:03:25	37.77471	712.0157	478.659	18.63511	1.58604	39.88666	0.672259
00:03:30	37.60502	693.4994	465.8077	18.68448	1.551545	38.81576	0.671677
00:03:35	37.72249	695.4678	465.7593	18.68512	1.546713	38.81172	0.669706
00:03:40	37.77471	716.3228	480.8126	18.62129	1.592951	40.06611	0.671223
00:03:45	37.82692	726.41	488.3285	18.59213	1.614907	40.69241	0.672249
00:03:50	37.70944	729.8255	490.5305	18.5739	1.626861	40.8759	0.67212
00:03:55	37.67028	718.7606	480.8557	18.60707	1.597367	40.0697	0.669007
00:04:00	37.72249	729.9246	490.5251	18.57439	1.626298	40.87546	0.672022
00:04:05	37.5006	730.3941	490.6166	18.559	1.635921	40.88308	0.671715
00:04:10	37.72249	717.0033	479.7573	18.61592	1.591696	39.97818	0.669115
00:04:15	37.44839	715.9997	479.8704	18.6023	1.603346	39.9876	0.67021
00:04:20	37.65723	748.8112	502.3965	18.50953	1.667244	41.8647	0.670925
00:04:25	37.51365	794.0229	532.6055	18.35421	1.771051	44.38201	0.670768
00:04:30	37.44839	771.9921	517.5575	18.42105	1.72534	43.12807	0.670418
00:04:35	37.55281	771.7078	516.4377	18.42892	1.717066	43.03475	0.669214
00:04:40	37.60502	769.9505	516.4162	18.43804	1.714682	43.03296	0.670713
00:04:45	37.51365	760.6429	511.07	18.46207	1.701461	42.58746	0.671892
00:04:50	37.44839	793.5276	533.7092	18.35134	1.777623	44.47399	0.672578
00:04:55	37.56586	800.8798	536.891	18.33565	1.782488	44.73913	0.670377
00:05:00	37.5006	750.8528	502.4612	18.49286	1.674208	41.87009	0.669187
00:05:05	37.55281	748.0187	500.2861	18.50539	1.664929	41.68884	0.668815
00:05:10	37.30481	812.8966	544.5362	18.27852	1.819454	45.3762	0.669871
00:05:15	37.5006	785.3096	528.3038	18.38148	1.757745	44.02355	0.672733
00:05:20	37.55281	764.1704	511.0538	18.45325	1.699687	42.58611	0.668769
00:05:25	37.25259	827.5752	554.2487	18.22705	1.853539	46.18554	0.669726
00:05:30	37.39618	761.9048	508.9649	18.45026	1.699825	42.41204	0.668016
00:05:35	37.51365	766.0267	511.07	18.44468	1.701461	42.58746	0.66717
00:05:40	37.29175	775.1104	518.6989	18.40042	1.736087	43.22318	0.669194
00:05:45	37.39618	788.8242	528.3468	18.363	1.762653	44.02714	0.66979
00:05:50	37.13512	813.7623	544.6061	18.26362	1.827768	45.38203	0.669245
00:05:54	37.35702	787.4503	526.2094	18.36478	1.757512	43.84903	0.668245
00:05:59	37.34396	805.6564	536.9826	18.30479	1.793079	44.74676	0.666516
00:06:04	37.34396	792.7351	529.4451	18.34673	1.768612	44.11866	0.667871
00:06:09	37.26565	801.8317	534.8613	18.31173	1.789842	44.56999	0.667049
00:06:14	37.18733	807.6979	540.2775	18.28712	1.811162	45.02132	0.66891
00:06:19	37.23954	785.4819	525.1811	18.36313	1.759551	43.76334	0.66861
00:06:24	37.18733	777.5482	518.742	18.3854	1.740962	43.22677	0.667151
00:06:29	37.22649	782.1525	521.9562	18.37307	1.749649	43.49461	0.667333
00:06:34	37.34396	763.6621	507.9096	18.4411	1.698707	42.32411	0.665097

00:06:39	37.23954	786.5587	523.0276	18.35962	1.752541	43.58389	0.664957
00:06:44	37.22649	777.8454	519.8026	18.3871	1.742637	43.31515	0.66826
00:06:49	37.23954	760.7161	507.9527	18.44374	1.70347	42.3277	0.66773
00:06:54	37.23954	763.9464	507.9527	18.43323	1.70347	42.3277	0.664906
00:06:59	37.29175	795.5691	529.4667	18.33392	1.771089	44.12046	0.665519
00:07:04	37.2787	763.1668	507.9366	18.43838	1.701681	42.32635	0.665564
00:07:09	37.29175	756.8052	504.7008	18.45992	1.690585	42.05672	0.666883
00:07:14	37.08291	760.6041	506.9405	18.43365	1.707145	42.24336	0.666497
00:07:19	37.23954	743.4877	495.0314	18.49982	1.661409	41.25097	0.665823
00:07:24	36.95238	792.9935	526.3763	18.31862	1.776757	43.86294	0.663784
00:07:29	37.13512	749.1558	498.3048	18.47452	1.676626	41.52374	0.665155
00:07:34	37.18733	752.7824	499.36	18.46613	1.677782	41.61167	0.663352
00:07:39	37.04375	748.4624	496.1889	18.47075	1.673714	41.34742	0.662944
00:07:44	37.18733	744.1682	495.0529	18.49421	1.663742	41.25276	0.665243
00:07:49	37.08291	743.3757	492.9425	18.48997	1.661387	41.07689	0.663114
00:07:54	37.17428	744.0691	493.9815	18.49368	1.660815	41.16348	0.663892
00:07:59	37.12207	729.6748	483.2353	18.53727	1.627989	40.268	0.662261
00:08:04	37.14817	728.7961	481.071	18.54181	1.619817	40.08765	0.66009
00:08:09	37.04375	753.8462	499.4193	18.45314	1.684285	41.61661	0.662495
00:08:14	37.17428	706.382	467.0622	18.61657	1.573034	38.92029	0.661203
00:08:19	36.99154	728.6842	482.2124	18.53211	1.630205	40.18276	0.661758
00:08:24	37.17428	725.7639	481.0602	18.55337	1.61868	40.08675	0.662833
00:08:29	37.06986	698.052	460.6446	18.63732	1.556338	38.38551	0.6599
00:08:34	37.09596	699.3269	459.557	18.63476	1.551724	38.29489	0.657142
00:08:39	37.13512	715.7758	472.4622	18.58348	1.592267	39.37027	0.66007
00:08:44	37.08291	713.226	470.3302	18.58853	1.587469	39.19261	0.659441
00:08:49	37.2787	686.7158	453.021	18.68697	1.523109	37.75024	0.659692
00:08:54	36.92628	738.9566	489.7767	18.49417	1.65783	40.8131	0.662795
00:08:59	37.08291	729.3776	482.1747	18.53573	1.626188	40.17962	0.661077
00:09:04	37.22649	698.164	459.5032	18.64656	1.546283	38.2904	0.658159
00:09:09	37.08291	684.153	449.8714	18.68356	1.520591	37.48779	0.65756
00:09:14	37.17428	687	451.9873	18.67978	1.523876	37.6641	0.657915
00:09:19	36.97849	686.5908	452.0681	18.66926	1.531945	37.67083	0.658424
00:09:24	37.17428	670.8484	440.1428	18.73244	1.485253	36.6771	0.656099
00:09:29	37.0307	644.9929	419.7433	18.8086	1.424039	34.97721	0.650772
00:09:34	37.14817	634.0399	412.1574	18.85102	1.39494	34.34507	0.65005
00:09:39	37.08291	511.8689	326.0422	19.24674	1.115804	27.1691	0.636964
00:09:44	37.82692	114.8015	49.00405	20.5521	0.207039	4.083508	0.426859
00:09:49	37.76165	55.08353	9.190279	20.74317	0.079502	0.765826	0.166843
00:09:54	37.82692	49.11819	4.856258	20.76259	0.065562	0.404672	0.098869
00:09:59	37.70944	46.07307	3.827937	20.77189	0.062305	0.318982	0.083084
00:10:04	37.76165	45.39255	2.729626	20.77428	0.058763	0.22746	0.060134
00:10:09	37.82692	46.96464	2.702707	20.7695	0.058661	0.225217	0.057548
00:10:14	37.82692	46.96464	2.702707	20.7695	0.058661	0.225217	0.057548

00:10:19	37.93134	46.68037	3.736411	20.77082	0.061941	0.311355	0.080042
00:10:24	37.98355	45.99985	3.714876	20.7732	0.061856	0.309561	0.080758
00:10:29	37.87913	47.3609	3.757947	20.76844	0.062026	0.31315	0.079347
00:10:34	37.93134	46.68037	3.736411	20.77082	0.061941	0.311355	0.080042
00:10:39	37.87913	47.3609	2.681171	20.76844	0.05858	0.223422	0.056611
00:10:44	37.82692	48.04142	3.779482	20.76605	0.062112	0.314944	0.078671
					Totals	**3763.66**	**0.67**
					% Error	**-1.47**	**-0.03**