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Liver function parameters, cholesterol, and phospholipid alpha linoleic acid are associated with adipokine levels in overweight and obese adults.

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Abbreviations

ALA: alpha linoleic acid

ALT: alanine transaminase

AST: aspartate transaminase

BMI: body mass index

CRP: C-reactive protein

CV: coefficients of variation

CVD: cardio vascular disease

GGT: gamma-glutamyl transpeptidase

T2DM: type 2 diabetes mellitus

WC: waist circumference

WHpR: waist:hip ratio

WHtR: waist:height ratio

Abstract

Dysregulation of adipose hormones in obesity has been associated with the hastened development of metabolic syndrome and associated chronic disease sequalae including cardiovascular disease and type 2 diabetes mellitus. This study aims to identify common biochemical and anthropometric markers that impact adipose hormones, including adiponectin and leptin. Based on previous literature, it was hypothesized that these would be adversely impacted by liver function parameters and adiponectin levels would be positively correlated with phospholipid omega-3 fatty acids. Forty non-diabetic adult subjects (body mass index (BMI)≥25.0kg/m²) were recruited. Fasting plasma samples were taken to assess adipokine levels, glucose metabolism, electrolytes, liver enzymes, and blood lipids. Basic anthropometric measurements were also recorded. Adiponectin levels were positively correlated with HDL cholesterol and negatively correlated with anthropometric measures, insulin, liver enzymes, triglycerides, and VLDL cholesterol but not BMI. Conversely, plasma leptin levels were positively correlated with anthropometric measures, C-reactive protein, HDL cholesterol, and plasma phospholipid proportions of omega-3 alpha linoleic acid, but inversely correlated with creatinine levels. These results support other data regarding correlations between adiponectin and relative adipose distribution. Correlations with specific liver enzymes may indicate that adiponectin levels are tied to fatty acid deposition in the liver; however, liver/kidney damage though further mechanistic clarification is required. Leptin levels were associated with measures of adiposity but not liver enzymes. Each of these variables, along with blood lipids, may serve as potential future therapeutic targets for the prevention and management of obesity and related co-morbidities.

Keywords: human, obesity, adipokines, liver enzymes, cholesterol, fatty acids.

1. Introduction

Overweight and obesity have been identified as major public health problems worldwide [1], resulting in increased mortality and relative risk of disease [2]. Estimates from 2008 suggest that 1.4 billion adults worldwide were overweight and of these, approximately 500 million were obese [3]; with approximately 2.8 million adults dying annually as a result of overweight and obesity [4].

Dieting, weight loss, and exercise constitute a multi-billion dollar industry [5], but unfortunately, most dieters inevitably regain weight as current methods to maintain weight loss have poor long-term success rates [6, 7]. There remains a limited understanding of the physiological processes regulating appetite control and energy expenditure, which has fundamentally hindered development of truly effective therapies to treat or prevent obesity [8]. Adipocytes produce a number of biologically active proteins that assist in the regulation of energy intake and expenditure; these are also referred to as adipokines. These adipose hormones, including adiponectin [9] and leptin [10, 11], have been identified as potential therapeutic targets in the prevention and treatment of obesity.

Low plasma adiponectin concentrations (hypoadiponectinemia) are linked to T2DM (male and female subjects, mean BMI 23.8kg/m^2) [12] and hypertension (male subjects, mean BMI 23.9kg/m^2) [13]. Low adiponectin levels were also independently associated with all causes of mortality, cardiac mortality, and myocardial infarction in male subjects presenting with chest pain [9]. This hormone has many regulatory actions on energy homeostasis, glucose balance, and fatty acid metabolism, and it enhances glucose metabolism in the liver and skeletal muscle [14-17].

Circulating levels of leptin change relative to food intake and adiposity [18, 19]. In non-obese subjects, leptin acts to inhibit feeding and increase thermogenesis [20]. In obesity, where weight is stable, higher plasma leptin levels do not induce the expected reduction in food intake and increase in energy expenditure, suggesting the development of resistance to the effects of endogenous leptin [21-23]. Where energy intake is restricted in obese subjects, administration of low doses of leptin is able to prevent decrease appetite and improve energy expenditure [24], factors that would otherwise hinder

weight loss [25]. To this extent, it appears that responsiveness to leptin in obesity is retained relative to a significant change in leptin levels and not to sustained circulating levels under basal conditions.

Significant alterations in circulating levels of adiponectin and leptin, and their physiological roles in the pathogenesis of obesity lead to the hypothesis that they may be involved in the progression towards metabolic syndrome, vascular disease, and T2DM. This study aims to identify additional anthropometric and biochemical markers correlated with circulating measures of adiponectin and leptin in obese subjects, with particular consideration given to the identification of potential therapeutic targets. Liver enzymes [26] and fatty acid indices [27] are recognized as components of metabolic syndrome and have led authors to further investigate potential correlations between these parameters and adipokine levels. Based on the current biochemical understanding of these hormones, it was hypothesized that adiponectin would be inversely correlated with liver function parameters and positively correlated with phospholipid omega-3 fatty acids. Conversely, it was hypothesized that leptin levels would be positively correlated with liver function parameters. With a focus on identifying parameters that may indicate increased risk of obesity and the progression towards metabolic syndrome within the general population, consideration was given to both male and female participants from a broad age range.

2. Methods and Materials

2.1 Participants

A total of forty subjects were recruited from within the greater Brisbane region. Even numbers of male and female participants were recruited, with 10 overweight and 30 obese subjects in total. All subjects were over the age of 18 with a BMI$\geq$25.0kg/m^2. Participants were non-diabetic, non-smokers, who were not pregnant/breastfeeding, reported no active substance abuse (alcohol or drug dependency), and were generally healthy. Subjects were not currently taking cholesterol medications or nutritional supplements. Written informed consent was obtained from all subjects.

2.2 Study Design

Anthropometric measures included height, weight, waist circumference (WC), and hip circumference. WC was measured to the nearest 0.1 cm at the umbilicus level. Hip circumference was measured to the nearest 0.1 cm at the widest point between the iliac crest and buttock. BMI was calculated as weight in kilograms divided by the square of the height in meters (kg/m^2). BMI groups were based on World Health Organization classifications for adults [28]. Overweight BMI range was categorized as $25.0kg/m^2 \leq BMI > 30.0kg/m^2$ and a $BMI \geq 30.0kg/m^2$ was classified as obese.

Fasting blood samples were taken, for the quantification of adipokine levels (total adiponectin and leptin), markers of glucose metabolism, electrolyte and liver function, cholesterol, and plasma fatty acid phospholipids. The study design was approved by Metro South Human Research Ethics Committee (HREC/10/QPAH/141) and The University of Queensland Medical Research Ethics Committee (2010001200).

2.3 Assays

Total adiponectin was measured in duplicate by Cardinal Bioresearch (Brisbane), utilizing Multiplex ELISA techniques on a Luminex platform with human adiponectin/Acrp30 [29] and following the standard procedures outlined in the corresponding Human Obesity MultiAnalyte Profiling Base Kit [30]. These assays demonstrated a mean minimum detectible dose of 6.4pg/mL and a CV of 9.2-14.6% for inter-assay variation and 2.8-5.6% for intra-assay variation. Total leptin was measured in duplicate using human leptin antibodies [31] supplied by R&D Systems and following the standard procedures outlined in the corresponding Human MultiAnalyte Profiling Base Kit B [32]. These assays demonstrated a mean minimum detectible dose of 7.69pg/mL and a CV of 7.2-11.2% for inter-assay variation and 4.6-8.0% for intra-assay variation. Plasma phospholipid concentrations were measured by gas chromatography based on one sample each, and in conjunction with FOODplus Institute at The University of Adelaide, Waite Campus [33]. Free fatty acids were measured in duplicate using a nonesterified free fatty acid assay from Wako Diagnostics in Osaka, Japan [34]. All other pathological markers were analyzed by Pathology Queensland (Princess Alexandra Hospital, Brisbane).

2.4 Statistical Analyses

All data are expressed as means ± standard deviation. Relationships between variables were assessed by nonparametric correlation analysis (Spearman's rank correlation coefficient). The sample size of 40 was determined to allow correlations between variables of 0.37 or more to be detected with a power of 0.80 at a level of significance of P=0.05. P values <0.05 were considered of statistical significance. All statistical analyses were performed in SPSS version 20.0.

3. Results

Anthropometric measures, adipokine levels, and metabolic parameters are summarized in Table 1. Subjects were between 23 and 79 years of age with a BMI between 26.4kg/m^2 to 46.9kg/m^2 (mean value 32.8kg/m^2). Indirect measures of adipose distribution including WC, waist:height ratio (WHtR), and waist:hip ratio (WHpR) were all higher in obese than overweight cohorts. There were no significant differences in adiponectin levels between overweight and obese participants (7.7 vs 7.1ug/mL; P=0.52). Leptin levels did not differ significantly between overweight and obese participants (23.2 vs 33.0ng/mL; P=0.14). The mean insulin level for all subjects was 7.9mU/L, mean total cholesterol was 5.mmol/L, and there were no significant differences between overweight and obese groups.

Table 2 shows Spearman's correlation coefficients (r) and coefficients of variation (CV) for adiponectin levels. Significant correlations between adiponectin and key anthropometric measures included WC (r=-0.61, P<0.01), WHtR (r=-0.39, P=0.01), and WHpR (r=-0.47, P<0.01). Of these, WC demonstrated the highest correlation with a CV of 37%. There was no significant correlation observed between adiponectin and BMI (r=-0.26, P=0.10). Insulin was inversely correlated with adiponectin levels (r=-0.49, P=0.01), as were alanine transaminase (ALT; r=-0.54, P<0.01), aspartate transaminase (AST; r=-0.44, P<0.01), gamma-glutamyl transpeptidase (GGT; r=-0.60, P<0.01), and urate (r=-0.55, P<0.01). In contrast, urea:creatinine was positively correlated with adiponectin levels (r=0.48, P<0.01). Significant inverse correlations were also observed between adiponectin and blood lipids, including triglycerides (r=-0.47, P<0.01), HDL:Total (r=-0.42, P=0.01), and VLDL (r=-0.44, P<0.01) but not total cholesterol. Conversely, HDL cholesterol levels were positively correlated with

adiponectin (r=0.52, P<0.01). Circulating adiponectin levels were also inversely correlated with plasma phospholipid percentages of saturated fatty acids (r=-0.66, P<0.01) and positively correlated with trans-saturated fatty acids (r=0.34, P=0.03). No direct correlation was observed between adiponectin levels and phospholipid percentages of omega-3 or omega-6 fatty acids.

Table 3 shows Spearman's correlation coefficients and CV for leptin levels. Positive correlations with anthropometric measures included BMI (r=0.44, P<0.01) and WHtR (r=0.34, P=0.03) but not WC or WHpR. Of these, BMI demonstrated the highest correlation with a CV of 18%. There were no significant correlations between markers of glucose metabolism and leptin levels, though creatinine (r=-0.51, P<0.01) and C-reactive protein (CRP; r=0.47, P=0.01) from the liver function and electrolytes panel were highly correlated. Leptin levels were positively correlated with HDL cholesterol (r=0.40, P=0.01) and inversely correlated with the HDL:Total ratio (r=-0.55, P<0.01). Of the plasma phospholipids, ALA was positively correlated with plasma leptin levels (r=0.33, P=0.04). No significant correlations were observed between levels of free fatty acids and adiponectin (r=0.03, P=0.88) or leptin (r=0.11, P=0.51).

Of further interest, insulin levels were positively correlated with anthropometric measures including WC (r=0.61, P<0.01) and WHtR (r=0.48, P≤0.01). Insulin was also inversely correlated with urea (r=-0.46, P=0.01) and positively correlated with estimated glomerular filtration rate (r=0.56, P=0.02), gamma-glutamyl transpeptidase (r=0.49, P<0.01), and ALT (r=0.44, P=0.02).

4. Discussion

Adiponectin and leptin are key regulators of energy homeostasis and may be key determinants of the progression and development of obesity [9-11]. This manuscript aims to identify anthropometric and biochemical markers that correlate with each of these hormones in overweight and obese subjects. This includes particular consideration of parameters that may serve as potential therapeutic targets in the future.

In relation to anthropometric parameters, the strongest association for adiponectin was WC. This inverse correlation has previously been observed by Shober et al. [35] in a combined cohort of

diabetic and non-diabetic adults (mean BMI 29.8kg/m^2, overweight). Contrary to previous data [36], no significant correlation to BMI was observed within this overweight/obese cohort. Inverse correlations were observed between adiponectin and WHtR as well as WHpR. This supports reports from other studies suggesting the relative distribution of body mass (as represented in this study by WC, WHtR, and WHpR) [37, 38] and not simply increased relative adiposity (approximated using BMI). Conversely, leptin levels were positively correlated with BMI and WHtR but not WC or WHpR. Previous studies have also observed correlations between leptin and both total and central fat mass [10, 11, 39]. Given the correlations between levels of these hormones and CVD risk [9], these data support previous thoughts regarding the role of BMI as a proxy for quantifying obesity risk as well as the significance of more recent indices of abdominal obesity, including WC, WHtR, and WHpR [40].

The relationship between obesity, insulin resistance and T2DM is not yet clearly understood, in particular the correlation with adipose tissues and adipokines. This study found that the strongest correlation for adiponectin relating to glycaemic control was insulin (inverse), whilst no significant associations were identified with leptin in this area. Previous data have also identified positive correlations between adiponectin, glycaemic control [36], and insulin sensitivity [41], as well as inverse associations with HbA1c [9] and insulin resistance [37, 38]. Cortisol [42] and insulin [42, 43] have also been correlated with leptin expression and secretion. The absence of these indications may be suggestive of changes in leptin sensitivity within an overweight/obese cohort.

Elevations in liver enzymes are common in obese subjects and have been associated with increased fatty acid deposition in the liver [44, 45]. Leptin, but not adiponectin, was significantly inversely correlated with creatinine, a metabolic by-product of skeletal muscle metabolism that is observed to be lower in subjects with lower muscle mass [46]. Significant inverse correlations were observed between plasma adiponectin levels and liver enzymes such as ALT, AST GGT. This is of particular interest given previous correlations between ALT and AST with liver injury, alcoholic cirrhosis, and liver congestion and non-alcoholic fatty liver disease [47-50].

Insulin levels were also negatively correlated with plasma adiponectin levels, although positively associated with ALT and GGT. Through correlations with fatty acid deposition in the liver, ALT and GGT have been identified as important biomarkers for the development of metabolic syndrome [51, 52] and elevated cardiovascular risk [51]. As adiponectin levels are also inversely correlated with obesity [53], these results indicate that adiponectin may provide a common denominator between elevations in liver enzymes and hyperinsulinemia in obesity and the subsequent development of metabolic syndrome.

In combination with negative correlations with triglycerides, positive correlations were observed between adiponectin and HDL cholesterol. These results concur with previous data [36, 41, 54] and are consistent with the role of adiponectin in the up regulation of lipid uptake and oxidation in skeletal muscle, inhibiting hepatic triglyceride synthesis. No significant correlations were observed between adiponectin and total or LDL cholesterol levels [27].

Similarly, leptin has been shown to increase hepatic fatty acid oxidation and plasma triglyceride as well as reduce triglyceride secretion into plasma [55, 56]. However, this study found minimal correlations between leptin and lipid profiles. No significant correlations were found between leptin and triglycerides or total cholesterol. This contrasted with previous data from Kalhan et al. [57] who reported significant correlations between plasma leptin and both of these levels in South American males of Asian descent (age 24.2±1.9 years). The discrepancy between these results may be attributable to differences in the mean BMI of these studies (22.0±2.9kg/m² vs 32.8±4.8kg/m²), with increased adipose tissue linked to abnormal lipid profiles (hyperlipidaemia) [58]. However, the present study did observe significant positive correlations between leptin and HDL cholesterol, with concurrent inverse correlations to HDL:total cholesterol ratios. Of further note is the absence of observed correlations between plasma leptin levels and free fatty acids. Given the association between leptin and changes in energy intake and expenditure, this contrasts with widely accepted literature which proposes that increased free fatty acid concentrations associated with obesity play a role in the subsequent adverse metabolic effects [59]. In contrast, more recent data suggests that the

concentration of free fatty acids per kilogram of adipose tissue decreases as total adiposity increases, in many cases leading to the normalization of free fatty acid levels in obese subjects [60].

In regards to cholesterol analyses, adiponectin levels were significantly correlated with plasma phospholipid percentages of trans-saturated fatty acids and inversely correlated to saturated fatty acid levels. Despite existing data indicating increased adiponectin levels associated with omega-3 supplementation [61, 62], no significant correlations were observed to plasma phospholipid levels of omega-3 or omega-6. Conversely, leptin was significantly positively associated with plasma phospholipid levels of alpha linoleic acid. Previous studies have shown reductions in leptin associated with conjugated linoleic acid supplementation in rats [63] and marine lipid (eicosapentaenoic acid and docosahexaenoic acid) consumption in humans [64].

By contrast, this study found a significant inverse correlation between adiponectin levels and the percentage of saturated fatty acids as well as a positive correlation with plasma phospholipid trans-saturated fatty acids. Leptin was also found to be positively correlated with the plasma phospholipid percentage of omega-3 ALA. Neither adiponectin nor leptin were significantly correlated with plasma phospholipid total omega-3, eicosapentaenoic acid, and docosahexaenoic acid percentages. This contrasts with data from other human studies reporting increased levels of adiponectin and leptin associated with omega-3 fatty acid intake (eicosapentaenoic acid/docosahexaenoic acid from marine lipid origin) in overweight/obese subjects [61, 65]. Clearly, these findings will need to be replicated in further studies in order to determine plausible reasons for this apparent contrast in results.

Results support correlations between adiponectin and relative adipose distribution, whilst leptin levels were associated with measures of both adipose distribution and relative adiposity (BMI). With correlations between these hormones and CVD [9], these results lend further credence and potential mechanistic understanding to the importance of both BMI and measures of abdominal obesity in quantifying the relative health risk of individuals.

Significant correlations between adiponectin and levels of hepatic enzymes (ALT and AST) are concurrent with previous literature indicating the role of adiponectin in hepatic fatty acid metabolism

[14-17]. Elevations in these enzymes have been associated with non-alcoholic fatty liver disease. These correlations may provide some indication of the role of adiponectin in the development of this condition.

Limitations are naturally inherent within the design and outcomes of any study. Although the sample size of this study was sufficient in meeting the desired aims, future trials incorporating a larger cohort would allow for additional subanalyses for variables such as age, gender, and menopausal status. Results are limited to overweight and obese subjects, so further studies would be needed for comparison to normal weight participants. The correlations between adipokines and anthropometric/biochemical parameters do not necessarily indicate the existence of a causal relationship or associated mechanisms. Thus, further intervention studies are necessary to investigate the extent of the clinical implications associated with these findings.

Accordingly, these results are found to partially support the hypothesis since adiponectin levels were observed to be inversely correlated with liver function parameters but not correlated with plasma phospholipid fatty acids. In contrast to the original hypothesis, leptin levels demonstrated minimal correlations to liver enzymes, although they were positively correlated with phospholipid omega-3 ALA.

These markers, along with blood lipids that were also correlated with these hormones (HDL cholesterol, triglycerides, saturated fatty acids, trans saturated fatty acids, and omega-3 alpha linoleic acid) may serve as potential future therapeutic targets for the prevention and management of obesity and related co-morbidities.

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Table 1: Baseline anthropometric and metabolic parameters for overweight and obese subjects

Metabolic Parameters	Overweight		Obese	
	Male	Female	Male	Female
Anthropometric Parameters				
Age (years)	42.97 ±10.97	48.62 ±6.86	40.86±9.96	46.41 ±13.15
BMI (kg/m^2)	28.18 ±0.51	27.81 ±1.21	33.44±3.66	35.27 ±4.96
WC (m)	1.00 ±0.10	0.89 ±0.08	1.15±0.09	1.03 ±0.16
WHtR	0.55 ±0.04	0.54 ±0.06	0.64±0.04	0.63 ±0.09
WHpR	0.93 ±0.09	0.79 ±0.07	0.99±0.06	0.85 ±0.11
Adipokines				
Adiponectin (ug/mL)	5.83 ±0.84	9.56 ±1.47	5.69±2.93	8.37 ±3.94
Leptin (ng/mL)	9.46 ±4.02	36.92 ±6.77	19.31±10.28	46.57 ±22.87
Insulin Sensitivity				
Glucose (mmol/L)	5.80 ±1.26	5.12 ±0.29	5.09±0.30	5.22 ±0.62
Insulin (mU/L)	7.70 ±5.37	5.60 ±1.35	9.25±5.00	7.20 ±3.79
HbA1C (NgSP; %)	5.48 ±0.25	5.24 ±0.05	5.53±0.39	5.60 ±0.55
HbA1C (IFCC; mmol/mol)	36.60 ±2.87	33.40 ±0.49	37.00±4.23	37.80 ±5.97
Electrolyte and Liver Function				
ALT (U/L)	23.80 ±5.91	20.80 ±6.58	43.20±27.55	27.80 ±19.01
AST (U/L)	18.80 ±5.38	16.40 ±2.80	26.67±9.43	22.73 ±10.34
GGT (U/L)	14.80 ±3.06	16.20 ±6.05	26.00±10.54	16.80 ±7.30
Urea (mmol/L)	5.46 ±1.91	5.76 ±1.14	5.44±1.09	4.73 ±0.93
Creatinine (mmol/L)	84.00 ±13.34	60.40 ±6.53	82.87±7.49	63.67 ±13.28
Urea:Creatinine	65.00 ±19.30	94.60 ±14.26	65.33±11.19	75.40 ±12.49
Urate (mmol/L)	0.39 ±0.06	0.27 ±0.07	0.42±0.08	0.30 ±0.05
CRP (mh/L)	1.13 ±0.05	2.05 ±0.84	4.18±3.56	6.50 ±4.48
Blood Lipids				
Total Cholesterol (mmol/L)	5.02 ±0.77	5.46 ±0.98	5.34±0.96	5.32 ±1.21
Triglycerides (mmol/L)	1.06 ±0.23	0.63 ±0.30	1.69±0.93	1.03 ±0.44
HDL (mmol/L)	1.04 ±0.16	1.70 ±0.42	1.02±0.16	1.31 ±0.27
HDL:Total	4.94 ±0.59	3.30 ±0.39	5.30±1.09	4.10 ±0.86
LDL(mmol/L)	3.52 ±0.65	3.44 ±0.52	3.54±0.70	3.52 ±1.08
VLDL (mmol/L)	0.48 ±0.12	0.28 ±0.15	0.77±0.43	0.47 ±0.21
Free Fatty Acids (mEq/L)	0.20 ±0.09	0.27 ±0.19	0.31±0.15	0.37 ±0.12
Plasma Phospholipid Levels (%)				
Saturated Fatty Acids	45.03 ±0.64	44.37 ±0.21	45.18±0.46	44.53 ±0.89
Transaturated Fatty Acids	0.56 ±0.14	0.55 ±0.11	0.55±0.15	0.60 ±0.13
Omega-3 Fatty Acids (total)	6.08 ±1.84	5.79 ±0.67	6.09±1.29	5.99 ±0.81
Alpha linoleic acid	0.15 ±0.05	0.22 ±0.08	0.16±0.04	0.23 ±0.09
Eicosapentaenoic acid	0.92 ±0.37	1.03 ±0.11	1.28±0.81	0.98 ±0.37
Docosahexaenoic acid	3.88 ±1.28	3.41 ±0.72	3.39±0.53	3.56 ±0.50
Omega-6 Fatty Acids (total)	35.48 ±3.30	35.23 ±1.92	34.40±1.95	34.61 ±1.28
Omega-3:Omega-6	0.18 ±0.06	0.17 ±0.03	0.18±0.05	0.17±0.03

10 overweight (5 male, 5 female; 25.0 kg/m^2 ≤ BMI>30.0 kg/m^2) and 30 obese (15 male, 15 female; BMI≥30.0kg/m^2) participants were included in the study. Due to insufficient plasma sample for some participants, one or more analytes may have been omitted. Data represents means ± SD. BMI, Body Mass Index; WC, waist circumference; WHtR, Waist:Height Ratio; WHpR, Waist:Hip Ratio; ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transpeptidase; CRP, C-reactive protein.

Table 2. Spearman's correlation coefficients and CVs for adiponectin levels

Metabolic Parameters	n	Spearman's correlation		CV (r^2 x 100)
		r	P Value	
Anthropometric Parameters				
BMI (kg/m^2)	40	-0.26	0.10	7%
WC (m)	40	-0.61	<0.01	37%
WHtR	40	-0.39	0.01	15%
WHpR	40	-0.47	<0.01	22%
Insulin Sensitivity				
Glucose (mmol/L)	40	-0.15	0.35	2%
Insulin (mU/L)	40	-0.49	0.01	24%
HbA1C (NgSP; %)	40	-0.20	0.21	4%
HbA1C (IFCC; mmol/mol)	40	-0.20	0.21	4%
Electrolyte and Liver Function				
ALT (U/L)	40	-0.54	<0.01	29%
AST (U/L)	40	-0.44	<0.01	19%
GGT (U/L)	40	-0.60	<0.01	36%
Urea (mmol/L)	40	0.25	0.12	6%
Creatinine (mmol/L)	40	-0.25	0.12	6%
Urea:Creatinine	40	0.48	<0.01	23%
Urate (mmol/L)	40	-0.55	<0.01	30%
CRP (mh/L)	40	-0.32	0.07	10%
Blood Lipids				
Total Cholesterol (mmol/L)	40	0.13	0.42	2%
Triglycerides (mmol/L)	40	-0.47	<0.01	22%
HDL (mmol/L)	40	0.52	<0.01	27%
HDL:Total	40	-0.42	0.01	18%
LDL(mmol/L)	40	0.17	0.29	3%
VLDL (mmol/L)	40	-0.44	<0.01	19%
Free Fatty Acids (mEq/L)	38	0.23	0.88	0%
Plasma Phospholipid Levels (%)				
Saturated Fatty Acids	39	-0.66	<0.01	44%
Transaturated Fatty Acids	39	0.34	0.03	12%
Omega-3 Fatty Acids (total)	39	-0.08	0.62	1%
Alpha linoleic acid	39	0.15	0.36	2%
Eicosapentaenoic acid	39	-0.16	0.34	11%
Docosahexaenoic acid	39	-0.04	0.80	0%
Omega-6 Fatty Acids (total)	39	0.11	0.50	1%
Omega-3:Omega-6	39	-0.09	0.58	1%

Data expresses correlations between adiponectin and various anthropometric and biochemical parameters measured in 40 subjects. Due to insufficient plasma sample for some participants, one or more analytes may have been omitted. BMI, Body Mass Index; WC, waist circumference; WHtR, Waist:Height Ratio; WHpR, Waist:Hip Ratio; HOMA, Homeostasis Model of Assessment scores for Insulin Resistance (IR) and pancreatic beta cell function (β); ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transpeptidase; CRP, C-reactive protein.

Table 3. Spearman's correlation coefficients and CVs for leptin levels

Metabolic Parameters	n	Spearman's correlation		CV (r^2 x 100)
		r	P Value	
Anthropometric Parameters				
BMI (kg/m^2)	40	0.44	<0.01	18%
WC (m)	40	0.09	0.60	1%
WHtR	40	0.34	0.03	12%
WHpR	40	-0.30	0.06	9%
Insulin Sensitivity				
Glucose (mmol/L)	40	0.19	0.23	4%
Insulin (mU/L)	40	0.09	0.65	1%
HbA1C (NgSP; %)	40	<0.01	0.99	0%
HbA1C (IFCC; mmol/mol)	40	<0.01	0.99	0%
Electrolyte and Liver Function				
ALT (U/L)	40	-0.12	0.48	1%
AST (U/L)	40	0.06	0.71	0%
GGT (U/L)	40	-0.06	0.70	0%
Urea (mmol/L)	40	-0.22	0.17	5%
Creatinine (mmol/L)	40	-0.51	<0.01	26%
Urea:Creatinine	40	0.16	0.33	3%
Urate (mmol/L)	40	-0.29	0.07	8%
CRP (mh/L)	40	0.47	0.01	22%
Blood Lipids				
Total Cholesterol (mmol/L)	40	-0.15	0.36	2%
Triglycerides (mmol/L)	40	-0.21	0.20	4%
HDL (mmol/L)	40	0.40	0.01	16%
HDL:Total	40	-0.55	<0.01	30%
LDL(mmol/L)	40	-0.21	0.18	4%
VLDL (mmol/L)	40	-0.19	0.24	4%
Free Fatty Acids (mEq/L)	38	0.11	0.51	1%
Plasma Phospholipid Levels (%)				
Saturated Fatty Acids	39	-0.17	0.30	3%
Transaturated Fatty Acids	39	-0.21	0.21	4%
Omega-3 Fatty Acids (total)	39	-0.14	0.38	2%
Alpha linoleic acid	39	0.33	0.04	11%
Eicosapentaenoic acid	39	-0.09	0.60	36%
Docosahexaenoic acid	39	-0.12	0.48	1%
Omega-6 Fatty Acids (total)	39	0.15	0.37	2%
Omega-3:Omega-6	39	-0.18	0.27	3%

Data expresses correlations between leptin and various anthropometric and biochemical parameters measured in 40 subjects. Due to insufficient plasma sample for some participants, one or more analytes may have been omitted. BMI, Body Mass Index; WC, waist circumference; WHtR, Waist:Height Ratio; WHpR, Waist:Hip Ratio; HOMA, Homeostasis Model of Assessment scores for Insulin Resistance (IR) and pancreatic beta cell function (β); ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transpeptidase; CRP, C-reactive protein.