Association between skeletal muscle fat content and very-low density lipoprotein-apolipoprotein B-100 transport in obesity: effect of weight loss

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Abstract

Aims: Ectopic deposition of fat in skeletal muscle is a feature of metabolic syndrome, but its specific association with very-low-lipoprotein (VLDL)-apolipoprotein (apo) B-100 metabolism remains unclear.

Methods: We examined the association between skeletal muscle fat content and VLDL-apoB-100 kinetics in 25 obese subjects, and the responses of these variables to weight loss. The fat contents of liver, abdomen and skeletal muscle were determined by magnetic resonance imaging and VLDL-apoB-100 kinetics were assessed using stable isotope tracers.

Results: In obese subjects who were insulin sensitive (HOMA score $\leq$ 2.6, n=12), skeletal muscle fat content was significantly associated with hepatic fat content (r=0.636), energy intake (r=0.694), plasma triglyceride (r=0.644), apoB-100 (r=0.529), glucose (r=0.622), VLDL-apoB-100 concentrations (r=0.860), VLDL-apoB-100 fractional catabolic rate (r=-0.581) and VLDL-apoB-100 secretion rate (r=0.607). These associations were not found in obese subjects who were insulin resistant (HOMA score >2.6, n=13). Of these 25 subjects, 10 obese subjects underwent a 16-week weight loss program. The low fat diet achieved significant reduction (P<0.05) in body weight, visceral and subcutaneous fat areas, liver and skeletal muscle fat, energy intake, triglyceride, insulin, HOMA score, VLDL-apoB100 concentrations and VLDL-apoB100 secretion rate. The percentage reduction of skeletal muscle fat with weight loss was significantly associated with the corresponding changes in VLDL-apoB100 concentration (r=0.770, P=0.009) and VLDL-apoB-100 secretion (r=0.682, P=0.030).

Conclusions: Skeletal muscle fat content is associated with VLDL-apoB-100 transport. Weight loss lowers skeletal muscle fat and VLDL-apoB-100 secretion.

Introduction

Disturbances in lipid metabolism are associated with insulin resistance and increased risk of cardiovascular disease in obesity [1]. However, the precise mechanism by which obesity leads to insulin resistance has not been fully established. Excess accumulation of fat in the abdominal visceral area, liver and skeletal muscle is a common feature of metabolic syndrome that contributes to the development of cardiometabolic risk in obesity. Increasing evidence indicates that ectopic fat deposition in skeletal muscle is associated with peripheral insulin resistance and development of type 2 diabetes [2].

Skeletal muscle is one of the primary sites for insulin-mediated glucose disposal. Excess accumulation of triglyceride within muscle may disturb glucose metabolism and impair insulin sensitivity through various mechanisms including substrate competition, inhibition of insulin signalling pathway, or modulation of gene transcription [3]. The factors determining accumulation of fat in the skeletal muscle are not clear, but may be partially attributed to an increased delivery of triglycerides. Very-low density lipoproteins (VLDLs) are synthesized and secreted by the liver. The principal role of the VLDL is to transport triglycerides, as a source of fatty acids, from the liver to peripheral tissues, including skeletal muscle. Oversecretion of VLDL-apoB-100 may, therefore, result in an increased deposition of triglycerides within muscle. However, a direct relationship between VLDL-apoB-100 transport and skeletal muscle fat content has not previously explored in subjects with obesity.

In the present study, we examined the association between VLDL-apoB-100 transport and skeletal muscle fat content in obese subjects according to insulin sensitivity status. We also explored these relationships after a period of moderate weight loss.

Methods

Subjects

Twenty-five non-smoking, centrally obese Caucasian subjects (15 men and 10 women) consuming *ad libitum*, weight-maintaining diets were recruited for the study. None of the subjects had diabetes mellitus (excluded by oral glucose tolerance test), apolipoprotein E2/E2 genotype, macroproteinuria, raised creatinine (>120 μmol/L), hypothyroidism, or abnormal liver enzymes; or consumed more than 20g alcohol/day. None reported a history of cardiovascular disease, nor was taking medication or other agents known to affect lipid metabolism. The study was approved by the Ethics Committee of the Royal Perth Hospital, and informed consent was obtained before the study was started.

Study design and clinical protocols

The details of study design and clinical protocols had been previously described [4]. Briefly, all subjects were admitted to the metabolic ward in the morning after a 14-hour fast. They were studied in a semi-recumbent position and allowed to drink only water. Venous blood was collected for measurements of biochemical analytes. Arterial blood pressure was recorded after 3 minutes in the supine position using a Dinamap1846 SX/P monitor (Critikon Inc, Tampa, FL, USA). Dietary intake was assessed for energy and major nutrients using at least two 24 hr dietary diaries and subsequently analysed using DIET 4 Nutrient Calculation Software (Xyris Software, Queensland, Australia). Body composition was estimated at rest in the supine position using a Holtain Body Composition Analyser (Holtain Ltd, Dyfed, UK) from which total body fat and fat free mass (FFM) were derived; FFM was calculated using a formula by Lukaski et al [5]: $FFM = (0.85 \times H^2/Z) + 3.04$, where H is height (cm) and Z is impedance. For this measure, subjects were asked to fast overnight and to refrain from alcoholic beverages for 24 hours; they were then studied in the

morning, after emptying the bladder, in a temperature-controlled room; technical error for FFM was < 3% calculated from 3 repeated measurements by the same operator.

A single bolus of [$^2H_3$]-leucine (5mg/kg of body weight) was administered intravenously within a 2-minute period into an antecubital vein via a 21G butterfly needle. Blood samples were taken at baseline and after injection of the isotope at 5, 10, 20, 30, 40 min, and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10 hours. Subjects were then given a snack and allowed to go home. Additional fasting blood samples were collected in the morning on the following morning of the same week (24hours).

Liver, skeletal muscle and abdominal fat measurements

Single voxel proton magnetic resonance spectroscopy (MRS) was performed with a 1.5 T Sonata system. Sequences and quantification procedures for liver and skeletal muscle fat were described previously [6, 7]. Magnetic resonance imaging (MRI) of eight trans-axial segments (field of view, 40-48 cm; 10 mm thickness) at intervertebral disc levels from T11 to S1 was carried out using a 1.0T Picker MR scanner (Picker International, Cleveland, OH), and a T1 weighted fast spin echo sequence with a high fat:water signal ratio. Visceral and subcutaneous abdominal adipose tissue areas were measured at the L3 vertebra.

Diet Intervention

Ten subjects (6 males and 4 females) were selected at random from 25 obese subjects [4]. They entered a weight reducing hypocaloric diet (low fat, low caloric diet) for 16 weeks, immediately followed by a 6-week weight stabilization period. For the first 16 weeks of weight loss, a dietitian provided dietary counselling based on calculated basal energy expenditure from which 2500 KJ was subtracted to estimate

the required dietary energy intake. We attempted to maintain the composition of the diet during the end of study, weight stabilization period similar to that in run-in phase. All kinetic and imaging procedures were repeated after interventions.

Measurements of VLDL-apo-B100 enrichments and calculation of kinetic parameters

Laboratory methods for isolation and measurement of isotopic enrichment apoB-100 have been fully described [4]. Briefly, apoB-100 in the VLDL fraction was separated by sequential ultracentrifugation, precipitated by isopropanol, delipidated, hydrolysed and derivatized. Isotopic enrichment was determined by ion monitoring of derivatized samples at a mass to charge ratio of 305 and 302. Tracer/tracee ratios were derived for each sample. The multicompartmental model used to describe VLDL-apoB100 leucine tracer/tracee ratios had been previously described [4]. The SAAM II program (The Epsilon Group, Charlottesville, VA) was used to fit the model to the observed tracer/tracee ratios. The fractional catabolic rate (FCR) of VLDL-apoB-100 was derived from the model parameters giving the best fit. Plasma volume was determined after adjusting for the decrease in relative plasma volume associated with an increase in body weight. VLDL-apoB-100 secretion rate was calculated as the product of VLDL-apoB-100 FCR and the corresponding pool size, and expressed as mg/kg/day.

Biochemical analytes

Plasma cholesterol and triglyceride concentrations were determined by standard enzymatic methods (Hitachi, Tokyo, Japan; Roche Diagnostic GmbH, Mannheim, Germany). HDL-cholesterol was measured by an enzymatic calorimetric method using a commercial kit (Boehringer Mannheim, Mannheim, Germany). LDL-cholesterol was calculated by the Friedewald calculation. Plasma non-esterified fatty

acids (NEFAs) were measured by an enzymatic colorimetric assay (Wako Pure Chemical, Osaka, Japan). VLDL-apoB100 concentration was measured using a modification of the method as described by Beghin et al [8]. Plasma total apoA-I and apoB-100 concentrations were determined by immunonephelometry (Dade Behring, Illinois, USA). Plasma insulin was measured by solid phase two site sequential chemiluminescent immunometric assay (Diagnostic Products Corporation, LA, CA, USA) and glucose by hexokinase method (Hitachi, Tokyo, Japan). Insulin resistance was estimated by the homeostasis model assessment (HOMA) score [fasting insulin (mU/L) x fasting glucose (mmol/L) / 22.5] [9]. Plasma retinol-binding protein-4 (RBP-4), adiponectin, interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) concentrations were determined using enzyme immunoassay kits (Quantikine, Research & Development Systems, Minneapolis, USA).

Statistical Analysis

All analyses were carried out using SPSS 15 (SPSS, Inc., Chicago, USA). Associations were examined by simple and multivariate linear regression methods. The cut-off point to define insulin resistance used in this current study corresponded with a HOMA score of 2.6 [10]. Comparisons between the insulin sensitive and insulin resistant groups were performed using an unpaired t test. Paired t test was used to determine the impact of weight loss treatment. Statistical significance was defined at the 5% level using a two-tailed test.

Results

The anthropometric and biochemical characteristics of the 25 subjects are summarized in Table 1. They were middle-aged and normotensive. Liver and skeletal muscle fat content was significantly higher ($P<0.01$ in both) in obese men compared with obese women (311+77cm2 vs 197+105cm2 and 30.0+6.1% vs 12.6+3.9%, respectively). Average daily energy and nutrient intake was: 8339+2190kJ, 37+6% energy from fat, 39+5% energy from carbohydrates, 21+4% energy from protein and 3+3% energy from alcohol. Using data from the same study cohort [4], we have previously reported that, compared with 15 (9 men and 6 women) non-obese age-matched subjects, our obese subjects had a significantly ($P<0.05$ for all) higher VLDL-apoB-100 concentration (137±52 vs 51±20 mg/L) and secretion rate (18.6±5.0 vs 15.6±4.4 mg/kg/day) and a reduced FCR (4.2±2.4 vs 7.5±2.9 pools/day). Furthermore, the 25 obese subjects had significantly higher visceral (265±104 vs 83.8±23.3 cm^2) and subcutaneous fat at L3 (310±128 vs 141±43.3 cm^2) compared with non-obese subjects [11] .

When the subjects were divided into two groups according to insulin sensitivity status (using a HOMA score of 2.6 as the cutoff [10]), the insulin resistant obese subjects IR group) had significantly higher plasma glucose and insulin concentrations and HOMA score. Liver fat content was significantly elevated in the IR group compared with the IS group ($P<0.05$). Plasma triglyceride and VLDL-apoB-100 concentrations and visceral and subcutaneous fat content at L3 were higher and plasma adiponectin and VLDL-apoB-100 FCR were lower in the IR group compared with the IS group; however, group differences were not statistically significant (all $P>0.05$). There were also no significant differences between the groups with respect to skeletal muscle fat content and VLDL-apoB-100 secretion rate.

Correlational Analysis

Table 2 shows the correlation between skeletal muscle fat and the anthropometric and biochemical characteristics in the subjects studied. In univariate analysis, skeletal muscle fat was significantly ($P<0.05$ for all) and positively associated with body weight ($r=0.415$), visceral fat area at L3 vertebra ($r=0.531$), energy intake ($r=0.531$), plasma non-esterified fatty acid ($r=0.428$) and glucose concentration ($r=0.477$). Skeletal muscle fat content was not associated with plasma cytokines, VLDL-apoB-100 concentrations, or the fractional catabolic and production rates of VLDL-apoB-100.

In the insulin sensitive group (HOMA score <2.6), skeletal muscle fat content was significantly associated with body weight ($r=0.649$), visceral fat area at L3 vertebra ($r=0.765$), hepatic fat content ($r=0.636$), energy intake ($r=0.694$), plasma triglyceride ($r=0.644$), apoB-100 ($r=0.529$), glucose ($r=0.622$), VLDL-apoB-100 concentrations ($r=0.860$), VLDL-apoB-100 FCR ($r=-0.581$) and VLDL-apoB-100 secretion rate ($r=0.607$). Figure 1 shows the correlations of skeletal muscle fat with VLDL-apoB-100 concentration, VLDL-apoB-100 FCR and secretion rate in the insulin sensitive subjects (n=12). The associations between skeletal muscle fat and the kinetics of VLDL-apoB-100 were lost after adjusting for visceral or hepatic fat content. In the obese insulin resistant subjects (HOMA score >2.6), plasma non-esterified fatty acid was associated with skeletal muscle fat ($r=0.578$, $P<0.05$). VLDL-apoB-100 concentration ($r= -0.224$), FCR ($r=0.297$) and secretion rate ($r=0.100$) were not significantly associated with skeletal muscle fat ($P>0.05$ for all).

Weight loss

Table 3 shows the effect of weight loss on the clinical, biochemical characteristics and the kinetic indices of VLDL-apoB100 metabolism following 16 weeks dietary intervention in 10 obese subjects (2 men and 2 women in the IS group and 4 men and 2 women in the IR group). The low fat diet achieved significant reduction (P<0.05) in body weight, BMI, waist circumference, total body fat, visceral and subcutaneous fat areas, liver and skeletal muscle fat, energy intake, triglyceride, insulin, HOMA score, RBP-4, VLDL-apoB100 concentration and VLDL-apoB100 secretion rate. There was a significant increase (P<0.05) in plasma adiponectin concentration with weight loss. The percentage reduction of skeletal muscle fat with weight loss was significantly associated with changes in VLDL-apoB100 concentration (r=0.770, P=0.009), VLDL-apoB-100 secretion (r=0.682, P=0.030) (Figure 2), and borderline associated with reduction in visceral fat area (r=0.561, P=0.091) and liver fat content (r=0.615, P=0.059). The association between the percentage reduction of skeletal muscle fat and VLDL-apoB-100 secretion rate remained significant (P<0.05 for all) after adjusting for corresponding changes in energy intake (r=0.675), HOMA score (r=0.677), subcutaneous fat area (r=0.654). However, the significant association was lost after adjusting for corresponding changes in visceral fat area (r=0.591, P=0.101) or liver fat content (r=0.472, P=0.200).

Discussion

Our major findings were that skeletal muscle fat, as quantified by magnetic resonance spectroscopy, was directly associated with plasma triglyceride and VLDL-apoB-100 concentrations, and was also positively correlated with the secretion of VLDL-apoB-100 in obese, insulin sensitive subjects. We also found that reduction of skeletal muscle fat, with weight loss, was significantly associated with corresponding reductions in VLDL-apoB-100 concentration and secretion. However, these associations were not independent of reductions in visceral or liver fat with weight loss.

This is the first report to examine the association of skeletal muscle fat with the kinetics of VLDL-apoB-100 in obese subjects. Previous studies have only examined the relationship of visceral and liver fat to VLDL-apoB-100 transport [4, 12]. We have previously found that visceral fat was significantly associated with VLDL-apoB-100 secretion in obese men [12]. We, and others, further demonstrated that elevated liver fat content was a significant predictor of VLDL-apoB-100 oversecretion in obese and/or type 2 diabetic individuals [4, 13]. Given that fat deposition in abdomen, liver and skeletal muscle are closely linked, we extend these previous studies by examining the association between skeletal muscle fat and VLDL-apoB-100 kinetics. We hypothesized that, in subjects with visceral obesity, changes in VLDL-apoB-100 secretion was associated with changes in skeletal muscle fat.

Skeletal muscle fat reflects the balance between FFA flux to the muscle, de novo lipogenesis and fatty acid oxidation within muscle [2,3]. Visceral fat accumulation in central obesity results in markedly increased flux of FFA in the portal vein to the liver [1, 14], which stimulates de novo lipogenesis. The net effect of these processes is

increased accumulation of fat in the liver, which may subsequently drive the oversecretion of VLDL-apoB-100. Increased delivery of triglycerides by VLDL to peripheral tissues results in ectopic fat accumulation in skeletal muscle. Consistent with the notion, we found that skeletal muscle fat was significantly and directly associated with visceral fat area, liver fat, plasma triglycerides, VLDL-apoB-100 concentration and VLDL-apoB-100 secretion rate in insulin sensitive obese subjects. Given visceral fat and liver fat liver fat content both play important roles in regulating VLDL-apoB-100 secretion, it is not unexpected that the associations between skeletal muscle fat content and the kinetics of VLDL-apoB-100 were not independent of visceral fat or hepatic fat content.

We found no significant difference in the fat content of abdominal (visceral and subcutaneous regions) and skeletal muscle and the secretion rate of VLDL-apoB-100 between insulin sensitive and insulin resistant groups (Table 1). These observations suggest that in the setting of similar levels of visceral fat deposition, insulin resistance alone does not alter VLDL-apoB-100 secretion and skeletal muscle fat deposition in obese subjects. When the subjects were divided into two groups according to visceral fat area (using a cut-off mean value of 256 cm^2, see Table 1), the obese subjects with higher visceral fat deposition (n=15) had significantly elevated VLDL-apoB-100 secretion rate (20±5 vs 16±4 mg/kg/day) and skeletal muscle fat content (20±6 vs 12±4 %) ($P<0.05$ for both) compared with those with lower visceral fat deposition (n=10). These observations reinforce our notion that increased visceral fat accumulation, and by implication elevated VLDL-apoB-100 secretion, were more likely to predict increased skeletal muscle fat deposition in obese subjects.

In insulin-resistant obese subjects (HOMA score >2.6), we failed to observe significant associations between skeletal muscle fat and VLDL-apoB-100 metabolic variables, suggesting that this metabolic relationship varies according to insulin resistant state. Given the diverse effect of insulin resistance on lipoprotein metabolism [15], it may be that, in the insulin resistant state, alteration in skeletal muscle fat might be masked by factors other than the VLDL delivery pathway. Insulin resistance may enhance de novo lipogenesis and concurrently diminish mitochondrial lipid oxidation, thereby increasing lipid accumulation in skeletal muscle. On the other hand, insulin resistance may enhance cholesteryl ester transfer protein (CETP) activity and subsequently favour the transfer of triglycerides to other lipoproteins (LDL or HDL). Taken together, less triglycerides are delivered to skeletal muscle via the VLDL pathway, thereby diminishing the strength of the association between skeletal muscle fat and VLDL concentration and secretion rate seen in insulin-sensitive obese subjects. However, this hypothesis remains to be formally tested.

Weight loss by dietary restriction is the most common approach to reduce abdominal and hepatic fat in the management of dyslipidaemia [16, 17]. Weight loss reduces FFA release from visceral fat to the liver. It also improves insulin sensitivity, thereby potentially diminishing novo lipogenesis. As reported previously, we found that moderate weight loss reduces abdominal fat and improves insulin sensitivity, and results in significant reductions in liver fat and VLDL-apoB-100 secretion [4]. In the present study, we further demonstrate that weight loss significantly reduced skeletal muscle fat. We also found that the percentage reduction in skeletal muscle fat was significantly associated with corresponding reductions in VLDL-apoB100 concentration and VLDL-apoB-100 secretion. <u>These data suggest that VLDL may</u>

have a role in the regulation of skeletal muscle fat, consistent with the earlier correlational findings. However, this association was not independent of changes in visceral or liver fat with weight loss, highlighting the importance of visceral and liver fat in regulating ectopic fat deposition in skeletal muscle.

In this study, we also observed an inverse association between skeletal muscle fat and VLDL-apoB-100 catabolism. It is unclear whether skeletal muscle fat deposition is causally or physiologically related to the catabolism of VLDL-apoB-100 or vice versa. Given the reciprocal relationship between VLDL-apoB-100 concentration and FCR ($r=-0.632$, $P<0.01$), it is possible that the observed association between skeletal muscle fat and VLDL-apoB100 FCR may be mathematically related secondary to the association between skeletal muscle fat and VLDL-apoB-100 concentration. This possibility is consistent with our findings that the change in skeletal muscle fat was not associated with the corresponding changes in VLDL-apoB-100 FCR with weight loss.

Our study does have limitations. The sample size was small. Hence, there is the possibility that our significant findings may be due to chance. However, our results are generally consistent with current understanding of the complex metabolic relationships between insulin resistance, ectopic fat accumulation and dyslipidaemia. Dividing our obese subjects into IS and IR might have increased within-group homogeneity, thereby limiting the ability to find statistically significant associations among the various clinical and metabolic characteristics. The lack of significant differences in visceral, subcutaneous and skeletal muscle fat content between our insulin sensitive and insulin resistant groups could also reflect low statistical power. The power of our multivariate analysis may also be limited given the small sample

size. However, this analysis provides evidence to support the inter-related contribution of visceral, hepatic and muscle fat to variation in VLDL-apoB-100 transport. Accordingly, our findings need to be interpreted with caution and confirmed with a larger sample size. Moreover, we did not have data on skeletal muscle fat content in non-obese individuals and cannot therefore make comparisons between obese and non-obese subjects; however, previous reports have consistently demonstrated that skeletal muscle fat is greater in obese than in lean individuals [18, 19]. Our weight loss study was not a controlled observation. However, it provides data to support the direct relationship between VLDL-apoB-100 secretion rate and skeletal muscle fat. Exercise has been shown to have a significant impact on insulin resistance and fat deposition in skeletal muscle [20]. Although our study did not control for exercise activity, our subjects were advised to maintain their level of exercise activity 1-week prior to the study and none of them had regularly participated in vigorous physical activity. Our estimate of insulin resistance was the HOMA score; a more accurate measurement of skeletal muscle insulin resistance would require use of the hyperinsulinemic-euglycemic clamp. Measurements of lipase and lipid transfer proteins in plasma, particularly, lipoprotein lipase, hepatic lipase and CETP, as well as the kinetics of VLDL-triglyceride may help to formally corroborate our findings.

Ectopic fat accumulation in skeletal muscle in obesity may predispose to the development of type 2 diabetes and cardiovascular disease. Our kinetic studies of VLDL metabolism suggest that accumulation of visceral and liver fat may regulate the deposition of fat in skeletal muscle via their effects on VLDL-apoB-100 transport. Our data support the role of VLDL-apoB-100 as an important driver of ectopic fat

accumulation in skeletal muscle and highlight the importance of therapies (e.g. weight loss) that targets visceral and liver fat in protecting against the consequences of dietary lipid overload in skeletal muscle.

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Table 1 Anthropometric and biochemical characteristics and VLDL-apoB100 kinetic parameters of the 25 subjects studied

Characteristics	Mean ± SD		
	Whole group (n=25)	IS group (n=12)	IR group (n=13)
Gender (M/F)	15/10	8/4	7/6
Age (yr)	57±8	58±9	55±7
Weight (kg)	96±15	96±13	97±16
Body mass index (kg/m^2)	33±4	32±2	33±4
Visceral fat (cm^2)	265±104	245±129	284±75
Subcutaneous fat (cm^2)	310±128	305±125	314±136
Total body fat (kg)	39±12	39±13	39±12
Fat free mass (kg)	57±11	57±12	58±11
Hepatic fat content (%)	20±13	15±13	24±12*
Skeletal muscle fat content (%)	17±6	17±6	17±7
Non-esterified fatty acid (mEq/L)	0.7±0.4	0.7±0.2	0.7±0.3
Cholesterol (mmol/L)	5.8±1.0	5.6±0.8	5.9±1.2
Triglyceride (mmol/L)	1.8±0.7	1.7±0.8	2.0±0.6
HDL-cholesterol (mmol/L)	1.2±0.4	1.3±0.6	1.1±0.3
LDL-cholesterol (mmol/L)	3.8±0.8	3.6±0.6	3.9±1.0
ApoA-I (g/L)	1.4±0.3	1.5±0.4	1.4±0.2
ApoB-100 (g/L)	1.2±0.3	1.1±0.3	1.2±0.3
Glucose (mmol/L)	5.5±0.6	5.2±0.6	5.8±0.6**
Insulin (U/L)	12±5.0	8.4±2.1	16±4.0**
HOMA score	3.0±1.4	1.9±0.4	4.0±1.3**
Retinol-binding protein-4 (mg/L)	27±6.1	28±6.6	26±5.5
Adiponectin (mg/L)	5.3±2.5	6.0±2.7	4.6±2.1
Interleukin-6 (ng/L)	1.0±0.4	1.0±0.4	1.0±0.3
TNF-alpha (ng/L)	5.9±1.8	6.1±2.0	5.7±1.6
VLDL-apoB-100 concentration (mg/L)	137±52	125±58	149±46
VLDL-apoB-100 FCR (pools/day)	4.2±2.4	5.0±3.1	3.5±1.0
VLDL-apoB-100 secretion rate (mg/ kg/day)	19±5.0	19±5.4	18±4.9

IS group: insulin sensitive group; IR group: insulin resistant group

The cut-off point to define insulin resistance used in this current study corresponded with a HOMA score of 2.6

Value significantly different from IS group: *P<0.05, **P<0.01

Table 2 Associations (Pearson correlation coefficients) of skeletal muscle fat content with the clinical, biochemical characteristics and the kinetic indices for VLDL-apoB-100 in the subjects studied

Characteristics	Pearson correlation coefficients		
	Whole group (n=25)	IS group (n=12)	IR group (n=13)
Age (yr)	-0.005	-0.161	0.153
Weight (kg)	0.415*	0.649*	0.273
Body mass index (kg/m^2)	0.017	0.293	-0.104
Visceral fat (cm^2)	0.531**	0.765**	0.328
Subcutaneous fat (cm^2)	-0.229	-0.405	-0.108
Total body fat (kg)	0.183	0.441	-0.025
Fat free mass (kg)	0.334	0.216	0.443
Hepatic fat content (%)	0.282	0.636*	0.018
Energy intake (kJ)	0.531**	0.694*	0.415
Non-esterified fatty acid (mEq/L)	0.428*	0.225	0.578*
Cholesterol (mmol/L)	0.084	0.049	0.102
Triglyceride (mmol/L)	0.236	0.644*	-0.159
HDL-cholesterol (mmol/L)	-0.343	-0.442	-0.300
LDL-cholesterol (mmol/L)	0.186	0.066	0.247
ApoA-I (g/L)	-0.215	-0.387	-0.030
ApoB-100 (g/L)	0.347	0.529*	0.227
Glucose (mmol/L)	0.477*	0.622*	0.454
Insulin (U/L)	0.058	-0.463	0.274
HOMA score	0.190	-0.172	0.411
Retinol-binding protein-4 (mg/L)	0.342	0.211	0.491
Adiponectin (mg/L)	-0.185	-0.213	-0.181
Interleukin-6 (ng/L)	-0.259	-0.065	-0.491
TNF-alpha (ng/L)	0.008	0.381	-0.343
VLDL-apoB-100 concentration (mg/L)	0.291	0.860**	-0.224
VLDL-apoB-100 FCR (pools/day)	-0.184	-0.581*	0.297
VLDL-apoB-100 secretion rate (mg/ kg/day)	0.326	0.607*	0.099

* P <0.05, ** P <0.01;
IS group: insulin sensitive group; IR group: insulin resistant group
The cut-off point to define insulin resistance used in this current study corresponded with a HOMA score of 2.6

Table 3. Clinical and biochemical characteristics before and after weight loss

	Weight Loss (n=10)	
	0	**22 wk**
Body Weight (kg)	100±6	94±5*
BMI (kg/m^2)	33±1	31±1*
Waist (cm)	107±4	98±4 *
Total body fat (kg)	39±4	35±4 *
Fat-free mass (kg)	60±3	59±3
Visceral fat at L3 vertebra (cm^2)	278±38	228±36 **
Subcutaneous fat at L3 vertebra (cm^2)	358±43	316±36 **
Hepatic fat content (%)	22±5	16±4*
Skeletal muscle fat content (%)	16±1	14±1**
Energy intake (kJ)	8269±789	5811±437**
Cholesterol (mmol/l)	5.8±0.2	5.5± 0.2
Triglyceride (mmol/l)	1.7±0.3	1.3±0.2 **
Non-esterified fatty acids (mEq/L)	0.6±0.1	0.6±0.1
HDL-cholesterol (mmol/l)	1.3±0.2	1.3±0.1
LDL-cholesterol (mmol/l)	3.7±0.2	3.7±0.2
Apolipoprotein A-I (g/l)	1.5±0.1	1.5±0.1
Apolipoprotein B-100 (g/l)	1.2±0.1	1.1±0.1
Glucose (mmol/l)	5.5±0.2	5.7±0.2
Insulin (mU/l)	12±2	10±2 **
HOMA score	3.1±0.5	2.6±0.4 **
Retinol-binding protein-4 (mg/L)	277±16	24±8*
Adiponectin (mg/L)	5.9±0.7	6.8±0.8*
Interleukin-6 (ng/L)	0.9±0.1	0.9±0.1
TNF-alpha (ng/L)	5.4±0.5	5.4±0.6
VLDL-apoB-100 concentration (mg/L)	142±12	123±19 *
VLDL-apoB-100 FCR (pools/day)	4.8±1.1	3.8±0.4
VLDL-apoB-100 secretion rate (mg/ kg/day)	20±2	15±1 **

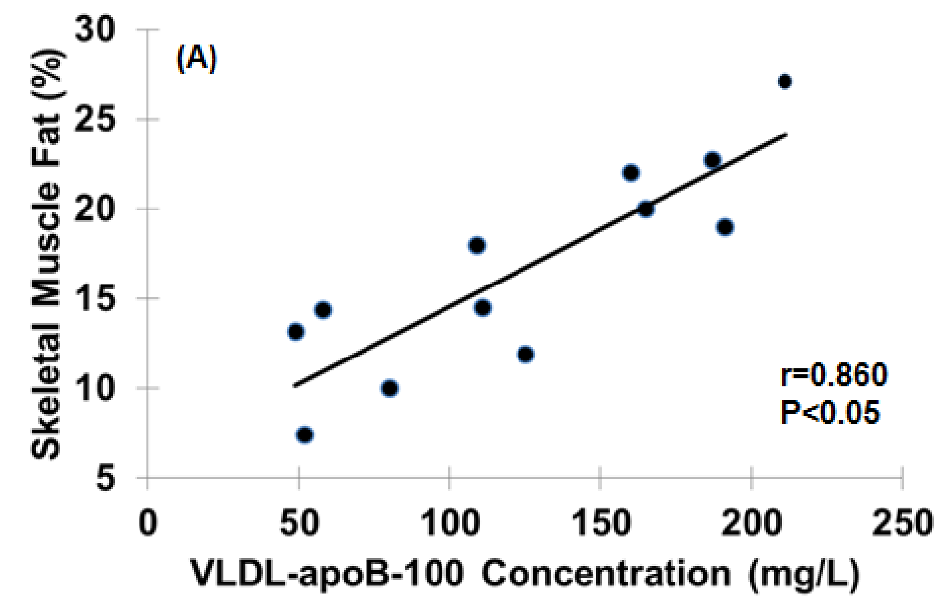
Values are expressed as Mean±SEM; There was no significant group difference in the variables at baseline.
Value significantly different from baseline value: *P<0.05, **P<0.01

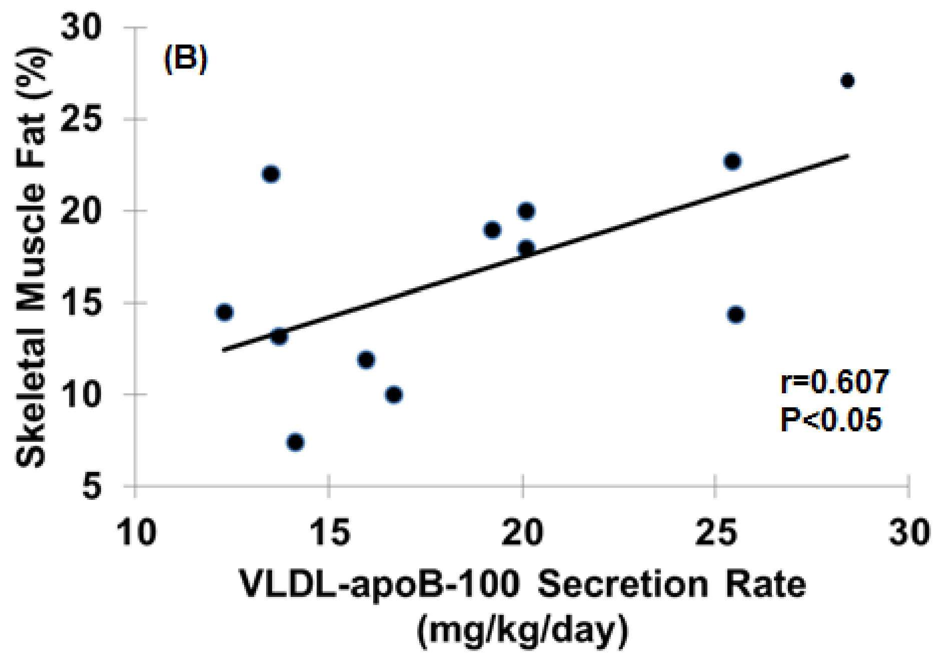
Legends

Figure 1 Association between skeletal muscle fat and VLDL-apoB-100 concentration (A), VLDL-apoB-100 secretion rate (B) and VLDL-apoB-100 fractional catabolic rate (C) in 12 insulin sensitive obese subjects

Figure 2 Association between changes in skeletal muscle fat and VLDL-apoB-100 concentration (A) and VLDL-apoB-100 secretion rate (B) in 10 obese subjects with weight loss

Figure 1





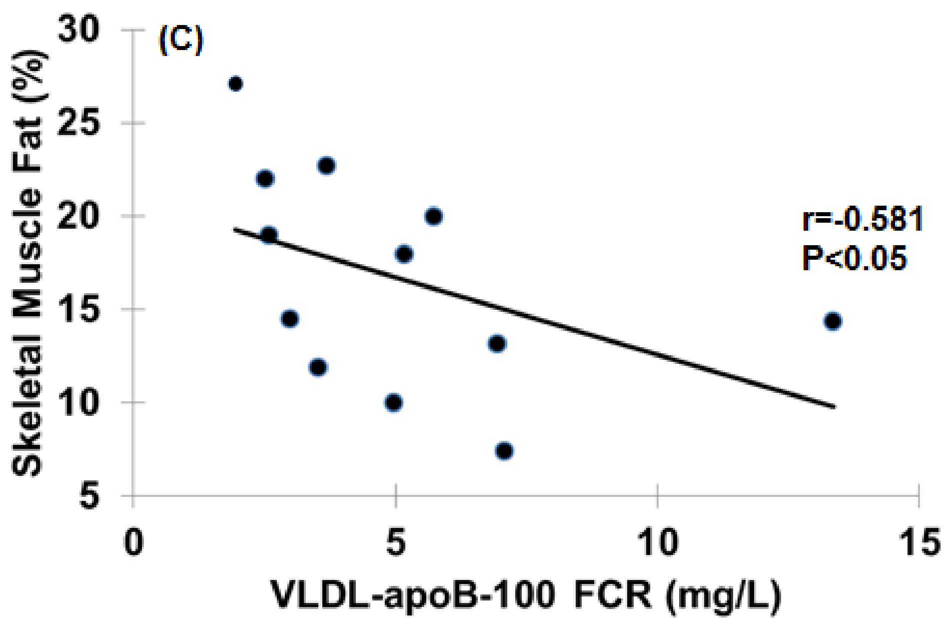


Figure 2