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Polycystic Ovary Syndrome and Nonalcoholic Fatty Liver in Obese Adolescents: Association with Metabolic Risk Profile.

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Abstract

Objective—To investigate the relationship between liver fat and *in vivo* insulin sensitivity, body composition, abdominal adiposity and lipid metabolism in obese adolescent girls with PCOS.

Design—Cross-sectional case-control study.

Setting—Pediatric Translational Research Center.

Patients—Thirty Tanner stage V obese girls with PCOS.

Intervention—None.

Main Outcome Measure(s)—Liver fat, abdominal adiposity, *in vivo* insulin-stimulated glucose disposal, whole-body lipolysis, fat oxidation and lipoprotein particle size and concentration and liver enzymes (ALT, AST). Fatty liver index (FLI) <1 is indicative of fatty liver.

Results—Fatty liver was present in 2 individuals (6.7%). ALT and AST were not different between those with fatty liver vs. without. FLI was associated with age (r=−0.53), BMI (r=−0.41), total (r=−0.43) and subcutaneous (r=−0.41) abdominal adiposity, insulin-stimulated glucose disposal (r=0.36), and small, medium small, and very small LDL concentrations (r>=−0.43). In a multiple regression analysis, age, total testosterone, race and insulin-stimulated glucose disposal explained 43% of the variance (R^2=0.43) in FLI, with age (R^2=0.28) and total testosterone (R^2=0.11) being independent contributors.

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Author Contribution

SFM first authored the manuscript, and contributed to the data analyses and interpretation; SJ reviewed the manuscript and analyzed the liver CT images; HT acquired data and reviewed the manuscript; SA provided the study concept and design, acquired data, obtained funding, provided administrative, technical and material support, supervised the study and critically reviewed/edited the manuscript.

Conclusion—Liver fat is associated with increasing age, even in the narrow adolescent age range, increasing abdominal adiposity, worsening insulin sensitivity and dyslipoproteinemia in obese adolescent girls with PCOS. Targeting these abnormalities early in the course of PCOS may halt future NAFLD in adulthood.

Keywords

Polycystic Ovary Syndrome; Hepatic Steatosis; Obesity

Introduction

Non-alcoholic fatty liver disease (NAFLD) commonly clusters with factors related to increased metabolic risk such as obesity (1, 2), dyslipidemia (3-8), insulin resistance (9, 10) and T2DM (1, 3-5). NAFLD is an umbrella term used to describe a spectrum of disorders from asymptomatic hepatic steatosis (here on out referred to as fatty liver) with or without elevated aminotransferases, to cirrhosis with complications of liver failure (11), and occurs when infiltration of free fatty acids (FFA) exceed the rate of hepatic fat oxidation. Clinically, fatty liver itself is generally a silent state of liver adiposity and is not usually discovered until aminotransferase levels are elevated or a radiographic study reveals a fatty liver (12). Thus, the natural progression of fatty liver can lead to nonalcoholic steatohepatitis (NASH) to cirrhosis and possibly liver disease (13). Consequently, several investigations have demonstrated that adults with NAFLD have higher mortality whereas adults with NASH have higher liver-related mortality compared with the general population (13, 14). However, similar investigations in women with PCOS are lacking.

Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder in females of reproductive age. PCOS is characterized by menstrual irregularities (oligomenorrhea or amenorrhea) and clinical and/or biochemical hyperandrogenism in the presence or absence of polycystic ovaries with more than half of the patients being overweight or obese (15). Coupled to the elevated rates of obesity is an increased risk for developing a number of obesity-related comorbidities including the metabolic syndrome, insulin resistance and impaired glucose tolerance (16, 17). Depending on the definition used, it is estimated that approximately 37-47% of adolescents with PCOS have the metabolic syndrome compared with 0.6-8.9% of adolescent girls within the general population (16, 18, 19). A hallmark of the metabolic syndrome, insulin resistance, is observed in approximately 50% to 80% of women with PCOS (20). We previously demonstrated that adolescents with PCOS had 50% lower insulin sensitivity compared with equally obese non-hyperandrogenic girls matched for age, body composition and Tanner stage (17). Moreover, insulin resistance may be more than a mere biomarker of PCOS; it may likely contribute to its pathogenesis. As thoroughly discussed in a recent systematic review (21), ovaries are abundant with insulin receptors and the dysregulation of insulin signaling in theca cells may augment the production of androgens. Irrespective, against the backdrop of severe insulin resistance, adolescents with PCOS also demonstrate evidence for dysregulated glucose metabolism. Approximately 30% of adolescents with PCOS, including lean PCOS, have impaired glucose tolerance (22) and in PCOS adolescents with impaired glucose tolerance, 50% demonstrate lower β-cell function (23). Collectively, the increased prevalence of these risk factors among adolescents with PCOS amplifies their risk for the development of T2DM. Since PCOS and NAFLD share common attributes in regards to their pathogenesis, insulin resistance, we hypothesized that liver fat would be associated with increased metabolic risk in adolescent girls with PCOS. Therefore, we investigated in obese adolescent girls with PCOS the relationship between liver fat and *in vivo* insulin sensitivity, body composition, abdominal adiposity and lipid metabolism.

Materials and Methods

Sample Population

The sample population consisted of 30 Tanner stage V obese adolescents with PCOS (Table 1). Participants were recruited from Children's Hospital of Pittsburgh PCOS center and through advertisements and flyers posted throughout the community, medical campus and pediatricians' offices. The diagnosis of PCOS was made based on the presence of clinical signs and symptoms of hyperandrogenism and/or biochemical hyperandrogenemia, oligoovulation and the exclusion of secondary etiologies as per the National Institute of Health 1990 (24) definition, as previously reported (17, 23, 25). The study was approved by the Institutional Review Board of the University of Pittsburgh. Informed consent and child assent was obtained from each participant and their legal guardians. Some participants were reported previously (25). Exclusion criteria included preexisting treatment for PCOS, pregnancy, existing systemic or psychiatric disease and the use of medications that influence glucose or lipid metabolism or blood pressure.

Clinical Examination

Other than a diagnosis of PCOS, participants had no other health condition and denied alcohol intake. All underwent complete medical history, physical examination and hematological and biochemical tests. Height and weight were assessed to the nearest 0.1 cm and 0.1 kg, respectively and used to calculate body mass index (BMI). Pubertal development was assessed using Tanner criteria (26). A fasting blood sample was obtained for lipoprotein particle size, plasma lipid concentrations [total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein and triglycerides], lipid subclass concentrations (total, large, medium, medium small, small and very small), adiponectin, leptin, high-sensitivity C-reactive protein (hs-CRP), and testosterone profile [total testosterone, free testosterone, sex hormone-binding globulin (SHBG) and dehydroepiandrosterone sulfate (DHEAS)] and liver enzymes [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)].

Hyperinsulinemic-euglycemic clamp

After a 10-12 hour overnight fast, endogenous hepatic glucose production (HGP) was measured with a primed constant-rate infusion of [6,6-$^{2}H_{2}$]glucose (Isotech, Miamisburgh, OH) as reported previously (17, 25). Whole-body lipolysis was measured at baseline and during a hyperinsulinemic-euglycemic clamp by the use of a primed (1.2 μmol/kg) constant rate (0.08 umol/kg^{-1}min^{-1}) infusion of [$^{2}H_{5}$]glycerol, as previously described (27). *In vivo* insulin-stimulated glucose disposal was evaluated with a 3-hr hyperinsulinemic-euglycemic clamp. Briefly, intravenous crystalline insulin (Humulin; Lilly, Indianapolis, IN) was infused at a constant rate of 80 mU m^{-2} min^{-1} to suppress HGP, as described before (17). Plasma glucose was clamped at 100 mg/dl with a variable rate infusion of 20% dextrose based on arterialized plasma glucose determined every 5 min. Indirect calorimetry by a ventilated hood system (Deltatrac metabolic monitor; SensorMedics, Anaheim, CA) was used to determine substrate utilization during the last 30 min of the baseline period and clamp as described by us (17, 27).

Biochemical measurements

Plasma glucose was measured with a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, OH) and insulin, leptin and adiponectin by RIA (25). hs-CRP was measured by COAG-Nephelometry (Esoterix Inc., (formerly Colorado Coagulation) Englewood, CO]. Plasma lipid concentrations were determined using the standards of the Centers for Disease Control and Prevention (28) and lipoprotein particle size and subclass

concentrations were determined using nuclear magnetic resonance spectroscopy (MRS) (LipoScience, Raleigh, NC) (28). Five participants are missing lipoprotein data due to insufficient sample volume. FFAs were determined by an enzymatic colorimetric assay (Wako nonesterified fatty acid C test kit; Wako, Osaka, Japan). Total testosterone and DHEAS were measured by HPLC-tandem mass spectroscopy (Esoterix Inc., Calabasas Hills, CA). Free testosterone was measured by equilibrium dialysis and SHBG by immunoradiometric assay.

Body Composition

Dual-energy x-ray absorptiometry was used to determined fat mass (FM), fat free mass (FFM) and percent total body fat (BF%). Computed tomography (CT) was used to determine subcutaneous (SAT), visceral (VAT) and total (TAT) adipose tissue as previously described by us (29). SAT and VAT data are missing for 3 subjects due to technical difficulties.

Liver fat determined by CT

CT was used to differentiate tissues on the basis of their attenuation characteristics (30, 31). Healthy livers are dense and characteristic of higher attenuation when compared with the spleen. Thus, a low liver attenuation relative to that of a spleen is indicative of a fattier liver. This ratio is clinically useful as an absolute CT number is not sensitive enough for predicting an abnormal liver (31). Fatty liver index (FLI) was represented as a ratio between liver to spleen Hounsfield attenuation units (HU) (liver HU /spleen HU). A single CT image that clearly displayed both the liver and the spleen was used to calculate the average attenuation values of 2 regions of interest within each organ. The regions of interest were placed in the parenchyma of the right lobe of the liver and a similar region within the spleen. Blood vessels, artifacts and other areas of inhomogeneity were avoided. FLI < 1.0 was indicative of fatty liver and correlated with hepatic fat volume percent (32).

Calculations

Fasting turnover calculations were made over the last 30 min of the basal post absorptive period. An index of hepatic insulin sensitivity (HIS) was calculated as the inverse of the product of HGP and the fasting plasma insulin concentration (33). Insulin-stimulated glucose disposal was calculated using the average exogenous glucose infusion rate during the final 30 min of the clamp to be equal to the rate of exogenous glucose infusion. Whole-body lipolysis was calculated from the rate of appearance (Ra) of glycerol in plasma according to steady-state tracer dilution equations (27). Basal and insulin-stimulated rates of glucose oxidation (GOX) and fat oxidation (FOX) were the calculated mean from indirect calorimetry measurements according to the equations developed by Frayn (34). Non-oxidative glucose disposal was estimated by subtracting the rate of GOX from the total-body insulin-stimulated glucose disposal during the last 30 min of the clamp. An index of adipose tissue insulin sensitivity (Adipo-IS) was calculated as a product of glycerol Ra (in μmol/kg/min) and fasting plasma insulin (in μU/mL) (35).

Statistical Analyses

Data are summarized as mean ± SEM. PASW Statistics (version 20, SPSS Inc., Chicago, IL) was used for statistical analyses and statistical significance was set at $p \leq 0.05$. To determine associations between FLI (liver attenuation/spleen attenuation) and metabolic risk factors, bivariate Pearson or Spearman correlation coefficients were computed when appropriate.

Results

Participant characteristics (Table 1)

All participants were overweight/obese girls in Tanner stage V puberty with a wide range of body composition, waist circumference and adiposity. Absolute liver attenuation was within a normal range for all subjects (29.5-75.3 HU). Fatty liver, FLI < 1, was present in 2 individuals (6.7% of the cohort). Those with fatty liver vs. without tended to be older (18.0±2.0 vs. 15.9±0.3yr; p=0.08), have higher VAT (118.9±14.0 vs. 70.5±4.8cm3; p<0.001) and lower insulin sensitivity (1.0±0.0 vs. 2.0±0.2mg/kg/min, p<0.001). However, ALT (28.0±5.0 vs. 27.4±1.7, p=0.93), AST (23.0±1.0 vs. 22.9±0.8, p=0.98), BMI (kg/m^2) (39.5±3.2 vs. 37.0±1.4, p=0.63) and BMI percentile were similar (98.0± 0.3 vs. 98.5± 1.0, p=0.72).

Associations between fatty liver index and body composition, insulin sensitivity and metabolic parameters (Table 2)

FLI correlated with increasing age, higher BMI, SAT and TAT (Table 2) and a tendency for higher FM (r=−0.32, p=0.08) and VAT (r=−0.36, p=0.07).

FLI correlated with insulin-stimulated glucose disposal and tended to associate with non-oxidative glucose disposal (r=0.35, p=0.07). There was no relationship between FLI and fasting glucose (r=0.11, p=0.56), insulin (r=−0.02, p=0.91), HGP (r=0.10, p=0.60) or HIS (r=−0.03, p=0.86).

FLI tended to associate with whole-body lipolysis (r=−0.32, p=0.09) but not FOX (r=−0.01, p=0.96) or Adipo-IS (r=−0.14, p=0.45).

FLI correlated with total, small, medium small and very small LDL concentrations (Table 2). There was no relationship between FLI and triglycerides (r=−0.07, p=0.71), HDL concentrations (r=0.09, p=0.65) or lipoprotein particle size (r≥0.24, p≥0.14).

FLI tended to associate with total testosterone (r= −0.34, p=0.07), estradiol (r= −0.33; p=0.07) and hs-CRP (r= −0.34; p=0.07). No significant associations were observed between FLI and free testosterone (r=−0.18, p=0.34), SHBG (r=−0.06, p=0.77), leptin (r=−0.16, p=0.41) or adiponectin (r=0.13, p=0.56).

In a multiple regression analysis, age, total testosterone, race and insulin-stimulated glucose disposal explained 43% of the variance (R^2=0.43, p=0.006) in FLI, with age (R^2=0.28, p=0.002) and total testosterone (R^2=0.11, p=0.03) being significant independent contributors.

Discussion

NAFLD is a comorbidity of obesity (1, 2), dysmetabolic syndrome (3-8), insulin resistance (9, 10) and T2DM (1, 3-5). NAFLD describes a spectrum of disorders from fatty liver to cirrhosis with complications of liver failure (11) and occurs as a result of an imbalance between triglyceride acquisition and removal. Clinically, fatty liver itself is generally a silent state of liver adiposity and is not usually discovered until aminotransferase levels are elevated or a radiographic study reveals a fatty liver (12). Thus, the natural progression of fatty liver can lead to NASH with evidence of inflammation, to cirrhosis and serious liver disease (13). Consequently, several investigations have demonstrated that adults with NAFLD have higher mortality rates whereas adults with NASH have higher liver-related mortality compared with the general population (13, 14). However, similar investigations in women with PCOS are lacking. The diagnostic methodologies used to assess fatty liver

include liver enzymes, ultrasonography, CT, magnetic resonance imaging (MRI) or MRS and liver biopsy. Among these methods, CT and MRI both provide an accurate and objective quantification of each body fat component. MRI is accurate although has many disadvantages, including high cost and long scan times whereas CT is simple and reproducible and thus, a widely used imaging modality that evaluates hepatic steatosis indirectly based on hepatic x-ray attenuation, although exposes the patient to radiation (36). Thus, clinically, liver enzymes and ultrasonography are often utilized for their ease of use. However, fatty liver can exist with or without elevated aminotransferases (11) and ultrasonography is relatively easy to use and inexpensive but has low reproducibility and it can only detect fatty liver when > 30% of the liver is affected (36).

Females with PCOS are characterized by obesity (15), the metabolic syndrome (20) and severe insulin resistance (17), conditions that enhance the risk of NAFLD. Schwimmer et al. evaluated medical charts of 73 women with PCOS and demonstrated that ~30% had elevated ALT and 12% had elevated AST which were primarily explained by their level of insulin resistance, assessed by QUICKI (37). A prospective investigation of 41 women with PCOS and 31 non-PCOS women similar in age, BMI and waist to hip ratio demonstrated that women with PCOS had higher ALT, fatty liver (assessed via ultrasound) and log HOMA-IR (39) whereas Gambarin-Gelwan et al. used ultrasonography to demonstrate that ~55% of PCOS women had fatty liver (42). With the juxtaposition of elevated rates of obesity and insulin resistance in PCOS girls, the finding that only two adolescent girls with PCOS had a FLI < 1 (6.7% of the cohort) was surprisingly lower than expected from data in the aforementioned studies of adult women. However, our findings are similar to a general population of children and adolescents where overall prevalence of fatty liver was ~9.6% (41). These observations in adolescents vs. adults may reflect the role that increasing age may play in fatty liver, especially considering the inverse relationship between age and FLI in our study. It is important to note that because fatty liver is a histological diagnosis, the true prevalence of fatty liver in the pediatric population is challenging. Though the FLI ratio correlates with the degree of fatty infiltration as measured by histomorphometry (43), some investigations have demonstrated fatty liver in spite of a normal FLI ratio (FLI ≥ 1) (32). Furthermore, it remains to be determined, if our findings are unique to PCOS or are driven by their level of obesity.

Several investigations within the general population have provided compelling evidence that fatty liver strongly correlates with obesity (1, 2), dyslipidemia (3-8), insulin resistance (8-10) and T2DM (1, 3-5). Since PCOS and NAFLD share common attributes in regards to their pathogenesis, we hypothesized that liver fat would be associated with increased metabolic risk in adolescent girls with PCOS. In accordance with our hypothesis, our investigation demonstrates that in obese adolescent girls with PCOS liver fat is associated with increasing abdominal obesity, worsening insulin sensitivity and dyslipoproteinemia. The reported methods of evaluating fatty liver in PCOS women are diverse, including liver enzyme determination (38, 44), liver ultrasound (42, 45-47), liver biopsy (46), CT method (45) and very recently, over the last year, magnetic resonance spectroscopy (48). To our knowledge, there are no reports of fatty liver in obese adolescents with PCOS. In adult women with PCOS, similar to the observations in the general public, abnormal AST activity was associated with lower HDL, higher triglycerides and higher fasting insulin (38). Independent from the contribution of age and total adiposity, abnormal ALT associated with impaired insulin sensitivity in both lean and obese women with PCOS (44). These findings are similar to Gambarin-Gelwan et al. who observed an association between fatty liver and BMI, HOMA-IR, HDL and impaired fasting glucose, impaired glucose tolerance and diabetes in both lean and obese women with PCOS (42). Using ultrasonography, Ma et al. showed that Chinese women with PCOS and severe fatty liver, had higher ALT activity, higher BMI, waist circumference, waist-hip ratio, fasting glucose, fasting insulin and 2-h

glucose (47). Sopher et al. showed that despite a higher prevalence of intrahepatic lipid content, measured by magnetic resonance spectroscopy in normal-weight adolescents with PCOS, fatty liver associated with luteinizing hormone and androstenedione but did not associate with triglycerides or insulin resistance measured by HOMA-IR (48). The lack of statistical association between fatty liver and insulin resistance in the aforementioned study (48) may be the result of a small sample size (n=24), a non-obese adolescent sample population, and/or the methodology used to estimate insulin resistance (i.e. HOMA-IR) which as an index is not a sensitive measure of *in vivo* insulin sensitivity as the hyperinsulinemic-euglycemic clamp. Moreover, the disparities in the associations between fatty liver and metabolic risk may also be attributable to the different methodologies used to assess fatty liver (i.e. liver enzymes, ultrasonography). Nonetheless, our investigation in obese adolescents with PCOS is in accordance with previous investigations in adult women with PCOS whereby liver fat associated with older age (49), higher BMI (42, 47, 49), LDL concentrations (49) and worsening insulin sensitivity assessed via the hyperinsulinemic-euglycemic clamp (44) and HOMA-IR (42, 49). Accordingly, age and total testosterone made the greatest contribution to liver fat as determined by multiple linear regression. Taken together, the findings in PCOS adult women and adolescents suggest that liver fat is associated with an amplified metabolic risk and with a hyperandrogenic profile.

Although the pathogenesis of fatty liver remains unclear, an infiltration of FFAs and a concomitant failure to adequately oxidize lipids would increase lipid accumulation. There is evidence that adults with NAFLD have increased FFA delivery from adipose tissue (50, 51) and reduced whole-body fat utilization (52). A recent study in obese adults with NAFLD demonstrated greater rates of palmitate release from adipose tissue and VLDL-TG secretion compared with obese controls matched for age, sex, BMI and percent body fat (51). In 18 morbidly obese women with high liver fat content, there was a 2-fold increase in visceral adipocyte derived glycerol release (50). In 20 overweight/obese adults with NAFLD, basal whole-body FOX significantly associated with the degree of steatosis and histological severity of the disease (52). In our study, fatty liver tended to correlate with higher rates of whole-body lipolysis but not with fat oxidation.

The data from our investigation demonstrate several practical implications to target therapeutic strategies to reduce fatty liver. In both adults (53-57) and youth (58-60), diet and/or exercise are integral components of lifestyle intervention able to ameliorate risk factors associated with the development of NAFLD. Van der Heijjden et al. demonstrated that a 12-week aerobic exercise intervention, without caloric restriction, significantly decreased liver fat, visceral adiposity and insulin resistance, assessed by HOMA-IR, by ~37%, ~9.3% and ~16% in obese adolescent boys and girls (59). Using ^{1}H-MRS and MRI, we demonstrated similar improvements in visceral adiposity (7%) and fatty liver (~40%) in obese adolescent boys (58). Following 8-weeks of circuit-training, insulin sensitivity improved by ~22.2%, independent of changes in body composition, in obese children (60). Collectively, these investigations show clear evidence for a benefit of exercise therapy on reducing visceral adiposity, increasing insulin sensitivity and reducing liver fat. These benefits appear to be apparent independent of changes in body composition. However, similar investigations are lacking in PCOS adolescents.

The strengths of this investigation are 1) that our subjects were not receiving any treatment while participating in this study; 2) the comprehensive examination of whole body, abdominal and liver adiposity; and 3) the state-of-the-art use of stable isotopes together with indirect calorimetry and the hyperinsulinemic-euglycemic clamp to assess *in vivo* whole-body lipolysis, substrate utilization and insulin sensitivity. A limitation is that our cross-sectional design does not allow us to determine a cause and effect relationship between fatty liver and metabolic risk and a comparison to non-PCOS obese girls. Another limitation is

that our findings in obese adolescents may not apply to normal-weight adolescents with PCOS. Lastly, unlike ultrasonography, CT attenuation is measured objectively and accurately; however, several factors other than fat deposition may affect CT attenuation. The presence of iron, copper, glycogen, fibrosis and edema as well as certain drugs such as amiodarone and gold are shown to affect CT attenuation (36). Grading fatty liver with imaging features alone has limited value without definitive pathologic confirmation although in children, we are ethically prohibited from obtaining liver biopsies. Lastly, it is difficult to distinguish between simple steatosis and nonalcoholic steatohepatitis (NASH) using imaging alone (61).

In summary, the present study demonstrates that in obese adolescent girls with PCOS, liver fat is associated with increasing age, even in the narrow adolescent age range, increasing abdominal adiposity, worsening insulin sensitivity and dyslipoproteinemia. Therapeutic strategies to decrease abdominal obesity and reduce insulin resistance and dyslipidemia early in the course of PCOS in youth may halt future NAFLD in adulthood.

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Table 1

Subject Characteristics

	Mean ± SEM	Range
N	30	
Age (yr)	16.1 ± 0.3	13 - 20
Race (%)		
Caucasian	66.7	
African American	20.0	
Biracial	13.3	
ALT (U/L)	27.4 ± 1.6	14.0 - 47.0
AST (U/L)	22.9 ± 0.8	15.0 - 33.0
DHEAS (ug/dl)	195.0 ± 18.9	53.0 - 470.0
T.Testos (ng/dl)	40.2 ± 3.0	14.0 - 84.0
F.Testos (pg/ml)	8.7 ± 0.8	3.2 - 19.0
Estradiol (pg/ml)	70.4 ± 9.7	4.0 - 254.0
SHBG (nmol/l)	24.2 ± 2.7	10.0 - 80.0
Anthropometric data		
BMI (kg/m^2)	37.1 ± 1.3	27.8 - 57.0
BMI percentile	97.6 ± 0.3	93.8 - 99.7
FM (kg)	47.1 ± 2.5	27.2 - 83.0
FFM (kg)	48.7 ± 1.3	36.1 - 57.9
BF (%)	46.9 ± 0.9	37.1 - 57.9
Waist circumference (cm)	104.5 ± 3.3	76.0 - 158.0
Abdominal CT data (L_{4-5}) *		
VAT (cm^2)	74.1 ± 5.2	28.3 - 132.9
SAT (cm^2)	600.0 ± 36.3	330.4 - 1098.0
TAT (cm^2)	674.0 ± 38.5	393.5 - 1158.3
Liver/spleen CT data		
Liver HU	63.6 ± 1.1	47.5 - 73.6
Spleen HU	51.3 ± 0.4	47.3 - 56.2
FLI	1.24 ± 0.02	0.9 - 1.5
Fasting Metabolic Parameters		
Fasting glucose (mg/dl)	93.6 ± 1.2	85.5 - 112.8
Fasting insulin (μU/ml)	36.1 ± 2.7	11.5 - 69.2
Hepatic glucose production (mg/kg/min)	2.1 ± 0.1	1.2 - 3.7
Hepatic insulin sensitivity (mg/kg/min per μU/ml)	16.4 ± 1.5	6.2 - 44.4
Glycerol Ra (umol/min)	320.9 ± 29.0	111.2 - 731.5
Adipo-IS (μmol/kg/min per μU/ml)	14.6 ± 1.7	4.2 - 45.5
Clamp Metabolic Parameters		
Insulin-stimulated glucose disposal (mg/kg/min)	5.7 ± 0.3	3.0 - 9.7
GOX (mg/kg/min)	2.4 ± 0.1	1.0 - 3.8

	Mean ± SEM	Range
Non-oxidative glucose disposal (mg/kg/min)	3.3 ± 0.3	0.98 - 7.21
Insulin sensitivity (mg/kg/min per μU/ml)	2.0 ± 0.2	0.80 - 3.6

T.Testos = total testosterone; F.Testos = free testosterone. FLI < 1.0 indicative of fatty liver.

* n = 27

Table 2

Significant correlations of fatty liver index with age, body composition, LDL subclass concentrations and *in vivo* insulin sensitivity

	Fatty Liver Index	
	r	**P**
Age (yrs)	−0.53	0.003
Body Composition		
BMI (kg/m^2)	−0.41	0.03
SAT (cm^2)	−0.41	0.04
TAT (cm^2)	−0.43	0.03
LDL (nmol/l) †		
Total LDL	−0.40	0.05
Small LDL	−0.44	0.03
Medium small LDL	−0.44	0.03
Very small LDL	−0.43	0.03
Hyperinsulinemic-Euglycemic Clamp		
Insulin-stimulated glucose disposal (mg/kg/min)	0.36	0.05

† n=25