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Relationship of Liver Enzymes to Insulin Sensitivity and Intra-abdominal Fat

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ABSTRACT

Objective: To determine the relationship between plasma liver enzyme concentrations, insulin sensitivity and intra-abdominal fat (IAF) distribution.

Research Design and Methods: Plasma gamma-glutamyl transferase (GGT), aspartate transaminase (AST), alanine transaminase (ALT) levels, insulin sensitivity (S_I), IAF and subcutaneous fat (SCF) areas were measured on 177 non-diabetic subjects (75M/102, 31-75 years) with no history of liver disease. Based on BMI (< or ≥27.5 kg/m^2) and S_I (< or ≥7.0x10^{-5} min^{-1} pM^{-1}) subjects were divided into lean insulin sensitive (LIS, n=53), lean insulin resistant (LIR, n=60) and obese insulin resistant (OIR, n=56) groups.

Results: All three liver enzymes were higher in men than women ($p<0.0001$ for each). In men, GGT levels were higher in insulin resistant than insulin sensitive subjects ($p<0.01$). In women, GGT levels were higher in the OIR vs. LIS ($p<0.01$) but no different in LIR. There was no difference in ALT and AST levels between LIS, LIR and OIR. GGT was associated with S_I ($r=-0.26$, $p<0.0001$), IAF ($r=0.22$, $p<0.01$), waist-hip ratio (WHR) ($r=0.25$, $p=0.001$), BMI ($r=0.17$, $p<0.05$), and SCF ($r=0.16$, $p<0.05$) after adjusting for age and gender. In men, only S_I ($r=-0.29$, $p<0.05$) remained independently correlated with GGT in multiple regression analysis. In women, IAF ($r=0.29$, $p<0.01$) and WHR ($r=0.29$, $p<0.01$) were independently associated with GGT, but S_I was not.

Conclusions: In non-diabetic men GGT levels, but not AST or ALT levels, are inversely related to insulin sensitivity independent of IAF. However in women, GGT is related to measures of central body fat rather than insulin sensitivity.

Relatively recently, the liver has been recognized as a major target of injury in patients with insulin resistance or the metabolic syndrome. Non-alcoholic fatty liver disease (NAFLD) is characterized by accumulation of hepatic fat in the absence of significant alcohol intake. In a proportion of patients, NAFLD may progress to non-alcoholic steatohepatitis (NASH), characterized by the presence of hepatic inflammation and hepatocellular damage, which may eventually progress to cirrhosis (1). The prevalence of NAFLD is about 20% and that of NASH is 2-3% in adults (2; 3).

NAFLD is strongly associated with insulin resistance, dyslipidemia, obesity and hypertension (4) and is probably the most common cause of abnormal liver function tests in diabetes (5). In non-diabetic subjects, elevated plasma liver enzyme levels are risk factors for the development of type 2 diabetes; however gamma-glutamyl transferase (GGT) may be a stronger predictor than aspartate transaminase (AST) or alanine transaminase (ALT) (6-8). Although GGT has been widely used as a marker of alcohol consumption, it has recently been found to be associated with an increased risk of developing type 2 diabetes independent of alcohol intake (9), as well as an increased risk of hypertension and cardiovascular mortality (10; 11).

Since diabetes, dyslipidemia, hypertension, cardiovascular disease and NAFLD have all been shown to be associated with central adiposity and insulin resistance (12), we hypothesized that differences in liver enzymes in healthy subjects are related in part to differences in fat distribution and insulin sensitivity. To test this hypothesis, we analyzed the relationship between liver enzymes, insulin sensitivity and body fat distribution in a large cohort of apparently healthy normal subjects.

RESEARCH DESIGN AND METHODS

Subjects

The data presented are baseline measurements from 177 subjects (75 men, 102 women) from a study population of 234 subjects in whom data on insulin sensitivity, body fat distribution, and plasma liver enzyme concentrations were available. There were no significant differences in subject characteristics between all 234 subjects and the 177 who form the basis of the current analysis. The subjects, who had been recruited by advertisement to participate in a study of the effect of egg consumption on plasma lipids in people with varying degrees of insulin sensitivity, were aged 31–75 years and apparently healthy, had no history of diabetes, dyslipidemia or uncontrolled hypertension, and had no known liver disease (13). Specific testing for liver disease was not performed at the time of the study. Subjects with fasting plasma glucose ≥6.4 mmol/l (≥115 mg/dl), biochemical evidence of renal disease, uncontrolled thyroid disease, coronary or other vascular disease, or anemia were excluded but formal oral glucose tolerance tests were not performed. The subjects were predominantly Caucasian: Caucasian (n=161), Asian (n=5), African American (n=7), Native American (n=2) and Hispanic (n=2). The study was approved by the Human Subjects Review Committee at the University of Washington and subjects provided written informed consent.

Subjects were divided *a priori* into three groups on the basis of body mass index (BMI) and the insulin sensitivity index (S_I) in order to analyze the relationship between liver enzyme concentrations, obesity and insulin sensitivity. These three groups were lean insulin sensitive (LIS): BMI <27.5 kg/m^2 and $S_I \geq 7.0 \times 10^{-5}$ min^{-1} pM^{-1}, lean insulin resistant (LIR): BMI <27.5 kg/m^2 and $S_I < 7.0 \times 10^{-5}$ min^{-1} pM^{-1} and obese insulin resistant (OIR): BMI ≥27.5 kg/m^2 and $S_I < 7.0 \times 10^{-5}$ min^{-1} pM^{-1}. The cut-off of 27.5

kg/m^2 was based on the criteria in place prior to the more recent definition of the criteria for overweight and obesity. The cut-off of 7.0 $x10^{-5}$ min^{-1} pM^{-1} for S_I represents the highest value for this parameter among a group of apparently healthy obese subjects studied in Seattle (14). Obese insulin sensitive subjects were excluded from this analysis because of their small number (n=8).

Measures of anthropometry and body fat distribution

The average of two weight and height measurements were used to calculate BMI as weight (kg)/[height (m)]^2. Waist and hip circumferences were calculated as the average of two measurements. Waist circumference was measured at the smallest circumference of the waist, and hip circumference was measured at the widest level of the buttocks, using a protocol described in the NHANES III Anthropometric Measurements Videotape (National Center for Health Statistics).

A computed tomography (CT) scan of the abdomen was performed at the level of the umbilicus to quantify subcutaneous fat (SCF) area and intra-abdominal fat (IAF) area as previously described (15). Fat area was computed as the area with an attenuation range of -250 to -50 Hounsfield units. IAF and SCF areas were quantified by delineating the border of the peritoneal cavity. These measurements were performed by a single observer using standard GE 8800 computer software. The variability of these measures made by a single observer was 1.5% (15).

Fasting plasma and insulin sensitivity measurements

Subjects underwent a tolbutamide-modified, frequently sampled intravenous glucose tolerance test (FSIGT) to quantify insulin sensitivity as the insulin sensitivity index (S_I) using Bergman's minimal model of glucose kinetics (16). Three basal blood samples were drawn at 15, 5, and 1 minute prior to the intravenous administration of glucose at time 0. Glucose (11.4 g/m^2 body surface area) was infused over 1 minute and tolbutamide (125 mg/m^2 body surface area) was injected intravenously over 30 seconds at time 20 minutes. Blood samples were taken at 32 time points over 240 minutes following commencement of the glucose injection. Fasting glucose and insulin concentrations were calculated as the average of the three basal samples. Liver function tests were determined on the 3-minute sample obtained during the FSIGT.

Alcohol intake

Alcohol intake was assessed using a standardized questionnaire and quantified as self-reported number of drinks per week.

Assays

Glucose was measured in duplicate using the glucose oxidase method. Immunoreactive insulin was measured in duplicate by radioimmunoassay using a modification of the double antibody technique. Samples for liver enzymes were assayed between 5 and 7 years after sampling. GGT was measured using an enzymatic colorimetric method (Modular P, Roche Diagnostics, Indianapolis, IN). AST and ALT were measured using the standardized kinetic method (Modular P, Roche Diagnostics, Indianapolis, IN). Samples were stored at -70°C prior to assay.

Calculations and statistics

Statistical analyses were performed using SPSS 12.0 (SPSS Inc, Chicago, Ill). For regression analysis, dependent variables were logarithmically transformed where appropriate to satisfy the statistical assumptions of linear regression. Multiple regression analysis was used to determine whether associations between the dependent (liver transaminase levels) and independent variables of interest remained

significant after adjusting for other potentially confounding independent variables. Model 1 contained S_I, IAF, BMI, and age for each gender. Model 2 contained S_I, waist/hip ratio, BMI, and age for each gender. Comparisons between groups were assessed by ANOVA with Tukey post-hoc analysis, Kruskal Wallis test, t test or Mann Whitney U test as appropriate. Data are presented as mean±SD unless specified. Non-normally distributed data with kurtosis were log transformed before applying parametric statistical tests. A $p<0.05$ was considered significant.

RESULTS

Demographic, anthropometric and metabolic characteristics

Subject characteristics are shown in Table 1 for all subjects (n=177) and subdivided into LIS (n=53), LIR (n=60) and OIR (n=56) subjects and into men (n=75) and women (n=102).

In this apparently healthy group of non-diabetic subjects, 66% were insulin resistant (defined as $S_I < 7.0 \times 10^{-5}$ min^{-1} pM^{-1}) and 32% were obese (defined as BMI >27.5 kg/m^2). In accordance with the *a priori* classification, the BMI of the obese group was significantly higher than that of both of the lean groups ($p<0.0001$; Table 1). S_I was 2.3-fold and 2.8-fold higher in the LIS group than in the LIR and OIR groups respectively ($p \leq 0.0001$). The mean age of the LIS subjects was slightly lower than that of the insulin resistant subjects.

LIR subjects were more centrally obese than LIS subjects as evidenced by higher waist-to-hip ratio ($p=0.009$) and IAF area ($p<0.0001$), despite a similar BMI in the two groups. LIR subjects were significantly less centrally obese (WHR $p=0.0005$; IAF $p<0.0001$) and more insulin sensitive ($p=0.0001$) than OIR subjects.

As listed in Table 1, fasting glycemia increased with increasing obesity and insulin resistance (LIS vs. LIR and LIR vs. OIR, $p<0.03$; LIS vs. OIR, $p<0.0001$) and a similar pattern was seen for triglycerides (LIS vs. LIR $p<0.006$, LIR vs. OIR, $p<0.05$; LIS vs. OIR, $p<0.0001$). Systolic blood pressure was significantly higher in OIR subjects than LIR and LIS subjects. There was no significant difference in alcohol intake between groups, reported as median (IQR) number of drinks per week.

Effect of gender on liver enzymes

There was no gender-based difference in age or BMI (Table 1). As expected, men had higher WHR ($p<0.0001$) and IAF area ($p<0.001$) than women, whereas women had more SCF area ($p<0.005$) than men (Table 1). Fasting glucose was higher in men ($p<0.001$) but S_I did not differ between men and women ($p=0.1$) (Table 1). All liver transferase levels [median (IQR)] were significantly higher in men compared to women: GGT 17 (14) vs. 10 (6) IU/L, ALT 16 (10) vs. 11 (6) IU/L and AST 21 (7) vs. 17.5 (5) IU/L ($p<0.0001$ for each).

Effect of obesity and insulin sensitivity on liver enzymes

Because transaminase levels were significantly higher in men compared to women, the effect of obesity and insulin sensitivity on transaminase levels was analyzed separately for each gender. In men, GGT levels were significantly higher in insulin resistant subjects (LIR and OIR) compared to LIS subjects (Figure 1A). GGT levels did not differ between LIR and OIR subjects ($p=0.6$). In women, GGT levels were also significantly higher in the OIR group compared to the LIS group and tended to be higher in the LIR vs. the LIS group ($p=0.09$) (Figure 1A). ALT and AST levels did not differ significantly between the LIS, LIR and OIR groups in either men or women (Figures 1B and 1C).

Relationship between liver enzymes, body anthropometrics, insulin sensitivity and gender

GGT was negatively associated with S_I and positively associated with IAF area, SCF area, WHR, and BMI (Table 2) after adjusting for age and gender. Waist circumference and alcohol consumption were not associated with GGT levels. ALT and AST were not associated with any of the variables and thus were not included in the multiple regression models.

Multiple linear regression analyses stratified by gender were performed with GGT as the dependent variable. In men, only S_I remained significantly associated with GGT levels independent of IAF and WHR (Table 3, Models 1 and 2), age, and BMI. In contrast, in women IAF and WHR (Table 3, Models 1 and 2) were significantly associated with GGT levels, but S_I was not.

DISCUSSION

We examined the relationship between body fat distribution, insulin sensitivity and liver enzymes in a cohort of 177 non-diabetic subjects of whom more than 97% had GGT levels within the normal range. It is well recognized that body fat distribution and insulin sensitivity are associated (17; 18) and in this cohort of apparently healthy individuals we found that GGT was negatively associated with insulin sensitivity in men, while in women GGT was associated with central obesity. In common with other studies (19), we found that men had higher GGT levels and increased central adiposity compared with women, and these differences may explain the different results in men and women. ALT and AST were not associated with insulin sensitivity or body fat measures in our study.

The association between elevated liver transaminases and insulin resistance in the context of NAFLD is well established (20). In the Tübingen Family Study, GGT was associated with insulin sensitivity and glucose tolerance in both men and women. In addition, in this same study GGT was positively correlated with hepatic lipid content measured by magnetic resonance spectroscopy (21). ALT has previously been shown to be inversely related to insulin sensitivity, determined by the euglycemic clamp, and it has also been shown to have this same relationship with endothelial function in subjects with type 2 diabetes (22). Recently two large studies have examined the role of liver transaminases in predicting the development of type 2 diabetes. In a study of 906 subjects, Hanley et al found that ALT, and to a lesser extent AST, were associated with the development of diabetes; however, they did not examine whether GGT predicted the development of hyperglycemia (6). In another study of 5974 non-diabetic subjects, Sattar et al found that ALT levels within the normal range predicted incident diabetes (23). In the Mexico City Diabetes Study, GGT was shown to be an independent risk factor for the development of IGT and diabetes (24) whereas Vozarova et al found that only ALT predicted progression to diabetes in Pima Indians (25).

Although GGT has been widely used as a marker of alcohol consumption, Lee et al have shown that GGT levels are also associated with an increased risk of developing type 2 diabetes independent of alcohol intake (9). In another study of over 4000 subjects, although an association between the incidence of diabetes and ALT levels was found, this was most strongly observed in the abnormal range of ALT and was weaker than the association with GGT levels (26). Others have found a strong, independent and graded association between GGT levels and type 2 diabetes but not ALT or AST (7; 8; 27). However, to our knowledge, no previous study has examined the relationship between GGT, intra-abdominal fat and insulin sensitivity in non-diabetic subjects.

The recent emergence of the potential protective role of GGT against oxidative stress may explain the inverse association between GGT levels and insulin sensitivity we found here. The basis of the proposed link between GGT and oxidative stress is that glutathione is a major intracellular defense against free radicals and peroxides. However, as intact glutathione cannot be taken up by cells, the intracellular synthesis of glutathione is dependent on the metabolism of extracellular glutathione by GGT to release cysteine, which is then transported into the cell and used as a substrate for the *de novo* intracellular synthesis of glutathione (28). *In vitro* studies have demonstrated a protective effect of GGT against oxidative stress and cell death (29). Thus increased GGT expression may initially represent an adaptive protective response to persistent oxidative stress. This would be consistent with the recent *in vivo* finding of a positive association between GGT and C-reactive protein levels (30). GGT levels have also been shown to predict future levels of inflammatory markers including CRP, fibrinogen and F2-isoprostanes (a biomarker of lipid peroxidation) (10).

Yki-Jarvinen's group has shown that fatty liver is associated with fasting insulin as a surrogate measure of insulin sensitivity independently of IAF and SCF. In their study ALT was more strongly correlated with liver fat than GGT (31). We found that in men GGT, but not ALT or AST, was associated with insulin sensitivity independently of body fat measures. As we quantified insulin sensitivity directly, we believe that our data raise the possibility that GGT may be a more sensitive marker of the liver's response to insulin sensitivity than ALT and AST. The finding that, even across the normal range, GGT levels are related to insulin sensitivity is of clinical relevance in the light of the emerging possible therapeutic role of the PPAR-γ agonists in the treatment of NASH. Promrat et al demonstrated an improvement in transaminases and amelioration of insulin resistance in subjects with NASH following 48 weeks of treatment with pioglitazone (32). Lifestyle changes with weight loss and increased exercise have also been shown to improve liver enzymes and histological findings in subjects with non-alcoholic fatty liver disease (4). Our data raise the possibility that increasing GGT levels (even within the normal range) in the context of insulin resistance may be an indication for lifestyle changes aimed at weight loss or treatment with PPAR-γ agonists.

The advantages of our analysis are that we have examined a large number of subjects in whom insulin sensitivity had been determined by the FSIGT and all of whom had fat distribution measured using CT scans. However, the lack of any direct measure of hepatic fat is a drawback. Another potential limitation is that as alcohol intake was assessed by self-reported questionnaire, consumption may have been underestimated. While liver enzyme measurements were made on a sample taken just after glucose administration, we doubt this affected our findings as nutrient intake has been shown not to affect liver enzymes (33). The transferase levels were uniformly lower than would be expected in a normal population, which may be due to the fact that transaminase levels tend to decrease slightly (about 8%) with time even when stored at temperatures of -80°C (34; 35). However, all samples were handled in the same manner. Although the absolute levels may have been affected, all samples should have been affected to the same degree, and therefore it is likely that while the absolute values may be lower, relative differences would have been robust and maintained.

In conclusion, GGT, but not ALT or AST, levels are inversely related to insulin sensitivity independently of central obesity in nondiabetic men. In contrast, in women GGT levels were positively associated with IAF and waist/hip ratio but were not associated with insulin

sensitivity. If GGT is a marker of hepatic fat accumulation, this gender difference suggests that body fat distribution may be a more important player in the development of hepatic steatosis in women than in men. This finding suggests that GGT is a more sensitive marker of insulin resistance,at least in men, but whether this liver enzyme will prove useful to guide treatment decisions related to insulin resistance will await further research.

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Table 1 Demographics and clinical variables in all subjects, lean insulin sensitive, lean insulin resistant and obese insulin resistant subjects, and men and women

	All	**Lean Insulin Sensitive**	**Lean Insulin Resistant**	**Obese Insulin Resistant**	**Men**	**Women**
N	177	53	60	56	75	102
Age (years)	52.3 (±9.9)	49.6 (±8.0)	53.8 (±11.4)*	53.2 (±9.6)	52.6 (±10.2)	52.0 (±9.8)
Gender (M/F)	75 / 102	18 / 35	27 / 33	25 / 31	-	-
Body mass index (kg/m^2)	26.4 (±4.3)	23.4 (±2.3)	24.3 (±1.8)	31.0 (±3.4)*†	26.8 (±3.5)	26.2 (±4.8)
Waist circumference (cm)	87.2 (±13.2)	77.9 (±8.5)	83.7 (±9.2)*	99.3 (±11.1)*†	94.9 (±10.6)‡	81.9 (±12.2)
Waist-to-hip ratio	0.84 (±0.09)	0.80 (±0.08)	0.83 (±0.09)*	0.89 (±0.08)*†	0.92 (±0.06)‡	0.78 (±0.06)
Subcutaneous fat area (cm^2)[a]	195.8 (135.6)	125.7 (102.7)	179.6 (114.1)*	299.1 (164.1)*†	166.9 (117.3)§	225.9 (162.9)
Intra-abdominal fat area (cm^2)[a]	88.4 (84.9)	43.3 (36.4)	76.8 (69.3)*	140.6 (57.9)*†	113.8 (86.9)‡	71.9 (79.1)
Systolic blood pressure (mm Hg)	118 (±12)	114 (±10)	117 (±10)	123 (±12)*†	120 (±11)¶	117 (±12)
Fasting plasma glucose (mmol/l)	5.4 (±0.4)	5.3 (±0.4)	5.4 (±0.4)*	5.6 (±0.5)*†	5.6 (±0.4)‡	5.3 (±0.4)
Triglycerides (mmol/l)[a]	1.4 (0.79)	0.99 (0.72)	1.4 (0.5)*	1.6 (0.75)*†	1.4 (0.82)	1.3 (0.75)
HDL-cholesterol (mmol/l)	1.4 (±0.4)	1.5 (±0.4)	1.3 (±0.4)*	1.2 (±0.4)*	1.2 (±0.3)‡	1.5 (±0.4)
Alcohol intake (drinks/week)[a]	1.0 (3.0)	1.0 (2.0)	1.0 (4.0)	1.5 (4.0)	2.0 (7.0)#	1.0 (2.0)

Data are Mean ± SD, [a]Median (IQR). Normal ranges: GGT <51 IU/L, ALT <40 IU/L, AST <38 IU/L.
LIS vs LIR vs OIR: ANOVA: *$p<0.05$ vs LIS; [†]$p<0.05$ vs LIR (8 obese insulin resistant subjects were excluded from this analysis due to the small number).
Men vs. women: t-test ‡ $p<0.0001$, § $p<0.005$, ¶ $p<0.05$; Mann Whitney U # $p<0.05$

Table 2. Linear regression analyses for liver transferases adjusted for age and gender

	Gamma-glutamyl transferase		**Alanine transaminase**		**Aspartate transaminase**	
	r	p	r	p	r	p
Insulin sensitivity (S_I)	-0.26	<0.0001	-0.07	0.326	0.02	0.754
Intra-abdominal fat area	0.22	0.003	0.12	0.127	0.02	0.844
Waist-hip ratio	0.25	0.001	0.15	0.054	0.08	0.292
Body mass index	0.17	0.027	0.14	0.066	0.02	0.766
Subcutaneous fat area	0.16	0.036	0.10	0.192	0.01	0.916
Waist circumference	0.04	0.569	0.07	0.403	0.05	0.501
Alcohol consumption	0.09	0.220	-0.03	0.688	-0.12	0.118

Data in bold are significant

Table 3. Multiple regression models with GGT as the dependent variable

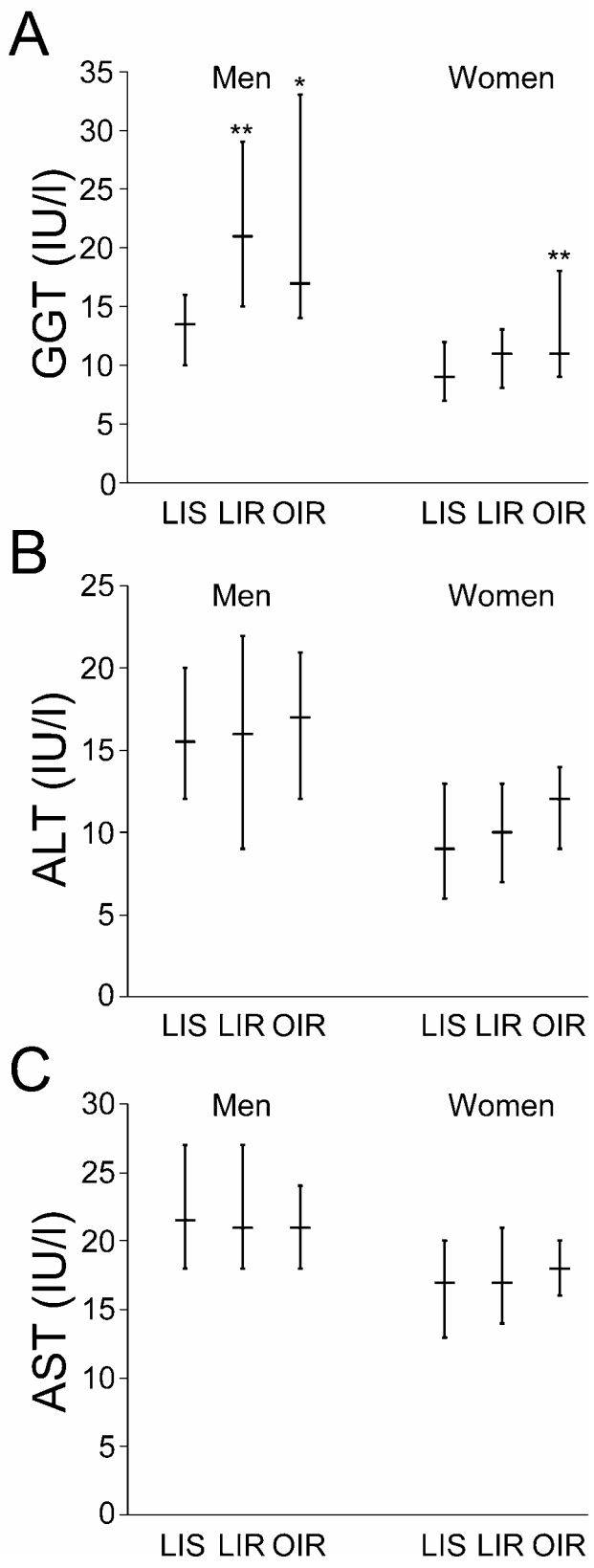
			Men			**Women**	
			Partial r	p		Partial r	p
Model 1	Insulin sensitivity (S_I)		-0.29	0.014		-0.08	0.449
	Intra-abdominal fat area		-0.15	0.206		0.29	0.004
	BMI		0.15	0.210		-0.15	0.137
	Age		0.01	0.921		-0.01	0.955
Model 2	Insulin sensitivity (S_I)		-0.32	0.010		-0.15	0.162
	Waist-hip ratio		0.06	0.647		0.29	0.005
	BMI		-0.06	0.660		-0.13	0.198
	Age		-0.02	0.896		0.02	0.821

Data in bold are significant

FIGURE LEGENDS

Figure 1. A) GGT B) ALT and C) AST levels in men (left): lean insulin sensitive (LIS, n=18), lean insulin resistant (LIR, n=27) and obese insulin resistant (OIR, n=25) subjects and women (right): LIS (n=35), LIR (n=33) and OIR (n=31). Data are median (IQR). *p<0.01 vs. LIS; **p<0.01 vs. LIS

Figure 1


A
GGT (IU/l)
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0
Men
Women
**
*
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LIS LIR OIR
LIS LIR OIR
B
ALT (IU/l)
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C
AST (IU/l)
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LIS LIR OIR
LIS LIR OIR