

LIVER FAT IS INCREASED IN TYPE 2 DIABETIC PATIENTS AND UNDERESTIMATED BY SERUM ALT COMPARED TO EQUALLY OBESE NON-DIABETIC SUBJECTS

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Short title: Liver fat and serum ALT as a marker of liver fat in type 2

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ABSTRACT

Objective: To determine whether type 2 diabetic patients have more liver fat than age-, gender-, and BMI matched non-diabetic subjects, and whether liver enzymes (S-ALT, S-AST) are similarly related to liver fat in type 2 diabetic and normal subjects.

Methods: 70 type 2 diabetic patients and 70 non-diabetic subjects matched for BMI, age and gender were studied. Liver fat ($^1\text{H-MRS}$), body composition (MRI), and biochemical markers of insulin resistance were measured.

Results. The type 2 diabetic patients had on the average 80% more liver fat and 16% more intra-abdominal fat than the non-diabetic subjects. The difference in liver fat between the two groups remained statistically significant when adjusted for intra-abdominal fat ($p < 0.05$). At any given BMI or waist circumference, the type 2 diabetic patients had more liver fat than the non-diabetic subjects. The difference in liver fat between the groups rose as a function of BMI and waist circumference. FS-insulin ($r = 0.55$, $p < 0.0001$), fP-glucose ($r = 0.29$, $p = 0.0006$), HbA_{1c} ($r = 0.34$, $p < 0.0001$), fS-triglycerides ($r = 0.36$, $p < 0.0001$), and fS-HDL cholesterol ($r = -0.31$, $p = 0.0002$) correlated with liver fat similarly in both groups. The slopes of the relationships between S-ALT and liver fat were significantly different ($p = 0.004$). Liver fat content did not differ between the groups at low S-ALT concentrations (10-20 U/l), but was 70-200% higher in type 2 diabetic patients as compared to controls at S-ALT concentrations of 50-200 U/l.

Conclusions: Type 2 diabetic patients have 80% more liver fat than age-, weight-, and gender-matched non-diabetic subjects. S-ALT underestimates liver fat in type 2 diabetic patients.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BP, blood pressure; BMI, body mass index; ECG, electrocardiogram; FFA, free fatty acid; fP, fasting plasma; fS, fasting serum; HbA_{1c}, glycosylated hemoglobin A_{1c}; HDL, high density lipoprotein; IA, intra-abdominal; LDL, low density lipoprotein; LFAT, liver fat; MRI, magnetic resonance imaging; NAFLD, non-alcoholic fatty liver disease; S, serum; SC, subcutaneous; TG, triglycerides; VLDL, very low density lipoprotein

It has been estimated that approximately 70-80% of type 2 diabetic patients have non-alcoholic fatty liver disease (NAFLD) (1, 2). In addition, nine of 12 prospective epidemiological studies have shown that elevated serum liver enzyme concentrations predict type 2 diabetes independent of obesity (3). The data would thus suggest that liver fat content is increased in patients with type 2 diabetes compared to equally obese non-diabetic subjects. To date, only one study has addressed this question (4). In this study, liver fat was measured qualitatively using the liver/spleen attenuation ratio (4). Liver fat was increased in type 2 diabetic patients compared to 10 weight-matched normal subjects (4). The small number of normal subjects prevents, however, any firm conclusions to be drawn.

Clinically, it would be helpful to have a simple measure of liver fat in patients with type 2 diabetes, as liver fat is closely correlated with insulin requirements (5, 6), and may be an important parameter to consider when choosing patients for PPAR γ -agonist therapy (7, 8). S-ALT correlates with liver fat content in non-diabetic subjects (9, 10), but whether S-ALT relates to liver fat similarly in type 2 diabetic patients has not been examined.

In the present study, we studied a group of 70 type 2 diabetic patients and a group of 70 non-diabetic subjects matched for age, gender and body mass index. Liver fat content was measured using proton magnetic resonance spectroscopy and body composition using MRI. In addition, measures of glycemia and insulinemia, serum lipids, and serum liver enzyme concentrations were determined. The data show that in type 2 diabetic subjects as compared to the non-diabetic subjects liver fat is increased independent of obesity and fat distribution, and that S-ALT underestimates the amount of liver fat.

METHODS

Subjects. A total of 70 non-diabetic subjects and 70 type 2 diabetic patients were recruited by newspaper advertisements and by

contacting occupational health services in Helsinki based on the following inclusion criteria: (i) age 18 to 70 years; (ii) no known acute or chronic disease based on history, physical examination and standard laboratory tests (blood counts, serum creatinine, thyroid-stimulating hormone, electrolyte concentrations and ECG); (iii) alcohol consumption less than 20g per day; (iv) no evidence of hepatitis A, B, or C, of autoimmune hepatitis, or clinical signs or symptoms of inborn errors of metabolism or history of use of toxins or drugs known to induce hepatitis. Exclusion criteria included proliferative retinopathy and use of antihypertensives possibly influencing glucose metabolism (β -blockers and thiazides), or of thiazolidinediones. A total of 13 non-diabetic subjects and 32 type 2 diabetic patients received antihypertensive medications (ACE-inhibitors or Ca-channel blockers). A total of 31 type 2 diabetic patients were treated with diet alone, 17 with metformin, and 22 with a combination of metformin and insulin. Five non-diabetic subjects and 22 type 2 diabetic patients were receiving statins. Regarding the insulin-treated patients, additional inclusion criteria included stable body weight and insulin dose for at least 6 months. Data on non-diabetic subjects (9) and 46 of the type 2 diabetic patients participating in treatment studies (5, 6, 11) have previously been reported. For the latter patients, only baseline data are included. The study protocol was approved by the ethics committee of the Helsinki University Central Hospital, and each participant provided written informed consent.

Liver fat content (proton spectroscopy). Liver fat content was measured by proton magnetic resonance spectroscopy as previously described (5). This measurement has been validated against histologically determined lipid content (12), and against estimates of fatty degeneration or infiltration by X-ray computer-assisted tomography (5). All spectra were analysed by a physicist in a blinded fashion. The reproducibility of repeated measurements of liver fat in non-diabetic

subjects studied on two occasions in our laboratory is 11% (13).

Measurements of body composition. Intra-abdominal and subcutaneous fat content were determined by magnetic resonance imaging (MRI) as previously described (5). The % body fat was determined using bioelectrical impedance spectroscopy (14). Waist circumference was measured midway between spina iliaca superior and the lower rib margin (15).

Analytical procedures. Plasma glucose, HbA_{1c}, serum free insulin and fS-C-peptide, fS-HDL cholesterol, fS-triglyceride, fS-LDL, fS-FFA, S-AST and S-ALT concentrations were measured as previously described (9).

Statistical analyses. Non-normally distributed data were used after logarithmic (base 10) transformation. If distributed normally, data are shown as mean \pm SEM, while non-normally distributed data are shown as median followed by the interquartile range (25th and 75th percentiles). The unpaired Student's *t* test was used to compare mean values between the groups. Analysis of covariance was used to compare slopes and intercepts of regression lines for the non-diabetic subjects and the type 2 diabetic patients, and for comparison of liver fat in the type 2 diabetic patients treated with diet, metformin or with insulin-metformin combination therapy. If neither the slopes nor the intercepts differed between the groups, a common regression equation was calculated. Correlation analyses were performed using Spearman's nonparametric rank correlation coefficient. Analysis of covariance was used to adjust for intra-abdominal fat content.

Calculations were made using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA), and SPSS 14.0 for Windows (SPSS, Chicago, IL). A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

Subject characteristics (Table 1). Non-diabetic subjects and type 2 diabetic patients were equally obese and similar with respect to age and gender. The type 2 diabetic patients had higher fS-insulin, fS-triglyceride, and fS-FFA concentrations, and lower fS-HDL cholesterol concentrations as compared to the non-diabetic subjects. The type 2 diabetic patients had approximately 16% more intra-abdominal and approximately 80% more liver fat than the non-diabetic subjects. The difference in liver fat remained significant even after adjusting for intra-abdominal fat content ($p < 0.05$). The type 2 diabetic patients treated with diet, metformin (MET), or the combination of insulin and metformin (INS+MET) differed slightly with respect to age (45 \pm 2, 55 \pm 2, 50 \pm 2 years, diet, MET, INS+MET, $p = 0.003$ for diet vs. MET), whereas BMI (32.0 \pm 0.7, 30.2 \pm 1.4, 32.2 \pm 1.0 kg/m², NS), liver fat content (15.5% (7.0-21.0%), 9.0% (4.2-19.5%), 14.0% (7.0-21.3%), NS), and serum ALT concentrations (42 (29-69), 31 (21-46), 38 (26-52) U/l, NS) were similar between the groups.

Relationships between body composition and liver fat content. Liver fat content was significantly correlated with BMI and waist circumference in the type 2 diabetic patients and in the non-diabetic subjects (Fig. 1). At any given BMI or waist circumference, the type 2 diabetic patients had significantly more liver fat than the non-diabetic subjects. The type of antihyperglycemic therapy had no impact on these relationships (data not shown). At a BMI of 25 kg/m², the type 2 diabetic patients had approximately 40% more liver fat than the non-diabetic subjects. This difference rose gradually as a function of BMI. For example, at a BMI of 40 kg/m², the type 2 diabetic patients had 80% more liver fat than the non-diabetic subjects. A similar phenomenon was observed when waist was plotted against liver fat. For example, at waist circumferences of 85 and 140 cm, liver fat content was 50% and 90% higher in the type 2 diabetic patients than in the non-diabetic subjects, respectively. Intra-abdominal fat was similarly related to liver fat in both non-

diabetic subjects and type 2 diabetic patients ($r=0.45$, $p<0.0001$). The volume of subcutaneous fat did not correlate with liver fat content in either group.

Relationships between insulin, glycemia, lipids, and liver fat content. Liver fat content was similarly related to fS-insulin ($r=0.55$, $p<0.0001$), C-peptide ($r=0.40$, $p<0.0001$), HbA_{1c} ($r=0.34$, $p<0.0001$), fP-glucose ($r=0.29$, $p=0.0006$), fS-triglyceride ($r=0.36$, $p<0.0001$) and fS-HDL cholesterol ($r=-0.31$, $p=0.0002$) concentrations in the non-diabetic subjects and the type 2 diabetic patients (Fig. 2).

Relationships between liver enzymes and liver fat content. S-AST and S-ALT correlated with liver fat content in both non-diabetic subjects and type 2 diabetic patients. The slopes of the regression lines between S-ALT concentrations and liver fat content differed significantly between the non-diabetic subjects and the type 2 diabetic patients ($p=0.004$). For any given ALT, the type 2 diabetic patients had more liver fat than the non-diabetic subjects (Fig. 3). Liver fat content did not differ between the groups at low S-ALT concentrations (10-20 U/l), but was 70%, 125%, and 200% higher in type 2 diabetic patients as compared to controls at S-ALT concentrations of 50 U/l, 100 U/l, and 200 U/l. At normal S-ALT concentrations (15-45 U/l), liver fat content was 30-70% higher in the type 2 diabetic patients than in the non-diabetic subjects. This increase rose gradually with increasing S-ALT concentrations, and averaged 80% and 130% at S-ALT concentrations of 60 U/l and 100 U/l. Serum liver enzyme concentrations did not differ between the subjects who were using statins as compared to those who were not (S-AST: 29 (22-40), 29 (23-46) U/l, NS; S-ALT: 35 (21-51), 33 (24-64) U/l, NS). The relationship between liver enzymes and liver fat content did not differ between subjects who were and were not using statins in either group (data not shown).

CONCLUSION

In the present study, we used state-of-the-art – methodology for quantification of liver fat in a

large group of type 2 diabetic patients and carefully age- and gender-matched equally obese non-diabetic subjects. Liver fat content was on the average 80% higher in the type 2 diabetic patients than in the non-diabetic subjects. This difference was not influenced by the type of antihyperglycemic treatment. Somewhat unexpectedly, S-ALT and S-AST were not related to liver fat similarly in the diabetic and non-diabetic subjects. Both S-ALT and S-AST underestimated liver fat content in type 2 diabetic patients. This underestimation became more pronounced at increasing concentrations of both S-ALT and S-AST.

In many previous studies, liver fat content, measured by proton magnetic resonance spectroscopy, has been found to exceed the upper limit of normal liver fat content (5.56%) (16) in type 2 diabetic patients. However, except for one study which included 10 non-diabetic controls (4), these studies had either no control group (5, 6, 11, 17-19) or the controls were not matched for body weight (20, 21). It has thus remained unclear whether the increase in liver fat content has just been a consequence of obesity in type 2 diabetic patients. The present study suggests that type 2 diabetic patients have more liver fat at any given BMI than non-diabetic subjects, and that the difference in liver fat content between the groups increases with increasing obesity. In keeping with Kelley et al (4), intra-abdominal fat was similarly related to liver fat in both type 2 diabetic patients and non-diabetic subjects, and the type 2 diabetic patients had more intra-abdominal fat than the non-diabetic subjects.

The higher liver fat content in the type 2 diabetic patients could contribute to diabetic dyslipidemia. The fatty liver overproduces VLDL particles in both non-diabetic subjects and type 2 diabetic patients, which, in the face of unchanged VLDL clearance, increases serum triglyceride concentrations (21). Hypertriglyceridemia in turn leads to low HDL-cholesterol concentrations (22). The similar relationship between triglycerides and HDL cholesterol concentrations and liver fat

content in the diabetic patients and non-diabetic subjects (Fig. 2) combined with the kinetic study (21) suggest that excess liver fat indeed contributes to diabetic dyslipidemia.

An intriguing observation in the present study was that the type 2 diabetic patients had 40 to 200% more liver fat at the same serum ALT and AST concentrations than the non-diabetic subjects (Table 2). Both enzymes thus underestimate liver fat in type 2 diabetes, and for any given amount of liver fat, ALT and AST are lower in type 2 diabetic patients than in normal subjects. Both serum ALT and AST increase in response to hepatocyte damage until hepatocytes are lost and cirrhosis develops (23, 24). To what extent the lower enzyme levels reflect a difference in hepatocellular damage cannot be determined from the present study.

It is important to develop tools to diagnose a fatty liver in type 2 diabetic patients, because NASH is more common in type 2 diabetic patients than in non-diabetic subjects (25, 26), and can progress to cirrhosis and liver failure (27). The present data suggest that if assessed

using S-ALT or S-AST, hepatic steatosis is underestimated in type 2 diabetic patients compared to equally obese non-diabetic subjects. There is thus a need to develop new serum markers of steatosis to complement S-AST and S-ALT which have been used in the clinic for almost 50 years (28).

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Table 1. Subject characteristics

	Non-diabetic subjects	Type 2 diabetic patients	p-value
N (women)	70 (33)	70 (33)	
Age (yrs)	48±1	49±1	NS
Body composition			
BMI (kg/m ²)	31.2±0.6	31.6±0.6	NS
Waist (cm)	105±1	107±1	NS
% fat	30±1	31±1	NS
SC fat (cm ³)	4600±270	4400±230	NS
IA fat (cm ³)	1900 (1300-2500)	2200 (1600-3500)	0.02
Liver fat (%)	7.3 (3.0-17.0)	13.0 (6.0-20.5)	0.005
Glycemic parameters			
fP-glucose (mmol/l)	5.6±0.1	9.3±0.3	<0.0001
HbA _{1c} (%)	5.6±0.1	7.6±0.2	<0.0001
fS-insulin (mU/l)	8.4 (6.0-12.7)	12.0 (8.0-18.0)	0.004
Serum lipids			
fS-triglycerides (mmol/l)	1.90±0.17	2.55±0.23	0.007
fS-HDL cholesterol (mmol/l)	1.36±0.04	1.17±0.04	0.0003
fS-LDL cholesterol (mmol/l)	3.18±0.11	2.75±0.11	0.0007
fS-FFA (μmol/l)	650±30	780±30	0.003
Blood pressure			
Systolic BP (mmHg)	136±2	135±2	NS
Diastolic BP (mmHg)	85±1	83±1	NS
Liver enzymes			
S-ALT (U/l)	31 (23-62)	38 (28-58)	NS
S-AST (U/l)	29 (22-44)	29 (24-48)	NS
AST/ALT	0.83 (0.59-1.04)	0.85 (0.68-1.05)	NS

Data are shown as mean ± SEM or, for non-normally distributed data, as median followed by the 25%th and 75%th percentiles.

Table 2. Liver fat content at increasing S-ALT and S-AST concentrations in the non-diabetic subjects and the type 2 diabetic patients.

	Liver fat content	
	Non-diabetic subjects	Type 2 diabetic patients
S-ALT (U/l)		
20	5.1%	6.0%
40	6.9%	10.7%
60	8.2%	15.0%
80	9.3%	19.0%
100	10.2%	22.9%
S-AST (U/l)		
20	5.2%	7.3%
40	7.5%	12.3%
60	9.3%	16.7%
80	10.9%	20.8%
100	12.3%	24.5%

FIGURE LEGENDS

Figure 1. The relationships between liver fat and body composition. Liver fat content correlates with (a) BMI [$r=0.45$, $p<0.0001$ for type 2 diabetic patients (regression equation: $\text{LFAT}\% = 10^{[-0.0524 \pm 0.27 + 0.034 \pm 0.01 * \text{BMI}]}$); $r=0.26$, $p=0.029$ for non-diabetic subjects ($\text{LFAT}\% = 10^{[0.003 \pm 0.37 + 0.0264 \pm 0.01 * \text{BMI}]}$); and (b) waist circumference [$r=0.45$, $p=0.0001$ for type 2 diabetic patients ($\text{LFAT}\% = 10^{[-0.42 \pm 0.36 + 0.014 \pm 0.003 * \text{Waist}]}$); $r=0.29$, $p=0.017$ for non-diabetic subjects ($\text{LFAT}\% = 10^{[-0.42 \pm 0.52 + 0.012 \pm 0.005 * \text{Waist}]}$)]. Open circles and grey lines denote non-diabetic subjects, and filled circles and black lines denote type 2 diabetic patients.

Figure 2. The associations between liver fat and fS-insulin concentrations and serum lipids. Liver fat content is related to (a) fS-insulin ($r=0.55$, $p<0.0001$ for both non-diabetic subjects and type 2 diabetic patients), (b) fS-triglycerides ($r=0.36$, $p<0.0001$ for both non-diabetic subjects and type 2 diabetic patients), and (c) fS-HDL cholesterol ($r=-0.31$, $p=0.0002$ for both non-diabetic subjects and type 2 diabetic patients). Dashed lines denote both non-diabetic subjects and type 2 diabetic patients. Symbols as in Figure 1.

Figure 3. The relationships between liver fat content and liver enzymes. Liver fat content associates with (a) S-AST [$r=0.49$, $p<0.0001$ for type 2 diabetic patients ($\text{LFAT}\% = 10^{[-0.11 \pm 0.25 + 0.75 \pm 0.16 * \log(\text{S-AST})]}$); $r=0.24$, $r=0.043$ for non-diabetic subjects ($\text{LFAT}\% = 10^{[0.01 \pm 0.40 + 0.54 \pm 0.26 * \log(\text{S-AST})]}$); and (b) S-ALT [$r=0.66$, $p<0.0001$ for type 2 diabetic patients ($\text{LFAT}\% = 10^{[-0.30 \pm 0.19 + 0.83 \pm 0.12 * \log(\text{S-ALT})]}$); $r=0.26$, $p=0.027$ for non-diabetic subjects ($\text{LFAT}\% = 10^{[0.15 \pm 0.30 + 0.43 \pm 0.19 * \log(\text{S-ALT})]}$)] concentrations. Symbols as in Figure 1.

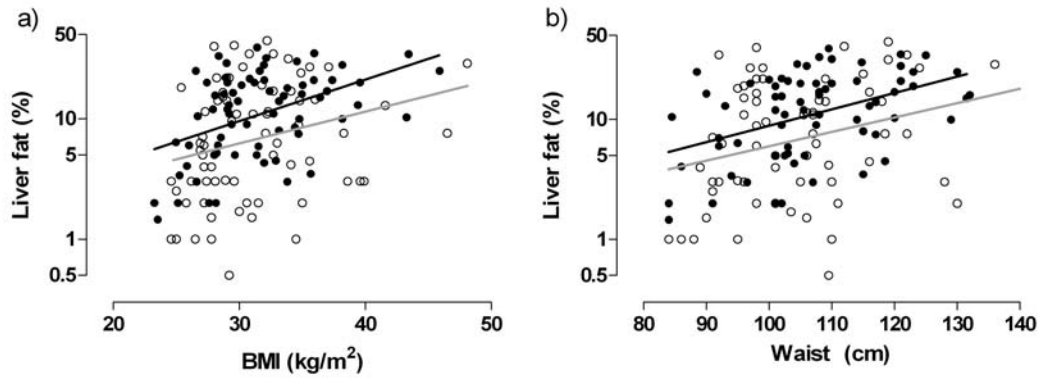
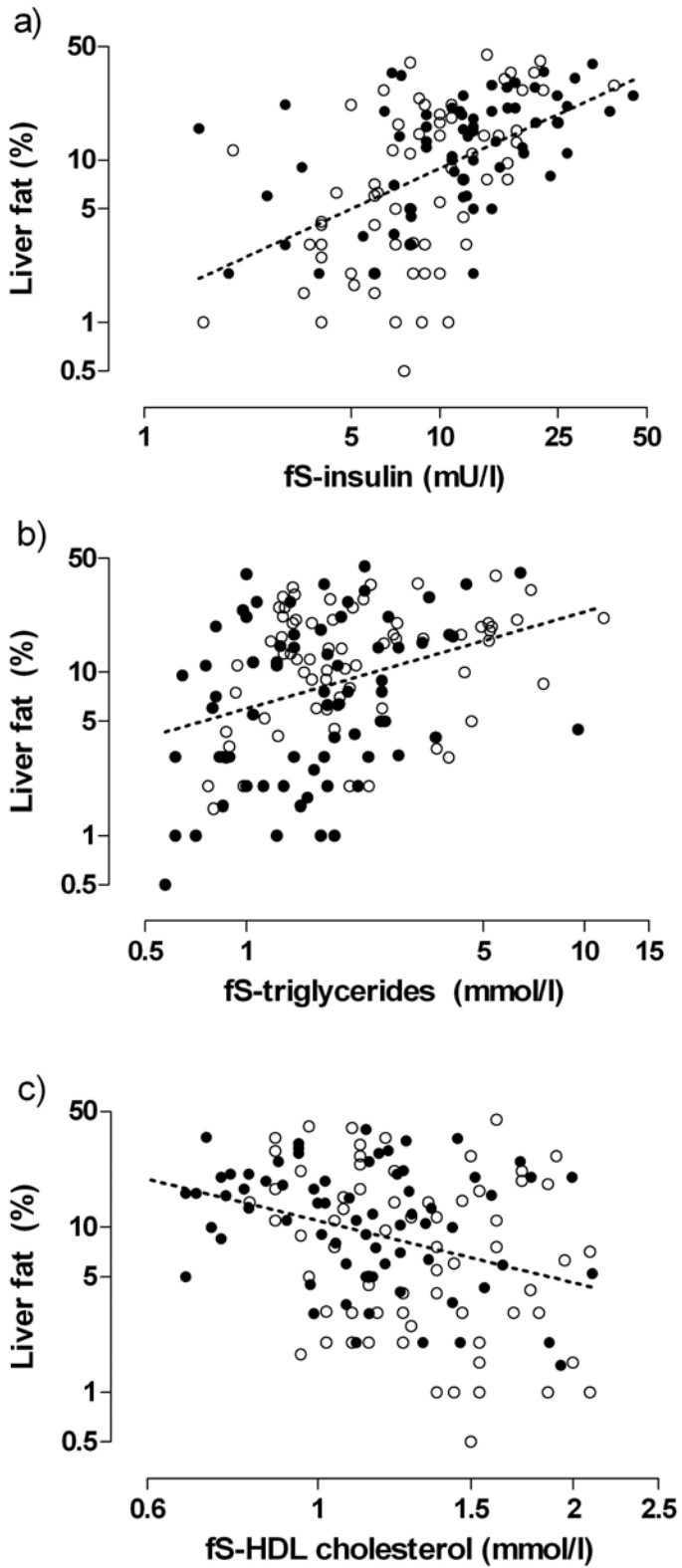


Fig. 1

Fig. 2



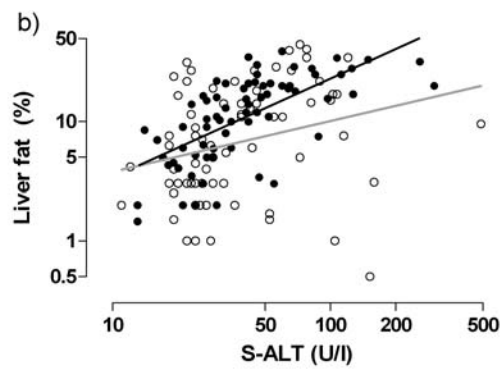
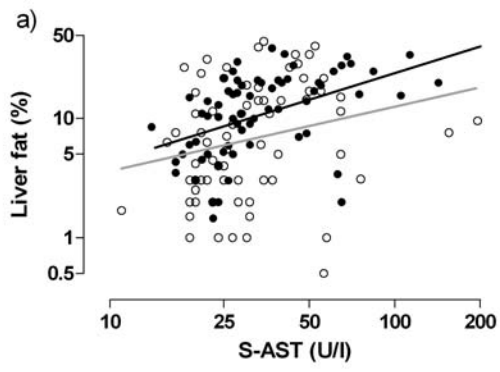


Fig. 3