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Hepatic fat is not associated with β-cell function or postprandial free fatty acid response

Josina M. Rijkelijkhuizen[a,*], Teddo Doesburg[b], Cynthia J. Girman[c], Andrea Mari[d], Thomas Rhodes[c], Amalia Gastaldelli[e], Giel Nijpels[a,f], Jacqueline M. Dekker[a]

[a]EMGO Institute, VU University Medical Center, 1081 BT Amsterdam, The Netherlands
[b]Department of Radiology, Westfries Gasthuis, 1624 NP Hoorn, The Netherlands
[c]Department of Epidemiology, Merck & Co., Inc., North Wales, PA 19454, USA
[d]Institute of Biomedical Engineering, National Research Council, 35127 Padua, Italy
[e]Institute of Clinical Physiology, National Research Council, 56124 Pisa, Italy
[f]Department of General Practice, VU University Medical Center, 1081 BT Amsterdam, The Netherlands

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Abstract

We evaluated the association of hepatic fat with β-cell function estimated from the oral glucose tolerance test. In addition, we tested the hypothesis that postprandial free fatty acid (FFA) suppression after a meal tolerance test (MTT) is linked to hepatic fat. Individuals with normal glucose metabolism (NGM; n = 10 with low and n = 10 with high insulin secretion, matched for insulin sensitivity and sex), impaired glucose metabolism (IGM; n = 14), and type 2 diabetes mellitus (DM; n = 14) underwent a 75-g oral glucose tolerance test and MTT. β-Cell function estimates were calculated from C-peptide using a mathematical model. Liver fat was quantified by proton magnetic resonance (^{1}H-MR) spectroscopy. Area under the curve (AUC) of triglycerides (TG) and FFA responses during MTT represented postprandial lipid responses. Linear regression models were adjusted for age, sex, and additionally for insulin sensitivity for IGM/DM subjects. Liver fat content was equal for the NGM groups with low and high insulin secretion: 4.5% (2.6-6.0) (median, interquartile range) and 4.9% (2.3-7.8), respectively; liver fat percentages of IGM and diabetic subjects were significantly higher: 11.2 (6.7-21.1) and 10.0 (7.8-24.5). Liver fat showed a fairly strong, significant negative association with insulin sensitivity, but was not associated with β-cell function. Significant associations of liver fat with fasting TG and AUC_{TG} were shown in the total study population and in IGM/DM subjects separately. No relationship existed between fasting FFA or AUC_{FFA} and liver fat. We conclude that fat accumulation in the liver is tightly linked to insulin sensitivity but not to β-cell function. Furthermore, liver fat is associated with circulating TG levels, but not with FFA concentrations.

1. Introduction

Excess (intra)abdominal fat is a key determinant of increased hepatic fat [1-4]. Recently, it has been shown that, in adolescents, strong correlations exist between body mass index (BMI) and liver, muscle, and pancreas fat content quantified by magnetic resonance imaging [5]. In patients with type 2 diabetes mellitus (DM), increased hepatic fat content is strongly related to the basal hepatic insulin resistance and decreased suppression of endogenous (hepatic) glucose production by insulin [6-8]. In individuals without diabetes, hepatic fat content is strongly correlated with hepatic insulin resistance [1-3,7-9] and hyperinsulinemia [3,5,10]. Insulin resistance in the liver results in the impaired ability of insulin to suppress hepatic glucose production [11]. However, individuals (initially) do not develop diabetes because the pancreatic β-cells have sufficient compensatory function to increase insulin secretion [12,13].

The conversion to type 2 DM is mainly determined by a deterioration of β-cell function [14,15]. Although there is a clear association between liver fat and insulin resistance, data on the associations between liver fat content and β-cell function are scarce. Some studies have determined the relationship between liver fat and *fasting* insulin, but only 1 study [16] included postload insulin secretion. Musso et al [16] reported that β-cell dysfunction estimated by the disposition index [17] and the adaptation index [18] during an intravenous glucose tolerance test was present in nonobese

* Corresponding author. Tel.: +31 20 444 9831; fax: +31 20 444 8181.
E-mail address: j.rijkelijkhuizen@vumc.nl (J.M. Rijkelijkhuizen).

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subjects with nonalcoholic steatohepatitis (NASH) before glucose intolerance appeared, suggesting that β-cell dysfunction is an early feature of NASH. However, β-cell function was determined during an intravenous glucose tolerance test (IVGTT), not reflecting all involved physiologic responses. Therefore, our objective was to study the relation between liver fat and β-cell function in subjects with different glucose status during a challenge that includes the gastrointestinal system: the oral glucose tolerance test (OGTT). We matched the individuals with a normal glucose metabolism (NGM) for (peripheral) insulin sensitivity because the interpretation of whether insulin secretion suffices for optimal glucose homeostasis depends on the ambient insulin sensitivity.

Dyslipidemia is tightly linked with insulin resistance, and fatty liver disease is characterized by increased accumulation of triglycerides (TG) in hepatocytes. Matikainen et al [3] showed that liver fat content is a close correlate of postprandial lipid disturbances. Because TG in the hepatocytes may be the result of increased free fatty acid (FFA) availability (either from adipose tissue or postprandial metabolism) and patients with type 2 DM or insulin-resistant individuals are known to have an impaired insulin-induced FFA suppression [19], we tested the hypothesis that (lack of) postprandial FFA suppression and postprandial TG expression after a meal might be linked to hepatic fat content.

In the present study, we studied the association between liver fat and β-cell function independently of insulin sensitivity during the OGTT in selected subjects of a population-based sample with a wide variation in β-cell function, including healthy and diabetic subjects. In addition, we investigated the relationship of liver fat content with postprandial TG and FFA exposure during a mixed meal test.

2. Subjects and methods

2.1. Selection of subjects and matching/correction procedure for insulin sensitivity

In a population study (n = 208), the OGTT-based glucose status (NGM, impaired glucose metabolism [IGM], or DM) of the participants, men and women aged 40 to 65 years, was classified according to the World Health Organization criteria of 2006 [20]. *Impaired glucose metabolism* was defined as impaired fasting glucose and/or impaired glucose tolerance. Three subjects could not be classified. The NGM subjects of the population (n = 163) were divided into quartiles based on their early insulin secretion (insulinogenic index [21]). Because we wanted to investigate the relationship between liver fat and β-cell function independently of insulin sensitivity, the NGM subjects from the highest and lowest quartile of the insulinogenic index were matched on insulin sensitivity and sex. The NGM subjects from the lowest quartile of the insulinogenic index were randomly selected and were matched to a subject from the highest quartile based on sex and insulin sensitivity. Both subjects of a matched pair had to be willing to participate in the liver spectroscopy procedure. Ten subjects with low and 10 with high insulin secretion participated in the liver spectroscopy measurements (n = 20). All subjects classified as DM (n = 23) or IGM (n = 19) were asked to participate in liver spectroscopy measurements, and 14 of each group agreed. Of the diabetic individuals, 3 were not using any oral antihyperglycemic agent (OHA), 8 were using metformin, and 3 subjects had a combined treatment of metformin and a sulfonylurea derivative. Because of the limited number of subjects in the diabetes and IGM groups, these individuals could not be matched on insulin sensitivity. Therefore, regression models for these groups were corrected for insulin sensitivity (see "Statistical analysis"). Thus, the total study population consisted of n = 48 individuals. Exclusion criteria were DM type 1, insulin therapy, malignant disease, history of drug or alcohol abuse, serious mental impairment, coronary heart disease, and pregnancy. All participants signed an informed consent. The study was approved by the ethical committee of the VU University Medical Center.

2.2. Study procedure

Participants received a standard 75-g OGTT and a standardized meal tolerance test (MTT) after a 10-hour overnight fast, on separate days, in random order, separated by less than 2 weeks. The approximate total nutrient content was 3487 kJ (74 g [36 energy%] carbohydrates, 49 g [52 energy%] fat of which 28.2 g was saturated, and 24 g [12 energy%] proteins).

The visits at the research center started between 7:30 and 9:00 AM to reduce the impact of diurnal variation on the interpretation of results. Subjects on statin therapy or OHA were asked to skip the dosage the night before (statins) and/or morning before (statins, OHA) the visits. Apart from the test meals and small amounts of water, participants refrained from food and drinks; and physical activity was limited. During both tests, blood samples were drawn from the antecubital vein in fasting state and at 15, 30, 60, 90, and 120 minutes after glucose or meal ingestion and additionally at 180, 240, 300, and 360 minutes after meal ingestion.

Physical (weight, height, waist and hip circumference) and blood pressure measurements were completed during the OGTT-visit.

2.3. β-Cell function and insulin sensitivity estimated from OGTT

2.3.1. Classic β-cell function parameters

The insulinogenic index (estimate of early insulin secretion) was calculated by dividing the increment in insulin during the first 30 minutes by the increment in glucose over the same period ($\Delta I_{30}/\Delta G_{30}$) during the OGTT [21]. Areas under the curve (AUCs) of insulin and glucose were calculated by the trapezium rule [22]. Overall glucose-stimulated insulin secretion was calculated as $AUC_{insulin}/AUC_{glucose}$ ratio and as the incremental AUC ratio ($\Delta AUC_{insulin}/\Delta AUC_{glucose}$).

2.3.2. Model-based β-cell function parameters

Model-based β-cell function parameters were calculated from the OGTT using a mathematical model developed by Mari et al [23,24]. In summary, glucose-mediated insulin secretion ($S[t]$) in this mathematical model is the sum of 2 components: $S(t) = P(t)f(G) + S_d(t)$, where $P(t)f(G)$ represents the product of the dose-response relation between insulin secretion and glucose concentration ($f[G]$) and the potentiation factor ($P[t]$), which modulates this dose-response relation. The slope of the dose-response is denoted as β-cell glucose sensitivity. The potentiation factor ratio is the ratio between potentiation at the end of the OGTT (100-120 minutes) and the initial value (0-20 minutes). $S_d(t)$ represents the enhancement of insulin secretion proportional to the rate of change of the plasma glucose concentration and is denoted as rate sensitivity.

2.3.3. Insulin sensitivity

Insulin sensitivity was estimated from the OGTT according to the method described by Mari et al (oral glucose insulin sensitivity) [25].

2.4. Postprandial lipid responses during MTT

Postprandial lipid responses were calculated as the 6-hour AUC of the TG and FFA measurements during MTT. Furthermore, the response relative to the baseline level was determined by calculating the incremental AUC (ΔAUC) of TG and FFA.

2.5. Laboratory analysis

Plasma glucose levels were determined with a glucose hexokinase method (Gluco-quant; Roche Diagnostics, Mannheim, Germany); serum insulin and C-peptide, with immunometric assays (ACS Centaur; Bayer Diagnostics, Mijdrecht, the Netherlands), and TG, total cholesterol, and high-density lipoprotein cholesterol, with enzymatic colorimetric assays (Roche). Low-density lipoprotein cholesterol was calculated according to the Friedewald-formula [26] except when fasting TG levels exceeded 5.0 mmol/L. Free fatty acid was measured by enzymatic colorimetric assays (WAKO Chemicals, Neuss, Germany); and alanine aminotransferase (ALAT), with the IFCC method (Roche Diagnostics).

2.6. Liver fat (^{1}H–MR spectroscopy)

Proton magnetic resonance (^{1}H-MR) spectroscopy was performed using a 1.5-T magnetic resonance device (Magnetom Avanto; Siemens, Erlangen, Germany). Subjects were in supine position on the Spine Matrix coil with a Body Matrix coil positioned over the upper abdominal region. Single-voxel (2 × 2 × 2 cm) proton spectra from the liver were obtained at 3 locations: high in the right lobe (segment 7 or 8), low in the right lobe (segment 5 or 6), and in the left lobe (segment 2 or 3), while avoiding large blood vessels, bile ducts, and the lateral margin of the liver. The voxel was positioned using T_2-weighted pictures in the coronal and transverse planes. Spatial localization was achieved using a spin echo sequence with the point-resolved spectroscopy method. A long repetition time (1500 milliseconds) and short echo time (30 milliseconds) were chosen to minimize the effects of T1 and T2 relaxation, respectively, on signal intensities, without water suppression. Liver fat was quantified by the sum of the AUCs of the peaks of the 3 lipid groups ([CH2]n, CH = CH-CH2 and CH3 with shifts of 1.1-1.5, 1.9-2.3, and 0.8-1.1 ppm relative to water signal intensity), relative to the AUC of the water signal. The mean liver fat content of the 3 locations was used in the statistical analyses. In 3 subjects, quantification on 1 or more locations did not succeed; the mean of the remaining locations was then used.

2.7. Statistical analysis

Variables are presented as mean (SD) or median (interquartile range) as appropriate. Differences between glucose status groups were tested with analysis of variance (with Bonferroni correction for multiple testing). Because men and women generally differ in body composition and the numbers of men and women in the IGM and DM groups were not equal, an adjustment for sex was performed for the variables waist, hip, and waist-hip ratio. Natural log-transformed variables were used in case of nonnormal distributions. For $\Delta AUC_{insulin}/\Delta AUC_{glucose}$, which was not normally distributed after transformation, a Kruskall-Wallis test was used to test overall differences between groups; and a Mann-Whitney test, to specify differences between groups. Spearman correlations (r_S) and linear regression models with adjustments for age and sex were used to establish the association between liver fat and body composition parameters, metabolic parameters, and insulin sensitivity. Regression models were performed for the total study population as well as for glucose status subgroups (NGM or IGM/DM) separately. Linear regression models investigating the relation between liver fat and β-cell function estimates were additionally adjusted for insulin sensitivity for IGM/DM subjects (not for NGM subjects because the 2 groups were matched for insulin sensitivity). Because it is not clear for some parameters in relation to liver fat which factor is cause or consequence, liver fat (natural log-transformed) was used as independent variable in most of the models, except for the models including BMI, waist or hip circumference, and waist-hip ratio, in which we stated that those parameters are determinants of liver fat. P less than .05 indicated statistical significance.

3. Results

3.1. Population characteristics, β-cell function parameters, insulin sensitivity, and liver fat

Subjects with diabetes (DM) had significantly higher waist circumference than both NGM–low insulin secretion and NGM–high insulin secretion groups, but not compared with IGM individuals (Table 1). Waist-hip ratio was higher in IGM and DM individuals compared with NGM subjects.

Both classic and model-based β-cell function parameters were lower in DM subjects compared with the other groups (Table 1). β-Cell function was less impaired for IGM than for DM subjects. A marked decrease in insulin sensitivity was observed in the IGM group: IGM and DM subjects had significantly lower insulin sensitivity than both NGM groups.

Liver fat was comparable in both NGM groups: 4.5% (2.6-6.0) and 4.9% (2.3-7.8) for the low insulin secretion and high insulin secretion group, respectively. The median liver fat percentages were significantly higher for IGM and DM subjects than for NGM subjects: 11.2 (6.7-21.1) and 10.0(7.8-24.5). Fig. 1 shows liver fat distribution in relation to waist-hip ratio, insulin sensitivity, β-cell glucose sensitivity, TG-AUC, and FFA-AUC and depicts a wide distribution in liver fat percentage for IGM and DM subjects, and a much lower and narrower distribution for all NGM individuals.

3.2. Liver fat in relation to estimates for β-cell function and insulin sensitivity

As expected, liver fat showed a fairly strong negative correlation with insulin sensitivity ($r_S = -0.60$, Table 2, Fig. 1). The association remained significant after adjustment for age and sex in a linear regression model (Table 2). Furthermore, fasting insulin and C-peptide, both markers of insulin resistance, showed fairly strong associations with liver fat in all subgroups together and in IGM/DM subjects separately. In all groups, β-cell function parameters failed to show a significant relationship with liver fat content in the

Table 1
Population characteristics (A), β-cell function parameters, insulin sensitivity (B), postprandial fat responses (C), and liver fat (D)

	NGM–low insulin secretion (n = 10)	NGM–high insulin secretion (n = 10)	IGM (n = 14)	DM (n = 14)
A. Population characteristics				
Sex (% male)	50	50	57	64
Age (y)	53.5 (6.4)	51.5 (9.7)	58.0 (5.0)	55.7 (4.4)
BP systolic (mm Hg)	143.5 (12.7)	142.5 (19.2)	145.0 (25.7)	138.9 (16.2)
BP diastolic (mm Hg)	81.8 (8.3)	81.3 (13.6)	80.3 (11.8)	77.3 (8.4)
BMI (kg/m^2)	27.5 (4.1)	26.6 (3.2)	28.0 (3.2)	30.1 (5.4)
Waist circumference (cm)[a]	93.0 (7.8)	94.7 (6.9)	102.0 (11.0)	107.2 (14.0)*,†
Hip circumference (cm)[a]	102.3 (7.1)	105.3 (4.3)	103.2 (6.4)	106.4 (9.7)
Waist-hip ratio[a]	0.91 (0.06)	0.90 (0.06)	0.99 (0.09)*,†	1.01 (0.09)*,†
ALAT (U/L)	9.0 (6.5-11.0)	7.0 (5.0-9.5)	13.0 (9.0-15.5)†	15.0 (13.0-23.3)*,†
Total cholesterol (mmol/L)	5.0 (1.0)	5.0 (1.0)	5.5 (1.1)	4.7 (0.7)
HDL cholesterol (mmol/L)	1.48 (0.51)	1.33 (0.44)	1.29 (0.22)	1.24 (0.46)
LDL cholesterol (mmol/L)	2.9 (0.9)	3.1 (0.9)	3.6 (1.0)	2.7 (0.7)
Fasting glucose (mmol/L)	5.4 (0.3)	5.1 (0.2)	6.1 (0.3)	8.3 (2.0)*,†,‡
Fasting insulin (pmol/L)	34.9 (27.9-41.5)	46.2 (34.1-75.9)	62.0 (43.8-81.6)*	59.3 (39.9-85.5)
Fasting C-peptide (nmol/L)	0.46 (0.07)	0.51 (0.13)	0.70 (0.19)	0.76 (0.34)*
Fasting FFA (mmol/L)	0.56 (0.18)	0.42 (0.10)	0.54 (0.20)	0.57 (0.11)
Fasting TG (mmol/L)	0.9 (0.8-1.9)	1.1 (0.8-1.7)	1.5 (1.2-1.8)	1.4 (1.1-2.4)
B. β-Cell function parameters and insulin sensitivity				
Insulinogenic index (pmol/mmol)	42.3 (35.7-53.5)	260.8 (189.0-395.2)*	112.1 (28.4-131.9)†	22.7 (17.6-35.4)†,‡
$AUC_{insulin}/AUC_{glucose}$ ratio (pmol/mmol)	25.1 (20.6-32.4)	55.8 (32.7-96.9)*	47.1 (33.7-76.2)	19.8 (8.1-29.7)†,‡
$\Delta AUC_{insulin}/\Delta AUC_{glucose}$ ratio (pmol/mmol)	84.0 (67.9-94.5)	366.0 (-249.7-556.2)	108.7 (53.0-208.2)	34.1 (21.1-58.1)*,‡
Glucose sensitivity ($pmol \cdot min^{-1} \cdot (m^2)^{-1} \cdot [mmol/L]^{-1}$)	61.1 (18.0)	156.2 (66.8)*	85.2 (50.1)†	38.5 (19.2)†
Rate sensitivity ($pmol/m^2/[mmol/L]$)	231.2 (94.5-639.4)	892.6 (544.6-1912.0)	207.6 (0.0-829.1)	65.7 (0.0-514.5)
Potentiation factor ratio (fold)	1.81 (0.57)	1.78 (0.90)	2.12 (0.71)	1.20 (0.78)‡
Insulin sensitivity ($mL/[min \cdot m^{-2}]$)	432.9 (52.0)	428.9 (52.5)	345.6 (27.4)*,†	309.2 (35.9)*,†
C. Postprandial fat responses				
AUC_{FFA} (mmol·h/L)	1.90 (0.51)	1.54 (0.29)	1.89 (0.50)	1.72 (0.30)
ΔAUC_{FFA}(mmol·h/L)	−1.30 (1.01)	−0.98 (0.79)	−1.50 (1.06)	−1.82 (0.59)
AUC_{TG} (mmol·h/L)	8.8 (6.7-14.9)	10.4 (7.9-14.3)	13.6 (10.8-16.0)	11.4 (9.3-15.1)
ΔAUC_{TG} (mmol·h/L)	2.8 (0.3-3.7)	3.2 (1.9-4.0)	3.4 (2.2-5.4)	2.2 (1.5-3.6)
D. Liver fat content				
Liver fat (%)	4.5 (2.6-6.0)	4.9 (2.3-7.8)	11.2 (6.7-21.1)*,†	10.0 (7.8-24.5)*,†

Values are mean (SD) or median (interquartile range). BP indicates blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

[a] Analysis of variance for waist, hip, and waist-hip ratio adjusted for sex.

* $P < .05$ compared with NGM–low index group.

† $P < .05$ compared with NGM–high index group.

‡ $P < .05$ compared with IGM group.

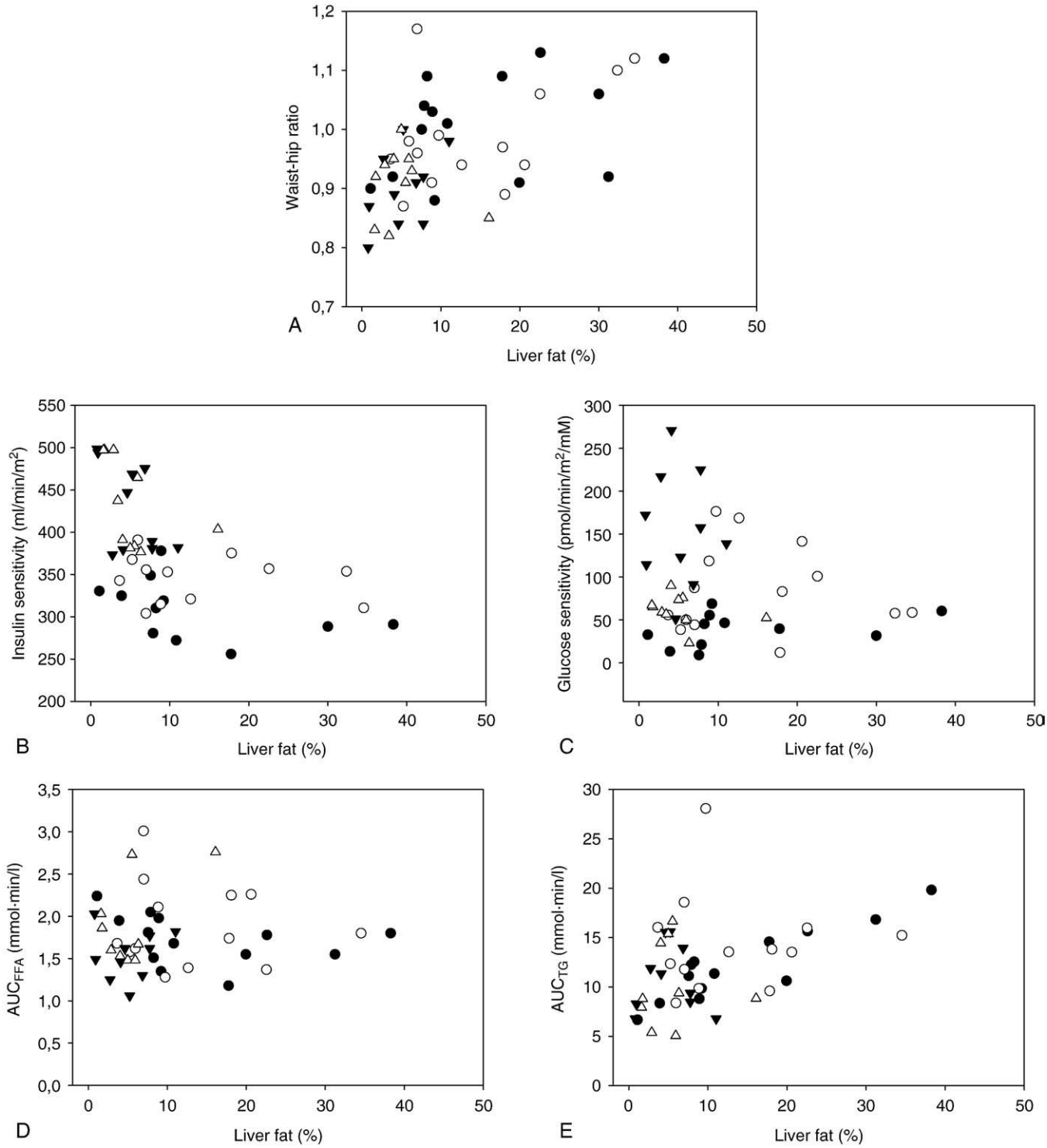

Fig. 1. Scatter plots of hepatic fat vs waist-hip ratio (A), insulin sensitivity (B), β-cell glucose sensitivity (C), AUC_{FFA} (D), and AUC_{TG} (E). Subgroups are represented by the following symbols: black circles, diabetic persons; white circles, IGM subjects; white triangles, NGM with low insulin secretion; black triangles, NGM with high insulin secretion.

age-, sex-, and (for IGM/DM) insulin sensitivity–adjusted models, with the exception of a positive association with $AUC_{insulin}/AUC_{glucose}$ ratio in IGM/DM subjects (Table 2). When β-cell function estimates were calculated with MTT data, regression analysis results were similar (data not shown). Spearman correlation coefficients for associations of liver fat and β-cell function parameters were low, ranging from −0.21 until 0.25. Poor associations were also observed between liver fat and blood pressure, cholesterol, and C-reactive protein (data not shown).

Table 2
Spearman correlations and regression models of fat distribution, β-cell function, insulin sensitivity, and postprandial fat in relation to liver fat

Dependent variable	Spearman correlation (r_S), all subjects	Regression coefficient (CI), all subjects	Regression coefficient (CI), NGM	Regression coefficient (CI), IGM + DM
BMI	0.54*	0.11 (0.05; 0.17)*	0.10 (−0.02; 0.21)	0.09 (0.20; 0.16)*
Waist circumference	0.62*	0.05 (0.03; 0.07)*	0.06 (0.01; 0.11)*	0.04 (0.01; 0.06)*
Hip circumference	0.34*	0.04 (0.00; 0.08)*	0.05 (−0.03; 0.12)	0.04 (−0.01; 0.07)
Waist-hip ratio	0.51*	8.55 (5.47; 11.64)*	11.38 (3.19; 19.58)*	5.78 (1.60; 9.95)*
ALAT[a]	0.57*	0.30 (0.16; 0.43)*	0.04 (−0.20; 0.29)	0.29 (0.09; 0.48)*
Insulinogenic index[a]	−0.19	−0.17 (−0.55; 0.20)	−0.21 (−0.96; 0.53)	0.37 (−0.19; 0.94)
$AUC_{insulin}/AUC_{glucose}$ ratio[a]	0.25	0.20 (−0.05; 0.44)	0.16 (−0.27; 0.59)	0.56 (0.15; 0.97)*
$\Delta AUC_{insulin}/\Delta AUC_{glucose}$ ratio[a]	0.05	0.07 (−0.34; 0.48)	0.11 (−0.63; 0.86)	0.56 (−0.10; 1.22)
Glucose sensitivity	−0.08	−8.27 (−28.4; 11.9)	−9.11 (−55.87; 37.65)	11.51 (−11.44; 34.45)
Rate sensitivity[a]	−0.21	[b]	−0.36 (−1.18; 0.45)	−1.44 (−8.97; 6.10)
Potentiation factor ratio	0.00	−0.001 (−0.28; 0.27)	0.28 (−0.21; 0.76)	−0.05 (−0.53; 0.42)
Insulin sensitivity	−0.60*	−42.81 (−61.20; −24.42)*	−39.00 (−64.97; −13.03)*	−9.41 (−29.67; 10.86)
Fasting glucose	0.48*	0.72 (0.22; 1.21)*	0.15 (−0.04; 0.34)	0.31 (−0.58; 1.21)
Fasting C-peptide	0.62*	0.22 (0.13; 0.32)*	0.05 (−0.08; 0.19)	0.26 (0.10; 0.41)*
Fasting insulin[a]	0.59*	0.28 (0.15; 0.42)*	0.12 (−0.17; 0.40)	0.36 (0.17; 0.56)*
Fasting TG[a]	0.38*	0.17 (0.06; 0.28)*	0.12 (−0.24; 0.26)	0.25 (0.11; 0.40)*
Fasting FFA	0.20	0.02 (−0.03; 0.07)	−0.04 (−0.14; 0.07)	0.03 (−0.05; 0.11)
AUC_{TG}[a]	0.41*	0.15 (0.05; 0.25)*	0.05 (−0.16; 0.26)	0.21 (0.07; 0.36)*
ΔAUC_{TG}[a]	−0.02	−0.01 (−0.20; 0.18)	0.06 (−0.27; 0.40)	−0.05 (−0.37; 0.27)
AUC_{FFA}	0.03	−0.02 (−0.17; 0.13)	0.05 (−0.20; 0.31)	−0.12 (−0.32; 0.09)
ΔAUC_{FFA}	−0.25	−0.22 (−0.52; 0.08)	−0.04 (−0.66; 0.57)	−0.20 (−0.66; 0.26)

CI indicates confidence interval.

[a] Ln-transformed. Liver fat (ln-transformed) was used as independent variable in the models, except for the models including BMI, waist or hip circumference, and waist-hip ratio (Ln[liver fat] was used as dependent variable). All models were adjusted for age and sex; models for IGM/DM subjects including β-cell parameters were additionally adjusted for insulin sensitivity.

[b] Relationship was not linear; therefore, linear regression was not performed.

* $P < .05$.

3.3. Liver fat in relation to body composition and postprandial lipid responses

Fairly high correlations existed between liver fat and ALAT, BMI, waist circumference, and waist-hip ratio (r_S = 0.50-0.65, Table 2, Fig. 1); the correlation with hip circumference was lower (Table 2). Linear regression showed significant positive associations between liver fat and all body composition parameters (Table 2).

Postprandial fat responses did not differ significantly between groups (Table 1). The correlations between liver fat and fasting TG and AUC_{TG} (Table 2, Fig. 1) were moderate (r_S = 0.38-0.41), and the regression models showed significant associations with fasting TG and the TG-AUC after the mixed meal for the total study population and for IGM/DM subjects. Correlations between liver fat and fasting FFA (fasting) or FFA response after the meal (AUC (Fig. 1) and ΔAUC) were low (with r_S ranging from −0.25 to 0.20). No significant relationships existed between fasting FFA or FFA responses and liver fat content.

4. Discussion

The results of this study suggest that liver fat content is not associated with either classic or model-based estimates of β-cell function in DM, IGM, and NGM subjects selected for high or low early insulin secretion and matched for insulin sensitivity, with the exception of the $AUC_{insulin}/AUC_{glucose}$ ratio in IGM/DM subjects. In the total study population and in IGM/DM subjects separately, relationships between liver fat and fasting insulin and C-peptide were apparent. We additionally confirmed earlier findings indicating that liver fat is negatively associated with insulin sensitivity and positively associated with body fat composition (BMI, waist, waist-hip ratio). Furthermore, liver fat was associated with fasting and postprandial TG but not with fasting or postprandial FFA.

4.1. Liver fat and β-cell function

The relation between hepatic fat and insulin resistance is the result of an excess fat in the hepatocytes, which interferes with insulin signaling, eventually leading to insulin resistance [5]. Concerning the association of liver fat and insulin secretion, several studies [5,7,8] have shown that liver fat content is associated with fasting insulin in both healthy subjects and patients with diabetes. We confirmed this finding in the total study population and in IGM/DM subjects, but not in the NGM subgroup. Furthermore, we did not find an association between hepatic fat and most β-cell function estimates calculated from the postload responses. This is not in agreement with the study of Musso et al [16], who reported a marked β-cell secretory dysfunction as reduced first-phase insulin secretion and low disposition index in subjects with NASH long before glucose intolerance appeared. This may be due to several reasons: First, none of the subjects in our study was diagnosed with NASH. Second,

in that study, β-cell function was estimated from an IVGTT, whereas we used an OGTT: IVGTT can better detect first-phase insulin secretion, but is less physiologic because it lacks the incretin response that can influence β-cell parameters. Third, we did not estimate the disposition index, that is, β-cell function relative to insulin sensitivity, because insulin sensitivity is correlated with liver fat and thus β-cell function parameters expressed relative to insulin sensitivity might logically be related to liver fat. Therefore, our NGM subjects were matched for insulin sensitivity; and the regression models for the IGM/DM subjects were adjusted for insulin sensitivity.

Gastaldelli et al [27] have shown that (magnetic resonance imaging–quantified) visceral fat is not related to β-cell function. Because abdominal (visceral) fat might logically be correlated with hepatic fat, this might lead to the expectation of our results, which show a lack of association between liver fat and β-cell function. However, the relationship between visceral and hepatic fat is not unequivocal. Tiikkainen et al [28] showed that a decrease in liver fat content as result of a weight loss intervention did not reflect changes in endogenous fat stores. It has been suggested that the reason for the variability in hepatic fat in obese subjects might be caused by variability in genetic susceptibility to lipotoxicity [5,29] and in lifestyle (nutrition and physical activity) [30]. It has been suggested that increases in hepatic and muscular fat might be associated with insulin resistance, whereas increased pancreatic fat might be related to impaired insulin secretion [5]. Indeed, a recent study has shown that pancreatic fat was significantly associated with various β-cell function parameters, but only in non-DM men [31]. Interestingly, pancreatic fat was also not associated with visceral fat, BMI, waist, TG, or FFA levels [31]. Possibly, the liver is more susceptible to fat accumulation than the pancreas, resulting in an earlier decrease in insulin sensitivity than in insulin secretion.

4.2. Liver fat and lipid responses after a mixed meal

Our second aim was to study if postprandial TG and FFA are determinants of liver fat content. In the liver, FFAs stimulate gluconeogenesis and very low-density lipoprotein secretion and ectopic TG accumulation, which may lead to a fatty liver. Several recent studies have reported the presence of fatty liver in patients with type 2 diabetes mellitus [32]. In addition, fatty liver has been shown to be associated with components of metabolic syndrome [32], including fasting hypertriglyceridemia [8]. Obesity, especially visceral adiposity, and meal-related increases in TG are sources for fatty acids. In obese subjects with IGM or DM, plasma FFA concentrations are often elevated in the fasting state and are not always suppressed after a meal or in response to insulin [33]. It has been shown in both healthy and DM subjects that liver fat is related to an impaired FFA suppression by insulin during a euglycemic-hyperinsulinemic clamp [7,8] or intravenous glucose infusion [34]. Whereas we found a significant association between fasting and postprandial TG response and liver fat after a mixed meal in IGM/DM subjects, fasting and postprandial FFA levels were not related to hepatic fat content. Our results were in concordance with those of another study [3]. The differences in results between studies using clamp techniques and meals might be caused by a lower FFA suppression as a result of the meal in comparison with a clamp. Furthermore, circulating FFA levels might not be similar to the concentrations to which the liver is exposed.

4.3. Study limitations

The subjects in this cross-sectional study were a selected subgroup of a population study representative of the general Dutch population. The population was mainly Caucasian. Therefore, generalization of the results to other populations must be performed with caution. Furthermore, our cross-sectional study design does not allow us to draw conclusions about the sequence of events in the development of fatty liver disease, lipid abnormalities, insulin sensitivity, and β-cell function. However, this is one of the first studies to perform liver spectroscopy in a population-based sample. We used relatively small but highly informative subgroups. By using this design, we were able to exclude any differences in insulin sensitivity explaining the differences in the possible association between liver fat and insulin secretion. Diabetic patients were allowed to take oral antidiabetic medication except on the day of the OGTT and meal tests. Therefore, agents like metformin and sulfonylureas might have influenced our results. Furthermore, the relatively small sample size may reduce power to detect associations. However, we did observe the known association between liver fat and waist, waist-hip ratio, and insulin sensitivity. Our observed associations do not suggest a clinically relevant link between liver fat and insulin secretion.

4.4. Conclusion and recommendations for future research

Future studies might evaluate the differences in (genetic) predisposition to fatty degeneration of the abdominal organs. Furthermore, the contribution of TGs or FFAs to the development of fatty liver disease might be explored in more detail by estimating the actual TG and FFA level to which the liver is exposed (as a result of release by adipocytes). We conclude that liver fat is tightly linked to insulin sensitivity but not to (classic or model-based) estimates of β-cell function. Furthermore, circulating (fasting and postprandial) FFA levels are not associated with liver fat content, in contrast with circulating (fasting and postprandial) TG levels, which show a positive relation with liver fat in IGM/DM subjects.

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References

[1] Chan DC, Watts GF, Ng TW, Hua J, Song S, Barrett PH. Measurement of liver fat by magnetic resonance imaging: relationships with body fat distribution, insulin sensitivity and plasma lipids in healthy men. Diabetes Obes Metab 2006;8:698-702.

[2] Eguchi Y, Eguchi T, Mizuta T, Ide Y, Yasutake T, Iwakiri R, et al. Visceral fat accumulation and insulin resistance are important factors in nonalcoholic fatty liver disease. J Gastroenterol 2006;41:462-9.

[3] Matikainen N, Manttari S, Westerbacka J, Vehkavaara S, Lundbom N, Yki-Jarvinen H, et al. Postprandial lipemia associates with liver fat content. J Clin Endocrinol Metab 2007;92:3052-9.

[4] Westerbacka J, Corner A, Tiikkainen M, Tamminen M, Vehkavaara S, Hakkinen AM, et al. Women and men have similar amounts of liver and intra-abdominal fat, despite more subcutaneous fat in women: implications for sex differences in markers of cardiovascular risk. Diabetologia 2004;47:1360-9.

[5] Kovanlikaya A, Mittelman SD, Ward A, Geffner ME, Dorey F, Gilsanz V. Obesity and fat quantification in lean tissues using three-point Dixon MR imaging. Pediatr Radiol 2005;35:601-7.

[6] Bajaj M, Suraamornkul S, Hardies LJ, Pratipanawatr T, Defronzo RA. Plasma resistin concentration, hepatic fat content, and hepatic and peripheral insulin resistance in pioglitazone-treated type II diabetic patients. Int J Obes Relat Metab Disord 2004;28:783-9.

[7] Gastaldelli A, Cusi K, Pettiti M, Hardies J, Miyazaki Y, Berria R, et al. Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. Gastroenterology 2007; 133:496-506.

[8] Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, Goto T, Westerbacka J, Sovijarvi A, et al. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. J Clin Endocrinol Metab 2002;87:3023-8.

[9] Burgert TS, Taksali SE, Dziura J, Goodman TR, Yeckel CW, Papademetris X, et al. Alanine aminotransferase levels and fatty liver in childhood obesity: associations with insulin resistance, adiponectin, and visceral fat. J Clin Endocrinol Metab 2006;91:4287-94.

[10] Ardigo D, Numeroso F, Valtuena S, Franzini L, Piatti PM, Monti L, et al. Hyperinsulinemia predicts hepatic fat content in healthy individuals with normal transaminase concentrations. Metabolism 2005;54:1566-70.

[11] Defronzo RA, Gunnarsson R, Bjorkman O, Olsson M, Wahren J. Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. J Clin Invest 1985;76: 149-55.

[12] Reaven GM, Hollenbeck CB, Chen YD. Relationship between glucose tolerance, insulin secretion, and insulin action in non-obese individuals with varying degrees of glucose tolerance. Diabetologia 1989;32:52-5.

[13] Tripathy D, Carlsson M, Almgren P, Isomaa B, Taskinen MR, Tuomi T, et al. Insulin secretion and insulin sensitivity in relation to glucose tolerance: lessons from the Botnia Study. Diabetes 2000;49:975-80.

[14] Nijpels G, Popp-Snijders C, Kostense PJ, Bouter LM, Heine RJ. Fasting proinsulin and 2-h post-load glucose levels predict the conversion to NIDDM in subjects with impaired glucose tolerance: the Hoorn Study. Diabetologia 1996;39:113-8.

[15] Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. Diabetologia 2003;46:3-19.

[16] Musso G, Gambino R, Biroli G, Carello M, Faga E, Pacini G, et al. Hypoadiponectinemia predicts the severity of hepatic fibrosis and pancreatic beta-cell dysfunction in nondiabetic nonobese patients with nonalcoholic steatohepatitis. Am J Gastroenterol 2005;100: 2438-46.

[17] Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, et al. Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function. Diabetes 1993;42:1663-72.

[18] Ahren B, Pacini G. Impaired adaptation of first-phase insulin secretion in postmenopausal women with glucose intolerance. Am J Physiol 1997;273:E701-7.

[19] Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 1988;37:1595-607.

[20] World Health Organization definition and diagnosis of diabetes mellitus and intermediate hyperglycemia—report of a WHO/IDF consultation, 2006.

[21] Seltzer HS, Allen EW, Herron Jr AL, Brennan MT. Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. J Clin Invest 1967; 46:323-35.

[22] Matthews JN, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research. BMJ 1990;300:230-5.

[23] Mari A, Tura A, Gastaldelli A, Ferrannini E. Assessing insulin secretion by modeling in multiple-meal tests: role of potentiation. Diabetes 2002;51(Suppl 1):S221-6.

[24] Mari A, Schmitz O, Gastaldelli A, Oestergaard T, Nyholm B, Ferrannini E. Meal and oral glucose tests for assessment of beta-cell function: modeling analysis in normal subjects. Am J Physiol Endocrinol Metab 2002;283:E1159-66.

[25] Mari A, Pacini G, Murphy E, Ludvik B, Nolan JJ. A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. Diabetes Care 2001;24:539-48.

[26] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18: 499-502.

[27] Gastaldelli A, Sironi AM, Ciociaro D, Positano V, Buzzigoli E, Giannessi D, et al. Visceral fat and beta cell function in non-diabetic humans. Diabetologia 2005;48:2090-6.

[28] Tiikkainen M, Bergholm R, Vehkavaara S, Rissanen A, Hakkinen AM, Tamminen M, et al. Effects of identical weight loss on body composition and features of insulin resistance in obese women with high and low liver fat content. Diabetes 2003;52:701-7.

[29] Stefan N, Machicao F, Staiger H, Machann J, Schick F, Tschritter O, et al. Polymorphisms in the gene encoding adiponectin receptor 1 are associated with insulin resistance and high liver fat. Diabetologia 2005; 48:2282-91.

[30] Schafer S, Kantartzis K, Machann J, Venter C, Niess A, Schick F, et al. Lifestyle intervention in individuals with normal versus impaired glucose tolerance. Eur J Clin Invest 2007;37:535-43.

[31] Tushuizen ME, Bunck MC, Pouwels PJ, Bontemps S, van Waesberghe JH, Schindhelm RK, et al. Pancreatic fat content and beta-cell function in men with and without type 2 diabetes. Diabetes Care 2007;30:2916-21.

[32] Ryysy L, Hakkinen AM, Goto T, Vehkavaara S, Westerbacka J, Halavaara J, et al. Hepatic fat content and insulin action on free fatty acids and glucose metabolism rather than insulin absorption are associated with insulin requirements during insulin therapy in type 2 diabetic patients. Diabetes 2000;49:749-58.

[33] Defronzo RA. Dysfunctional fat cells, lipotoxicity and type 2 diabetes. Int J Clin Pract Suppl 2004:9-21.

[34] Carpentier A, Mittelman SD, Bergman RN, Giacca A, Lewis GF. Prolonged elevation of plasma free fatty acids impairs pancreatic beta-cell function in obese nondiabetic humans but not in individuals with type 2 diabetes. Diabetes 2000;49:399-408.