CLINICAL STUDY

Lack of association of liver fat with model parameters of β -cell function in men with impaired glucose tolerance and type 2 diabetes

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Abstract

Objective: Hepatic steatosis and obesity are components of the metabolic syndrome and risk factors for developing type 2 diabetes (T2DM). We studied how liver fat and body fat distribution relate to various aspects of β -cell function.

Methods: In 12 men with T2DM, 10 men with impaired glucose tolerance (IGT), and 14 age- and body mass index-matched controls, we measured body fat distribution and liver fat by magnetic resonance imaging and spectroscopy. An oral glucose tolerance test was performed to calculate insulin secretory rate (ISR) by C-peptide deconvolution, and β -cell function using a mathematical model that describes ISR as a function of absolute glucose levels (insulin secretory tone and glucose sensitivity), the glucose rate of change (rate sensitivity), and a potentiation factor.

Results: Waist circumference and the various body fat compartments did not differ among groups. IGT had the highest total and late phase insulin secretion ($P < 0.001$), whereas patients had the lowest insulinogenic index adjusted for insulin resistance ($P = 0.006$). In spite of the hypersecretion, IGT had β -cell glucose sensitivity, rate sensitivity, and potentiation similar to controls. Liver fat content was highest in diabetic patients ($P = 0.004$) and showed the strongest association with total and late phase of insulin secretion in the IGT group ($r = 0.657$, $P = 0.039$ and $r = 0.732$, $P = 0.016$ respectively). Model β -cell function variables showed no association with liver fat or body fat compartments.

Conclusions: These data suggest that, in spite of the association of central adiposity and liver fat with T2DM risk, additional, hitherto unknown factors may contribute to β -cell dysfunction in susceptible humans.

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Introduction

Central adiposity and liver steatosis are considered key features underlying the insulin resistance or metabolic syndrome and its associated cardiometabolic abnormalities (1). Furthermore, central obesity and liver steatosis are associated with a high risk for the development of type 2 diabetes (T2DM) (1, 2). However, evidence indicates that T2DM only develops in insulin-resistant subjects with the onset of β -cell dysfunction (3–5). In addition, recent data from the Relationship between Insulin Sensitivity and Cardiovascular risk (RISC) study demonstrate that each of obesity, insulin resistance, abdominal obesity, and insulin response can be found in isolation despite their strong tendency to cluster (6). The relationship of insulin sensitivity and β -cell function is therefore not unequivocal, confirming the notion that T2DM is a heterogeneous disease. Thus, the mechanisms that link central obesity and liver fat content to β -cell dysfunction

are at present incompletely understood. Chronic exposure of pancreatic islets to high circulating levels of non-esterified fatty acids (NEFA), due to unsuppressed lipolysis in insulin-resistant adipose tissue, is considered as a potential primary cause of β -cell dysfunction (4). Recently, we reported that fat localized in the pancreas was inversely associated with dynamic measures of β -cell function in non-diabetic men (7). Previous reports described an association of intra-abdominal fat with β -cell function (8, 9); however, this was not confirmed by others (10–12). This seeming discrepancy may be due to the differences in populations studied and the methods by which β -cell function parameters were estimated. In particular, if the magnitude of the insulin secretory response to acute glucose stimulation is used to estimate β -cell function, an association with visceral adipose tissue (VAT) may rather reflect impaired insulin sensitivity (11). Similarly, although liver steatosis is associated with hepatic insulin resistance and inflammation, and was

shown to predict T2DM in many different populations (1, 2), its relation with β -cell dysfunction is unclear. Insulin-resistant subjects with liver steatosis, when compared with insulin-sensitive individuals, have greater insulin responses and lower hepatic insulin clearance, leading to hyperinsulinemia (4, 13).

However, at present, it is unknown whether liver fat contributes to abnormalities in dynamic aspects of β -cell function that can be derived from a physiological model, describing the relationship between insulin secretion and glucose concentration (6, 14, 15). Therefore, we assessed liver fat and various aspects of β -cell function, by performing proton magnetic resonance spectroscopy (^1H -MRS) measurements and a modified oral glucose tolerance test (OGTT) respectively, in men with normal (NGT) and impaired glucose tolerance (IGT) and in men with uncomplicated T2DM.

Subjects and methods

Subjects

After obtaining written informed consent, 36 Caucasian males, aged 35–65 years, with known T2DM ($n=12$), IGT ($n=10$), and with NGT ($n=14$) were studied. Patients were treated by diet ($n=3$), sulfonylurea (SU; $n=3$), and/or metformin ($n=6$) only. Claustrophobia, excess alcohol intake (>20 units/week), history of hepatitis and/or pancreatitis, abnormal liver and renal function tests (>2 times upper limits of normal), recent (<3 months) changes in weight ($\geq 5\%$) and/or medication, history or current use of steroids, and insulin and/or thiazolidinediones, were exclusion criteria. The local ethics committee approved the study and the investigation conformed to the principles outlined in the Declaration of Helsinki.

Study protocol

Following an overnight fast, participants arrived at the research centre in the early morning and underwent MR examinations and an OGTT during one single visit. They were instructed to omit their medication in the morning of the examination and to refrain from heavy physical activities during the previous 24 h.

MR examinations, both imaging (MRI) for quantification of abdominal fat compartments and ^1H -MRS to assess liver fat content, were performed in the supine position using a 1.5 T whole-body system (Sonata; Siemens, Erlangen, Germany). For ^1H -MRS, the body array coil was positioned at the upper abdominal region as described previously (7). Briefly, three volumes-of-interest in the liver (right anterior, right posterior, and medial or left anterior) of 15 cc ($2.5 \times 2.5 \times 2.5 \text{ cm}^3$) were measured with stimulated-echo acquisition mode (STEAM) (repetition time 5000 ms, echo time 20 ms, 8 separate acquisitions), avoiding major blood vessels,

intra-hepatic bile ducts, and the lateral margin of the liver. Spectral quantification was performed with LCModel (version 6.1) (16). Lipid content is expressed as the percentage of the area under the methyl (0.9 ppm) and methylene peaks (1.3 ppm) relative to that under the water peak (4.65 ppm). Mean lipid content of three volumes-of-interest was used. The coefficient of variation of liver fat in two subsequent MRS sessions, determined in eight subjects, was 4.7%.

VAT area and subcutaneous abdominal adipose tissue area (SAT) were measured by MRI as described previously (17). Briefly, sagittal and coronal slices were used to localize anatomic sites for image acquisition (L4–L5). Three transverse images (spacing, 20 mm) were obtained. Quantification of visceral and s.c. fat areas was performed using an image analysis program, running on a Sparc10 workstation (Sun Microsystems, Palo Alto, CA, USA). A seed point is placed in a fat depot, and using a seed-growing procedure, this fat depot can be circumscribed by the selection of a pixel intensity range. The areas were expressed in cm^2 and the average area of the three transverse images was used for statistical analyses. Processing of MRI data and calculations of VAT and SAT were performed using a single experienced investigator (S B).

Following the MR examination, a 75 g OGTT was performed (14, 15). Whole blood samples were drawn at 0, 10, 20, 30, 60, 90, and 120 min to measure glucose (YSI 2300 STAT Plus Glucose Analyzer, Yellow Springs, Ohio, USA), serum C-peptide, and insulin concentrations (both by IRMA (Centaur, Bayer Diagnostics)). Baseline plasma glucose concentrations were measured by hexokinase-based technique (Roche Diagnostics). HbA1c was measured with cation exchange chromatography (Menarini Diagnostics, Florence, Italy; reference values 4.3–6.1%). Plasma total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides were determined by enzymatic methods (Modular, Hitachi). Low-density lipoprotein cholesterol was calculated by the Friedewald formula. NEFA were assessed by enzymatic colorimetric method (WAKO chemicals, Neuss, Germany).

β -cell function parameters

Various β -cell function parameters were calculated. Insulin secretion rates (ISR) were determined from C-peptide deconvolution (18), since hepatic insulin resistance may influence the hepatic clearance of insulin and the clearance of C-peptide is liver independent. The insulinogenic index was calculated as the insulin increment at 30 min above basal divided by the corresponding glucose increment, without and with subsequent adjustment for homeostasis model assessment (19) insulin resistance ($\Delta I_{0-30}/\Delta G_{0-30}$ and $\Delta I_{0-30}/\Delta G_{0-30}/\text{HOMA-IR}$) (14). An oral glucose insulin sensitivity (OGIS) index was derived from OGTT glucose and insulin values and has been demonstrated to give a very

similar assessment of insulin sensitivity in type 2 diabetic subjects as the glucose clamp (20). Early and late phase ISR were calculated as the ISR integrals from 0 to 30 min (ISR AUC_{0–30}) and 30 to 120 min (ISR AUC_{30–120}) respectively. Dynamic aspects of β -cell function were assessed using a model describing the relationship between insulin secretion and glucose concentration, which has been detailed previously (7, 14, 15). In particular, β -cell glucose sensitivity (i.e., the slope of the dose–response function relating ISR to glucose concentration), ISR at a reference (close to basal) glucose level (calculated from the β -cell dose–response), and parameters quantifying the ability of the β -cell to anticipate a phase of rising insulin secretion (or rate sensitivity) and to memorize the glucose stimulus as well as reading incretin signals (potentiation) were calculated.

Modeling of β -cell function parameters

The model consists of three blocks: a) a model for fitting the glucose concentration profile, the purpose of which is to smooth and interpolate plasma glucose concentrations; b) a model describing the dependence of insulin (or C-peptide) secretion on glucose concentrations; and c) a model of C-peptide kinetics, i.e. the two-exponential model proposed by Van Cauter *et al.* (18), in which the model parameters are individually adjusted to the subject's anthropometric data.

The model expresses insulin secretion as the sum of two components. The first component represents the relationship between insulin secretion and absolute glucose concentration at any time point, i.e. a dose–response function. The mean slope of the dose–response function is taken to represent β -cell glucose sensitivity. Insulin secretion at reference glucose levels representing the average basal glucose concentration (5 mmol/l in non-diabetic and 7 mmol/l in diabetic subjects) was

calculated from the β -cell dose–response. The dose–response function is modulated by a time-varying factor, expressing a relative potentiation effect on insulin secretion. The potentiation factor, which encompasses various potentiating signals (glucose-induced potentiation, incretins, neural factors), was set to have a mean value equal to unity over the 2 h of the study. As the potentiation factor typically increases during an OGTT, the ratio between the potentiation factor value at 2 h and that at time 0 was used as a parameter quantifying potentiation. The second insulin secretion component represents a dynamic dependence of insulin secretion on the rate of change of glucose concentration. It is proportional to the time derivative of glucose concentration when glucose rises and is zero otherwise. The proportionality constant, termed rate sensitivity, accounts for anticipation of insulin secretion as glucose levels rise. The potentiation factor and total insulin secretion (expressed in pmol/min per square meter of body surface area) were calculated every 5 min for the whole 2-h period, as detailed (14, 15).

Statistical analysis

Results are presented as the means $\pm$ S.E.M. or medians (interquartile range, IQR). Non-normally distributed data were log transformed. Differences between groups were calculated by ANOVA. The association of liver fat content and body fat compartments with variables of β -cell function was assessed by univariate correlation. We performed regression analysis to assess the association of β -cell function parameters and liver fat content, adjusting for OGIS alone, and in combination with body mass index (BMI) and age. All statistical analyses were run on SPSS for Windows version 15.0 (SPSS, Chicago, IL, USA). A $P < 0.05$ was considered statistically significant.

Table 1 Baseline characteristics of the study population.

	T2DM	IGT	Controls	P
N	12	10	14	–
Age (years)	54.6 $\pm$ 2	58.0 $\pm$ 2	53.6 $\pm$ 3	NS
BMI (kg/m ²)	31.3 $\pm$ 1	29.0 $\pm$ 1	29.2 $\pm$ 1	NS
Waist (cm)	111.8 $\pm$ 2	107.0 $\pm$ 2	106.2 $\pm$ 3	NS
Systolic blood pressure (mmHg)	136 $\pm$ 4	140 $\pm$ 5	123 $\pm$ 3	0.01
Diastolic blood pressure (mmHg)	82 $\pm$ 2	84 $\pm$ 3	77 $\pm$ 2	NS
HbA1c (%)	7.2 $\pm$ 0.3	5.6 $\pm$ 0.1	5.5 $\pm$ 0.1	<0.001
Fasting plasma glucose (mmol/l)	8.2 $\pm$ 0.5	5.6 $\pm$ 0.2	5.3 $\pm$ 0.1	<0.001
HOMA-IR	2.0 $\pm$ 0.3	1.2 $\pm$ 0.1	0.9 $\pm$ 0.1	<0.01
OGIS (ml/min/m ²)	328 $\pm$ 12	383 $\pm$ 17	458 $\pm$ 10	<0.001
NEFA (mmol/l)	0.68 $\pm$ 0.08	0.69 $\pm$ 0.06	0.55 $\pm$ 0.05	NS
Total-cholesterol (mmol/l)	4.7 $\pm$ 0.3	5.3 $\pm$ 0.2	4.9 $\pm$ 0.2	NS
HDL-C (mmol/l)	1.05 $\pm$ 0.1	1.26 $\pm$ 0.1	1.44 $\pm$ 0.1	<0.01
LDL-C (mmol/l)	2.8 $\pm$ 0.3	3.5 $\pm$ 0.2	2.9 $\pm$ 0.2	NS
Triglycerides (mmol/l)	2.4 $\pm$ 0.5	1.3 $\pm$ 0.2	1.2 $\pm$ 0.1	<0.01
ALT (U/l)	32.0 $\pm$ 3	31.1 $\pm$ 4	25.1 $\pm$ 2	NS

Values are mean $\pm$ S.E.M. or median (interquartile range). T2DM, type 2 diabetes mellitus; IGT, impaired glucose tolerance; BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; OGIS, oral glucose insulin sensitivity; NEFA, non-esterified fatty acids; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; ALT, alanine aminotransferase.

Results

Table 1 lists the baseline characteristics of the three groups, which were well matched for age, BMI, and waist circumference. As expected, patients had the highest HbA1c, fasting plasma glucose, triglyceride levels, and HOMA-IR, whereas HDL-C levels and OGIS were lowest. Median liver fat content was highest in patients versus controls, whereas men with IGT had intermediate values (T2DM: 16.6 (IQR 9.7–29.8), IGT: 9.3 (6.9–21.3), NGT: 5.4 (1.8–10.8), $P=0.004$; Fig. 1). Interestingly, VAT and SAT did not differ among patients, IGT and NGT (VAT: 301 ± 31 , 263 ± 20 , 255 ± 28 cm²; $P=0.454$; SAT: 312 ± 32 , 291 ± 2 , 305 ± 37 ; $P=0.899$; VAT/SAT ratio: 1.1 ± 0.1 , 0.9 ± 0.1 , 0.9 ± 0.1 ; $P=0.639$). In diabetic, when compared with non-diabetic individuals, empirical and model-derived parameters of β -cell function were significantly impaired (Table 2). IGT had the highest total and late phase insulin secretion ($P<0.001$), whereas patients, relative to IGT and NGT, had the lowest insulinogenic index adjusted for insulin resistance (11 ± 3 , 82 ± 14 , and 110 ± 30 ; $P=0.006$). In spite of the hypersecretion, IGT had β -cell glucose sensitivity, rate sensitivity, and potentiation similar to controls (Table 2). The model-derived β -cell parameters did not differ between SU and non-SU users.

In univariate analysis, initially performed in the entire population, liver fat was significantly associated with HOMA-IR ($r=0.459$; $P=0.005$), OGIS ($r=-0.648$; $P<0.001$), and VAT ($r=0.359$; $P=0.034$). Liver fat content showed the strongest association with total and late phase of insulin secretion in the IGT group (Table 3); however, model-derived β -cell function variables showed no association with liver fat or with VAT or SAT. This lack of association remained after adjustment for OGIS alone and in combination with age and BMI (data not shown).

Discussion

These data confirm observations by others, who found no association of abdominal fat with dynamic aspects of

β -cell function (10), and extend these findings by showing the absence of a relationship between liver fat content and dynamic β -cell function variables in men with IGT, T2DM, and age- and BMI-matched NGT controls. An association was present between liver fat and exaggerated and prolonged secretion of insulin after a glucose load, which may in part reflect a measure of β -cell function, but should rather be regarded as an indicator of impaired insulin sensitivity (13, 21). Previous studies found insulin hypersecretion in obese, relative to lean individuals, whereas dynamic β -cell function parameters were comparable in obese and lean subjects (22). Lifestyle interventions leading to the reduction of VAT in glucose intolerant subjects did not result in improvement of β -cell function (23). Similarly, weight loss after lifestyle changes in obese women was associated with a decrease in VAT, SAT, and liver fat and with reduced fasting hyperinsulinemia (24). The changes in liver fat and abdominal fat depots were unrelated and confirmed the observations showing that local fat accumulation, including the liver, may be different in people with similar BMI and waist circumference. Adipose tissue-derived proinflammatory adipokines and NEFA have been proposed to link (central) obesity to T2DM by inducing pancreatic islet dysfunction through inflammation and lipotoxicity respectively (13, 25). However, the association of inflammatory markers and incident T2DM is not unequivocal (26, 27), indicating a more complex interplay among genetic susceptibility, obesity, and β -cell dysfunction.

The association of hepatic steatosis, as estimated by ALT, and the risk of T2DM were reported by some (28–30), but were not found by us and others (31, 32). Theoretically, there are several mechanisms by which fatty liver could indirectly contribute to β -cell dysfunction, including the recently reported induction of proinflammatory genes in steatotic versus non-steatotic livers (33), by glucose-lipotoxicity, due to unsuppressed hepatic glucose production and output of triglyceride-rich lipoproteins and oxidative stress injury (4, 32). Previously, we showed that pancreatic fat

Figure 1 (A) ¹H-MR spectra (CH₂ peak at 1.3 ppm is main signal of lipids) were obtained from abdominal MRI scans indicating volume-of-interest in the liver. (B) Median (interquartile range) lipid content of the liver in men with normal (NGT), in those with impaired glucose tolerance (IGT), and in men with type 2 diabetes (T2DM).

Table 2 Characteristics of β -cell function parameters.

	Non-T2DM subjects			T2DM subjects
	NGT subjects		IGT subjects	
Empirical parameters of β -cell function				
Insulinogenic index ($\Delta I_{0-30}/\Delta G_{0-30}$)	152 $\pm$ 25	148 $\pm$ 38	158 $\pm$ 28	34 $\pm$ 8*
Early phase insulin secretion (nmol/m ²)	8.9 $\pm$ 0.7	8.0 $\pm$ 0.8	10.3 $\pm$ 1.1	5.3 $\pm$ 0.4 [†]
Late phase insulin secretion (nmol/m ²)	40.1 $\pm$ 3.5	30.3 $\pm$ 2.8	53.9 $\pm$ 4.5	28.1 $\pm$ 2.6 [†]
Total insulin secretion (nmol/m ²)	49.0 $\pm$ 3.7	38.2 $\pm$ 3.1	64.2 $\pm$ 4.8	33.4 $\pm$ 2.8 [†]
Model parameters of β -cell function				
Insulin secretion at the reference glucose level (pmol/min/m ²) ^a	161 $\pm$ 18	178 $\pm$ 30	138 $\pm$ 13	124 $\pm$ 13
β -cell glucose sensitivity (pmol/min/m ² /mM)	122 $\pm$ 15	125 $\pm$ 24	118 $\pm$ 14	30 $\pm$ 3*
Rate sensitivity (pmol/m ² /mM)	1107 $\pm$ 131	926 $\pm$ 120	1341 $\pm$ 245	194 $\pm$ 42 [†]
Potential factor	1.5 $\pm$ 0.2	1.6 $\pm$ 0.3	1.5 $\pm$ 0.3	1.0 $\pm$ 0.1

For calculations of the various β -cell parameters, see Subjects and methods. Values for β -cell parameters are mean $\pm$ S.E.M. * P < 0.01; [†] P < 0.001 (calculated by ANOVA between NGT, IGT, and T2DM groups). NGT, normal glucose tolerance; IGT, impaired glucose tolerance; T2DM, type 2 diabetes mellitus.

^aParameter calculated at 5 and 7 mmol/l glucose for non-T2DM and T2DM respectively.

content was an independent determinant of abnormal model parameters of β -cell function in non-diabetic men (7). In view of the absence of an association of these β -cell function measures and other fat depots, it seems that fat accumulation in close vicinity to the islets, acting through locally released NEFA and adipokines, may be the culprit in causing β -cell dysfunction, rather than more distant fat depots (7). Genetic and environmental aspects as well as factors determining the rate of fat accumulation and local fatty replacement in the various organs may contribute to the individual differences in local fat deposition and the differential susceptibility to develop β -cell dysfunction. Accordingly, once T2DM develops the rate of β -cell function decline during the course of disease may be

unpredictable due to the individual susceptibility of β -cells to diabetes-related hyperglycemia and its sequelae, including oxidative stress, inflammation, and endothelial dysfunction.

Although the relatively small size of this study may be regarded as a limitation and therefore may not be generalized, the limited number of subjects was sufficient to detect relevant findings such as the significant difference in liver fat between the groups as well as a significant association of liver fat and estimates of insulin resistance.

We conclude that, in spite of the reported association of central adiposity and liver fat with T2DM risk, we found no association of liver fat content or abdominal fat depots, with dynamic β -cell function parameter

Table 3 β -cell function parameters and their univariate correlation with liver fat.

	Liver fat content (log %)									
	Non-T2DM subjects									
	All subjects		NGT subjects		IGT subjects		T2DM subjects			
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
Empirical parameters of β -cell function										
Insulinogenic index ($\Delta I_{0-30}/\Delta G_{0-30}$)	-0.243	NS	-0.039	NS	-0.045	NS	-0.101	NS	-0.033	NS
Early phase insulin secretion (nmol/m ²)	-0.121	NS	0.102	NS	0.048	NS	-0.159	NS	0.498	NS
Late phase insulin secretion (nmol/m ²)	0.381	0.022	0.668	<0.001	0.556	0.039	0.732	0.016	0.477	NS
Total insulin secretion (nmol/m ²)	0.318	NS	0.634	0.001	0.525	NS	0.657	0.039	0.494	NS
Model parameters of β -cell function										
Insulin secretion at the reference glucose level (pmol/min/m ²) ^a	NA ^b	NA ^b	-0.019	NS	0.007	NS	0.375	NS	0.159	NS
β -cell glucose sensitivity (pmol/min/m ² /mM)	-0.187	NS	0.102	NS	0.071	NS	0.355	NS	0.087	NS
Rate sensitivity (pmol/m ² /mM)	-0.274	NS	-0.003	NS	-0.404	NS	0.089	NS	0.008	NS
Potential factor	-0.111	NS	-0.014	NS	-0.035	NS	0.114	NS	-0.264	NS

NGT, normal glucose tolerance; IGT, impaired glucose tolerance; T2DM, type 2 diabetes mellitus; NA, not applicable, because of different reference glucose level; *R*, Pearson's correlation coefficient, and only significant correlations are in bold; NS, non significant.

^aParameter calculated at 5 and 7 mmol/l glucose for non-T2DM and T2DM respectively.

^bNot comparable with control subjects because of different reference glucose level.

variables in men with IGT, T2DM, and age- and BMI-matched NGT controls. Thus, additional, hitherto unknown factors may contribute to β -cell dysfunction in susceptible humans, which require further study.

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