# Cardiac Steatosis Associates With Visceral Obesity in Nondiabetic Obese Men

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**Background:** Liver fat and visceral adiposity are involved in the development of the metabolic syndrome (MetS). Ectopic fat accumulation within and around the heart has been related to increased risk of heart disease. The aim of this study was to explore components of cardiac steatosis and their relationship to intra-abdominal ectopic fat deposits and cardiometabolic risk factors in nondiabetic obese men.

**Methods:** Myocardial and hepatic triglyceride (TG) contents were measured with 1.5 T magnetic resonance spectroscopy, and visceral adipose (VAT), abdominal subcutaneous tissue (SAT), epicardial and pericardial fat by magnetic resonance imaging in 37 men with the MetS and in 40 men without the MetS.

**Results:** Myocardial and hepatic TG contents, VAT, SAT, epicardial fat volumes, and pericardial fat volumes were higher in men with the MetS compared with subjects without the MetS ($P < .001$). All components of cardiac steatosis correlated with SAT, VAT, and hepatic TG content and the correlations seemed to be strongest with VAT. Myocardial TG content, epicardial fat, pericardial fat, VAT, and hepatic TG content correlated with waist circumference, body mass index, high-density lipoprotein cholesterol TGs, very low-density lipoprotein-1 TGs, and the insulin-resistance homeostasis model assessment index. VAT was a predictor of TGs, high-density lipoprotein cholesterol, and measures of glucose metabolism, whereas age and SAT were determinants of blood pressure parameters.

**Conclusions:** We suggest that visceral obesity is the best predictor of epicardial and pericardial fat in abdominally obese subjects. Myocardial TG content may present a separate entity that is influenced by factors beyond visceral adiposity. **(*J Clin Endocrinol Metab* 98: 1189–1197, 2013)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2012-3190 Received August 28, 2012. Accepted January 11, 2013.
First Published Online February 15, 2013

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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; BSA, body surface area; CRP, C-reactive protein; EF, ejection fraction; HDL-C, high-density lipoprotein cholesterol; $^1$H MRS, proton magnetic resonance spectroscopy; HOMA, homeostasis model assessment; LV, left ventricular; MetS, metabolic syndrome; MR, magnetic resonance; SAT, subcutaneous adipose tissue; TG, triglyceride; TR, repetition time; VAT, visceral adipose tissue; VLDL, very low-density lipoprotein cholesterol; WC, waist circumference.

Obesity is a worldwide epidemic associated with excess cardiovascular deaths (1–3). Recent data highlight that central obesity has deleterious consequences on cardiovascular health (4). The inability of adipose tissue to expand and to store fat results in lipid overflow to other organs in the setting of excess caloric intake and physical inactivity. Metabolically deleterious and life-threatening forms of obesity associate with an excess accumulation of fat in the visceral adipose tissue (VAT) and with ectopic fat deposition in organs that usually contain minor amounts of fat, such as skeletal muscle, liver, pancreas, and heart (5, 6). This aberrant fat accumulation strongly correlates with unfavorable metabolic profile comprising insulin resistance, dyslipidemia, and the development of a chronic inflammatory state (7). Thus, the site of fat accumulation seems to have important metabolic implications with respect to rates of cardiovascular morbidity and mortality and has raised the concept of healthy vs unhealthy obesity.

Proton magnetic resonance spectroscopy ($^1$H MRS) has become the gold standard to quantitate liver fat content. Recently, $^1$H MRS has been tested as a valid and reproducible technique for noninvasive measurement of myocardial triglyceride (TG) content in humans (8, 9). This technique has revealed also that myocardial TG is a component of ectopic fat depots and a cardiometabolic risk marker. Thus, myocardial TG content is reported to be higher in obesity (10), in subjects with impaired glucose tolerance (11), and in persons with type 2 diabetes mellitus (12, 13). Elevation of myocardial fat has detrimental metabolic consequences, including impaired lipid oxidation, oxidative stress, and mitochondrial defects (14–16). This cardiac lipotoxicity is considered to reflect the deleterious effects of the accumulation of lipid and other toxic products of free fatty acid metabolism within myocardial tissue. Data accumulating from animal studies have provided consistent evidence that cardiac lipotoxicity impairs left ventricular (LV) function and promotes cardiac fibrosis and apoptosis (14, 15, 17).

Hepatic lipid overloading is the hallmark of nonalcoholic liver disease, which is highly prevalent in obesity and associates strongly with visceral adiposity (18). The coexistence of these ectopic fat depots associates with multiple cardiometabolic risk factors, including insulin resistance, hypertension, hyperglycemia, hypertriglyceridemia, reduced high-density lipoprotein cholesterol (HDL-C), and intra-abdominal obesity. This constellation of metabolic abnormalities has been nominated as the metabolic syndrome (MetS) (19), which is widely accepted as a practical tool to identify centrally obese persons at high risk. It should be noted, however, that the MetS as such does not exceed the risk associated with the individual components (20, 21).

So far, limited information exists on the interrelationship between different ectopic fat depots and cardiac fat stores in the MetS subjects free of clinical cardiovascular disease. Therefore, the present study focused on different components of cardiac steatosis and their relationship to intra-abdominal ectopic fat deposits, including liver fat, and also on their association with a broad panel of cardiometabolic risk factors in nondiabetic men with and without the MetS.

## Materials and Methods

### Study population

A total of 77 men were recruited for the study by advertisements in local newspapers. Subjects were allocated into 2 groups (cardiometabolic risk factors present or absent, ie, with or without the MetS) based on the following criteria: 1) waist circumference (WC) $\geq$94 cm, and 2) having $\geq$2 abnormal findings as per the harmonized definition of the MetS (19). Exclusion criteria included no known acute or chronic disease based on history, physical examination, and standard laboratory tests (blood counts, creatinine, aspartate aminotransferase [AST], alanine aminotransferase [ALT], thyroid-stimulating hormone), type 2 diabetes mellitus (based on a 2-h oral glucose tolerance test), significant alcohol consumption defined as more than 20 g per day, and treatment with lipid-lowering therapy except for statins. Only men were included because the hormonal status or use of contraceptives modify lipid metabolism in women. Elevated liver enzymes were allowed. Five subjects were receiving medications for hypertension; 3 subjects were receiving medications for dyslipidemia (statins), and 1 subject was receiving medications for both hypertension and dyslipidemia.

Thirty-seven participants fulfilled the criteria for the MetS. In these participants myocardial ischemia was excluded by means of adenosine stress magnetic resonance (MR) perfusion. The Helsinki University Central Hospital Ethics Committee approved the study design, and each subject provided written informed consent.

### Demographic variables and biochemical investigations

Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared (kg/m$^2$). WC was determined midway between spina iliaca superior and the lower rib margin. Blood pressure was measured by BPM-200 (Quick Medical, Snoqualmie, Washington) in the sitting position after a 5-minute rest and the mean of 5 measurements was recorded. The subjects were classified as present, past, or nonsmokers.

Blood samples were collected after overnight fasting. Total serum cholesterol, TG, very low-density lipoprotein cholesterol (VLDL-1) TG, HDL-C, free fatty acids, apolipoprotein A-I, apolipoprotein B, and high-sensitivity C-reactive protein (CRP) were automatically analyzed by Konelab analyzer 60i (Thermo Fisher Scientific Oy, Vantaa, Finland) with Konelab TM kits. The concentration of low-density lipoprotein cholesterol was calculated using the Friedewald formula (22). Measurement of AST, ALT, creatinine, and thyroid-stimulating hormone was performed using standard laboratory techniques. Fasting and postload glu-

cose were determined by the hexokinase method (Roche Diagnostic Gluco-quant, Mannheim, Germany) using either a Hitachi 917 or a Modular analyzer (Hitachi Ltd, Tokyo, Japan). Serum insulin concentration was assessed by double-antibody radioimmunoassay (Pharmacia RIA kit; Pharmacia, Uppsala, Sweden). The insulin-resistance homeostasis model assessment (HOMA) index was calculated using the following formula: (fasting plasma glucose × fasting plasma insulin)/22.5 (23).

## Determination of myocardial and hepatic triglyceride content

Myocardial and hepatic TG content and fat depot volumes were measured and cardiac imaging was performed with a 1.5-T (MAGNETOM Avanto; Siemens AG, Erlangen, Germany) whole-body MR imager. For measuring myocardial TG content, the spectroscopic volume of interest was positioned within the interventricular septum using the end-systolic cardiac cine images in 3 planes. A carefully positioned standard flex-coil was used for signal reception. The localizer images and spectroscopic data acquisition were double-triggered to end-exhalation and end-systole, using Prospective Acquisition Correction navigator echoes (PACE, WIP-sequence, program version B17, Medical Solutions USA Inc, New York, New York) to control for respiratory movement and electrocardiograph-derived R wave to control for cardiac pulsation. Spectral localization and data collection were performed with the PRESS sequence with 35-ms echo time, while repetition time (TR) (TR > 3000 ms) did not fall below the respiratory cycle length. Navigator echoes were collected from the lung-diaphragm interface and the end-systole triggering was set at about 80% of the resting heart rate of the subject. Spectra were collected with and without water suppression, using 32 and 4 acquisitions, respectively. Spectra were analyzed with jMRUI v3.0 software (www.mrui.uab.es/mrui/) (24) using the AMARES algorithm (25) to determine water (4.7 ppm), methylene (1.3 ppm), and methyl (0.9 ppm) resonance areas. The myocardial TG content was expressed as a ratio of fat to water (%). Relaxation was not corrected for as no reliable data are available for cardiac methylene T2 relaxation.

The measurement of hepatic TG content was performed as previously described (26). The hepatic MRS data were collected using a standard body coil and liver spectra were analyzed with jMRUI v3.0 software (24) using the AMARES algorithm (25), as was done for the myocardial spectra. Areas of water signal (4.7 ppm) and methylene (1.3 ppm) were determined using a line-fitting procedure. Hepatic TG content was calculated as methylene/(water + methylene) signal area × 100 and the values were further converted to mass fractions (27). Nonalcoholic fatty liver disease was defined as hepatic TG content ≥5.56% of liver tissue weight (28).

## Determination of abdominal fat volumes

The distribution of VAT and subcutaneous adipose tissue (SAT) was measured using a series of 16 T1-weighted transaxial images acquired from a region extending from 8 cm above to 8 cm below the fourth and fifth lumbar intervertebral disks (26), using a standard body coil for image acquisition. MR images were analyzed using SliceOmatic v4.3 (Tomovision, Montreal, Quebec, Canada) segmentation software. The areas of SAT and VAT were measured for each slice using a region-growing routine. The results were expressed as total volumes of SAT and VAT. We also calculated the VAT/SAT ratio as a metric of abdominal fat distribution (29).

## Cardiac MR imaging and LV analysis

For cardiac imaging a multichannel body coil was used for reception. Cine series were acquired in 4-chamber, 2-chamber, and LV short-axis orientations during breath-hold using a retrospectively electrocardiographically gated steady-state free precession gradient echo sequence. A stack of short-axis cine series (typically 12 slices) was acquired from base to apex covering the whole LV. Typical imaging parameters were TR/echo time/flip angle 50 ms/1.18 ms/69°, matrix 186 × 220, field-of-view 355 × 420 mm, slice thickness 8 mm, gap 2 mm, and temporal resolution 32–53 ms.

Volumetric analysis of the LV was scrutinized using dedicated postprocessing software (Argus; Siemens). LV ejection fraction (EF), mass, and end-diastolic volume were reported, and mass and end-diastolic volume were indexed to the subject's body surface area (BSA). LV mass was also indexed to height$^{2.7}$ (30).

## Determination of epicardial and pericardial fat

Segmenting the epicardial and pericardial fat planes is very time consuming and standardized measurement is challenging due to anatomical variety. In our preliminary data, epicardial and pericardial fat areas measured in a single 4-chamber-view slice showed good correlation with epicardial and pericardial fat volumes measured with conventional Simpson method covering both right and left ventricles in a stack of short-axis image slices ($n = 6$, $r = 0.895$, $P < .02$). Therefore we used epicardial and pericardial adipose tissue areas measured in a 4-chamber-oriented image to estimate the amount of epicardial and pericardial fat, a method also used by others (31).

Epicardial fat is by definition the layer of adipose tissue between the myocardium and the visceral sheet of pericardium. This epicardial fat shows in MR as a high-intensity layer between the lower intensity myocardium and the low-signal-intensity visceral pericardium. In determining the area of the epicardial fat layer, all phases of the cine images were inspected and measure-

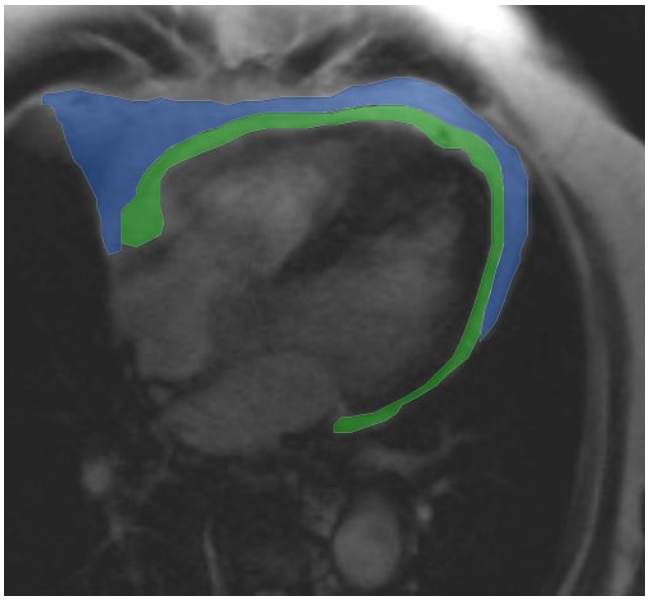

**Figure 1.** Contours of the epicardial (shown in green) and pericardial (shown in blue) fat in end-diastolic 4-chamber-oriented image.

ment was performed in the end-diastolic image using standard radiologic workstation (Impax 5.5 software; Agfa Healthcare, Mortsel, Belgium). Pericardial fat, which is a continuum of the thoracic or mediastinal adipose tissue outside the parietal pericardium, was measured in the same end-diastolic 4-chamber-oriented image as epicardial fat (Figure 1).

### Statistical analyses

All statistical analyses were performed with SPSS 19.0 for Windows (SPSS, Inc, Chicago, Illinois). Normality of continuous variables was analyzed by the Kolmogorov-Smirnov test. Logarithmic transformation of variables was performed, if necessary. Correlation analyses were adjusted for both age and smoking status. Data are presented as frequencies or percentages for categorical variables, as means ± SD for normally distributed continuous variables, and as medians (range) for skewed variables. Between-group differences were assessed by the Mann-Whitney *U* test, unpaired *t* test, and the $\chi^2$ test, as appropriate. To detect determinants of myocardial TG content, epicardial fat, pericardial fat, hepatic TG content, visceral fat, and VAT/SAT ratio univariate regression analyses were performed. Stepwise multivariable linear regression analyses were used to evaluate the impact of fat depots on individual cardiometabolic factors as dependent variables. To adjust for confounding, age was included in all models. A *P* value $<.05$ was considered statistically significant.

## Results

The clinical and biochemical characteristics of the study population are summarized in Table 1. Subjects with the MetS were older than those without the MetS. In the MetS group, 24 subjects had normal glucose tolerance, 4 had impaired fasting glucose, and 9 had impaired glucose tolerance. All individuals in the non-MetS group had normal glucose tolerance. There were more current smokers in the MetS group than the group without the MetS. Total cholesterol, low-density lipoprotein cholesterol, apolipoprotein B, high-sensitivity CRP, free fatty acids, AST, ALT, fasting glucose, fasting insulin, and the HOMA index were higher in individuals with the MetS compared with those without the MetS. The lipid profile showed robust differences between the 2 study groups. Most subjects with the MetS had elevated TGs (n = 24; 65%) representing par-

**Table 1.** Clinical and Biochemical Characteristics of the Study Groups

| | MetS Present | MetS Absent | *P* |
|---|---|---|---|
| n (males) | 37 | 40 | |
| Age, y | 47 ± 6 | 40 ± 8 | <.001 |
| BMI, kg/m$^2$ | 30.9 (24.2–42.5) | 23.4 (17.6–29.8) | <.001 |
| WC, cm | 107.0 (94.0–135.0) | 87.0 (71.0–93.5) | <.001 |
| Systolic BP, mm Hg | 132 ± 14 | 115 ± 10 | <.001 |
| Diastolic BP, mm Hg | 88 ± 9 | 74 ± 6 | <.001 |
| Current smokers, n (%) | 13 (35) | 5 (12) | .019 |
| Total cholesterol, mmol/L | 5.25 ± 0.74 | 4.41 ± 0.79 | <.001 |
| Low-density lipoprotein cholesterol, mmol/L | 3.25 ± 0.71 | 2.54 ± 0.66 | <.001 |
| HDL-C, mmol/L | 1.02 ± 0.26 | 1.50 ± 0.39 | <.001 |
| TGs, mmol/L | 2.20 (0.65–6.26) | 0.78 (0.35–1.57) | <.001 |
| VLDL1-TGs, mmol/L | 1.52 (0.29–4.65) | 0.47 (0.07–1.29) | <.001 |
| Apolipoprotein A-I, mg/dL | 132 ± 18 | 142 ± 20 | .033 |
| Apolipoprotein B, mg/dL | 117 ± 25 | 74 ± 17 | <.001 |
| High-sensitivity CRP, mg/L | 1.8 (0.2–11.8) | 0.2 (0.0–5.8) | <.001 |
| Fasting free fatty acids, μmol/L | 524 ± 172 | 450 ± 198 | .089 |
| fP-ALT, U/L | 39 (20–259) | 21 (7–59) | <.001 |
| fP-AST, U/L | 30 (23–119) | 29 (19–53) | .076 |
| fP-glucose, mmol/L | 5.8 (4.6–6.9) | 5.0 (4.4–6.0) | <.001 |
| fS-insulin, mU/L | 9.3 (3.3–36.9) | 2.9 (0.9–7.7) | <.001 |
| HOMA index | 2.6 (0.8–8.0) | 0.6 (0.2–2.0) | <.001 |
| Myocardial TG content, % | 0.90 (0.31–2.33) | 0.44 (0.14–1.39) | <.001 |
| Epicardial fat, mm$^2$ | 838 (385–1753) | 520 (251–1129) | <.001 |
| Pericardial fat, mm$^2$ | 1905 (615–6131) | 562 (66–1582) | <.001 |
| Hepatic triglyceride content, % | 6.59 (0.40–31.74) | 0.73 (0.17–4.45) | <.001 |
| Visceral fat, cm$^3$ | 3304 (1257–5743) | 843 (67–3170) | <.001 |
| Subcutaneous fat, cm$^3$ | 4816 (2216–9354) | 1773 (284–4017) | <.001 |
| VAT/SAT ratio | 0.63 (0.26–1.64) | 0.46 (0.13–1.23) | .001 |
| LV EF, % | 61 ± 6 | 62 ± 4 | .641 |
| LV end-diastolic volume/BSA, mL/m$^2$ | 65 ± 13 | 84 ± 11 | <.001 |
| LV mass, g | 128 ± 22 | 123 ± 15 | .207 |
| LV mass/BSA, g/m$^2$ | 58 ± 9 | 62 ± 7 | .033 |
| LV mass/height$^{2.7}$ | 26 ± 5 | 24 ± 5 | .083 |

Abbreviations: fS, fasting serum; fP, fasting plasma. Data are expressed as means (±SD), medians (range), or frequencies (%).

**Table 2.** Univariate Correlation Analyses Between Myocardial Triglyceride Content, Epicardial Fat, Pericardial Fat, Hepatic TG Content, Visceral fat, VAT/SAT Ratio, and Different Study Parameters Adjusted for Age and Smoking Status

| | Myocardial TG Content | Epicardial Fat | Pericardial Fat | Hepatic TG Content | Visceral Fat | VAT/SAT Ratio |
|---|---|---|---|---|---|---|
| Demographic variables | | | | | | |
| Age | 0.369[b] | 0.221 | 0.323[b] | 0.221 | 0.416[c] | 0.510[c] |
| WC | 0.426[c] | 0.571[c] | 0.464[c] | 0.607[c] | 0.842[c] | 0.129 |
| BMI | 0.405[c] | 0.587[c] | 0.445[c] | 0.584[c] | 0.807[c] | 0.063 |
| Systolic BP | 0.089 | 0.395[b] | 0.358[b] | 0.308[b] | 0.401[c] | 0.087 |
| Diastolic BP | 0.170 | 0.419[c] | 0.395[c] | 0.413[c] | 0.493[c] | 0.118 |
| Lipid profile | | | | | | |
| Total cholesterol | 0.159 | 0.128 | 0.231[a] | 0.037 | 0.146 | 0.091 |
| HDL-C | −0.292[a] | −0.322[b] | −0.382[b] | −0.321[b] | −0.590[c] | −0.286[a] |
| LDL cholesterol | 0.136 | 0.075 | 0.214 | 0.051 | 0.211 | 0.162 |
| TGs | 0.315[b] | 0.374[b] | 0.424[c] | 0.254[a] | 0.486[c] | 0.183 |
| VLDL-1 TG | 0.356[b] | 0.406[c] | 0.443[c] | 0.229[a] | 0.442[c] | 0.165 |
| Apolipoprotein A-I | −0.185 | −0.079 | −0.154 | −0.161 | −0.336[b] | −0.152 |
| Apolipoprotein B | 0.312[b] | 0.299[a] | 0.392[b] | 0.221 | 0.488[c] | 0.189 |
| Glucose tolerance and fatty acids | | | | | | |
| Fasting plasma glucose | 0.537[c] | 0.195 | 0.304[b] | 0.369[b] | 0.445[c] | 0.143 |
| Fasting plasma insulin | 0.373[b] | 0.564[c] | 0.484[c] | 0.508[c] | 0.648[c] | 0.213 |
| HOMA-IR | 0.463[c] | 0.562[c] | 0.494[c] | 0.536[c] | 0.761[c] | 0.228[a] |
| Free fatty acids | 0.020 | −0.028 | 0.074 | −0.011 | 0.017 | −0.128 |
| Inflammation biomarker | | | | | | |
| High-sensitivity CRP | 0.082 | 0.261[a] | 0.128 | 0.229 | 0.373[b] | −0.110 |
| Liver enzymes | | | | | | |
| ALT | 0.064 | 0.437[c] | 0.358[b] | 0.402[c] | 0.386[b] | 0.059 |
| AST | −0.057 | 0.307[b] | 0.281[a] | 0.222 | 0.241[a] | −0.053 |
| Fat depots | | | | | | |
| Myocardial TG content | — | 0.198 | 0.141 | 0.353[b] | 0.463[c] | 0.219 |
| Epicardial fat | 0.198 | — | 0.673[c] | 0.581[c] | 0.645[c] | 0.269[a] |
| Pericardial fat | 0.141 | 0.673[c] | — | 0.552[c] | 0.655[c] | 0.497[c] |
| Hepatic TG content | 0.353[b] | 0.581[c] | 0.552[c] | — | 0.779[c] | 0.477[c] |
| Visceral fat | 0.463[c] | 0.645[c] | 0.655[c] | 0.779[c] | — | 0.537[c] |
| Subcutaneous fat | 0.343[b] | 0.550[c] | 0.380[b] | 0.514[c] | 0.764[c] | −0.028 |
| VAT/SAT ratio | 0.216 | 0.269[a] | 0.497[c] | 0.476[c] | 0.537[c] | — |
| LV function | | | | | | |
| LV EF, % | −0.044 | −0.094 | −0.057 | −0.225 | −0.098 | −0.221 |
| LV end-diastolic volume/BSA, mL/m$^2$ | −0.329[b] | −0.433[c] | −0.454[c] | −0.411[c] | −0.639[c] | −0.332[b] |
| LV mass (g) | 0.106 | 0.001 | −0.128 | 0.022 | 0.082 | −0.280[a] |
| LV mass/BSA, g/m$^2$ | −0.098 | −0.291[a] | −0.345[b] | −0.246[a] | −0.317[b] | −0.355[b] |
| LV mass/height$^{2.7}$ | 0.092 | 0.049 | −0.069 | 0.087 | 0.116 | −0.294[a] |

[a] $P < .05$; [b] $P < .01$; [c] $P < .001$. — indicates the variable cannot be correlated with itself.

ticipants with hypertriglyceridemic waist. Notably, both TGs and VLDL-1 TGs were about 3-fold higher in the group with the MetS compared with the group without the MetS. As expected, BMI and WC were higher in subjects with the MetS than in those without the MetS.

Visceral and subcutaneous fat masses were markedly higher in the group with the MetS being increased by 3.9-fold and 2.7-fold compared to the group without the MetS. In participants with the MetS the hepatic TG content was ~10-fold higher than in those without the MetS. Myocardial TG content was increased by 2-fold ($P < .001$). Similarly, the epicardial and pericardial fat were clearly higher in subjects with the MetS compared with subjects without the MetS. All study subjects had a normal LV EF. LV end-diastolic volume indexed to BSA was decreased in the MetS group compared with the group without the MetS (Table 1).

Visceral fat correlated significantly with hepatic TG content as well as with WC and BMI. Although a significant correlation existed between hepatic TG content and VAT/SAT ratio, this was less strong than with visceral fat. Further analyses revealed that all components of cardiac steatosis correlated with subcutaneous and visceral fat masses, and hepatic TG content and the correlations seemed to be stronger with visceral fat than with subcutaneous fat or hepatic TG content (Table 2 and Figure 2). The VAT/SAT ratio correlated with hepatic TG content, epicardial fat, and pericardial fat, but not as strongly as with visceral fat. The VAT/SAT ratio was not correlated with myocardial TG content (Table 2).

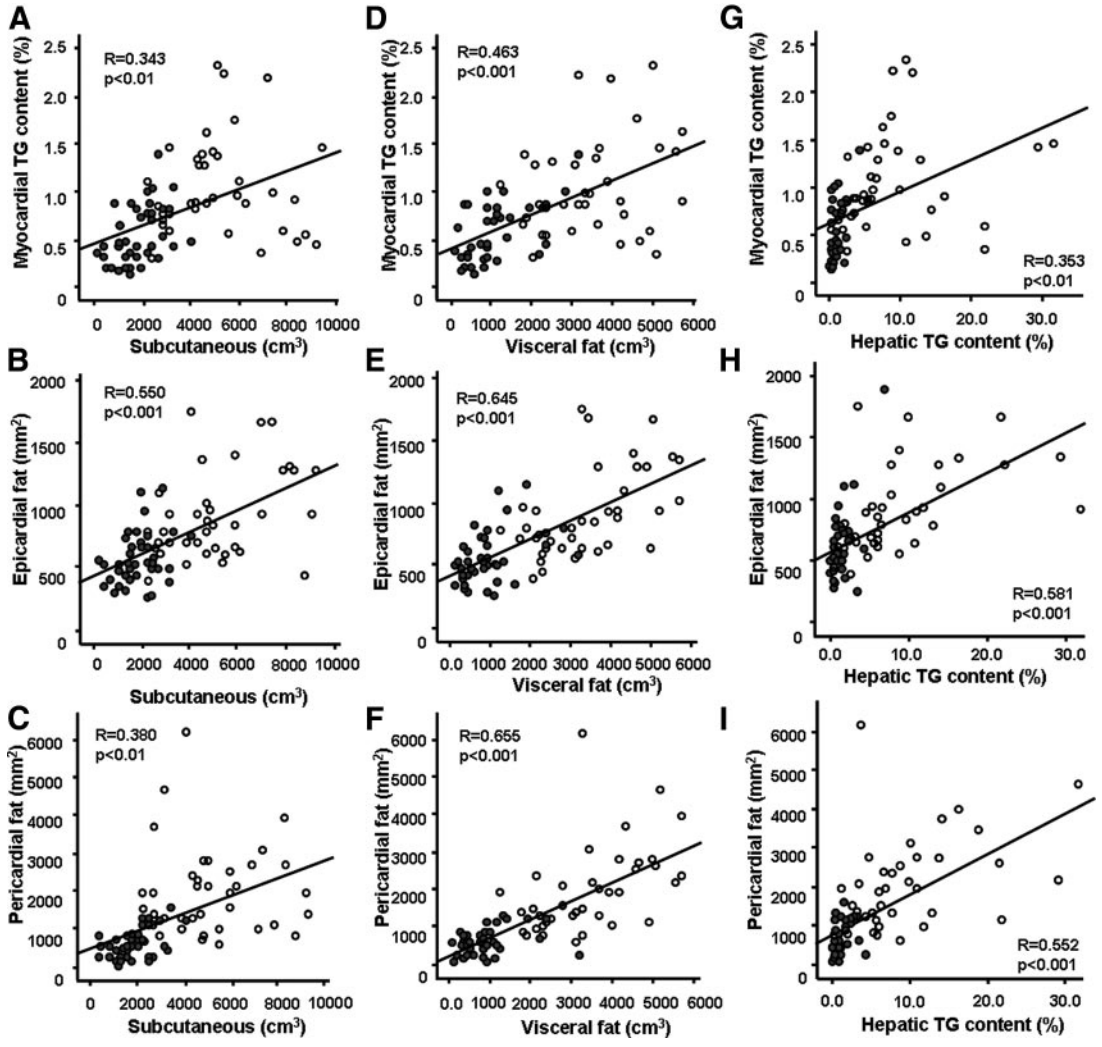


**Figure 2.** A–I, Correlations between different ectopic fat depots. The values in figures are adjusted to age and smoking status. The open circles indicate subjects in the MetS group and the filled circles indicate subjects in the group without the MetS.

Myocardial TG content, epicardial fat, pericardial fat, hepatic TG content, and visceral fat showed significant correlations with WC, BMI, HDL-C, TGs, VLDL-S TGs, plasma insulin, and the HOMA index. High-sensitivity CRP was linked to epicardial and visceral fat. The VAT/SAT ratio showed moderate correlations with HDL-C and the HOMA index. LV end-diastolic volume as well as LV end-diastolic volume indexed to BSA correlated with epicardial fat, pericardial fat, hepatic TG content, visceral fat, and the VAT/SAT ratio. Only myocardial TG content was associated with LV end-diastolic volume indexed to BSA (Table 2).

Next we used stepwise multivariable regression analyses to evaluate the impact of fat depots on individual cardiometabolic risk factors. Visceral fat was an independent predictor of TGs, HDL-C, plasma glucose, plasma insulin, and the HOMA index, whereas age and subcutaneous fat were independent determinants of systolic and diastolic blood pressure (BP) (Table 3).

## Discussion

To the best of our knowledge, this is the first study to use MR technology to examine cardiac steatosis by quantifying simultaneously all 3 cardiac fat stores as well as to relate these data to intra-abdominal fat depots, including liver fat also in a large nondiabetic group of abdominally obese men free of clinical cardiovascular disease.

The definition of MetS was used as a tool to identify obese subjects at high cardiometabolic risk in this study. We consistently found an ~2-fold elevation in myocardial and epicardial fat, respectively, and an ~3-fold elevation in pericardial fat depot in subjects with the MetS. The data show that myocardial TG content correlated with visceral fat, hepatic TG content, and multiple parameters of overall obesity. In multivariable regression analyses, visceral fat remained an independent predictor of TGs, HDL-C, plasma glucose, plasma insulin, and the HOMA index, whereas age and subcutaneous fat were independent de-

**Table 3.** Results of Stepwise Multivariable Regression Analyses

| Independent Variables | β | P |
|---|---|---|
| Dependent variable: TGs (log) | | |
| Age | −.107 | .252 |
| Myocardial triglyceride content (log) | −.088 | .419 |
| Epicardial fat (log) | −.083 | .479 |
| Pericardial fat (log) | −.213 | .091 |
| Hepatic TG content (log) | −.203 | .215 |
| Visceral fat (log) | −.654 | **<.001** |
| Subcutaneous fat (log) | −.265 | .097 |
| Adjusted $R^2$ | **.488** | **<.001** |
| Dependent variable: HDL-cholesterol | | |
| Age | .242 | .021 |
| Myocardial TG content (log) | .123 | .311 |
| Epicardial fat (log) | .029 | .821 |
| Pericardial fat (log) | .155 | .272 |
| Hepatic triglyceride content (log) | .268 | .141 |
| Visceral fat (log) | −.686 | **<.001** |
| Subcutaneous fat (log) | −.098 | .584 |
| Adjusted $R^2$ | **.364** | **<.001** |
| Dependent variable: fasting plasma glucose (log) | | |
| Age | .030 | .777 |
| Myocardial TG content (log) | .243 | **.050** |
| Epicardial fat (log) | −.070 | .595 |
| Pericardial fat (log) | .037 | .797 |
| Hepatic triglyceride content (log) | .170 | .373 |
| Visceral fat (log) | .411 | **.002** |
| Subcutaneous fat (log) | .215 | .238 |
| Adjusted $R^2$ | **.345** | **<.001** |
| Dependent variable: fasting plasma insulin (log) | | |
| Age | −.009 | .892 |
| Myocardial TG content (log) | −.040 | .598 |
| Epicardial fat (log) | .087 | .282 |
| Pericardial fat (log) | −.033 | .708 |
| Hepatic triglyceride content (log) | .078 | .496 |
| Visceral fat (log) | .618 | **<.001** |
| Subcutaneous fat (log) | .291 | **.011** |
| Adjusted $R^2$ | **.752** | **<.001** |
| Dependent variable: HOMA index (log) | | |
| Age | .004 | .946 |
| Myocardial TG content (log) | −.012 | .875 |
| Epicardial fat (log) | .073 | .351 |
| Pericardial fat (log) | −.022 | .795 |
| Hepatic triglyceride content (log) | .100 | .362 |
| Visceral fat (log) | .613 | **<.001** |
| Subcutaneous fat (log) | .301 | **.006** |
| Adjusted $R^2$ | **.769** | **<.001** |
| Dependent variable: systolic BP | | |
| Age | **.328** | **.001** |
| Myocardial TG content (log) | .030 | .797 |
| Epicardial fat (log) | .126 | .289 |
| Pericardial fat (log) | .149 | .222 |
| Hepatic triglyceride content (log) | .177 | .220 |
| Visceral fat (log) | .159 | .420 |
| Subcutaneous fat (log) | **.452** | **<.001** |
| Adjusted $R^2$ | **.358** | **<.001** |
| Dependent variable: diastolic BP | | |
| Age | .238 | .013 |
| Myocardial TG content (log) | .047 | .688 |
| Epicardial fat (log) | .120 | .304 |
| Pericardial fat (log) | .145 | .227 |
| Hepatic triglyceride content (log) | .187 | .189 |
| Visceral fat (log) | .207 | .284 |
| Subcutaneous fat (log) | .534 | <.001 |
| Adjusted $R^2$ | .379 | <.001 |

terminants of systolic and diastolic BP. The association of multiple cardiometabolic risk factors with myocardial TG content, pericardial fat, and epicardial fat agrees with previous studies (32, 33).

So far, most studies have measured either pericardial or epicardial fat depots because their quantification is technically easier than that of lipids in cardiomyocytes, the latter requesting a more sophisticated technique. Overall, both pericardial and epicardial fat depots associate with the amount of visceral fat that is increased in obesity, subjects with MetS, impaired glucose tolerance, and subjects with type 2 diabetes mellitus (32). It should be recognized that the 3 cardiac sites differ in their capacity to accumulate fat with the pericardial site having the greatest capacity and cardiomyocytes having the lowest capacity. Thus, increase of cardiac fat depots in men with the MetS in context of visceral adiposity is not a surprising finding. In our cohort, the VAT/SAT ratio as a measure of organ-specific fat distribution did not provide any clear benefits beyond visceral fat mass.

Our data confirm and expand the results by Gaborit et al (34), who reported that epicardial fat volume and myocardial TG content were increased in individuals with the MetS. Notably, the 2 studies show differences in participants because the cohort of Gaborit et al (34) exhibited extreme obesity (BMI range, 33 to 52 kg/m$^2$), including only 18 subjects with the MetS, and reported no data on hepatic TG content. Our results expand previous observations of increased myocardial lipid accumulation in insulin-resistant states in subjects with impaired glucose tolerance and type 2 diabetes mellitus to abdominally obese men with cardiometabolic risk factors (10–12). All 3 cardiac fat depots showed broadly similar significant correlations with measures of apolipoprotein B containing lipoproteins, HDL-C, and parameters of glucose homeostasis. Visceral fat was an independent predictor of TGs, HDL-C, and measures of glucose metabolism, but subcutaneous fat was the determinant of BP parameters. The data highlight that visceral adiposity lies at the root of the systemic effects linked to ectopic fat depots quantified by MR technology, as proposed also by Després (18).

The inefficient storage capacity of SAT mass in the face of extra caloric intake results in excessive flow of free fatty acids into systemic circulation, driving the overload of free fatty acids into ectopic fat depots (18). In this concept, expansion of visceral, pericardial, and epicardial fat depots represents visceral components and the preferential places to store excess dietary fats. Our data support the concept that visceral fat depot is indeed the priority site for excess fat storage. Moreover, pericardial and epicardial fat depots are interrelated with visceral fat and good markers of ectopic fat deposition. The constellation of these 3 fat depots is linked to the development of the cardiometabolic risk profile, including disturbances of both glucose and lipid metabolism. In addition, products (like cytokines) of epicardial fat can have local effects on myocardial metabolism.

The liver plays a central role in controlling lipid and free fatty acid metabolism. Notably, the liver is exposed to first-pass free fatty acids from visceral fat depot. Recognizing the strong correlation between visceral fat and hepatic TG content, it was not surprising that hepatic TG content showed a correlation with myocardial TG content. Likewise, McGavock et al (12) reported a modest correlation between hepatic and myocardial TG content. However, we did not find any correlation between myocardial TG content and epicardial or pericardial fat depots. This finding suggests that fat accumulation in cardiomyocytes may also be regulated by other factors than those linked to systemic fat depositions.

The study design does not allow us to evaluate the molecular mechanisms underlying cardiac steatosis. It should be recognized that lipid accumulation in cardiomyocytes reflects the imbalance between uptake and utilization of free fatty acids. Excessive uptake of free fatty acids by cardiomyocytes associates with accelerated fatty acid oxidation instead of glucose oxidation and may elicit lipotoxicity reflected as impaired cardiac function (17, 35). Another source for increased lipid uptake by cardiomyocytes is the uptake of lipids from TG-rich lipoproteins mediated by VLDL receptor and lipoprotein lipase catalyzed hydrolysis of TGs (36, 37). Recent data suggest a link between VLDL receptor expression and lipid droplets in human cardiomyocytes (37). Interestingly, both concentrations of TG in serum and large VLDL-1 particles correlated with each cardiac fat depot and liver fat. We have previously reported that hepatic TG represents the driving force for VLDL assembly and secretion of large VLDL-1 particles (38). This may explain in part the correlation between hepatic and myocardial TG content. If the oxidative capacity of mitochondria is exceeded, cardiac steatosis is ensued, compromising cardiac function. The objective of this report was not to examine the parameters of cardiac function in-depth; however, LV end-diastolic volume index was decreased despite comparable LV EF, suggesting diastolic dysfunction in subjects with the MetS. This is in agreement with previous reports (10, 13, 34).

### Study limitations

A limitation of the methodology in the study is the narrow range of myocardial TG content measured by $^{1}$H MRS in healthy subjects, which has been reported to be less than or about 1% (8, 39). In line with this, myocardial TG content was low in most lean subjects and this may underestimate its association with metabolic parameters as compared to pericardial fat. Both gender and age may influence the magnitude of cardiac steatosis, although the data are not consistent (33, 34). Accordingly, only men were included in this study and the data were adjusted to age. However, recent reports have documented the increase of myocardial TG content in insulin-resistant obese women also (40). Finally, this study has a cross-sectional design that limits our ability to infer any causality.

## Conclusions

Our study is the first to investigate cardiac lipotoxicity in a large nondiabetic group of abdominally obese men. We conclude that visceral obesity is the best predictor of epicardial and pericardial fat in abdominally obese subjects. We suggest that all 3 depots comprise a common constellation in face of extra free fatty acid flux. Myocardial TG content may present a separate entity that is also influenced by other factors than the excess visceral obesity. Furthermore, longitudinal studies with a specific focus on parameters of cardiac function are needed to elucidate potential differences in organ-specific lipid accumulation in the setting of excess caloric intake.

## Acknowledgments

The authors gratefully acknowledge the skillful laboratory work by Hannele Hildén, Virve Naatti, Helinä Perttunen-Nio, and Sirpa Stick. We thank Marja Piitulainen, Miika Paukkunen, and Laura Impivaara for their excellent technical assistance.

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This work was supported by Helsinki University Central Hospital Research Foundation Grants TLD8100096 and TYH2009235, the Finnish Foundation for Cardiovascular Research, the Finnish Medical Foundation, and the Wilhelm and Else Stockmann Foundation.

Disclosure Summary: The authors have no conflicts of interest to disclose.

## References


1. **Poirier P, Giles TD, Bray GA, et al.** American Heart Association; Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation*. 2006;113:898–918.
2. **Mottillo S, Filion KB, Genest J, et al.** The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. *J Am Coll Cardiol*. 2010;56:1113–1132.
3. **Cornier MA, Marshall JA, Hill JO, Maahs DM, Eckel RH.** Prevention of overweight/obesity as a strategy to optimize cardiovascular health. *Circulation*. 2011;124:840–850.
4. **Coutinho T, Goel K, Correa de Sa D, et al.** Central obesity and survival in subjects with coronary artery disease: a systematic review of the literature and collaborative analysis with individual subject data. *J Am Coll Cardiol*. 2011;57:1877–1886.
5. **Després JP, Lemieux I, Bergeron J, et al.** Abdominal obesity and the metabolic syndrome: contribution to global cardiometabolic risk. *Arterioscler Thromb Vasc Biol*. 2008;28:1039–1049.
6. **Shimabukuro M.** Cardiac adiposity and global cardiometabolic risk: new concept and clinical implication. *Circ J*. 2009;73:27–34.
7. **Eckel RH, Grundy SM, Zimmet PZ.** The metabolic syndrome. *Lancet*. 2005;365:1415–1428.
8. **Szczepaniak LS, Dobbins RL, Metzger GJ, et al.** Myocardial triglycerides and systolic function in humans: in vivo evaluation by localized proton spectroscopy and cardiac imaging. *Magn Reson Med*. 2003;49:417–423.
9. **Kellman P, Hernando D, Arai AE.** Myocardial fat imaging. *Curr Cardiovasc Imaging Rep*. 2010;3:83–91.
10. **Kankaanpää M, Lehto HR, Pärkkä JP, et al.** Myocardial triglyceride content and epicardial fat mass in human obesity: relationship to left ventricular function and serum free fatty acid levels. *J Clin Endocrinol Metab*. 2006;91:4689–4695.
11. **Iozzo P, Lautamaki R, Borra R, et al.** Contribution of glucose tolerance and gender to cardiac adiposity. *J Clin Endocrinol Metab*. 2009;94:4472–4482.
12. **McGavock JM, Lingvay I, Zib I, et al.** Cardiac steatosis in diabetes mellitus: a 1H-magnetic resonance spectroscopy study. *Circulation*. 2007;116:1170–1175.
13. **Rijzewijk LJ, van der Meer RW, Smit JW, et al.** Myocardial steatosis is an independent predictor of diastolic dysfunction in type 2 diabetes mellitus. *J Am Coll Cardiol*. 2008;52:1793–1799.
14. **Szczepaniak LS, Victor RG, Orci L, Unger RH.** Forgotten but not gone: the rediscovery of fatty heart, the most common unrecognized disease in America. *Circ Res*. 2007;101:759–767.
15. **Wende AR, Abel ED.** Lipotoxicity in the heart. *Biochim Biophys Acta*. 2010;1801:311–319.
16. **Szendroedi J, Roden M.** Ectopic lipids and organ function. *Curr Opin Lipidol*. 2009;20:50–56.
17. **Brindley DN, Kok BP, Kienesberger PC, Lehner R, Dyck JR.** Shedding light on the enigma of myocardial lipotoxicity: the involvement of known and putative regulators of fatty acid storage and mobilization. *Am J Physiol Endocrinol Metab*. 2010;298:E897–E908.
18. **Després JP.** Body fat distribution and risk of cardiovascular disease. An update. *Circulation*. 2012;126:1301–1313.
19. **Alberti KG, Eckel RH, Grundy SM, et al.** Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the study of obesity. *Circulation*. 2009;120:1640–1645.
20. **Mente A, Yusuf S, Islam S, et al.** INTERHEART Investigators. Metabolic syndrome and risk of acute myocardial infarction a case-control study of 26,903 subjects from 52 countries. *J Am Coll Cardiol*. 2010;55:2390–2398.
21. **Bayturan O, Tuzcu EM, Lavoie A, et al.** The metabolic syndrome, its component risk factors, and progression of coronary atherosclerosis. *Arch Intern Med*. 2010;170:478–484.
22. **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.
23. **Wallace TM, Levy JC, Matthews DR.** Use and abuse of HOMA modelling. *Diabetes Care*. 2004;27:1487–1495.
24. **Naressi A, Couturier C, Castang I, de Beer R, Graveron-Demilly D.** Java-based graphical user interface for MRUI, a software package for quantification of in vivo/medical magnetic resonance spectroscopy signals. *Comput Biol Med*. 2001;31:269–286.
25. **Vanhamme L, van den Boogaart A, Van Huffel S.** Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *J Magn Reson*. 1997;129:35–43.
26. **Westerbacka J, Corner A, Tiikkainen M, 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;47:1360–1369.
27. **Kotronen A, Peltonen M, Hakkarainen A, et al.** Prediction of nonalcoholic fatty liver disease and liver fat using metabolic and genetic factors. *Gastroenterology*. 2009;137:865–872.
28. **Szczepaniak LS, Nurenberg P, Leonard D, et al.** Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab*. 2005;288:E462–E468.
29. **Kaess BM, Pedley A, Massaro JM, Murabito J, Hoffmann U, Fox CS.** The ratio of visceral to subcutaneous fat, a metric of body fat distribution, is a unique correlate of cardiometabolic risk. *Diabetologia*. 2012;55:2622–2630.
30. **de Simone G, Daniels SR, Devereux RB, et al.** Left ventricular mass and body size in normotensive children and adults: assessment of allometric relations and impact of overweight. *J Am Coll Cardiol*. 1992;20:1251–1260.
31. **Sicari R, Sironi AM, Petz R, et al.** Pericardial rather than epicardial fat is a cardiometabolic risk marker: an MRI vs echo study. *J Am Soc Echocardiogr*. 2011;24:1156–1162.
32. **Iacobellis G, Bianco AC.** Epicardial adipose tissue: emerging physiological, pathophysiological and clinical features. *Trends Endocrinol Metab*. 2011;22:450–457.
33. **Iozzo P.** Myocardial, perivascular, and epicardial fat. *Diabetes Care*. 2011;34:S371–S379.
34. **Gaborit B, Kober F, Jacquier A, et al.** Assessment of epicardial fat volume and myocardial triglyceride content in severely obese subjects: relationship to metabolic profile, cardiac function and visceral fat. *Int J Obes*. 2012;36:422–430.
35. **van de Weijer T, Schrauwen-Hinderling VB, Schrauwen P.** Lipotoxicity in type 2 diabetic cardiomyopathy. *Cardiovasc Res*. 2011;92:10–18.
36. **Bharadwaj KG, Hiyama Y, Hu Y, et al.** Chylomicron- and VLDL-derived lipids enter the heart through different pathways: in vivo evidence for receptor- and non-receptor-mediated fatty acid uptake. *J Biol Chem*. 2010;285:37976–37986.
37. **Perman JC, Boström P, Lindbom M, et al.** The VLDL receptor promotes lipotoxicity and increases mortality in mice following an acute myocardial infarction. *J Clin Invest*. 2011;121:2625–2640.
38. **Adiels M, Taskinen MR, Packard C, et al.** Overproduction of large VLDL particles is driven by increased liver fat content in man. *Diabetologia*. 2006;49:755–765.
39. **Liu CY, Redheuil A, Ourwerkerk R, Lima JA, Bluemke DA.** Myocardial fat quantification in humans: evaluation by two-point water-fat imaging and localized proton spectroscopy. *Magn Reson Med*. 2010;63:892–901.
40. **Utz W, Engeli S, Haufe S, et al.** Myocardial steatosis, cardiac remodelling and fitness in insulin-sensitive and insulin-resistant obese women. *Heart*. 2011;97:1585–1589.