Liver fat content is related to postprandial triglyceride-enrichment of HDL particles in association with endothelial and HDL dysfunction

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ABSTRACT

Background & aims: Liver fat associates with postprandial hypertriglyceridemia, thus potentially contributing to postprandial triglyceride-enrichment of HDL (HDL-TG), and subsequent HDL dysfunction and finally cardiovascular disease.

Methods: We assessed HDL composition and function, and endothelial function as flow-mediated dilation (FMD), before and following 3 consecutive meals, during a 16h period in 36 men with T2DM, men with the MetS, and controls. Blood sampling was performed before each meal, and 12h and 16h after breakfast. Liver fat was measured by magnetic resonance-spectroscopy.

Results: Triglycerides increased after the meals (P<0.001 in all, compared to baseline), and remained elevated after dinner in T2DM and MetS men (both P<0.05). Fasting HDL-TG was highest in T2DM, relative to MetS and controls (0.15±0.02, 0.11±0.01, 0.08.3±0.01 mmol/mmol, respectively; P=0.002), and increased postprandially in all groups (P<0.001). Postprandial HDL function was negatively associated with the increase in HDL-TG following 3 consecutive meals (r=-0.32, P<0.05). Liver fat was associated with HDL-TG-content after 3 meals (r=0.65, P<0.001). HDL-TG was an independent predictor of postprandial FMD, both measured at t=4h after dinner (r= -0.477, P=0.003).

Conclusions: In men with T2DM and the MetS following exposure to 3 consecutive meals, liver fat is associated with HDL-TG enrichment which was closely related with endothelial and HDL dysfunction.
INTRODUCTION

Increased liver fat accumulation is common in type 2 diabetes mellitus (T2DM), and closely related with features of the metabolic syndrome (MetS) and increased risk of cardiovascular disease (CVD). The pro-atherogenic lipid profile in subjects with increased liver fat is characterized by elevated levels of fasting and postprandial triglycerides, low high-density-lipoprotein (HDL)-cholesterol, and an increase in small dense low-density-lipoprotein (LDL) particles. In hypertriglyceridemic states, the net transfer of triglycerides from triglyceride-rich lipoproteins (VLDLs and chylomicrons) to HDL particles, as mediated by cholesteryl-estertransfer-protein (CETP), is enhanced, yielding in (the formation of) large, triglyceride-rich, cholesterolester-core-depleted HDL particles. These HDL particles are the preferred substrate for the enzyme hepatic lipase, which hydrolyzes HDL triglycerides and promotes hepatic HDL uptake. Liver fat content has been shown to adversely affect fasting levels of the anti-atherogenic HDL subfractions. However, the independent association of liver fat content and postprandial HDL compositional changes has hitherto not been addressed. Although the association of postprandial triglyceride elevations and impaired vascular endothelial function has been established, it is currently unclear whether this relationship is (partly) mediated by the associated HDL compositional and functional changes. Previous studies showed that triglyceride-enrichment of HDL may alter anti-atherogenic capacities of this lipoprotein class, including anti-oxidative and anti-inflammatory properties, adversely affecting the ability of HDL to protect the endothelium and vascular reactivity.

In the present study, we first investigated whether liver fat content is associated with postprandial altered HDL composition, especially triglyceride content, and its anti-oxidative function. Second, we assessed the interrelationship of liver fat, physicochemical properties of HDL particles and endothelial function in vivo, measured as flow mediated dilatation (FMD), following 3 consecutive meals during a 16h period in males with T2DM, males with the MetS and healthy controls.

METHODS

Subjects

Caucasian males, aged 40-65 years, with type 2 diabetes (n=12) or with the MetS (n=12), and 12 age-matched healthy males were recruited by advertisement and studied after obtaining written informed consent. Diet, sulphonylurea and/or metformin were the only glucose-lowering treatments allowed in the type 2 diabetic group. Males with the MetS...
had to meet 3 out of 5 inclusion criteria based on NCEP/ATP III-criteria, without having hyperglycemia during a 75g oral glucose tolerance test. Claustrophobia, excess alcohol intake (>20 units/wk), history of hepatitis and/or pancreatitis, abnormal liver and renal function tests (>2 times upper limits of normal), recent (<3 months) changes in weight (≥5%) and/or medication, history or current use of glucocorticosteroids, lipid-lowering drugs, insulin and/or thiazolidinediones, were exclusion criteria. Participants were instructed to omit their medication during the examination and to refrain from heavy physical activities during the previous 24h. The local ethics committee approved the study and the investigation conformed to the principles outlined in the Declaration of Helsinki.

**Study design**

After an overnight fast, participants were admitted in the research unit for a 16h period and received 3 consecutive meals (75g carbohydrates, 50g fat (60% saturated), 35g protein), at time points 9.00 AM, 1.00 PM, and 5.00 PM. Blood samples were drawn before and 2, 4, 6, 8, 12, and 16h after breakfast. To avoid lipoprotein lipase (LPL) activation by physical activity, participants remained in the semi-recumbent position during the whole testing day.

**Biochemical measurements**

Plasma glucose concentrations were measured by hexokinase-based technique (Roche diagnostics, Mannheim, Germany) and insulin concentrations by immunoradiometric assay (Centaur, Mijdrecht, The Netherlands). Plasma total cholesterol, HDL-cholesterol and triglycerides were determined by enzymatic methods (Modular, Hitachi, Japan). LDL-cholesterol was calculated by the Friedewald formula. Glycated hemoglobin was measured with cation exchange chromatography (Menarini Diagnostics, Florence, Italy).

**HDL composition and function**

HDL was isolated from EDTA-plasma by ultracentrifugation for measurement of triglyceride, cholesterol, and protein content. HDL-triglyceride and HDL-cholesterol content was expressed as nmol per gram protein (nmol/g). HDL-TG was calculated as the HDL triglyceride : total cholesterol ratio and expressed as mmol/mmol. The antioxidative function of HDL was assessed by monitoring its capacity to inhibit the oxidation of dichlorodihydrofluorescein (Invitrogen) by oxidized 1-palmitoyl-2-arachidonoyl-phosphatidylcholine (Avanti Lipids). Briefly, in a total volume of 790 µL phosphate buffered saline (pH 7.4), 35 µL of a normal LDL solution (final concentration of 18 µmol/L cholesterol), 35 µL of test HDL-cholesterol (final concentration of 11 µmol/L cholesterol),
20 µl oxidized 1-palmitoyl-2-arachidonoyl-sn-phosphatidylcholine (final concentration 50 µmol/L), and 10 µL of dichlorofluorescein solution (final concentration of 40 µmol/L) were incubated in glass tubes for 2 hours at 37°C. Afterwards the fluorescence intensity was determined with a HTS 7000 plate reader (Perkin Elmer) at 485 and 530 nm (excitation and emission wavelength, respectively). The intra-assay CV was 3.4%.

**Endothelial function**

Before each blood collection, FMD was measured at the right brachial artery by a single observer using ultrasound (Wall-track System, PieMedical, Maastricht, The Netherlands), as previously described.

**Liver fat content**

Using a 1.5-T whole-body system MRI (Sonata; Siemens, Erlangen, Germany), liver fat content was measured at 3 positions in the liver and calculated by user-independent spectral quantification as previously described in detail.

**Statistical analysis**

Results are presented as the mean±SE or medians (interquartile range). Sixteen hour area under the curve (AUC\textsubscript{0-16h}) were calculated according to the trapezoid rule. Differences between groups were calculated using ANOVA and post-hoc analyses (Bonferroni). Non-normally distributed data were log transformed. The associations of liver fat content, FMD and HDL-cholesterol composition and function were assessed by univariate and multivariate linear regression analyses. A \( P < 0.05 \) was considered statistically significant.

**RESULTS**

The baseline characteristics of the T2DM, MetS and healthy groups are listed in Table 4.1. Figure 4.1A-D depicts the 16h course and AUC\textsubscript{0-16h} of the postprandial metabolic responses. Fasting and AUC\textsubscript{0-16h} plasma glucose concentrations were similar in both non-diabetic groups, but differed significantly from T2DM males (\( P < 0.001 \)) (Figure 4.1A). Plasma triglycerides increased significantly after the meals in all groups. HDL-cholesterol concentrations decreased in all groups following the consecutive meals, however, AUC\textsubscript{0-16h} of HDL was significantly lower in the 2 dysmetabolic groups, as compared to controls. Postprandial HDL-TG increased significantly in all groups, especially at time point 12h.
Liver fat and postprandial HDL

(i.e. 8 hours after breakfast and 4 hours after lunch), all \( P<0.001 \) (Figure 4.1E). At baseline, there was no difference in anti-oxidative function of HDL between the 3 groups. The postprandial decrease in anti-oxidative capacity of HDL, as observed in T2DM, did not reach statistical significance \( (P=0.12) \). Postprandial HDL function, adjusted for baseline, was negatively associated with the increase in HDL-TG following 3 consecutive meals \( (r=-0.32, P<0.05) \).

Baseline FMD differed between groups, and was correlated with fasting HDL-TG \( (r=-0.43, P<0.01) \). FMD deteriorated postprandially in all 3 groups (Figure 4.1F) and AUC\(_{0-16h}\) FMD was impaired in MetS and T2DM males vs. controls \( (P=0.002) \). During the postprandial state, FMD at time points 8 and 12h following breakfast was negatively associated with corresponding HDL-TG (both \( r=-0.48, P=0.003 \) (Figure 4.1H).

### Table 4.1  Baseline characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>T2DM</th>
<th>MetS</th>
<th>Controls</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54.6 ± 1.0</td>
<td>57.2 ± 1.8</td>
<td>55.3 ± 2.2</td>
<td>0.556</td>
</tr>
<tr>
<td>Body Mass Index (kg/m(^2))</td>
<td>32.6 ± 1.3</td>
<td>30.6 ± 1.0</td>
<td>27.1 ± 0.8</td>
<td>0.002</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>111.8 ± 2.9</td>
<td>110.9 ± 2.9</td>
<td>100.7 ± 2.5</td>
<td>0.012</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>137 ± 4</td>
<td>140 ± 4</td>
<td>121 ± 2</td>
<td>0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>83 ± 1</td>
<td>84 ± 2</td>
<td>74 ± 1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.2 ± 0.3</td>
<td>5.9 ± 0.1</td>
<td>5.6 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>8.9 ± 0.7</td>
<td>5.6 ± 0.1</td>
<td>5.4 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Post 75 g OGTT glucose (mmol/L)</td>
<td>15.6 ± 1.2</td>
<td>6.2 ± 0.2</td>
<td>5.0 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.1 ± 0.2</td>
<td>5.4 ± 0.3</td>
<td>5.1 ± 0.3</td>
<td>0.534</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>3.0 ± 0.5</td>
<td>3.3 ± 0.8</td>
<td>3.1 ± 0.9</td>
<td>0.643</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>2.2 ± 0.4</td>
<td>2.2 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>0.002</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.0 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Fasting HDL-TG (mmol/mmol)</td>
<td>148 ± 16</td>
<td>108 ± 13</td>
<td>78 ± 8</td>
<td>0.002</td>
</tr>
<tr>
<td>Fasting FMD (%)</td>
<td>4.9 ± 0.5</td>
<td>5.7 ± 0.7</td>
<td>7.8 ± 0.5</td>
<td>0.003</td>
</tr>
<tr>
<td>Liver fat content (%)</td>
<td>17.8 (9.4-39.0)</td>
<td>9.2 (3.4-11.5)</td>
<td>3.4 (1.8-9.3)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are means±SE or median (interquartile range). T2DM, type 2 diabetes mellitus; MetS, metabolic syndrome; BMI, body mass index; OGTT, oral glucose tolerance test; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; FMD, flow-mediated dilatation.
Figure 4.1  The 16-h course of plasma glucose (A), insulin (B), triglyceride (C), HDL-cholesterol (D), HDL-TG concentrations (E), and flow mediated dilatation (FMD)(F) after 3 high-fat mixed meals in T2DM (open circles), MetS (asterisk) and healthy males (solid circles). Bars (black, healthy males; grey, metabolic syndrome (MetS); white, type 2 diabetes (T2DM)) in the insets represent respective 16h-AUC values. The \( P \) value given for 16h-AUC difference (ANOVA). Data are mean±SE. Scatter plots representing the relationship between liver fat content (G) and FMD (H), and HDL-TG-enrichment following 3 high-fat mixed meals in the whole study population. Pearson correlation coefficients are shown.
Liver fat content was positively associated with AUC\(_{0-16h}\) glucose (r=0.44, P<0.01), triglycerides (r=0.62, P<0.001), insulin (r=0.62, P<0.001), and inversely with AUC\(_{0-16h}\) HDL-cholesterol (r=-0.56, P<0.001) and FMD (r=-0.44, P<0.01). As shown in Figure 4.1G, fasting HDL-TG and postprandial HDL-TG following 3 meals was strongly associated with liver fat content (r=0.61, P<0.001 and r=0.65, P<0.001, respectively).

Multivariate analysis was performed in the pooled groups to study the independent interrelationship of postprandial FMD, liver fat content, HDL-TG, and HDL-function. FMD at 12h following breakfast was entered as a dependent variable, and age, HDL-TG, plasma triglyceride and glucose concentration at corresponding time point (t=12h), liver fat content, HDL-function at t=12h were entered as independent variables into the model. Stepwise regression analyses, revealed HDL-TG at the corresponding time point as independent predictor of postprandial FMD; r=-0.477, P=0.003 (Coefficient B -15.220 (-25.007 to -5.433)).

**DISCUSSION**

In the present study, we demonstrated that liver fat content is associated with HDL-TG enrichment after 3 consecutive meals in men with T2DM and men with the MetS. Furthermore, postprandial HDL-TG enrichment was independently related to endothelial dysfunction measured by FMD and closely associated with change in the anti-oxidative capacity of HDL following 3 consecutive meals.

Our present findings are in line with and extent recent results by Patel et al. who demonstrated that infusion of an artificial fat emulsion results in HDL-TG-enrichment with impaired endothelial function, as assessed by inhibition of (in vitro) endothelial cell adhesion molecule expression, in young healthy males. Our results suggest that liver fat accumulation is related to exaggerated and prolonged postprandial dysmetabolism, including HDL-TG-enrichment that independently is associated with postprandial endothelial dysfunction. We could only speculate whether this is the result of an impaired clearance of TG-enriched HDL by the liver, or an increased TG-enrichment of HDL by, for example, CETP.

A limitation of the present study is that we isolated and examined the total class of HDL instead of the HDL\(_2\) and HDL\(_3\) subclasses.

In conclusion, in men with T2DM and the MetS exposure to 3 consecutive meals produces exaggerated HDL-TG enrichment, which was closely associated with liver fat content, and HDL and endothelial dysfunction. Our findings may link liver fat accumulation and
postprandial dysmetabolism to the high CVD risk present in T2DM and the MetS. Future studies should elucidate whether liver fat regression by therapeutic intervention may lead to an improvement of CVD risk.

REFERENCES
