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Liver fat content is associated with an increase in cholesterol synthesis independent of statin therapy use in patients with type 2 diabetes☆

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ABSTRACT

We investigated how liver fat content (LFC) influences cholesterol metabolism by quantifying liver fat using proton magnetic resonance spectroscopy and by measuring the serum concentrations of lathosterol, a marker of cholesterol synthesis, and sitosterol and campesterol, two markers of cholesterol absorption. We also evaluated whether this relationship could be modified by statin therapy. The study was conducted in 263 patients with type 2 diabetes, 137 of whom (52.0%) received statin therapy.
Results: One hundred and sixty-five patients (62.7%) had steatosis (LFC>5.5%). We performed specific analyses in patients without statin therapy and in patients treated with statin therapy. In both groups, the lathosterol to cholesterol ratio correlated positively with LFC, and in multivariate analysis, the lathosterol to cholesterol ratio was associated with LFC independently of age, gender and BMI. Sitosterol and campesterol concentrations were not associated with LFC.
Conclusions: Our study suggests that in patients with type 2 diabetes, LFC is associated with an increase in cholesterol synthesis that is independent of obesity or diabetes mellitus. Statin therapy does not modify this relationship.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of conditions ranging from simple steatosis to non-alcoholic steatohepatitis and is commonly associated with type 2 diabetes. Most studies found that 60–76% of patients with type 2 diabetes have steatosis [1,2]. NAFLD is strongly associated with lipid abnormalities. In diabetic patients, the presence of hepatic steatosis was associated with elevated serum triglycerides, small, dense LDL, and reduced HDL [3]. The influence of liver fat content on cholesterol metabolism has been less frequently studied. Recently, it has been shown that cholesterol synthesis in non-diabetic patients with NAFLD is increased [4]. On the other hand, insulin resistance, commonly observed in obese and/or type 2 diabetic patients, is associated with increased cholesterol synthesis [5]. However, no study has evaluated either the influence of liver fat content (LFC) on cholesterol synthesis in patients with type 2 diabetes, or whether this relationship could be modified by statin therapy. Lathosterol is an intermediate of cholesterol synthesis, it is thus a marker of hepatic cholesterol formation [4,5]. It has been demonstrated that liver cholesterol synthesis is proportional to plasma levels of lathosterol. In this study, we used serum concentrations of lathosterol as a marker of cholesterol synthesis, and sitosterol and campesterol as markers of cholesterol absorption.

2. Research design and methods

We investigated how LFC influences cholesterol metabolism by quantifying liver fat using proton magnetic resonance spectroscopy (1H-MRS) and by measuring the serum concentrations of lathosterol, a marker of cholesterol synthesis, and sitosterol and campesterol, two markers of cholesterol absorption. This single-centre

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study was approved by our regional ethics committee, and written informed consent was obtained from all patients. Between February 2008 and November 2010, patients were screened prospectively at the endocrinology department for the following inclusion criteria: type 2 diabetes, the absence of acute or chronic disease based on the patient's medical history, physical examination, and standard laboratory tests (blood counts, electrolyte concentrations); and alcohol consumption of less than 20 g/day. Patients who had been treated with thiazolidinediones, ezetimibe or fibrates were excluded.

3. Evaluated parameters

3.1. Liver fat content

LFC was evaluated using 1HMR Spectroscopy with a 3.0 T Magnetom TRIO TIM whole body system (Siemens, Erlangen, Germany) as previously described [6]. Hepatic steatosis was defined as LFC>5.5% [7].

3.2. Lathosterol and phytosterol analyses

To measure levels of lathosterol, sitosterol and campesterol, plasma was mixed with epicoprostanol, which was used as the control standard. Potassium hydroxide saponification was followed by lipid extraction with hexane. Levels of lathosterol, sitosterol and campesterol were measured in the trimethylsilyl ether state by GC–MS using a Hewlett Packard HP6890 Gas Chromatograph equipped with an HP7683 Injector and an HP5973 Mass Selective Detector.

3.3. Biochemical measurements

Plasma glucose, HA1c, fasting serum HDL cholesterol, fasting LDL cholesterol, fasting serum triglycerides, and plasma liver enzymes were determined by standard procedures.

3.4. Statistical analyses

Statistical correlations were determined by the nonparametric Spearman test. A p value of <0.05 was considered statistically significant. To determine which factors were associated with the lathosterol to cholesterol ratio, a multiple linear regression model was fitted with the following factors: age, BMI, liver fat content and gender. Multivariate analyses were performed in the group of patients treated with statin therapy and in the group of patients without statin therapy. In the statin-treated subgroup, lathosterol to cholesterol ratios were log-transformed. In the non-statin-treated subgroup, lathosterol to cholesterol ratios were square-root transformed. Statistical analyses were performed with STATA software, version 11 (Statacorp, Texas, USA).

4. Results

The study was conducted in 263 patients with a mean age of 60.1+/−10.0 years. Table 1 shows the clinical and biochemical characteristics of the study population. One hundred and twenty-six patients were treated without statin therapy and 137 received statin therapy. Seventy (51.0%) patients were treated with atorvastatin, 24 (17.5%) with rosuvastatin, 17 (12.4%) with pravastatin and 26 (18.9%) with simvastatin. Patients treated with statin therapy were older (61.9+/−9.7 vs 57.9+/−10.1 years, $p = 0.001$) and had lower levels of LDL cholesterol (2.49+/−0.86 vs 3.11+/−0.94 mmol/l; $p < 0.001$) than patients without statin.

One hundred and sixty-five patients (62.7%) had steatosis (hepatic triglyceride content greater than 5.5%). Patients with steatosis had a higher body mass index (BMI), higher plasma alanine amino-transferase (ALT) levels, and higher plasma triglyceride levels than did patients without steatosis (Table 1). Lathosterol did not correlate with HbA1c. The LFC in patients treated with statin therapy was lower than in those without statin therapy (8.3% +/−7.2 vs 12.9% +/−9.4; $p < 0.001$). Patients treated with statin therapy had lower levels of lathosterol and higher levels of sitosterol and campesterol than did patients without statin (0.72+/−0.59 vs 1.66+/−0.71 mg/l, $p < 0.001$; 2.36+/−1.34 vs 2.02+/−1.32 mg/l, $p = 0.001$, 2.90+/−1.59 vs 2.70+/− 1.66 mg/l, $p = 0.07$ respectively).

4.1. Patients without statin therapy

All non-cholesterol sterols correlated positively with total cholesterol ($r = 0.26$, $p = 0.002$; $r = 0.42$, $p < 0.001$ and $r = 0.37$, $p < 0.001$ for lathosterol, campesterol and sitosterol respectively). BMI correlated positively with lathosterol levels ($r = 0.29$, $p = 0.0008$) and negatively with campesterol and sitosterol levels ($r = -0.25$, $p = 0.004$ and $r = -0.30$, $p = 0.0006$ respectively). The lathosterol to cholesterol ratio correlated positively with LFC ($r = 0.26$, $p = 0.002$), (Fig. 1). In multivariate analysis, the lathosterol to cholesterol ratio was associated with LFC independently of age, gender and BMI ($p = 0.01$).

Table 1
Subjects' characteristics according to statin therapy and the presence of steatosis.

	Without statins		p	With statins		p
	No steatosis	Steatosis		No steatosis	Steatosis	
n	35	91		63	74	0.003
Age	58.6+/−11.0	57.7+/−9.7	0.73	63.3+/−9.3	61.1+/−9.9	0.14
BMI	32.7+/−6.7	35.2+/−6.2	0.02	32.5+/−5.9	35.3+/−6.2	0.009
Total cholesterol mmol/l	5.18+/−2.19	5.16+/−1.06	0.93	4.35+/−1.01	4.54+/−1.06	0.45
HDL mmol/l	1.13+/−0.33	1.09+/−0.25	0.70	1.09+/−0.27	1.08+/−0.24	0.75
LDL mmol/l	3.15+/−1.11	3.09+/−0.87	0.78	2.50+/−0.81	2.49+/−0.91	0.66
Triglycerides mmol/l	1.85+/−1.16	2.55+/−1.81	0.009	1.72+/−0.97	2.34+/−1.69	0.001
ASAT (UI/l)	20.9+/−10.6	26.8+/−16.8	0.12	18.3+/−8.4	23.6+/−17.5	0.03
ALAT (UI/l)	38.8+/−51.3	46.3+/−26.3	0.001	27.4+/−15.0	38.5+/−15.7	<0.001
HbA1c (%)	8.8+/−2.1	8.7+/−1.7	0.91	8.7+/−1.7	8.5+/−1.8	0.59
Campesterol (mg/l)	2.78+/−1.73	2.67+/−1.64	0.63	2.88+/−1.33	2.91+/−1.79	0.58
Sitosterol (mg/l)	2.26+/−1.53	1.92+/−1.22	0.18	2.35+/−1.16	2.37+/−1.49	0.76
Lathosterol (mg/l)	1.38+/−0.67	1.76+/−0.70	0.01	0.57+/−0.39	0.85+/−0.69	0.01
Lathosterol/cholesterol	272+/−124	350+/−149	0.01	131+/−80	181+/−130	0.01

ASAT, aspartate amino-transferase; ALAT, alanine amino-transferase.