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Tissue-specific stable isotope measurements of postprandial lipid metabolism in familial combined hyperlipidaemia

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Abstract

Objective: The metabolic defects underlying familial combined hyperlipidaemia (FCHL) are not clearly understood. We used stable isotope techniques combined with tissue-specific measurements in adipose tissue and forearm muscle to investigate fatty acid handling by these tissues in the fasting and postprandial states.
Results: Patients were insulin resistant as shown by higher glucose and insulin concentrations and lower muscle glucose extraction than controls. Plasma triacylglycerol (TAG) concentrations were higher in patients. Adipose tissue TAG extraction was not lower in patients than controls, although TAG clearance was lower, probably representing saturation. Following a test meal, patients showed a greater increase in chylomicron-TAG concentrations. There were no differences between FCHL patients and controls in postprandial suppression of non-esterified fatty acid (NEFA) concentrations or postprandial NEFA release, but patients had greater trapping of exogenous fatty acids in adipose tissue. 3-Hydroxybutyrate concentrations were lower in patients indicative of decreased hepatic fatty acid oxidation.
Conclusions: In this group of patients with FCHL, the major defect appeared to be overproduction of TAG by the liver due to decreased fatty acid oxidation, with fatty acids directed to TAG synthesis. We found no evidence of decreased lipoprotein lipase action or impaired fatty acid re-esterification in adipose tissue.

Keywords: Fatty acids; Hyperlipoproteinemia; Metabolism; Postprandial

1. Introduction

Familial combined hyperlipidaemia (FCHL) is one of the most common hereditary disorders among survivors of myocardial infarction. FCHL carries considerable coronary heart disease (CHD) risk, disproportionate to the degree of elevation of cholesterol concentration.

Factors which may play a role in FCHL include hyper-apobetalipoproteinaemia (hyper-apoB), reflecting overproduction of very-low-density lipoproteins (VLDL), decreased lipoprotein lipase (LPL) action, the presence of small, dense, low-density lipoprotein particles, impaired chylomicron remnant clearance, insulin resistance, altered postprandial non-esterified fatty acid (NEFA) metabolism and impaired fatty acid trapping by adipose tissue.

Hyper-apoB is a characteristic feature of FCHL [1]. This may result from an increased rate of secretion of apolipoprotein B-containing particles by the liver [2], but a more proximal defect is thought to be increased fatty acid supply to the liver [3]. Hepatic VLDL secretion is strongly influenced by the delivery to the liver of fatty acids, the main substrate for VLDL-triacylglycerol (TAG) synthesis [4]. Therefore, the major underlying defect in hyper-apoB may lie in the

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regulation of fatty acid supply [3]. However, it may be reinforced by clearance defects as seen in insulin resistance [5] and reported in some cases of FCHL [6]. In recent years considerable evidence has accumulated to suggest that in hyper-apoB there is a defect in the ability to entrap fatty acids in adipose tissue in the postprandial period [7].

The activity of LPL can have significant effects on the concentration of cholesterol and TAG in the plasma. In normal and dyslipidaemic subjects, there is a clear relationship between LPL activity and lipid concentrations [8], and further, defects in LPL activity accentuate the development of dyslipidaemias [9]. LPL activity is positively correlated with high-density lipoprotein (HDL)-cholesterol concentrations, probably a result of enhanced TAG lipolysis. The heterozygote state for LPL deficiency may represent a subset of FCHL [10]. The dyslipidaemia associated with FCHL may therefore result from defects in lipoprotein clearance from the circulation, defects in fatty acid trapping or increased hepatic VLDL synthesis.

Recently, the Upstream Factor-1 (USF-1) gene was identified as linked to FCHL in a genome-wide scan of a Finnish cohort of patients [11]. USF-1 is known to regulate a number of metabolic events in the liver, such as glucokinase and apolipoprotein CIII expression, suggesting that the lipid and carbohydrate metabolic defects observed in FCHL might have a common denominator.

Stable isotope techniques combined with tissue-specific measurements can be used to determine LPL action, fatty acid entrapment and release of NEFA into the circulation [12]. We have therefore used these techniques to study postprandial lipid metabolism in patients with FCHL. Our hypothesis was that differences in adipose tissue handling of fatty acids would underlie the lipid phenotype of FCHL, with decreased fatty acid re-esterification leading to increased NEFA delivery to the liver, and consequent increased hepatic VLDL production.

2. Subjects and methods

2.1. Subjects and protocol

Eight healthy volunteers (four male) and eight patients (five male) with FCHL were studied following an overnight fast. Previous studies of adipose tissue fatty acid metabolism have shown clear differences between groups of this size [5]. Patients and controls were well matched for age and sex, although body mass index was higher in patients (Table 1). Patients were recruited from the lipid clinic according to the following criteria—primary combined hyperlipidaemia (fasting TAG 2–5 mmol/L, total cholesterol 6–8 mmol/L), family history of CHD in a first-degree relative and plasma apolipoprotein B > 1.1 g/L, which corresponds to the top quartile of a healthy population-based reference sample. We did not perform family tracing of lipid phenotypes, but at least five of the eight patients had a first-degree relative with hyperlipidaemia. Insulin resistance and/or metabolic syndrome were not part of the selection criteria, but the patients did have many features of the metabolic syndrome, and five of the eight patients met the National Cholesterol Education Program criteria for metabolic syndrome [13]. Patients receiving lipid-lowering treatment had their treatment stopped for 4 weeks before the study. One patient had a normal lipid profile and apolipoprotein B on the day of the study but had previously had a combined hyperlipidaemia. Two patients had fasting plasma TAG concentrations greater than 5 mmol/L on the day of the study but had originally fulfilled the entry criteria. Subjects refrained from strenuous exercise and alcohol for 24 h before the study and were given instructions to consume a low-fat meal on the evening before the study and then to fast from 08:00 h. None of the subjects were smokers. The study was approved by the Central Oxford Research Ethics Committee, and all subjects gave written informed consent. Data from a group of control subjects which partially overlaps with the present control group have been published previously [12].

Table 1
Subject characteristics

	Controls	Patients	*P* value
n	8	8	
Sex	4M, 4F	5M, 3F	
Age, years	45.6 (2.2)	47.3 (3.8)	0.7
Body mass index (kg/m^2)	25.9 (0.7)	29.1 (1.4)	0.05
Waist circumference (cm)	85.6 (3.6)	101.0 (4.6)	0.02
Fasting plasma values			
Glucose (mmol/L)	5.2 (0.1)	5.7 (0.2)	0.05
Insulin (pmol/L)	43 (5)	81 (15)	0.03
Total cholesterol (mmol/L)	4.7 (0.4)	6.7 (0.4)	0.003
High-density lipoprotein cholesterol (mmol/L)	1.52 (0.18)	0.90 (0.08)	0.007
Triacylglycerol (mmol/L)	1.0 (0.2)	4.6 (1.1)	0.001
Non-esterified fatty acids (μmol/L)	578 (86)	613 (50)	0.7
Apolipoprotein B (mg/L)	78.2 (5.8)	132.9 (13.4)	0.002
3-Hydroxybutyrate (μmol/L)	125.1 (19.8)	60.9 (5.9)	0.02
HOMA-R	9.80 (1.04)	21.40 (4.70)	0.01

Values are mean (S.E.). *P* values are for *t*-tests except TAG and 3-hydroxybutyrate, which are Mann–Whitney *U*-tests.

2.2. Experimental methods

A 10 cm, 22-gauge Hydrocath catheter (Becton Dickinson, UK) was introduced over a guide wire into a superficial vein on the anterior abdominal wall with its tip just superior to the inguinal ligament. This provided access to the venous drainage from the subcutaneous abdominal adipose tissue [14]. A retrograde cannula was placed in a vein draining the hand, which was warmed in a hot-air box maintained at 60 °C to obtain arterialised blood. A further cannula was placed retrogradely in the other arm in a deep antecubital vein draining the forearm muscle. The cannulae were kept patent by a slow infusion of 0.9% (w/v) saline.