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Biomarkers and prediction of myocardial triglyceride content in non-diabetic men

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Abstract *Background and aims:* Lipid oversupply to cardiomyocytes or decreased utilization of lipids leads to cardiac steatosis. We aimed to examine the role of different circulating metabolic biomarkers as predictors of myocardial triglyceride (TG) content in non-diabetic men.
Methods and results: Myocardial and hepatic TG contents were measured with 1.5 T magnetic resonance (MR) spectroscopy, and LV function, visceral adipose tissue (VAT), abdominal subcutaneous tissue (SAT), epicardial and pericardial fat by MR imaging in 76 non-diabetic men. Serum concentration of circulating metabolic biomarkers [adiponectin, leptin, adipocyte-fatty acid binding protein 4 (A-FABP 4), resistin, and lipocalin-2] including β-hydroxybuturate (β-OHB) were measured. Subjects were stratified by tertiles of myocardial TG into low, moderate, and high myocardial TG content groups. Concentrations of β-OHB were lower ($p = 0.003$) and serum levels of A-FABP 4 were higher ($p < 0.001$) in the group with high myocardial TG content compared with the group with low myocardial TG content. β-OHB was negatively correlated with myocardial TG content ($r = -0.316$, $p = 0.006$), whereas A-FABP 4 was not correlated with myocardial TG content ($r = 0.192$, $p = 0.103$). In multivariable analyses β-OHB and plasma glucose levels were the best predictors of myocardial TG content independently of VAT and hepatic TG content. The model explained 58.8% of the variance in myocardial TG content.
Conclusion: Our data showed that β-OHB and fasting glucose were the best predictors of myocardial TG content in non-diabetic men. These data suggest that hyperglycemia and alterations in lipid oxidation may be associated with cardiac steatosis in humans.

Abbreviations: A-FABP 4, adipocyte-fatty acid binding protein; β-OHB, β-hydroxybuturate; CVD, cardiovascular disease; FFA, free fatty acid; HOMA-IR, the insulin-resistance homeostasis model assessment index; LV, left ventricular; MRS, magnetic resonance spectroscopy; T2DM, type 2 diabetes mellitus; TG, triglyceride; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue.

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Introduction

Intramyocardial triglyceride (TG) content has been reported to be higher in obesity [1], in subjects with the metabolic syndrome [2], impaired glucose tolerance [3], and in type 2 diabetes mellitus (T2DM) [4,5], suggesting that cardiac steatosis is relatively common in humans. Furthermore, increases in intramyocardial TG levels precede the development of cardiac dysfunction, suggesting a causative role of myocardial fat in the development of cardiac dysfunction in these disorders [5,6].

Adipose tissue, apart from its traditional role as energy storage, acts as a source for the production of multiple bioactive molecules including adipokines. These have important roles in the regulation of angiogenesis, blood pressure, glucose homeostasis, lipid metabolism, and vascular haemostasis [7]. The secretion of these molecules is altered in adipose tissue dysfunction that has consequences on vascular health and also cardiac function.

Adipocyte-fatty acid binding protein (A-FABP), leptin, resistin, and lipocalin-2, possess proinflammatory properties, whereas others, such as adiponectin, have an anti-inflammatory effect [8]. A-FABP and adiponectin are the two most abundant adipokines produced by adipocytes. A-FABP 4, a member of FABP family, is a carrier protein that facilitates intracellular FA trafficking from cell membranes to lipid droplets or mitochondria in adipocytes and macrophages. A-FABP 4 has been associated with levels of adiposity, the metabolic syndrome, non-alcoholic fatty liver disease, endothelial dysfunction, atherosclerosis, and cardiovascular disease (CVD) mortality [9].

Limited information exists on the links between adipocyte derived metabolic biomarkers and ectopic fat accumulation in the heart assessed by magnetic resonance spectroscopy (MRS) or magnetic resonance imaging (MRI). Adiponectin has been inversely related with hepatic TG content [10,11], myocardial TG content, epicardial and pericardial fat depots [3,12]. Leptin has been associated with the soleus intramyocellular lipid content [10].

Ketone bodies serve as important energy sources to cardiac and skeletal muscle and nervous tissue during fasting state. β-hydroxybuturate (β-OHB) belongs to the group of ketone bodies and is formed during FA oxidation by reduction of acetoacetate in the liver. Prolonged exposure to β-OHB has shown to inhibit insulin-stimulated glucose uptake in adult rat cardiomyocytes [13]. In humans, lower basal β-OHB concentrations have been found in obese than in lean individuals [14,15] and in patients with non-alcoholic fatty liver disease [16].

The purpose of the current study was to determine differences in the concentration of different circulating biomarkers potentially modifying lipid and energy homeostasis with increasing amount of intramyocardial TG in a large non-diabetic group of men free of CVD. Our second objective was to examine the association of different biomarkers with different ectopic fat depots. We also evaluated the independent predictors of myocardial TG content.

Methods

Study population

A total of 77 men were examined using the same study cohort as have been previously described [2]. A detailed description of the study recruitment can be found in Supplemental Material. Briefly, the subjects were categorized into tertiles based on myocardial TG content: group 1 (n = 25) = myocardial TG low, group 2 (n = 26) = myocardial TG moderate, and group 3 (n = 25) = myocardial TG high. One subject in the group 2 was excluded and considered as outlier due to a highly elevated serum β-OHB concentration. Thirty-seven participants fulfilled the criteria for the metabolic syndrome [17]. In these participants, myocardial ischemia was excluded by means of adenosine stress MR perfusion test. The study was approved by the Helsinki University Central Hospital Ethics Committee and conforms to the principles outlined in the Declaration of Helsinki. Each subject provided written informed consent.

Demographic variables and biochemical investigations

Waist circumference was determined midway between iliac crest and the lower rib margin. Blood pressure was measured by BPM-200 (Quick Medical, WA, USA) in the sitting position after a 5 min rest, and the mean of five measurements was recorded. The subjects were classified as present, past or non-smokers.

Blood samples were collected after overnight fasting. Total serum cholesterol, TGs, very low-density, high-density lipoprotein cholesterol, FFAs, apolipoprotein B, and high-sensitivity C-reactive protein were analyzed as previously described [2]. The concentration of low-density lipoprotein cholesterol was calculated using the Friedewald formula [18]. Fasting and postload glucose were determined by the hexokinase method (Roche Diagnostic Gluco-quant) 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-IR) index was calculated using the following formula: (fasting plasma glucose × fasting plasma insulin)/22.5 [19].

We used commercially available enzyme-linked immunosorbent assays for measuring serum levels of adiponectin (R&D Systems, Minneapolis, MN, USA), leptin, resistin (Mediagnost, Reutlingen, Germany), A-FABP 4 (Biovendor, Brno, Czech Republic), and lipocalin-2 (R&D Systems), according to the manufacturer's protocol. Serum β-OHB concentration was determined enzymatically (DiaSys Diagnostic Systems, Holzheim, Germany).

Determination of myocardial and hepatic triglyceride content

Myocardial and hepatic TG content were measured by ^{1}H-MRS with a 1.5 T (MAGNETOM Avanto; Siemens AG,