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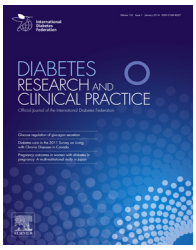

Human serum acylcarnitine profiles in different glucose tolerance states

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ABSTRACT

Aims: To understand the relationship between serum acylcarnitine profiles and glucose tolerance status.

Methods: We analyzed 61 subjects who were divided into three groups based on their glucose tolerance status: normal glucose tolerance (NGT; $n = 20$, M/F = 9/11, mean age 48 years), pre-diabetes (Pre-DM; $n = 20$, M/F = 11/9, mean age 51 years), or newly diagnosed type 2 diabetes mellitus (T2DM; $n = 21$, M/F = 8/13, mean age 49 years). Fasting serum free carnitine and acylcarnitine concentrations were determined using isotope dilution electrospray ionization mass spectrometry coupled with high performance liquid chromatography.

Results: In comparison with NGT subjects, Pre-DM and type 2 diabetes subjects showed serum metabonomic changes highlighted by dysregulation of mitochondrial fatty acid combustion. Of the long-chain carnitine esters, significantly higher palmitoylcarnitine (C16), 3-OH-hexadecanoylcarnitine (C16-OH), carnitine C20, carnitine C22, and carnitine C24 concentrations (all $P < 0.05$) were noted in the newly diagnosed type 2 diabetes group, and even the pre-diabetes group.

Conclusions: This research provides further evidence of alterations in serum acylcarnitine profiles being associated with worse glucoseintolerance. The findings may suggest different degrees of involvement of dysregulated mitochondrial function and incomplete long-chain fatty acid oxidation pathways in the natural course of type 2 diabetes.

1. Introduction

The global epidemic of type 2 diabetes mellitus (T2DM) is one of the most challenging problems of the 21st century. Cardiovascular events are known to account for a large proportion of the excess mortality related to diabetes [1], and individuals with diabetes are up to 4 times more likely to have cardiovascular events as age- and sex-matched individuals without diabetes [2,3]. The concept of diabetes as a coronary risk factor has led to widespread investigation of the mechanisms of the increased cardiovascular risk associated with the disease. The development of type 2 diabetes is a lengthy process involving metabolic abnormalities and an underlying absolute or relative deficiency of insulin. It is well known that in the pathogenesis of type 2 diabetes, disturbances of glucose and lipid metabolism (glucotoxicity and lipotoxicity, respectively) interact. Many studies reported in

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the literature have shown that dysregulated fatty acid metabolism and tissue lipid accumulation are associated with the development of insulin resistance and type 2 diabetes [4–7].

Carnitine is an endogenous metabolite and exogenous nutrient that has a pivotal role in lipid metabolism by facilitating fatty acid transport between the cytosol and mitochondria [8–12]. The essential role of acylcarnitine in fatty acid oxidation has prompted numerous metabolic studies over several decades to identify the potential mechanism of its effects on metabolic diseases. Increasing evidence suggests that carnitine is crucial in the regulation of carbohydrate metabolism in addition to its role in the oxidation of fatty acids [13–16], and several studies have shown that carnitine also increases the utilization of glucose by peripheral tissues and increases the sensitivity of cells to insulin [17,18]. In hyperinsulinemic–euglycemic clamp studies, carnitine infusions improved glucose metabolism in healthy volunteers, mainly by a non-oxidative mechanism that resulted in the accumulation of glycogen [17,19,20]. It has been documented in healthy humans and animals that levocarnitine supplementation enhances maintenance of skeletal muscle contractile force and delays muscle fatigue [17,21]. In addition, Power et al. [22] reported that carnitine supplementation reduces lipid overload and glucose intolerance in diabetic mice by enhancing the mitochondrial efflux of excess acyl groups from insulin-responsive tissues, without altering body weight or food intake. Overall, these studies suggest that acylcarnitine may represent a link between fatty acids and carbohydrate metabolism.

Divergent studies of carnitine metabolism in diabetic patients have, however, been reported. Plasma total carnitine and acylcarnitine concentrations have been found to be normal, increased, or decreased in type 2 diabetes, and to vary in different ethnic groups [20–27]. Some studies have indicated that carnitine administration increases glucose oxidation [20,22], while others suggest that inefficient β-oxidation of long-chain fatty acids (LCFAs) promotes accumulation of acylcarnitine by-products that may interfere with insulin sensitivity [27]. Most of these data are derived from investigations of patients with diabetes of long duration and even chronic complications such as diabetic retinopathy and neuropathy [23,24], and few studies have been conducted in individuals with newly diagnosed diabetes or pre-diabetes. Emerging technologies such as tandem mass spectrometry (MS) have allowed the identification of acylcarnitine profiles with the mass-to-charge ratio reflecting the length and composition of the acyl chain. These techniques have become the preferred screening method to investigate the impact of acylcarnitine on fat metabolism and insulin sensitivity.

In this study, we analyzed the serum acylcarnitine profiles in Chinese subjects with different glucose tolerance states, including normal glucose tolerance, pre-diabetes, and newly diagnosed type 2 diabetes, using a MS method coupled with high performance liquid chromatography. The aims of the study were to explore the relationships between serum acylcarnitine profiles and the states of glucose tolerance with a view to providing further insights into the biochemical networks that underlie metabolic homeostasis.

2. Methods

2.1. Subjects

A total of 61 adult subjects were enrolled in the study at our institution in Beijing, China and were divided into 3 age- and body mass index (BMI)-matched groups according to the World Health Organization (WHO) criteria for the diagnosis of diabetes [28]: (1) normal glucose tolerance (NGT group; $n = 20$, M/F ratio = 9/11, mean age 48 years); (2) pre-diabetes, including subjects with impaired fasting glucose and/or impaired glucose tolerance (Pre-DM group; $n = 20$, M/F ratio = 11/9, mean age 51 years); and (3) newly diagnosed type 2 diabetes (T2DM group; $n = 21$, M/F ratio = 8/13, mean age 49 years). Exclusion criteria were clinical existence of renal and/or hepatic diseases, gastrointestinal tract diseases, psychiatric disorders, neoplasia, and any other disease that could interfere with the conduct of the study. None of the participants had taken pharmacological doses of hormones, antiobesity agents, lipid-lowering drugs, or antihyperglycemic agents, and none had received carnitine supplementation before the study. The study complied with the principles expressed in the Declaration of Helsinki and was approved by the Ethics Committee of Peking University People's Hospital. Written informed consent was obtained from each participant of the study.

2.2. Sample collection and processing

Venous blood samples were taken from the study participants after an overnight fast for at least 10 h, and serum samples were obtained in the usual manner. An oral glucose tolerance test (OGTT) was performed in each participant by measuring plasma glucose concentrations at 0 and 2 h after oral ingestion of 75 g glucose. Aliquots of fasting serum samples were snap frozen in liquid nitrogen, and stored at −80 °C until analyzed.

2.3. Clinical chemistry measurements

Plasma glucose concentrations were measured by the glucose oxidase method. Serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and liver function and renal function indicators were measured using an automatic biochemical analyzer. Hemoglobin A1c (HbA1c) was measured by high-performance liquid chromatography [Ultra2 HbA1c Detector, PRIMUS Corporation, USA; normal range: 4% to 6%]. Fasting insulin (FINS) and 2-h insulin (2HINS) concentrations were determined by an electrochemiluminescence immunoassay (Elecsys 2010 system, Roche Diagnostics Ltd, Basel, Switzerland).

Insulin resistance index (IR) was calculated from the fasting plasma glucose (FPG, mmol/L) and insulin (FINS, μU/ml) concentrations as: homeostasis model of assessment–insulin resistance (HOMA-IR) = FPG • FINS/22.5 [29].

2.4. LC-MS/MS analysis

Serum carnitine concentrations were determined using the isotope dilution mass spectrometry (MS) method in an