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Dietary fat differentially modulate the mRNA expression levels of oxidative mitochondrial genes in skeletal muscle of healthy subjects

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KEYWORDS
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Abstract *Background and aims:* Different types of dietary fats exert differential effects on glucose and lipid metabolism. Our aim was to evaluate the impact of different dietary fats on the expression of skeletal muscle genes regulating mitochondrial replication and function in healthy subjects.
Methods and results: Ten healthy subjects (age 29 ± 3 years; BMI 25.0 ± 3 kg/m^2) received in a random order a test meal with the same energy content but different composition in macronutrients and quality of fat: Mediterranean (MED) meal, SAFA meal (Lipid 66%, saturated 36%) and MUFA meal (Lipid 63%, monounsaturated 37%). At fast and after 180 min, a fine needle aspiration was performed from the vastus lateralis for determination of mitochondrial gene expression by quantitative PCR. No difference in glucose and triglyceride response was observed between the three meals, while NEFA levels were significantly higher following fat-rich meals compared to MED meal ($p < 0.002–0.0001$). MED meal was associated with an increased expression, albeit not statistically significant, of some genes regulating both replication and function. Following MUFA meal, a significant increase in the expression of PGC1β ($p = 0.02$) and a reduction in the transcription factor PPARδ ($p = 0.006$) occurred with no change in the expression of COX and GLUT4 genes. In contrast, SAFA meal was associated with a marked reduction in the expression of COX ($p < 0.001$) PFK ($p < 0.003$), LPL ($p = 0.002$) and GLUT4 ($p = 0.009$) genes.

Abbreviations: CHO, carbohydrate; COX, cytocrome c oxidase; NEFA, non esterified fatty acids; FNA, fine needle aspiration; MED, Mediterranean; MUFA, monounsaturated fat; OXPHOS, oxidative phosphorylation; PGC1α – PGC1β, proliferator-activated receptor gamma coactivator α – β; SAFA, saturated fat.

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Conclusion: Dietary fats differentially modulate gene transcriptional profile since saturated, but not monounsaturated fat, downregulate the expression of genes regulating muscle glucose transport and oxidation.

Introduction

Skeletal muscle plays a key role in regulating insulin-stimulated glucose metabolism since it takes up a large proportion of glucose. In fact, under condition of insulin stimulation skeletal muscle shifts from reliance upon fat oxidation to glucose oxidation, as demonstrated by the increase of the respiratory quotient toward 1 [1]. This capacity, known as "metabolic flexibility", is characteristic of lean, insulin-sensitive healthy subjects. In obese or type 2 diabetic subjects, insulin's ability to stimulate glucose oxidation and suppress fat oxidation is blunted giving rise to the concept of insulin resistance [1]. Being the major site of fuel oxidation, mitochondria have recently gained much attention in an attempt to elucidate the link between mitochondrial oxidative capacity and insulin action [2]. Mitochondrial activity is under control of genes regulating the oxidation of substrates and genes regulating mitochondrial biogenesis [3]. The oxidative genes encode for mitochondrial transport chain complexes, among which is the cytocrome c oxidase (COX) or complex IV, the main regulator of mitochondrial oxidative activity [4,5]. The main genes of mitochondrial biogenesis are the proliferator-activated receptor gamma coactivator α and β (PGC1α and PGC1β), which are largely expressed in tissues with high oxidative capacity, such as heart, muscle and brown adipose tissue [6,7]. PGC1α [8] and possibly PGC1β [9] also activate oxidative phosphorylation (OXPHOS) gene expression, increase the transcription of enzymes necessary for electron transport and ATP synthesis, and induce the expression of the insulin-responsive glucose transporter GLUT-4 [10].

Among environmental factors, dietary pattern as well as physical activity are known to influence mitochondrial function [11]. There is evidence that experimental lipid oversupply or high-fat diet are able to downregulate mitochondrial oxidative genes as well as mitochondrial biogenesis in skeletal muscle [12,13]. Increased fatty acid metabolites concentrations can exert deleterious effects on muscle mitochondrial ATP synthesis and, in turn, the reduced mitochondrial oxidative capacity further promotes lipid storage within muscle cells [14].

However, different types of dietary fat exert different effects on glucose and lipid metabolism [15,16]. Aim of the present study is to compare the impact of meals rich in saturated or monounsaturated fatty acids on the expression of skeletal muscle mitochondrial genes regulating mitochondrial replication and function in healthy subjects.

Methods

Subjects

The study subjects consisted of 10 healthy volunteers, 7 males and 3 females, mean age (29 $\pm$ 3 years) (mean $\pm$ SD), BMI (25.0 $\pm$ 3 kg/m^2), recruited among students at the Federico II Medical School. All participants had normal glucose tolerance and normal lipid profile (Total Chol 154 $\pm$ 22 mg/dl; HDL-Chol 51 $\pm$ 19 mg/dl; Triglycerides 52 $\pm$ 16 mg/dl).

None of them had a family history of arterial hypertension, hyperlipidemia, diabetes or cardiovascular disease. The study was approved by the Institutional Ethics Committee of Federico II University, Naples and was in accordance with the Declaration of Helsinki. All subjects gave their written informed consent before study participation.

Experimental procedure

On three different occasions, participants consumed a mixed meal with the same energy content but different composition in macronutrients. The order of the meals was randomized and the studies were performed at least two weeks apart. Participants followed a standard maintenance diet (CHO 50%, Protein 15% and Fat 30%) and abstained from physical activity in the three days preceding the test. The participants' energy intake in the days preceding the test was evaluated by a 24-h dietary record. The meals were prepared in a metabolic kitchen and were consumed in the morning after an overnight fast. A 20-G cannula was inserted into an antecubital vein to collect blood samples for determination of glucose, free fatty acids, triglycerides in the fasting state and every 60 min for 180 min. A fine needle aspiration (FNA) of vastus lateralis was performed before and 180 min after each test meal.

Composition of the test-meal

The meals had the same energy content (970 Kcal) but different composition in macronutrients and quality of fat (Table 1). The Mediterranean (MED) meal (reference meal) was composed of: CHO 53%, Protein 16%, Lipid 30% of which 6% saturated fat. The meal rich in saturated fat (SAFA) was composed of CHO 22%, Protein 12%, Lipid 66% of which 36% saturated fat. The meal rich in monounsaturated (MUFA) fat was composed of CHO 24%, Protein 13%, Lipid 63% of which 37% monoinsaturated fat.

Analytical methods

Plasma glucose, lipids, free fatty acids (NEFA) and were measured by commercially available kits. Serum LDL-cholesterol was calculated with the Friedewald formula.

Muscle fine needle aspiration (FNA)

Skeletal muscle was obtained by fine needle aspiration (FNA) from the vastus lateralis muscle. Muscle FNA was performed with a 22-G spinal needle (Becton Dickinson, Madrid) under ultrasound guidance as previously described