Nutrition, Metabolism & Cardiovascular Diseases (2014) 24, 198–204

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/nmcd

Nutrition, Metabolism & Cardiovascular Diseases

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

A.A. Turco [a], M. Guescini [b], V. Valtucci [a], C. Colosimo [a], P. De Feo [c], M. Mantuano [b], V. Stocchi [b], G. Riccardi [a], B. Capaldo [a,*]

[a] Department of Clinical and Experimental Medicine, Federico II University, Naples, Italy
[b] Department of Biomolecular Sciences, University of Urbino "Carlo Bo", Urbino, Italy
[c] Department of Internal Medicine, University of Perugia, Italy

Received 15 January 2013; received in revised form 1 July 2013; accepted 1 July 2013
Available online 22 December 2013

KEYWORDS
Dietary fat;
Mitochondrial gene expression;
Oxidative genes

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.

* Corresponding author. Department of Clinical Medicine and Surgery, Federico II University, Napoli, Italy. Tel.: +39 (0)817462311; fax: +39 (0)815466152.
E-mail address: bcapaldo@unina.it (B. Capaldo).

0939-4753/$ - see front matter
http://dx.doi.org/10.1016/j.numecd.2013.07.001

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

Table 1 Composition of the three meals.

	Protein (g)	Total fat (g)	SAFA (g)	MUFA (g)	PUFA (g)	CHO (g)	Kcal
MED meal							
Pasta 70 g	7.6	0.9	0.1	0.1	0.5	55.3	247.1
Beans 110 g	25.9	2.2	0.4	0.1	1.1	55.8	333.3
Ham 37 g	9.6	8.2	2.7	3.7	0.7	–	113.2
Olive oil 20 g	–	19.9	2.8	14.5	1.5	–	179.8
Apple 200 g	0.6	0.2	–	–	–	27.4	106.0
Total	**43.8**	**31.6**	**6.2**	**18.5**	**3.8**	**138.6**	**979.4**
SAFA meal							
Egg 120 g	9.3	5.5	2.2	–	1.5	51.2	279.1
Bread 110 g	14.8	10.4	3.8	3.1	0.6	–	153.6
Cheese 20 g	5.1	6.4	4	1.8	0.1	–	78.4
Butter 60 g	0.4	50	29.2	14.2	1.6	0.6	454.8
Total	**29.8**	**72.3**	**39.3**	**19.1**	**3.9**	**51.9**	**965.9**
MUFA meal							
Potato 300 g	6.3	3	0.5	0.1	1.8	54	255
Egg 60 g	7.4	5.2	1.9	1.5	0.8	–	76.8
Parmesan 25 g					**0.2**		
Semiskimmed milk 30 g	1	0.4	0.2	0.1	0.02	1.5	13.8
Olive oil	–	49.9	7.2	36.4	3.8	–	449.5
Bread crumbs 10 g	1.1	0.1	–	–	0.1	7.7	35.4
Baked ham 30 g	6.6	1.3	0.4	0.5	0.2	0.3	39.6
Total	**30.9**	**67.1**	**14**	**38.1**	**6.8**	**63.5**	**966.8**

by Guescini et al. [17]. Briefly, the needle was kept firm in the middle of the muscle, and samples were collected keeping a constant vacuum for a period of 2 min through a 60 ml plastic syringe maintained at its maximal extension by a firm support, this procedure was repeated twice. No local anesthesia was required because the procedure is painless. Muscle sample, trapped in the fine needle following aspiration process, was recovered by repeated needle washing with 1 ml of RLT solution (Qiagen GmbH, Hilden, Germany) diluted 1:3 by diethyl pyrocarbonate treated water. The material was immediately frozen and kept at −80 °C for subsequent analysis.

Nucleic acid extraction

Muscle tissue was rapidly thawed and incubated with 0.2 mg/ml of Proteinase K at 55 °C for 10 min. Subsequently, genomic DNA and total RNA were co-purified using silica micro columns by the RNeasy Micro Kit (Qiagen) according to the manufacturer's instructions [17].

Multiplexed Tandem real-time RT-PCR

Gene expression analysis was achieved by Multiplexed Tandem real-time RT-PCR strategy as reported by Guescini et al. [17]. GAPDH, ACTIN and B2M expression levels were used for gene expression normalization. From analysis of gene expression levels we found that GAPDH expression levels significantly changed in response to different meals; hence it was removed from the genes used for normalization. Real-time PCR amplifications were conducted using Light-Cycler 480 SYBR Green I Master (Roche, Basilea, Switzerland) according to the manufacturer's instructions, with 300 nM primers and a variable amount of DNA standard in 20 μl of final reaction volume. Thermocycling was conducted using a LightCycler 480 (Roche) initiated by a 10 min incubation at 95 °C, followed by 40 cycles (95 °C for 5 s; 60 °C for 5 s; 72 °C for 10 s) with a single fluorescent reading taken at the end of each cycle. Each reaction was conducted in triplicate and completed with a melt curve analysis to confirm the specificity of amplification and lack of primer dimers.

Statistics

Results are expressed as mean ± SD. Postprandial areas of substrates were calculated by the trapezoidal method as the area under the curve from 0 to 180 min (AUC).

Differences in substrate concentration and in gene expression within each meal were tested by paired *t*-test. Differences between the three meals were tested by two-way ANOVA. Furthermore, in order to check the homogeneity of variance among MED, MUFA and SAFA and to verify that the different groups have a similar variance, F max Hartley's test was applied. Only one gene showed heteroscedasticity, (GLUT4), whilst other genes had variance not significantly different. To investigate whether the administered meals resulted in co-regulation of multiple genes, we performed the linear multiple discriminant analysis [18]. The advantage of this analysis is that an algorithm is generated that predicts the group membership of a newly encountered condition (the meal in our case). Analysis of mRNA expression values of all the genes studied yielded two discriminant functions. These two functions significantly contributed to the prediction of group membership (Wilk's lambda, $p < 0.01$), although the first accounted for ~80% of the total variance explained. The genes included

in the model were the following: PGC-1α, PGC-1β, PPARα, PPARδ, PPARγ, COX2, COX5b, PFK, GLUT-4, LPL and CPT-1. Statistical analysis was carried out using SPSS for Windows release 8.0 (SPSS, Inc., Chicago, IL). Spearmen's correlation analysis was performed to evaluate possible association between changes in gene expression and circulating substrates.

Statistical significance was defined at a value of $p < 0.05$ (two-tailed).

Results

Glucose, NEFA and triglyceride during meals

As shown in Fig. 1, blood glucose transiently decreased after meals with no difference between meals. NEFA levels decreased at 1 h and remained below basal values with all meals; as expected, NEFA were significantly higher in response to fat-rich meals compared to the reference meal, as expressed by AUC (AUC $_{MUFA}$ vs. AUC$_{MED}$: $p < 0.0001$; AUC$_{SAFA}$ vs. AUC$_{MED}$ $p < 0.002$). Plasma triglyceride almost doubled postprandially with no difference between meals both at single time points and as AUC.

Analysis of muscle mRNA expression levels

The transcriptional profile of the main mitochondrial genes in response to different meals is shown in Fig. 2. After the reference (MED) meal there was a tendency toward an increased expression of genes regulating both biogenesis and oxidation, which, however, did not reach the statistical significance. The MUFA meal was associated with a significant increase in the expression of PGC1β ($p = 0.02$) and a reduction in the transcription factor PPARδ ($p = 0.006$). Moreover, neither the expression of COX genes nor the expression of GLUT4 gene changed with the MUFA meal. A completely different pattern was observed after the SAFA meal. The expression of both PGC1α and PGC1β and PPARα genes tended to decrease reaching the statistical significance for PPARα ($p < 0.001$). In addition, the expression of COX genes was markedly reduced ($p < 0.001$ for COX2 and COX5b) together with a significant decrease in mRNA transcript of PFK ($p < 0.003$), LPL ($p = 0.002$) and GLUT4 ($p = 0.009$).

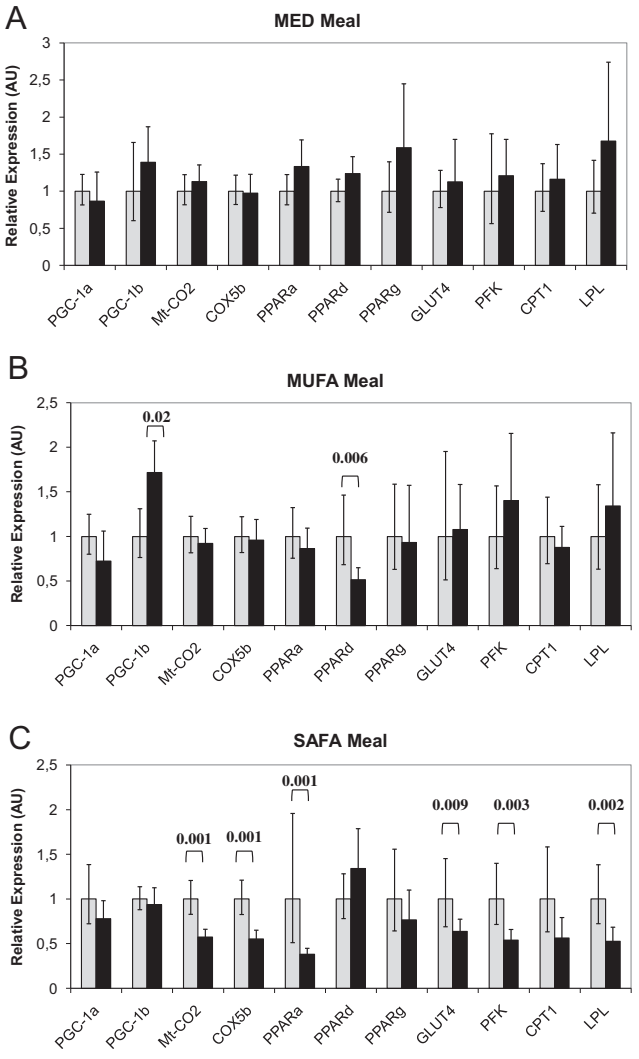

Figure 2 Analysis of muscle mRNA expression levels in response to meals. T0, T180 (a. $p = 0.006$; b. $p = 0.02$; c. $p < 0.001$; d. $p < 0.03$; e. $p = 0.002$; f. $p = 0.009$).

We subsequently performed linear multiple discriminant analysis to investigate whether the administered meals resulted in co-regulation of multiple genes. The segregation of the three different types of meal through the two

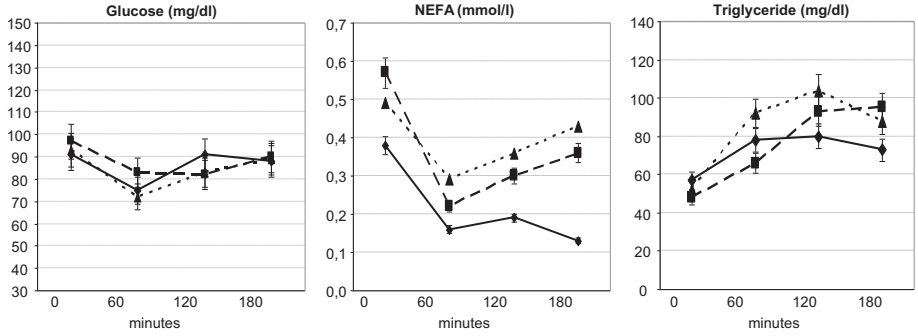

Figure 1 Glucose, NEFA and triglyceride concentrations in response to meals (MED meal, MUFA Meal, SAFA Meal) *$p = 0.03$ MED vs. MUFA **$p = 0.04$ MED vs. SAFA; $p = 0.003$ MED vs. MUFA ***$p < 0.0001$ MED vs. SAFA; $p < 0.0001$ MED vs. MUFA.

discriminant functions is illustrated in Fig. 3, where starting from normalized mRNA expression levels, the function 1 and 2 correctly classified the 100% of MED, MUFA and SAFA meals. No significant association was found between changes in gene expression and circulating substrates.

Discussion

The current study demonstrates that meals with different composition are able to acutely modulate the expression of genes involved in mitochondrial biogenesis and function in healthy subjects. After the MED meal there was a tendency toward an increased mitochondrial gene expression suggesting the activation of metabolic pathways in response to an increased substrate availability. The MUFA meal was associated with some changes in the biogenesis and transcriptional genes while no changes occurred in the expression of oxidative genes. In contrast, the SAFA meal resulted in downregulation of almost all analyzed genes with a marked reduction in mRNA transcripts of mitochondrial oxidative activity. Actually, the expression of COX2 and COX5b encoding the cyt c oxidase — the key enzyme of the electron transport chain - was reduced by 70% after the SAFA meal. Although we failed to detect significant differences in terms of single gene expression between meals, the linear multiple discriminant analysis highlighted the occurrence of three different co-regulated gene programs in response to the meals. These adaptations probably consist of multiple, albeit slight, mRNA regulations not detectable when gene expression is analyzed individually. On the whole, this analysis indicates that the mRNA expression program induced by each meal is clearly discernible. In particular, the change in the expression of PGC-1β showed the highest correlation with the linear multiple discriminant function 1, while the changes in the expression of PGC-1α, COX5b and Mt—CO_2 genes showed the highest correlation with the linear multiple discriminant function 2.

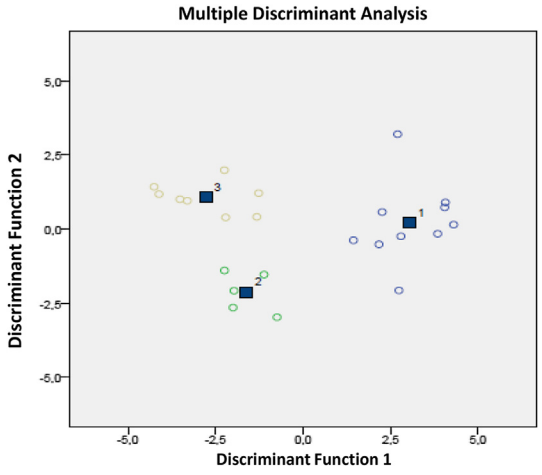

Figure 3 Territorial distribution of the different types of meals between the two discriminant functions 1 and 2. Functions were obtained as a result of multiple discriminant analysis using PGC-1α, PGC-1β, PPARα, PPARδ, PPARγ, COX2, COX5b, PFK, GLUT-4, LPL and CPT-1 gene expression levels after MED (1), MUFA (2) and SAFA (3) meals.

Lipid infusion or high-fat diet has been shown to cause marked changes in mitochondrial function both in experimental animals [12,19] and in humans [12,13,20]. Some studies, but not all [21], demonstrated that feeding mice a high-fat diet resulted in the downregulation of OXPHOS genes [13]. In healthy subjects the consumption of a high-fat diet (50% fat) for 3 days decreased the expression of PGC1α and PGC1β as well as of oxidative genes compared to a reference diet [13]. Subsequent studies demonstrated that fat-induced mitochondrial dysfunction was paralleled by concomitant impairment of insulin sensitivity and a reduction in muscle glucose uptake/phosphorylation and intracellular glucose utilization [2,22]. In addition, Richardson et al. reported a marked increase in the expression of extracellular matrix related-genes, including collagens, fibronectin, tissue inhibitor of metalloproteinases following prolonged (48-h) lipid infusion [12], indicating a pro-inflammatory response. Collectively, these data support the concept that high fat intake, through downregulation of skeletal muscle oxidative genes and upregulation of inflammatory genes, reduces insulin sensitivity with a consequent impairment of insulin stimulated glucose disposal in skeletal muscle.

In the present study we compared the effects of monounsaturated vs. saturated fat on muscle mitochondrial gene expression since these two types of dietary fats are known to exert different metabolic effects [15,16]. With regard to insulin sensitivity, in vitro [23,24] and in vivo [15] evidence show that saturated NEFA induce insulin resistance whereas unsaturated NEFA oleate improves insulin sensitivity. Although the precise mechanisms underlying these differential effects have not been fully elucidated, a number of factors could play a role, i.e., increased production and accumulation of ceramide and diacylglycerol [23], mitochondrial dysfunction [26], and reduced cellular oxygen consumption [24].

Data on the effects of different dietary fats on mitochondrial gene expression are scarce and conflicting [27]. Our study provides the novel finding that in human muscle cells saturated fat reduces the expression of mitochondrial genes, particularly those involved in oxidative processes. We also found a significant reduction in GLUT4 gene expression in response to SAFA meal suggesting an impairment of muscle glucose uptake, which is consistent with the reported impairment of insulin sensitivity following high saturated-fat exposure [15,23,25]. The lack in our study of a statistically significant reduction in the expression of PGC1, as observed in previous studies [12,13], may be explained by the fact that we performed a single meal while in previous studies the nutritional intervention was much more prolonged (3days of high-fat diet or 48-h lipid infusion).

The increased expression of PGC1β in response to the MUFA meal is not of straightforward interpretation. A similar finding was obtained in murine muscle cells by Rodriguez et al. who examined the time-course of gene expression to exposure of oleic/linoleic mixture and found an early upregulation of PGC1β at 4-h with return to baseline after 8-h, suggesting that this transient increase may be an adaptive response to lipid overload [28].

Our finding of a reduced transcription of oxidative genes after SAFA meal is in line with a recent study by Jans et al. [29] who compared the acute effects of different fatty meals

(MUFA, PUFA, and SAFA) on fatty acid metabolism in the skeletal muscle. In that study, SAFA meal resulted in greater downregulation of the genes of the respiratory chain compared to PUFA meal [29].

The current study presents some limitations that need to be discussed. First, changes in gene expression do not necessarily reflect changes in the related metabolic function. However, the coordinated reduction of transcriptional regulators, coactivators and oxidative and glucose transport genes after SAFA meal consistently support a reduction in oxidative processes. Second, we did not evaluate insulin sensitivity with the different meals; however, the metabolic perturbations induced by the meals are too short to expect changes in insulin sensitivity. Indeed, short-term (5 days) high-fat overfeeding increases hepatic glucose production but not impact peripheral glucose disposal [30]. Finally, the small sample size may limit our ability to draw firm conclusions.

In conclusion, the present study shows that mitochondrial gene expression in the skeletal muscle of healthy subjects can be modulated by acute changes of nutrient intake. Dietary fats have a differential impact on the gene transcriptional profile since saturated, but not monounsaturated fat, downregulate the expression of genes involved in oxidative processes and glucose transport. This observation, if confirmed in larger samples, could provide further support to the recommendation by major nutritional guidelines to reduce the intake of meals rich in saturated fat.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We are grateful to volunteers who participated in the study. This study was funded in part by a grant "SID — FO.RI.SID 2009 of the Italian Society of Diabetes (SID)."

References

[1] Kelley DE. Skeletal muscle fat oxidation: timing and flexibility are everything. J Clin Invest 2005;115:1699—702.
[2] Brehm A, Krssac M, Schmid AI, Nowotny P, Waldhäusl W, Roden M. Increased lipid availability impairs insulin stimulated ATP synthesis in human skeletal muscle. Diabetes 2006;55:136—40.
[3] Chanséaume E, Morio B. Potential mechanism of muscle mitochondrial dysfunction in aging and obesity and cellular consequences. Int J Mol Sci 2009;10:306—24.
[4] Lomax MI, Hsieh CL, Darras BT, Francke U. Structure of the human cytochrome c oxidase subunit Vb gene and chromosomal mapping of the coding gene and seven pseudogenes. Genomics 1991;10:1—9.
[5] Hill BC. The sequence of electron carriers in the reaction of cytochrome c oxidase with oxygen. J Bioenerg Biomembr 1993;25:115—20.
[6] Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 1999;98:115—24.
[7] Handschin C, Choi CS, Chin S, Kim S, Kawamori D, Kurpad AJ, et al. Abnormal glucose homeostasis in skeletal muscle-specific PGC-1 knockout mice reveals skeletal muscle-pancreatic cell crosstalk. J Clin Invest 2007;117:3463—74.
[8] Puigserver P, Spiegelman BM. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. Endocr Rev 2003;24:78—90.
[9] Meirhaeghe A, Crowley V, Lenaghan C, Lelliott C, Green K, Stewart A, et al. Characterization of the human, mouse and rat PGC1 beta (peroxisome-proliferator-activated receptor-gamma co-activator 1 beta) gene in vitro and in vivo. Biochem J 2003;373:155—65.
[10] Miura S, Kai Y, Ono M, Ezaki O. Overexpression of peroxisome proliferator-activated receptor coactivator- 1 down-regulates GLUT4mRNAin skeletal muscles. J Biol Chem 2003;278: 31385—90.
[11] Ling C, Poulsen P, Carlsson E, Ridderstråle M, Almgren P, Wojtaszewski J, et al. Multiple environmental and genetic factors influence skeletal muscle PGC-1alpha and PGC-1beta gene expression in twins. J Clin Invest 2004;114:1518—24.
[12] Richardson DK, Kashyap S, Bajaj M, Cusi K, Mandarino SJ, Finlayson J, et al. Lipid infusion decreases the expression of nuclear encoded mitochondrial genes and increase the expression of extracellular matrix genes in human skeletal muscle. J Biol Chem 2005;180:10290—7.
[13] Sparks LM, Xie H, Koza RA, Mynatt R, Hulver MW, Bray GA, et al. A high-fat diet co-ordinately downregulates genes required for mitochondrial oxidative phosphorylation in skeletal muscle. Diabetes 2005;54:1926—33.
[14] Abdul-Ghani MA, Muller FL, Liu Y, Chavez AO, Balas B, Zuo P, et al. Deleterious action of FA metabolites on ATP synthesis: possible link between lipotoxicity, mitochondrial dysfunction, and insulin resistance. Am J Physiol Endocrinol Metab 2008; 295:E678—85.
[15] Vessby B, Unsitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC, et al., KANWU Study. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: the KANWU Study. Diabetologia 2001;44:312—9.
[16] Ferretti G, Bacchetti T. Effect of dietary lipids on paraoxonase-1 activity and gene expression. Nutr Metab Cardiovasc Dis 2012;22:88—94.
[17] Guescini M, Fatone C, Stocchi L, Guidi C, Potenza L, Ditroilo M, et al. Fine needle aspiration coupled with real-time PCR: a painless methodology to study adaptive functional changes in skeletal muscle. Nutr Metab Cardiovasc Dis 2007;17(5):383—93.
[18] Sosulina L, Graebenitz S, Pape HC. GABAergic interneurons in the mouse lateral amygdala: a classification study. J Neurophysiol 2010;104:617—26.
[19] Bonnard C, Durand A, Peyrol S, Chanseaume E, Chauvin MA, Morio B, et al. Mitochondrial dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice. J Clin Invest 2008;118:789—800.
[20] Hoeks J, Hesselink MK, Russell AP, Mensink M, Saris WH, Mensink RP, et al. Peroxisome proliferator activated receptor gamma coactivator-1 and insulin resistance: acute effect of fatty acids. Diabetologia 2006;49:2419—26.
[21] Turner N, Bruce CR, Beale SM, Hoehn KL, So T, Rolph MS, et al. Excess lipid availability increases mitochondrial fatty acid oxidative capacity in muscle: evidence against a role for reduced fatty acid oxidation in lipid-induced insulin resistance in rodents. Diabetes 2007;56:2085—92.
[22] Roden M, Price TB, Perseghin G, Petersen KF, Rothman DL, Cline GW, et al. Mechanism of free fatty acid-induced insulin resistance in humans. J Clin Invest 1996;97:2859—65.
[23] Powell DJ, Turban S, Gray A, Hajduch E, Hundal HS. Intracellular ceramide synthesis and protein kinase Czeta activation play an essential role in palmitate-induced insulin resistance in rat L6 skeletal muscle cells. Biochem J 2004;382:619—29.

[24] Lovejoy JC, Smith SR, Champagne CM, Most MM, Lefevre M, DeLany JP, et al. Effects of diets enriched in saturated (palmitic), monounsaturated (oleic), or trans (elaidic) fatty acids on insulin sensitivity and substrate oxidation in healthy adults. Diabetes Care 2002;25:1283—8.

[25] Coll T, Jové M, Rodríguez-Calvo R, Eyre E, Palomer X, Sànchez RM, et al. Palmitate-mediated downregulation of peroxisome proliferator-activated receptor-gamma coactivator 1alpha in skeletal muscle cells involves MEK1/2 and nuclear factor kappa B activation. Diabetes 2006;55:2779—87.

[26] Gao D, Griffiths HR, Bailey CJ. Oleate protects against palmitate induced insulin resistance in L6 myotubes. Br J Nutr 2009;102:1557—63.

[27] Staiger H, Staiger K, Haas C, Weisser M, Machicao F, Häring HU. Fatty acid-induced differential regulation of the genes encoding peroxisome proliferator-activated receptor-γ coactivator-1α and -1β in human skeletal muscle cells that have been differentiated in vitro. Diabetologia 2005;48:2115—8.

[28] Rodriguez AM, Sanchez J, Tobaruela A, Priego T, Picò C, Palou A. Time course effects of increased fatty acid supply on the expression of genes involved in lipid/glucose metabolism in muscle cells. Cell Physiol Biochem 2010;25:337—46.

[29] Jans A, Konings E, Goosens GH, Bouwman FG, Moors CC, Boekschoten MV, et al. PUFAs acutely affect triagylglycerol-derived skeletal muscle fatty acid uptake and increase postprandial insulin sensitivity. Am J Clin Nutr 2012;95:825—36.

[30] Brøns C, Jensen CB, Storgaard H, Hiskock NJ, Withe A, Appel JS, et al. Impact of short-term high-fat feeding on glucose and insulin metabolism in young healthy men. J Physiol 2009;587:2387—97.