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Influence of a diet rich in linoleic acid on mRNA levels for enzymes of branched chain amino acids metabolism in rat's adipose tissue: a pilot study

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Abstract: White adipose tissue plays an important role in the catabolism of branched chain amino acids (BCAAs). Two initial regulatory steps in BCAAs catabolism are catalyzed by branched chain aminotransferase (BCAT) and branched chain α-keto acid dehydrogenase complex (BCKDH complex), respectively. It has been demonstrated that synthetic ligands for PPARγ receptors increased mRNA levels for enzymes involved in BCAAs catabolism. We hypothesized that feeding rats with diet rich in linoleic acid (LA), a natural PPARγ agonist modifies mRNA levels for enzymes catalyzing BCAAs degradation in adipose tissue. The current pilot study was aimed at the investigation of the effect of diet rich in LA on mRNA levels for BCATm, branched chain α-keto acid dehydrogenase (E1 component of the BCKDH), and mRNA levels for the regulatory enzymes of BCKDH complex, a specific kinase (BDK) and a specific phosphatase (PPM1K) in epididymal white adipose tissue (eWAT). Wistar male rats were fed with high unsaturated fat diet containing mainly linoleic acid (study group) or with the high saturated fat diet (control group). The relative mRNA levels were quantified by reverse transcription PCR. We have found that in rats fed diet rich in LA mRNA level for BCATm decreased, while mRNA amount for BDK increased. There was no difference between mRNA levels for BCKDH E1 and PPM1K. It is conceivable that changes in mRNA levels for enzymes involved in BCAAs metabolism in eWAT may lead to modification of BCAAs catabolic rate. Further studies are required to fully elucidate this issue.

Keywords: branched chain amino acids (BCAAs), branched chain α-ketoacid dehydrogenase (BCKDH), linoleic acid, adipose tissue, rat.

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Introduction

Essential branched chain amino acids (BCAAs), leucine, isoleucine and valine, exert several functions in the body, the primary one being substrates for protein synthesis. In addition, BCAAs (mainly leucine) act as nutrient signals regulating different metabolic processes such as protein turnover (particularly in skeletal muscles), glucose and lipid metabolism and hormones secretion [1]. Thus, maintaining BCAAs homeostasis is crucial for proper body functions.

The primary mechanism for maintaining body BCAAs homeostasis is regulation of their catabolism. Unlike other essential amino acids, leucine, isoleucine and valine bypass the liver and are predominantly catabolized by extrahepatic tissue, including adipose tissue [2]. Initially, BCAAs catabolism involves the same reactions. At first BCAAs follow reversible transamination catalyzed by the branched chain aminotransferase (BCAT). The products of this reaction are branched chain α-ketoacids (BCKA). There are two known mammalian BCAT isozymes: mitochondrial (BCATm) and cytosolic (BCATc). BCATm is expressed ubiquitously, whereas BCATc is found almost exclusively in nervous tissue [3]. The second step of BCAAs catabolism involves irreversible oxidative decarboxylation of BCKA. This key step in BCAAs metabolism is catalyzed by the branched chain α-ketoacid dehydrogenase complex (BCKDH complex) [4]. Subsequently, each amino acid follows a unique pathway to amphibolic intermediates. Products of BCAAs metabolism can be used for glucose or/and ketone bodies synthesis or can be used as energy source by oxidation to CO_2 and H_2O in Krebs cycle [5].

BCKDH is composed of multiple copies of 3 catalytic subunits. Branched chain α-ketoacid dehydrogenase (E1 subunit) is responsible for the rate limiting step in the overall reaction catalyzed by BCKDH complex. In addition, two regulatory enzymes, a specific branched chain α-ketoacid dehydrogenase kinase (BDK) and a specific protein phosphatase, Mg^{2+}/Mn^{2+} dependent 1K (PPM1K) can be associated with BCKDH complex, depending on the metabolic conditions [5, 6].

Adipose tissue can metabolize substantial amounts of BCAAs [7]. The key transcriptional regulators of metabolic processes in adipose tissue are the peroxisome proliferator-activated receptors (PPAR), mainly PPAR γ [8]. Linoleic acid (LA, 18:2ω6) and other polyunsaturated fatty acids are considered as natural modulators of PPARγ. Essential fatty acids through direct interactions with PPAR act as signal molecules regulating expression of different genes, including enzymes of biochemical pathways [9].

It has been demonstrated that pharmacological activation of PPARγ with thiazolidinediones, synthetic PPARγ ligands that are used as therapeutic agents in the treatment of type 2 diabetes, increased mRNA levels for enzymes of leucine, isoleucine and valine catabolism in white adipose tissue (WAT) [10]. Therefore, we hypothesized that feeding rats with diet rich in LA, a natural PPARγ agonist, modifies mRNA levels for enzymes involved in BCAAs catabolism. The current pilot study was aimed at the

investigation of the in vivo effect of diet rich in LA on mRNA levels for: BCATm, branched chain α-ketoacid dehydrogenase (E1 subunit of BCKDH complex), BDK and PPM1K in white epididymal adipose tissue (eWAT).

Material and Methods

Experimental design

Male albino Wistar rats (n = 10) from inbred strain weighting 151 ± 3 g were purchased from the breeding facility of the Jagiellonian University Faculty of Pharmacy. Animals were multicaged and maintained under standardized conditions of artificial 12 h light/dark cycle at constant room temperature (20 ± 4°C) and humidity (55 ± 10%). The rats were given *ad libitum* access to food and water. The animals were randomized into two groups fed with diets containing 20% fat for 16 days. The only difference between these diets was fatty acid profile. Study group (UNSAT; n = 5) was fed with high unsaturated fat diet containing mainly linoleic acid (78% of all fatty acids) (MP Biomedicals, 960243, USA), while control group (CT; n = 5) was fed with the high saturated fat diet containing primarily lauric acid, myristic acid and stearic acid (75% of all fatty acids) (MP Biomedicals, 960241, USA). The experiment time-frame was chosen based on previous studies on the effect of different diets on BCKDH complex [11, 12]. At the end of experiment rats were sacrificed and eWAT was excised and freeze clamped with aluminum tongs precooled in liquid nitrogen. All samples were stored at –80°C until analysis. Animal experiments were conducted in accordance with the guidelines of the Animal Research Committee and were approved by the Jagiellonian University Ethic Committee.

RNA isolation and RT-PCR

The mRNA relative levels of selected genes were quantified using semi-quantitative reverse transcription PCR (RT-PCR) analysis. Each gene was amplified together with a housekeeping gene β-actin. Frozen eWAT samples were ground into fine powder using a nitrogen-cooled mortar and pestle, homogenized in RNA Extracol (EURx, Poland) and total RNA was extracted according to the manufacturer's protocol. Concentration and the quality of RNA was measured using Cary 100 Bio UV-VIS spectrophotometer (Varian, Australia). RT reaction was performed with High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, USA). Each PCR reaction was carried out with rat-specific primers that were designed for the genes of interest using Primer-BLAST software (NCBI) (Table 1). The PCR products were subjected to agarose gel electrophoresis (2% agarose gel), and the bands were analyzed by densitometry using the Quantity One software (Bio Rad). Obtained data were first normalized to β-actin (in the same sample) and presented as arbitrary units.

Table 1. Primers characteristics.

Gene amplified (product bp)	Primer sequence	Primer concentration (pmol/µl)	Primer annealing temperature (°C)
E1α (191)	5'-TGCTGCCACCTTGGAGTGTCCT-3' 5'-GCCGTCGGGCCTCCTTAGTG-3'	**10** **10**	**65**
PPM1K (229)	5'-GCTGATGCAAGCCTCCTGACCTC-3' 5'-TTACATGGGGCTGTCCCAGGCT-3'	**4** **4**	**65**
BDK (547)	5'-ACATCAGCCACCGATACACA-3' 5'-TGTCTTCATGTAGCGCCAAG-3'	**6** **6**	**60**
BCATm (189)	5'-CAGGACTTGGGGTGAGTTCC-3' 5'-AGTTCCGGCCCATTTTCCAT-3'	**4** **4**	**60**
β-actin (374)	5'-CAGCCTTCCTTCCTGGGTATG-3' 5'-AGGGTGTAAAACGCAGCTCA-3'	**1** **1**	**55**

Statistics

Statistical calculations and graphs were done using the GraphPad Prism version 5.0 (GraphPad Software, Inc., USA). Data are presented as mean ± SD (from two independent experiments). Data were analyzed using Student's t-test for unpaired samples. Acceptable significance level was set at $p < 0.05$.

Results

Effect of diet rich in LA on rats body weight

The body weight of all tested rats increased as compared to the initial body weight. There was no difference in mean final body weight between UNSAT and CT groups (255 ± 8 g vs. 253 ± 4 g).

Effect of diet rich in LA on mRNA for BCATm

Mean mRNA abundance for BCATm was lower in UNSAT rats than in control group (0.93 ± 0.02 vs. 1.15 ± 0.03) (Fig. 1). The difference was statistically significant ($p < 0.0001$).

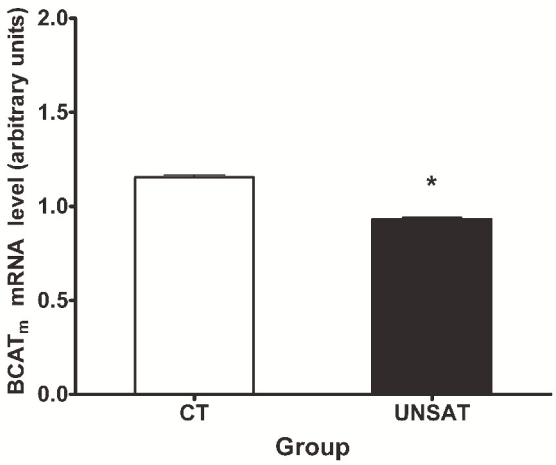

Fig. 1. Effect of diet rich in LA on mRNA level for BCATm in rat's epididymal white adipose tissue. The relative mRNA levels for BCATm was quantitated by RT-PCR then normalized to β-actin mRNA (in the same sample) and expressed as arbitrary units. Data are presented as mean ± SD. UNSAT — study group (n = 5), CT — control group (n = 5). *p <0.0001.

Effect of diet rich in LA on mRNA level for E1 catalytic subunit

There was no difference in mean mRNA level for E1 catalytic subunit of BCKDH complex between UNSAT and CT groups (1.50 ± 0.07 vs. 1.51 ± 0.09; p >0.05) (Fig. 2).

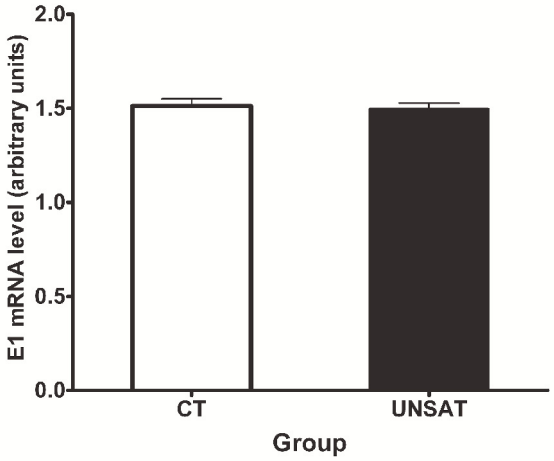

Fig. 2. Effect of diet rich in LA on mRNA level for E1subunit in rat's epididymal white adipose tissue. The relative mRNA levels for E1 was quantitated by RT-PCR then normalized to β-actin mRNA (in the same sample) and expressed as arbitrary units. Data are presented as mean ± SD. UNSAT — study group (n = 5), CT — control group (n = 5).

Effect of diet rich in LA on mRNA levels for BDK and PPM1K

In UNSAT rats BDK mRNA level was higher than in control group (1.41 ± 0.06 vs. 1.25 ± 0.05; p <0.01) (Fig. 3A). Similarly, the level of mRNA for PPM1K increased in response to diet rich in LA (0.33 ± 0.12 vs. 0.38 ± 0.11). However, the difference was not statistically significant (p >0.05) (Fig. 3B).

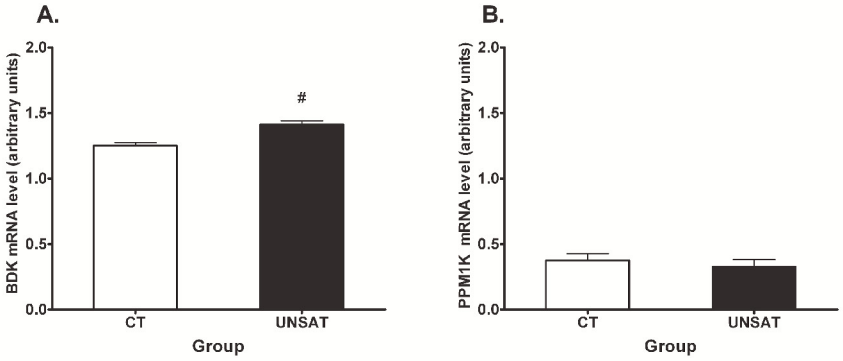

Fig. 3. Effect of diet rich in LA on mRNA level for regulatory enzymes, BDK (A) and PPM1K (B) in rat's epididymal white adipose tissue. The relative mRNA levels for BDK (A) and PPM1K (B) were quantitated by RT-PCR then normalized to β-actin mRNA (in the same sample) and expressed as arbitrary units. Data are presented as mean ± SD. UNSAT — study group (n = 5), CT — control group (n = 5). #p <0.01.

Discussion

It has been demonstrated that pharmacological activation of PPARγ stimulates BCAAs catabolism in adipose tissue. Blanchard *et al.* [13] have found that administration of synthetic PPARγ agonist rosiglitazone to rats fed high fat diet resulted in the increase of mRNA level for genes encoding catalytic subunits of BCKDH complex and have no effect on BDK and BCATm mRNA levels in inguinal WAT (which belongs to subcutaneous white adipose tissue). We have demonstrated that in eWAT (which belongs to visceral white adipose tissue) of rats fed diet rich in LA mRNA level for BCATm decreased. In contrast, mRNA level for BDK increased. There was no difference between mRNA levels for BCKDH E1 catalytic subunit and PPM1K.

Adipose tissue is not a single homogeneous compartment, but rather a tissue with specific regional depots which have different structural organization, morphologic features and metabolic characteristics. It has been shown that the expression of genes for key enzymes of lipid metabolism is related to the morphologic and metabolic differences between adipose tissue depots [14]. Taking into account the results of the present study and those obtained by Blanchard *et al.* [13] it cannot be excluded

that the changes in mRNA levels of genes for BCAAs catabolic enzymes occurring in response to different PPARγ agonists are dependent not only on agonist used but also on the adipose tissue type.

It can be assumed that diet rich in LA affected mRNA level for BDK in eWAT tissue indirectly by changing insulin and adiponectin levels. It has been demonstrated that insulin upregulated BDK mRNA and protein levels in cultured rat liver Clone 9 cells with a concomitant decrease in BCKDH activity [15]. Lai *et al.* [16] have found that LA stimulated insulin secretion from the rat's pancreas. It is possible that the elevation of insulin level results in the increase of BDK mRNA amount in eWAT. Adiponectin is another hormone that regulates BCAAs metabolism by downregulation of mRNA level for BDK [17]. It has been shown that LA inhibited adiponectin secretion from primary rat adipocytes in the presence of insulin [18]. It can be presumed that in rats fed diet rich in LA adiponectin secretion is reduced. In consequence, the mRNA level for BDK in eWAT increases.

The activity of the BCKDH complex is regulated by different mechanisms: by changes in the gene expression of BCKDH complex enzymes, allosteric inhibition and by covalent modification. It is widely accepted that the most important regulatory mechanism is covalent modification achieved by a reversible phosphorylation of E1 subunit catalyzed by BDK and PPM1K [19, 20]. Phosphorylation of E1 subunit of BCKDH complex catalyzed by BDK inactivates BCKDH, while dephosphorylation mediated by PPM1K activates the complex [21]. Different exogenous and endogenous factors that modify the expression of genes for BDK and PPM1K influence BCKDH activity and BCAAs metabolism [22]. Thus, the increase of BDK mRNA amount may lead to inhibition of BCKDH activity. Present results also indicate that rats fed diet rich in LA had lower mRNA level for BCATm. Products of reaction catalyzed by BCAT are substrates for BCKDH complex. Therefore, changes of mRNA levels for BCATm and BDK in response to feeding diet rich in LA may result in inhibition of BCAAs catabolism in rat's eWAT.

BCAAs play crucial role in adipose tissue metabolism. BCAAs catabolism accounts for as much as 30% of lipogenic acetyl-CoA pool that is used for fatty acid synthesis in adipocytes [23]. It has also been shown that leucine acting as regulatory molecule inhibits lipogenesis and promotes lipolysis [24, 25]. We can suspect that one of the consequences of feeding rats diet rich in LA is modification of TG content in adipocytes. Moreover, decrease in BCKDH activity may also result in lower level of 3-hydroxyisobutyrate (3-HIB) which is the metabolite of valine. 3-HIB regulates glucose and fatty acid transport to adipocytes [26]. Reduction of its concentration in adipocytes may affect these processes.

The major limitation of our study was the number of animal included. Because we intended to perform a pilot study we used a small number of rats from inbred strain.

In summary, feeding rats with diet rich with LA changes mRNA levels for some enzymes involved in BCAAs catabolism leading potentially to changes in BCKDH activity. Further studies are required to fully elucidate this issue.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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