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Original Article

Isoleucine and aerobic training regulated the hepatic metabolism in obese mice.

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Abstract

Background: Obesity harmfully affects all physiological functions of the body and public health. Leucine, isoleucine, and valine. Isoleucine could influence protein metabolism, apoptosis, regeneration of hepatocytes, and insulin resistance. Previous studies demonstrated that elevated circulating isoleucine are strongly associated with metabolic disorders, such as obesity, metabolic syndrome, and type 2 diabetes mellitus. This study examines the effect of Isoleucine supplements on hepatic lipogenesis and obesity following an acute bout of exercise in adult mice.

Methods: Thirty male mice of type C57BL/6 were distributed randomly into five groups: Regular diet group, High-fat diet group, Exercise group, Regular diet+20% Isoleucine diet group, and 20% Isoleucine diet+Exercise group. Next, each group was sacrificed and then, the liver was collected; Real-time qPCR investigated the expression of mRNA levels.

Results: Our data indicated that the Exercise group, 20% Isoleucine, and 20% Isoleucine +Exercise groups, significantly amplified the levels of *Ucp2*, *Ppar-γ*, *Ppar*-α, and *Pgc1-α* mRNA compared to the control group. In contrast, the expression level of *Ppar-α* in the high-fat diet group compared to the control group was decreased.

Conclusion: Interestingly, a high-fat diet was due to down-regulated expression levels of *Pgc1-α*, *Ppar-γ*, and *Ucp2* in the liver, but *Ppar-α* increased.

Keywords: Isoleucine, Liver, Aerobic exercise, *Ppar-γ*, *Pgc1*, *Ucp2*

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Introduction

Energy imbalance caused by an increasing rate of caloric intake to energy expenditure leads to obesity (1). Physical exercise increases energy expenditure and has been correlated with improved weight control (2, 3). Obesity is well known as a state of chronic low- grade inflammation, particularly in adipose tissue and liver (4-6). Many studies point to this inflammatory state as an underlying mechanism for the development of insulin resistance in obesity and T2DM (7).

Three of nine amino acids that cannot be synthesized by animals and humans and are essential nutrients are including leucine (Leu), and isoleucine (Ile); therefore, isoleucine have to be obtained from foods (8). Isoleucine play critical roles in the regulation of energy homeostasis (9), nutrition metabolism, gut health, immunity, and disease in humans and animals (10). Abundance studies suggested isoleucine could be biomarkers non-communicable diseases (10). Circulating cytokine levels are increased in obesity and diabetes mellitus (7). Free fatty acids could promote inflammation by indirectly binding to TLR4 and TLR2 through the adaptor protein fetuin-A, resulting in NF-κB (11) and JNK1 activation (12). Once activated, these pathways might increase the synthesis and secretion of chemokines such as monocyte chemoattractant protein–1 (MCP1) from adipocytes or hepatocytes, leading to the infiltration of proinflammatory macrophages (12). Peroxisome proliferator-activated receptors (PPARs) family is thought to be involved in the control of energy homeostasis. Isoleucine-rich diets have shown to be an overexpression of Peroxisome proliferator-activated receptor (*Ppar-γ*) coactivator-1α (*Pgc-1α*), a master regulator of mitochondrial biogenesis (13, 14). Uncoupling proteins (*Ucps*) are also involved in the regulation of energy expenditure in mammals (15). Isoleucine have been shown to up-regulate *Ppar-γ* and uncouple (*Ucp2*), reducing triglyceride concentrations in mouse livers (14, 16). Fatty acid oxidative *Ppar-α* target genes and *Ppar-α* expression are suppressed in obese mice, resulting in hypertriglyceridemia and hypercholesterolemia (17). An abundance of hepatic de novo lipogenesis and resultant abnormal lipid Sedimentation contribute to liver injury in obese individuals (18). Suppression of hepatic lipogenesis improves the metabolic profile and insulin sensitivity (19). They were gradually more concentrated on treating advanced malignancies via inhibition of the dysregulated signaling network, such as the PI3K-AKT-mTOR signal pathway (20, 21). (14).. Several studies have shown that Isoleucine supplementation is effective in downregulating protein metabolism in cirrhosis,

improving nitrogen balance, and finally resulting in better clinical outcomes (22). In this manuscript, we attempt to explore the effect of exercise and isoleucine in liver mice fed high-fat diets.

Material and methods

Thirty young male mice with an average weight of 16 ± 2 g were selected. Four-week-old wild type male C57BL/6 mice were used in this experiment. They were housed in a temperature-controlled room (24 ± 3 °C) with a humidity of 65% (±5%) and 12 hour-light/dark cycle. Male mice of type C57BL/6 were distributed randomly into five groups: standard diet group, high-fat diet group, exercise group, standard diet + 20% Isoleucine diet group, 20% Isoleucine diet + exercise group.

Isoleucine supplementation and food intake mice were fed with free access to standard food and tap water. Isoleucine were dissolved in distilled water and applied by gavage (500μL) once per day for eight weeks (20 mg/ mL/day for 20 ile group).

Ex was played briefly as a type of moderate-high intensity exercise on a treadmill for six weeks (5 days/week). After being acclimated to treadmill exercises over two weeks, running speed and duration of exercise were progressively increased to reach ~ 70% VO2 max. At the same time, exercise training was 45 min. After six weeks of exercise and oral consumption of Isoleucine, mice fasted for six hours before euthanasia. Mice were euthanized under the combined administration of xylazine (10 mg/kg body weight per mouse) and ketamine (80 mg/kg body weight per mouse).

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from the liver using TRIzol reagent (Sigma, USA). DNase treatment was performed with DNase I (TaKaRa) to remove contaminating genomic DNA. cDNA synthesis was performed with 1 μg of total RNA by cDNA synthesis kit according to the producer's instruction (TaKaRa). qRT-PCR was performed with CYBR Green (TaKaRa, Japan) using Step One Plus Real-Time PCR System (Applied Biosystems, United States). Assessment of gene expression was performed according to ΔΔCT method. The expression level of genes was reported comparative to the 18s rRNA expression level as already was implemented in similar studies. Primers were designed by oligo-7 and ordered from micro-gene (South Korea), and their sequences are listed in Table 1.

Table 1. Primer sequence

Gene	Forward primer	Reverse primer	Annealing temperature (°C)	Accession no.
PPAR-γ	TGAGACCAACAGCCTGAC	GTTCACCGCTTCTTTCAAATC	58	NM_001127330.1
PGC1-α	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCTGTTTTC	60	NM_008904.2
UCP2	TTCCTCTGTCTCGTCTTG	TCTGATTTCCTGCTACCTC	60	NM_011671.5
PPAR-α	ACTTGCTCACTACTGTCCTT	ATGCTGGTATCGGCTCAATA	59	Gene ID: 19013
18srRNA	CGGACACGGACAGGATTG	TCGCTCCACCAACTAAGAAC	60	NR_003278.1

Statistical Analysis

Statistical analysis was performed using GraphPad Prism Software (Version 8.0 Graph Pad Software Inc., La Jolla, CA). Kolmogorov–Smirnov test was used for normalizing the distribution, and variables were normally distributed. Results are presented as mean ± standard error of the mean (SEM). Differences at $p < 0.05$ were considered to be significant in all studies.

Result

We measured the levels of mRNA encoding *Ppar-α* and *Pgc1-α* and *Ucp2* in livers of regular diet group, HFD group, Isoleucine group, treadmill-trained group, and isoleucine + treadmill-trained group mice. Thus, we tested the effect of exercise on the expression of the hepatic *Ucp2* gene, which is involved in the regulation of energy expenditure. Isoleucine +Ex significantly increased the levels of *Ucp2* mRNA compared with high fat diet group. These results suggest that exercise stimulates fatty acid oxidation through hepatic *Ppar-α* and *Ucp2* (Fig2A). In addition, Isoleucine +Ex group up-regulated *Pgc1-α*, *Ppar-α*, and *Ppar-γ* mRNA levels in liver tissue (Fig2 B-D). These results could be indicated that biogenesis mitochondrial enhanced by consumption Isoleucine accompanied by exercise.

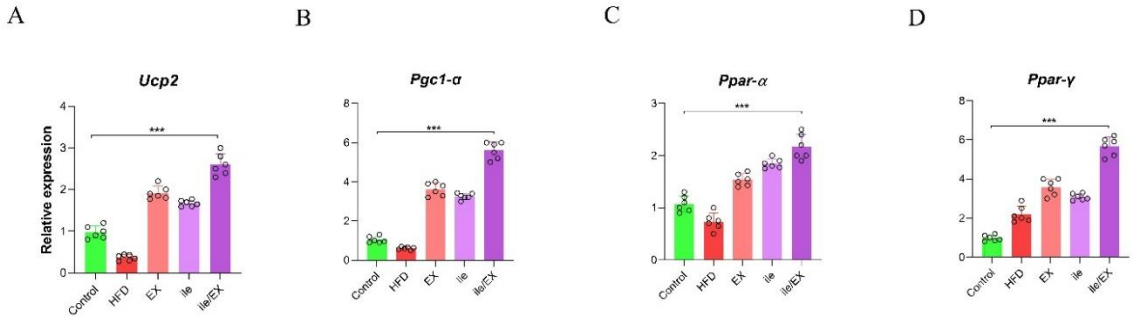

Figure1. Expression level of Ucp2, Pgc1-α, Pparα, and Pparγ

Discussion

Through the involvement of *Ppar-α* and *Ucps* in energy homeostasis, we considered that treadmill training might activate *Ppar-α* and *Ucp2* in the liver, leading to reductions in body weight gain, adiposity, and dyslipidemia caused by leptin receptor deficiency in male mice. Simultaneously, hepatic mRNA levels of peroxisome proliferator-activated receptor α (*Ppar-α*) target enzymes responsible for mitochondrial and peroxisomal fatty acid β-oxidation were significantly increased by moderate-high intensity exercise on a treadmill. Furthermore, mRNA levels of uncoupling protein 2 (*Ucp2*) in the liver were also markedly increased by treadmill training. D'Antona *et al.* described the Isoleucine.

Supplement diet in mice were correlated with increased mitochondrial biogenesis in the fat or liver. Our results confirmed such a concept of exercise could increase the expression level of *Ppar-γ*, *Ppar-α*, *Pgc1-α*, and *Ucp2* in liver tissue. In this study, the significance of dietary isoleucine and exercise in the association between circulating I Isoleucine indexes and obesity or T2DM risk was investigated. We discovered 20% Isoleucine diet remarkably was inversely and positively in the relationship with overweight/obesity and T2DM risk, respectively. Furthermore, recently limited reviews show the possible effects of the Isoleucine supplementations diet on circulating Isoleucine profiles (8, 23-30). However, investigations on the long-term effects of the Isoleucine diet on the circulation of Isoleucine factors are essential to confirm these results better. The significant result of our examination was that tissue fat mass is increased in the control group compared with the 20% Isoleucine diet group.

In a previous study, the activation of PPAR- α was found to prevent the high fat diet-induced increase in body weight and adipose tissue mass without influencing food intake, and also that insulin resistance was improved by the same treatment concomitantly. In contrast, knockout of *Ppar- α* in mice was shown in the further report to become obese when fed high-fat diet (31). These findings point up the significance of our present data on the effects of ISOLEUCINEon *Ppar-* α and the related molecular parameters for lipid mobilization, including fatty acid oxidation, in WAT skeletal muscle, and liver(31-34).

Competing interests

There is no competing of interest to disclose

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