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Research Article

Effect of Cinnamon Hydro Alcoholic Extract on Ucp3 Expression in Adipose Tissue of High Fat Dietary Obese Male Wistar Rats

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Abstract

UCPs are the mitochondrial inner membrane proteins, which their primary role in the mitochondria is to guide hydrogen ions into the matrix. Ucp3 is a member of UCP family and is one of the proteins that is in muscle and adipose tissue, helping metabolism and fat oxidation by increasing in proton transfer and frees up energy and generates heat and adjust the level of basal metabolism then it could regulate adipose tissue construction and fat mass accumulation.

Goal: In this study the effect of cinnamon hydro alcoholic extract on Ucp3 gene expression in adipose tissue has been discussed in order to obesity prevention.

Materials and methods: 42 adult male Westar rats divided into 6 groups of 7 including control group, the high-fat diet group that was receiver of 1/5 mgKg-1 of high-fat diet, the experimental group that in addition to the high-fat diet respectively received 50, 100, 200 mgKg-1 doses of cinnamon extracts and the sham group recipient of 200 mgKg-1 of the cinnamon extract. All rats in this period freely had rodent's food and water. After 6 weeks mice were dissected and RNA extracted from adipose tissue, cDNA synthesized Ucp3 gene expression level was examined by quantitative real-time PCR.

Discussions and conclusion: In adipose tissue of Obese group a significant decrease in UCP3 gene expression were observed, treatment with 50mgKg-1 cinnamon extract in experimental group increased UCP3 expression level and as we investigated the effect of cinnamon extract was dose dependent and only in low dose significant increased level were observed.

Keywords: Cinnamon hydro alcoholic extract; High-fat diet; Obesity; Ucp

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Introduction

Obesity is one of the important problems menace public health in all around the world. By understanding molecular base of obesity synchronous with epidemiologic study could provide the best opportunities for Obesity prevention [1].

Obesity occurs by white adipose tissue mass growth, with body weight gains and increased BMI (body mass index) but adipose tissue in both lean and Obese individuals is an important organ that regulates metabolism and some other physiological effects [2]. White adipose tissue mitochondria involve in regulation of whole- body energy homeostasis [3].

Uncoupling proteins (UCPs) are mitochondrial transporters, they regulate Oxidative phosphorylation by proton transportation from mitochondrial membranes and adjust energy metabolism and thermogenesis [4].UCP3 is one of the important proteins of UCP family could increase mitochondrial membrane proton leak and it could decrease production of reactive Oxygen and influence insulin secretion, lipogenesis and energy consumption [5].UCP3 expressed in skeletal muscle, cardiac muscle and white and brown adipose tissue and it act more in regulation of cellular fatty acid metabolism rather than to mitochondrial uncoupling of oxidative phosphorylation then it is one of the molecular factors in weight control and fat mass accumulation related to Obesity [6].

Cinnamon is one of the ancient medical herbs that used for treatment of some metabolic disorders like diabetes and obesity. Evidences shown that cinnamon extract improve glucose and lipid metabolism [7]. Cinnamon betterment insulin action and could increase glucose metabolism up to 20-fold in adipose tissue [7].

In Obese mice models with high-calorie feeding Cinnamon extract could be improved insulin resistance and lipid metabolism [8]. Cinnamon changed expression level of some genes that involved in fat metabolism and insulin signaling in adipose tissue [9].

In this study we examined cinnamon extract and obesity effects on UCP3 gene expression in omental white adipose tissue of high fat dietary obese male Westar rats.

Methods

Rats and Diets

42 Male Wistar rats (6-8 weeks old weighing approximately 150–200 g) were purchased and maintained in the Central Animal House, Zanjan Islamic Azad university biology research center. The animals

were housed under controlled temperature(25°C) and health conditions by 12h dark and 12h light cycle. Before initiation of the dietary treatments, the rats were allowed a 1-week acclimation period.

All experimental protocols were approved by the Biosafety and Ethics Committee of the Zanjan Islamic Azad University Research Center.

Research managed on 42 male Wistar rats were randomly divided into 6 experimental groups: The control group with a normal diet, three experimental groups received a high-fat diet and Cinnamon 70% hydro alcoholic extract in 50mgKg^{-1}, 100mgKg^{-1} and 200mgKg^{-1} doses.

Obese group received high-fat diet and Sham group received normal diet and 200mgKg^{-1} cinnamon extract. All groups treated for 6 weeks with oral gavage of high-fat emulsion as 1.5ml (Table 1) once per day and Cinnamon hydro alcoholic extract. Food and water intakes and body weight were controlled daily for evaluation of Rats health.

components	Weight in emulation
corn oil	400 g
Sucrose	150 g
milk powder	80 g
Cholesterol	100 g
Tween 80	36.4 g
Propylene glycol	31.1 g
multi vitamine	2.5 g
Salt	10 g
Distilled water	300 g

Table 1: The composition of high-fat emulation.

At the end of the experimental period, the rats were fasted for 12 h, anesthetized with Somnopentyl® (pentobarbital sodium 64.8mg/mL solution), and euthanized by total blood collection from the left ventricle. The adipose tissue was collected, weighed and immediately used for RNA extraction. Cinnamon 70% Hydroalcoholic extract was ordered from Iranian biological resource center, Karaj, Iran.

Gene expression

Total RNA was isolated from adipose tissue using RNX kit (Cinnagene). RNA samples concentration was determined using biophotometr Spectrophotometer (eppendorf). Messenger RNA was reverse transcribed into cDNA using a Takara cDNA Synthesis kit (Takara) according to manufacturer's instructions. cDNA was quantified using the biophotometr Spectrophotometer to ensure equivalent concentrations for real-time analysis. Quantitative real-time polymerase chain reaction was used to validate a subset of differentially expressed genes. Gene-specific primers were designed using the Primer3 program (http://frodo.wi.mit.edu/). Primer design criteria included a base-pair length of 100 to 200 and a guanine-cytosine (GC) content of 40% to 60%. Primers were designed to span exon/intron boundaries where possible and were tested using the same cDNA sample pool, to ensure that there was no genomic contamination. We used UCP3 f 5`- GACTCACAGGCAGCAAAGGAA- 3` and UCP3 r 5`- GAGGAGATCAGCAAAACAGGC-3` specific primers that produced 131bp products and Rpl13 a primers F 5`- GGATCCCTCCACCCTATGACA-3` and R 5`- CTGGTACTTCCACCCGACCTC-3` as refrence gene specific primers.

Real-time PCR analysis was performed using SYBR Green (Ampliqon- Danmark). Each 10 µLreaction contained 2X SYBR Green Master Mix I, 0.5 µmol/L gene specific forward and reverse primers, and 100 ng cDNA. Polymerase chain reaction analysis was accomplished using the Rotor-Gene Q PCR System with the following cycle conditions: 95°C for 15 minutes followed by 40cycles of 95°C for 10 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. A melt curve analysis confirmed the amplification of a single cDNA product. Relative quantification of UCP3, toward the samples, were calculated by using the comparative2$^{-\text{ delta-delta-Ct}}$ method. Rpl13a is the most stable reference genes for adipose tissue. Statistical tests of differential expression were conducted using the moderated t test.

Results

In our study mean body mass index in sham which was only treated with a dose of 200mg/kg Cinnamon Was significantly decreased also the ultimate weight gain after 6 weeks treatment with high-fat diet alcoholic extract of cinnamon the weight difference is significant between all groups. To the group of Receiving high fat diet and C200 has a significant weight gain (P=00/0) compared to the control group C100;C50 and sham groups were decreased significantly (P = 00/0) compared to controls and this indicates that cinnamon at a dose of 200 mg/kg does not weight reduction And vice versa can also cause weight gain but the doses of 100 mg/kg and 50 mg/kg in the experimental group and dose 200 mg/kg in sham groups had a significant effect on rats weight loss.

According to statistical analysis of adipose tissue weight difference between the groups after 6 weeks of treatment with high-fat diet and alcoholic extract of cinnamon adipose tissue weight in Ob and C200 groups than the control group did not significantly increase. Also in the C100 and C50 and sham groups there was no significant weight loss and fat tissue. According to statistical analysis to examine the difference in Ucp3 gene expression in adipose tissue between the groups after 6 weeks of treatment with high-fat diet and alcoholic extract of cinnamon Ucp3 gene expression in the sham group and receiving the high-fat diet group was significantly lower than the control group (P=0/01) .But the reduction in ucp3 gene expression compared to control group was not significant in the C200 group (P=0/5). But the increase in Ucp3 gene expression between the C100 group (P=0/02) and C50 group (P=0/00) Compared to the control group was statistically significant.

Discussion

Uncoupling proteins (UCPs) facilitate proton transport inside the mitochondria and decrease the proton gradient, leading to heat production [10]. The uncoupling proteins 2 and 3(UCP2 and UCP3) are probable candidates for underlying the variability in human energy metabolism. The uncoupling protein genes encode for mitochondrial protein carriers, which uncouple mitochondrial respiration from ATP production and thus stimulates heat production [11].In a study that was conducted in 2001 on Regulation of UCP1, UCP2, and UCP3 mRNA Expression in Brown Adipose Tissue, White Adipose Tissue, and Skeletal Muscle in Rats by Estrogen Showed that In WAT, both estrogen-deficient groups had significantly lower UCP2 mRNA expression compared to the control rats and estrogen- treated rats; In contrast, the UCP3 mRNA expression in WAT was similar in all four groups. Finally, in skeletal muscle the OVX group on mild energy restriction had reduced UCP3 mRNA expression compared to control, OVX, and estrogen-treated rats [12]. In a study that was conducted in

1998 on Role of UCP homologues in skeletal muscles and brown adipose tissue: mediators of thermogenesis or regulators of lipids as fuel substrate? Showed that In IBAT (a tissue highly dependent on lipids for thermogenesis), the pattern of mRNA expression of UCP2 and UCP3 closely follows that of UCP1: it was markedly down- regulated during food deprivation (when this tissue's thermogenesis and lipid fuel requirements are decreased) and restored to control levels by day 5 of refeeding. Together, these tissue-dependent differential mRNA expressions of the UCP homologues in IBAT during food deprivation and refeeding are much more consistent with a role for UCP2 and UCP3 in the regulation of lipids as fuel substrate rather than as mediators of regulatory thermogenesis [13]. In a study that was conducted in 1997 on UCP3: An Uncoupling Protein Homologue Expressed Preferentially and Abundantly in Skeletal Muscle and Brown Adipose Tissue showed that UCP3 is distinguished from UCP1 and UCP2 by its abundant and preferential expression in skeletal muscle in humans, and brown adipose tissue and skeletal muscle in rodents. Since skeletal muscle and brown adipose tissue are believed to be important sites for regulated energy expenditure in humans and rodents, respectively, UCP3 may be an important mediator of adaptive thermogenesis. Since UCP3 is minimally expressed in human heart and other critical organs, it is a promising target for anti-obesity drug development aimed at increasing thermogenesis [14, 15]. In this study, the group receiving the high-fat diet Ucp3 gene expression in adipose tissue significantly decreased and in groups that are receiving high-fat diet and treated with alcoholic extract of Cinnamon had led to Ucp3 gene expression significant increase in the dose of 100 and 50 cinnamon.

Conclusion

Laurence and et al By studying the Increased Uncoupling Protein-2 and -3 mRNA Expression during Fasting in Obese and Lean Humans in 1997 Concluded that UCP2 and – 3 mRNA levels were not correlated with body mass index (BMI) in skeletal muscle, but a positive correlation ($r=0.55$, $P<0.01$, $n=22$) was found between UCP2 mRNA level in adipose tissue and BMI. The effect of fasting was investigated in eight lean and six obese subjects maintained on a hypocaloric diet (1,045 kJ/d) for 5 d. Calorie restriction induced a similar 2-2.5-fold increase in UCP2 and – 3 mRNA levels in lean and obese subjects.

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