PINE NUT OIL INTAKE UPREGULATES mRNA EXPRESSION OF UNCOUPLING PROTEIN 2 (UCP2) IN BROWN ADIPOSE TISSUE OF HIGH FAT DIET-FED MICE

Le Ngoc Hoan1,*, Duong Thi Anh Dao1, Le Thi Tuyet1, Nguyen Thi Hong Hanh1, Ho Thi Hong Van2

Abstract. Thermogenesis plays an important role in body energy homeostasis and thus contributes in the progression of a high-fat diet-induced obesity and obesity-related metabolic disorders. In the current study, we researched the effect of pine nut oil (PN) extracted from Korean pine (Pinus koraiensis) on the expression of several thermogenic genes in brown adipose tissue (BAT) of the high-fat diet (HF)-fed mice. Mice were fed a HF consisted with 10% calorie from lard and 30% calorie from pine nut oil (HF-PN) or and 30% calorie from soybean oil (HF-SB) for 12 weeks. As a result, the HF-PN-fed mice showed a significantly increase in mRNA expression of uncoupling protein 2/UCP2 (a key marker of thermogenesis) in BAT of the mice compared to that of the HF-SB-fed control mice. mRNA expression of uncoupling protein 3/UCP3 and nuclear respiratory factor-1/NRF-1 (a key marker of thermogenesis and a key mediator of mitochondrial biogenesis, respectively) was mildly higher in the BAT of the HF-PN-fed mice than that of the HF-SB-fed control mice. However, this change was not significantly different. Together, these data suggested that PN has a possible effect on induction expression of UCP2 that enhances thermogenesis in BAT and thus, contributing to inhibit the HF-induced obesity.

Keywords: Brown adipose tissue, high-fat diet, NRF-1, pine nut oil, UCP2, UCP3.

1. INTRODUCTION

Increasing the incidence of obesity and its related metabolic disorders (such as cardiovascular diseases, liver diseases, and diabetes) has been recognized as a considerable issue of the modern world (Saklayen, 2018). High calorie food intake and low energy expenditure are main factors leading to overweight and obesity. Thus, strategies to lowered food intake and/or increased energy expenditure play important roles in the combat against obesity and its related metabolic diseases (Caudwell, Gibbons, Finlayson, Näslund, & Blundell, 2013). Classically, there have been studies about increased feeling of satiety to reduce food intake. For example, the peptide YY (PYY), is a gut hormone that regulates feeling of satiety in the brain, has been shown to have roles in controlling obesity. Mice without PYY are hyperphagic and develop obesity. In contrast, increased plasma PYY in mice reduces diet-induced obesity (Karra, Chandarana, & Batterham, 2009).
In another hand, recent studies have focused on effects of molecules or food nutrients on increased energy expenditure and also shown marked effectiveness on reduction of obesity and its related metabolic dysfunctions. Unsaturated fats such as omega 3 and 6 supplementation increased energy expenditure and reduced metabolic disorders (D’Angelo, Motti, & Meccariello, 2020). Notably, pine nut oil (PN) extracted from Korean pine (Pinus koraiensis) has been recently recognized as a plenty of pinolenic acid source, an unsaturated fat, (Pasman et al., 2008) and thus, PN may have protective effect on high-fat diet-induce obesity and its related metabolic disorders. As a consequence, in the present study, we fed mice with a high-fat diet containing PN or a high-fat diet containing soybean oil (SB) and tested the mRNA expression of genes, including uncoupling protein 2 (UCP2), uncoupling protein 3 (UCP3) and nuclear respiratory factor-1 (NRF-1) which are important molecules regulating thermogenesis, mitochondria biogenesis, as well as controlling energy expenditure.

2. MATERIALS AND METHODS

2.1. Animals and Diets

Five-week-old male C57BL/6 mice were fed a high-fat diet containing pine nut oil (30 % energy from PN + 15 % energy from lard, HF-PN) and the control mice were fed a high-fat diet containing soybean oil (30 % energy from SB + 15 % energy from lard, HF-SB). The mice were arbitrarily fed with the food and water. After 12 weeks of the feeding, the animals were sacrificed and brown adipose tissues were dissected. The mice and PN were in Korea.

2.2. Quantitative real time PCR (qRT-PCR)

Total RNA was extracted from each 20 mg brown adipose tissue sample. Each two-microgram sample of RNA were reverse transcribed to cDNA using M-MLV reverse transcriptase. Real-time PCR (RT-PCR) amplification was performed on a Thermal Cycler Dice Real Time system using SYBR premix Ex Taq (TaKaRa bio Inc). The samples were hold at 95 °C for 10 s, followed by 45 cycles of 95 °C for 5 s and 60 °C for 30 s. The results were read with Real Time System TP800 software, and all values were normalized to the levels of the control gene β-actin. The primers are shown in Table 1.

Table 1. Mouse primers used for qRT-PCR tests

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer (5’ → 3’)</th>
<th>Reverse primer (5’ → 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-actin</td>
<td>CATCCGTAAAGACCTCTAGGCAAC</td>
<td>ATGGGACAACCCGATCCACA</td>
</tr>
<tr>
<td>UCP2</td>
<td>ACCGAAGGCACACATGGAC</td>
<td>AGGATAGCCAGGCTACAGAGA</td>
</tr>
<tr>
<td>UCP3</td>
<td>CTGAAGATGGTGTCAGAGA</td>
<td>CGCAGTACCTGGACTTTCATT</td>
</tr>
<tr>
<td>NRF-1</td>
<td>GACCTTGGCCACAGGCAGTTAA</td>
<td>CGCCTGCTCCATGAACACTC</td>
</tr>
</tbody>
</table>

2.3. Statistical analysis

The results were shown as means ± standard error of the mean (SE). Variables were compared by using Student’s t test. The P values < 0.05 were named as significant differences in comparisons. Data are analyzed by using Excel.
3. RESULTS AND DISCUSSION

3.1. The effect of Pine nut oil (PN) on expression of UCP2 mRNA in Brown adipose tissue

Uncoupling protein 2 (UCP2) is an important protein of mitochondria. This molecule increased proton flow through inner membrane of mitochondria to produce heat. Thus, UCP2 markedly contribute to regulation of thermogenesis (Faggioni, Shigenaga, Moser, Feingold, & Grunfeld, 1998). Firstly, we check whether pine nut oil (PN) induced expression of UCP2 in brown adipose tissue (BAT) of the HF-fed mice. As shown in Figure 1A and 1B, expression of UCP2 mRNA was significantly increased in BAT of the HF-PN-fed mice compared to that in BAT of the HF-SB-fed control mice. Because, BAT is the most important site of thermogenic regulation in the body, therefore, an increase in UCP2 mRNA expression in BAT of the HF-PN-fed mice shows a potential positive effect of PN on thermogenesis.

Figure 1. Effects of pine nut oil (PN) on expression of UCP2 mRNA in BAT. Real time RT-PCR analysis for expression of UCP2 mRNA. Levels of UCP2 mRNA were normalized to levels of β-actin mRNA. (A) data analysis of UCP2 mRNA levels. (B) comparison of UCP2 mRNA levels. Data are presented as means ± SE; n = 5 in each group. *P < 0.05 compared between the HFD-PN- and HFD-SB-fed mice

3.2. The effect of Pine nut oil (PN) on expression of UCP3 mRNA in Brown adipose tissue

Beside UCP2, UCP3 (Uncoupling protein 3), is also well-known as a key regulator of mitochondrial thermogenesis (Azzu, Jastroch, Divakaruni, & Brand, 2010). Thus, the next examination was to investigate whether PN also induced expression of UCP3 mRNA in BAT of the HF-fed mice. The present data showed that the expression of UCP3 mRNA in BAT of the HF-PN-fed mice is tendency higher than that of the HF-SB-fed control mice (Figure 2A and 2B). However, this comparison is not significantly different. UCP3 has known as to be highly expressed in skeletal muscle tissues and it importantly contributes to skeletal energy metabolism. In contrast, UCP3 may not strongly affect energy metabolism in BAT (Brauner et al., 2003). Consistent with this, PN enhances thermogenesis in BAT of the HF-fed mice may be via increased expression of UCP2 rather than UCP3.
Figure 2. Effects of pine nut oil (PN) on expression of UCP3 mRNA in BAT. Real time RT-PCR analysis for expression of UCP3 mRNA. Levels of UCP3 mRNA were normalized to levels of β-actin mRNA. (A) data analysis of UCP3 mRNA levels. (B) comparison of UCP3 mRNA levels. Data are presented as means ± SE; n = 5 in each group. n.s. is not significant between the HF-PN- and HF-SB-fed mice

3.3. The effect of Pine nut oil (PN) on expression of NRF-1 mRNA in Brown adipose tissue

Figure 3. Effects of pine nut oil (PN) on expression of NFR-1 mRNA in BAT. Real time RT-PCR analysis for expression of NFR-1 mRNA. Levels of NFR-1 mRNA were normalized to levels of β-actin mRNA. (A) data analysis of NFR-1 mRNA levels. (B) comparison of UCP3 mRNA levels. Data are presented as means ± SE; n = 5 in each group. n.s. is not significant between the HF-PN- and HF-SB-fed mice

Following forementioned data, we then asked whether PN affect expression of nuclear respiratory factor-1 (NRF-1) which is also a crucial regulator of mitochondrial biogenesis (Kiyama et al., 2018). As shown in Figure 3A and 3B, expression of NRF-1 mRNA in BAT of the HF-PN fed mice was not significantly higher than that of the HF-SB fed control mice. It is worth to note that NRF1 works as a transcription factor controlling expression of several nuclear genes as well as of mitochondrial factors (Evans & Scarpulla, 1990). Moreover, PGC1α, a master regulator, triggers mitochondrial biogenesis and respiration through induction of NRF-1 gene expression. In addition, PGC1α enhances expression of UCP2 gene (Barbe et al., 2001). Since NRF-1 works as a co-regulator of
UCP2, increased UCP2 mRNA expression in BAT of the HF-PN fed mice may be contributed by at least partly upregulation of NRF-1 expression.

4. CONCLUSIONS

Obesity is often caused by imbalance of food intake and energy expenditure. Here, the current study shown that PN significantly upregulated expression of UCP2 mRNA level and partly upregulated expression of UCP3 and NRF-1 mRNA levels in BAT of the HF-PN fed mice compared to those in the HF-SB fed control mice. Because, UCP2, UCP3 and NRF-1 are important molecules that enhance mitochondrial oxidative phosphorylation leading to increases in consumption of nutrients (such as glucose and fatty acids) and production of heat. Therefore, PN would have potential effect(s) on protection against obesity and its related metabolic disorders. And further in vitro studies about effect of PN on thermogenesis should be needed to show clearer mechanism(s).

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REFERENCES


BẢO CÁO KHOA HỌC VỀ Nghiên cứu và Giảng dạy Sinh học ở Việt Nam


BỔ SUNG DẦU HẠT THÔNG TRONG CHẾ ĐÔ ĂN LÀM TĂNG BIỂU HIỆN MỨC mRNA CỦA PROTEIN KHÔNG BẮT CAPPING 2 (UCP2) Ở MÔ MÔ NÀU CỦA CHUỘT ĂN CHẾ ĐÔ ĂN GIÀU CHẤT BÉO

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Tóm tắt. Sự sinh nhiet động và trở quan trọng trong sự cần bằng năng lượng của cơ thể và do đó có tác động lên quá trình gay béo phi của chế độ ăn giàu chất béo và các rối loạn chuyển hóa liên quan béo phì. Ở nghiên cứu này, chúng tôi kiểm tra tác động của dầu hạt thông được chiết từ hạt quả thông Han Quốc (Pinus koraiensis) lên sự biểu hiện của một số gen điều hòa sinh nhiet ở mô mỡ nâu (BAT) của chuột ăn chế độ ăn giàu chất béo (HF). Chú trọng cho ăn chế độ ăn HF chứa 10% năng lượng từ mô mỡ lớn và 30% năng lượng từ dầu hạt thông (HF-PN) hoặc chế độ ăn đối chứng chứa 10% năng lượng từ mô mỡ lớn và 30% năng lượng từ dầu dầu nành (HF-SB) trong 12 tuần. Kết quả cho thấy nhom chuột HF-PN thầy hiện tăng lên có ý nghĩa thống kê lúc mRNA của UCP2 (marker chi khoa của sự sinh nhiet) ở mô mỡ nâu khi so sánh với nhom HF-SB đối chứng. Mức mRNA của UCP3 và NRF-1 (marker chi khoa khác của sự sinh nhiet và yếu tố điều hòa chi khoa của sinh học ti thể, theo thuyết) có sự tăng lên nhẹ ở BAT của nhom chuột HF-PN so với nhom đối chứng. Tuy nhiên, sự tăng lên này không có ý nghĩa thống kê. Như vậy, những số liệu này cho thấy PN có tác động tiềm năng lên sự biểu hiện của UCP2 nên làm tăng sự sinh nhiet ở BAT và có thể giúp ức chế sự béo phi gây ra bởi HF.

Từ khóa: Chế độ ăn giàu chất béo, dầu hạt thông, NRF-1, mô mỡ nâu, UCP2, UCP3.

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