Conference Paper

Blunted Expression of PPARa in Mice with FABP-4 and -5 Deficiency under Acute Cold Exposure

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Abstract

Brown Adipose Tissue (BAT) is a nonshivering thermogenesis organ during cold exposure. Peroxisomal proliferator activated receptor alpha (PPARa) is the member of the nuclear hormone receptor superfamily and primarily expressed in BAT and liver. PPARa is coordinated with uncoupling protein 1 (UCP1) to regulate fatty acid metabolism in BAT. Fatty acid binding protein (FABP)-4 and-5 play role in adaptive response under fasting and cold exposure. The purpose of this study was to investigate the expression of PPARa in mice with FABP4/5 deficiency (DKO). Wildtype (WT) and DKO mice were exposed to cold for 2 hours under fed or 20 hours-fasted conditions. BAT was collected and further mRNA level of PPARa was examined using quantitative real-time PCR. As the result, PPARa gene expression in WT mice were increased 50% and 100% in fed and fasted condition respectively after cold exposure. There was no alteration in PPARa expression in BAT of DKO mice. As conclusion, The functional FABP-4 and -5 are necessary to modulate PPARa gene expression in Brown Adipose Tissue under acute cold exposure.

Keywords: Acute cold exposure; FABP4; FABP5; Fasting PPARa.

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1. Introduction

Under cold environment, mammals activate a system to maintain body temperature in order to survive. Thermogenesis refers to the metabolic process to produce heat from two organs, brown adipose tissue (BAT) and skeletal muscle. BAT, a nonshivering thermogenesis, is distributed in mouse body, including interscapular, periaortic, perirenal and intercostal region [1]. Skeletal muscles increase the metabolic heat by involuntary and rhythmic contraction, known as shivering thermogenesis organ [2]. Coordination of these organs is necessary to maintain metabolic process in vital organ during cold exposure.

The ability to produce heat in BAT is facilitated by activating uncoupling protein 1 (*UCP1*) gene. In the most of the tissues, ATP is formed from the oxidative process of substrates. Although BAT consumes a large amount of glucose, triglycerides and free fatty acid during cold induction, only less amount of ATP is produced due to the low capacity of ATP synthase [3]. In this condition, an excessive amount of protons is accumulated in the outer mitochondrial membrane. Activated UCP1 promotes the free-flow of protons across the inner mitochondrial membrane, resulting in the rapid dissipation of chemical energy as heat [4]. As the results, activated BAT can produce 300 times of heat energy compare to most of the tissues5.

Peroxisomal proliferator activated receptor alpha (PPARa) is a member of the nuclear hormone receptor super family and primarily expressed in BAT and liver [6]. PPARa is involved in regulating mitochondrial and peroxisomal fatty acid oxidation [6a]. In brown adipocytes, coordination between carbohydrate response element binding protein (ChREBP) and PPARa plays an important role in the regulation of lipogenesis [7]. PPARa is also coordinated with UCP1 to regulate fatty acid metabolism [8]. PPARA-null mice shown maladaptive response in fasting condition, such as excessive fatty liver, severe hyperglycemia, hyperketonemia and hypothermia [6a]. PPARa deletion also reduce the expression of *UCP1* gene under basal or cold temperature [9]. Thus, the expression of *PPARα* is necessary to maintain fatty acid metabolism in the BAT.

Fatty acid binding proteins (FABPs) are 14-15 kDa proteins that bind hydrophobic ligands, such as fatty acid, and facilitate several biological process (See review [10]). Among of FABPs isoforms, adipocyte FABP is the best-known to play role in metabolic and immunology processess. Another FABPs isoform, Epidermal FABP (E-FABP, FABP5, mal1), has similarity in 52% of amino acid sequence, tissue distribution, and selectivity and affinity in fatty acid bound with FABP4. Thus, under single knock-out condition,

FABP4 and FABP5 can compensate each other. To study the real function of FABP4 and FABP5, FABP-4 and -5 double knockout mice (DKO) was established [11].

We previously proposed an important role of FABP-4 and-5 expression in capillary endothelium of heart and red skeletal muscle to facilitate fatty acid transport from circulation into tissue [12]. Deletion of FABP-4 and -5 inhibits fatty acid uptake and robustly increases glucose uptake in heart and red skeletal muscle. DKO mice also shown the maladaptive response in prolonged fasting and acute cold exposure [3c, 13]. The purpose of current study is to investigate the expression of PPARa in BAT of DKO mice under acute cold exposure and fasting.

2. Materials and Methods

2.1. Animal Experiment and Cold Tolerance Test

Cold tolerance test was conducted as previously described [3b, 13a]. All study protocols were approved by The Institutional Animal Care and Use Committee (Gunma University Graduate School of Medicine). Mice were placed in air circulation and 12 hours day-light/night cycle controlled-room. Before the experiment, mice were habituated for seven days and gave fed and water ad libitum. Fed or overnight fasted wild-type (WT) mice and FABP4/5 DKO mice were caged individually in the temperature-controlled room or 4˚c-controlled room for two hours. At the end of the test, mice were sacrificed, BAT was collected and stored in -80˚c.

2.2. Gene Expression Measurement and Statistical Analysis

The total RNA from BAT was isolated using the RNAiso Plus reagent (Takara, Japan). Semi-quantitative RT-PCR was performed with an RT-PCR kit (Takara, Japan) according to the manufacturer's protocol. Quantitative real-time PCR was performed using the SYBR Green PCR Master Mix (Applied Biosystems) according to the manufacturer's instructions. The expression level of *PPARα* was normalized to the mRNA level of TATA-binding protein (*TBP*). The primer sequences of PPARa and TBP are previously described [3c, 13a].

Results were analyzed using one-way ANOVA for samples in fed and fasted groups separately. Bonferroni's posthoc multiple comparison tests were performed to evaluate the differences between the control and experimental groups. The data are presented as the means±S.E A p-value <0.05 was considered as statistically significant.

3. Result and Discussion

Mice were caged in room temperature or cold room (4°C) for two hours, with fed ad libitum or fasted overnight. At the end of the experiment, mice were sacrificed and BAT was collected and checked for the gene expression of *PPARα*. The result is showed in Figure 1.

The expression of *PPARα* in BAT was comparable in basal condition. After 2 hours acute cold induction, the expression *of PPARα* in BAT of WT mice were 1.5 times higher compare to the basal condition. On the other hand, there was no different in PPARa gene expression even after cold induction.

The expression of *PPARα* after overnight fasting was lower in all groups. It was 80% lower in WT mice under room temperature compare to WT mice under fed ad libitum. This expression was comparable with DKO mice under the same condition. After cold induction for two hours, the expression of *PPARα* in BAT of WT mice was almost three times higher compare to DKO mice.

Our study showed that the availability of energy substrates influence the expression of PPARa in BAT of mice. The pattern of PPARa is different for each organ. For example, *PPARα* gene expression 4 times higher in the liver of 24 hours-fasted mice [13a]. Fasting promotes energy storage breakdown, including glycogen and lipid droplet in the liver and lipid droplet in adipose tissue. It also enhances production of new energy substrate, including glucose from gluconeogenesis and ketone bodies from partial fatty acid oxidation. The enhancement of PPARa in the liver is important to ensure the process of energy storage breakdown and/or energy substrate production. In our study, the expression of *PPARα* in BAT of WT mice was decreased after 20 hours fasting. After cold induction, it increased significantly both under fed or fasted condition. This result indicates lipid in BAT is not utilized although the breakdown of energy substrate storages is massively enhanced during fasting condition. the weight and triglyceride concentration of BAT is not significantly different before and after fasted [3c]. We speculated that the reduction of *PPARα* gene expression prevents lipid breakdown in BAT. In the other words, lipid in BAT is exclusively used to generate heat and not to fulfill energy demand during fasting.

The expression of *PPARα* gene in BAT of DKO mice was not different with WT mice. It is also reduced after 20 hours fasted. Considering the simillar BAT weight and other parameters between WT and DKO mice, it is suggested there is no disturbance in function or capability to maintain body temperature under basal condition. However, acute cold induction fails to increase the *PPARα* expression, both under fed or fasted

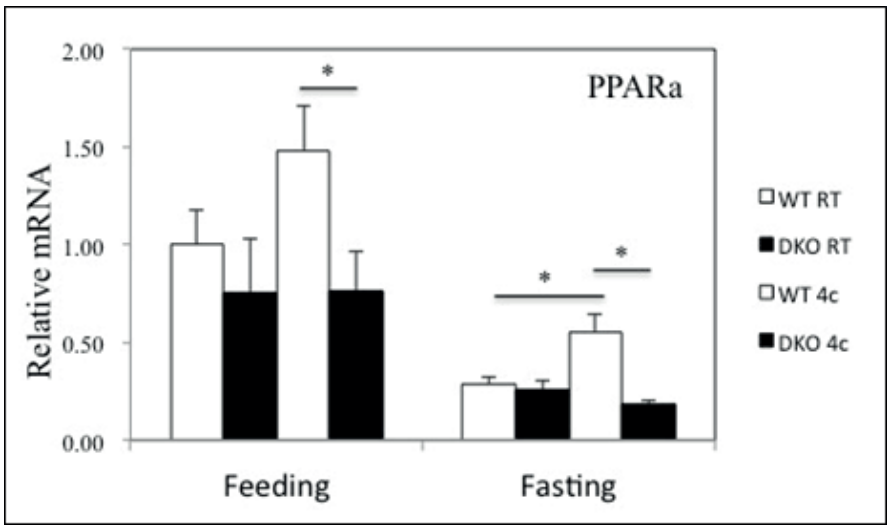

Figure 1: A blunted expression of PPARa gene in DKO mice after acute cold exposure. BAT was isolated and further mRNA P*PARα* was examined by quantitative real time-PCR. n = 4–5/group. *p<0.05.

condition. DKO mice are cold intolerance, particularly under fasted condition [3c]. Thus, dull expression of *PPARα* might partially contribute to the inability to adapt to cold environment.

4. Conclusion

The functional FABP-4 and -5 is necessary to modulate *PPARα* gene expression under acute cold exposure.

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