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RESEARCH ARTICLE

The Upregulation of *Carnitine Palmitoyltransferase 1a* (*CPT1a*) Expression under Prolonged Fasting in CD36 Knockout Mice

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Abstract

Food deprivation is one of the extreme conditions that mammals have to survive. The majority of the tissues, excluding the brain and red blood cells, depend on the fatty acids (FA) utilization to produce energy. We recently showed in mice lacking for CD36 ($CD36^{-/-}$), the uptake of FA is limited with dramatically increased of glucose uptake in heart and skeletal muscle in fasted condition, indicating a compensatory mechanism of organ to fulfill an energy demand. The liver is the central tissue maintaining metabolic homeostasis in fasted state. Synthesize adenosine triphosphate (ATP) in the mitochondria via beta-oxidation was mediated by *carnitine palmitoyltransferase 1a* (*CPT1a*). The objective of this research was to explore the role of CD36 in *CPT1a* expression in the fasted state. This research was conducted at Gunma University Japan in 2015. The method was in vivo-experimental, that we used $CD36^{-/-}$ and wild-type (WT) mice, as a control. The gene expression of *CPT1a* was measured by real-time PCR. Fasting condition up regulated mRNA expression of *CPT1a* in both WT and $CD36^{-/-}$ mice in 24 h and 48 h. However in $CD36^{-/-}$ mice, the mRNA expression of *CPT1a* in 24 h fasted state was lower very significantly than WT mice ($p<0.01$). We demonstrate that CD36 deficiency up regulate *CPT1a* gene expression, suggested that CD36 is essential for nutrient homeostasis when requirement for FA is increased and obtainability of nutrient is inadequate.

Keywords: CD36, *CPT1a*, fasted, fatty acid

Peningkatan Ekspresi Gen *Carnitine Palmitoyltransferase 1a* (*CPT1a*) pada C*D36 Knockout Mice* dalam Keadaan Puasa

Abstrak

Kekurangan makanan adalah salah satu kondisi ekstrem yang harus dihindari oleh mamalia. Sebagian besar jaringan, kecuali otak dan sel darah merah sangat bergantung pada pemanfaatan langsung asam lemak untuk menghasilkan energi. Penelitian kami sebelumnya menunjukkan pada mencit dengan defisiensi CD36 ($CD36^{-/-}$), serapan asam lemak terbatas karena peningkatan pengambilan glukosa hati dan otot rangka secara signifikan dalam kondisi puasa yang mengindikasikan mekanisme kompensasi organ untuk memenuhi kebutuhan energi. Hati adalah jaringan sentral yang menjaga homeostasis metabolik tubuh dalam keadaan berpuasa. Sintesis *adenosine triphosphate* (ATP) di mitokondria melalui beta-oksidasi dimediasi oleh *carnitine palmitoyltransferase 1a* (*CPT1a*). Tujuan penelitian ini mengetahui peran CD36 dalam ekspresi *CPT1a* dalam keadaan puasa. Penelitian ini dilakukan di Universitas Gunma Jepang pada tahun 2015. Metode penelitian ini adalah eksperimental *in vivo* dengan menggunakan mencit $CD36^{-/-}$ dan *wild type* (WT) sebagai kontrol. Ekspresi gen *CPT1a* diukur dengan *real-time* PCR. Puasa meningkatkan ekspresi mRNA *CPT1a* pada mencit WT dan $CD36^{-/-}$ baik setelah puasa selama 24 jam dan 48 jam. Namun, pada mencit $CD36^{-/-}$, ekspresi mRNA *CPT1a* dalam keadaan setelah dipuasakan 24 jam lebih rendah daripada mencit WT ($p<0,01$). Penelitian ini menunjukkan bahwa defisiensi CD36 mengatur ekspresi gen *CPT1a* sehingga CD36 sangat diperlukan untuk homeostasis nutrisi ketika kebutuhan asam lemak meningkat dan kemungkinan ketersediaan nutrisi terbatas.

Kata kunci: Asam lemak, CD36, *CPT1a*, puasa

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Introduction

Food deprivation is one of the extreme conditions that mammals have to survive. This action includes many metabolic processes at numerous levels. In generating energy, mainly the tissues in mammals rely greatly on the direct utilization of fatty acids (FA), except the brain and red blood cells. Elongated fasting stimulates the hydrolysis of triacylglycerol (TG) in adipose tissue; followed by up regulating of the non-esterified FA (NEFA) concentration in plasma, and then liver takes up the circulating FAs.[1]

Heart, adipose tissues and skeletal muscle (SkM) are FA consuming organs, where a membrane protein, namely CD36 or FA translocase (FAT), transport long-chain FA from circulation into tissues.[2] Our previous study showed that the FA uptake by CD36 is essential for thermogenesis with prior fasting. In mice deficient for CD36 ($CD36^{-/-}$), the uptake of FA is limited with dramatically increased uptake of glucose in heart and skeletal muscle in fasted condition, suggesting a compensatory mechanism of organ to fulfill an energy demand.[3] Moreover, fasted $CD36^{-/-}$ mice showed the disturbance of thermogenesis related genes expression in brown adipose tissue (BAT), an organ that responsible to generate heat.[4]

The liver is the central tissue preserve metabolic homeostasis in the fasted state, where NEFA will through several processes. Either re-esterification process from NEFA to TG and secreted as very low-density lipoprotein (VLDL), adenosine triphosphate (ATP) synthesis in the mitochondria of hepatocytes by beta-oxidation or change into ketone bodies that is utilized by organs.[5–8] The increase in serum ketone bodies is resulted from the increased of capacity for FA flux via beta oxidation and shunting of acetyl-CoA continue to synthesize of ketone body, marked by increasing carnitine palmitoyltransferase 1a (*CPT1a*). The flux of FA by beta-oxidation and acetyl-CoA through the tricarboxylic acid cycle increases production of hepatic mitochondrial nicotinamide adenine dinucleotide (NADH).[1,8,9]

CPT1a is a mediator for beta-oxidation. The capacity for mitochondrial beta oxidation is primarily regulated at the level of *CPT1* in gene level in reaction to various stimuli either physiologic or pathologic example, as fat feeding, fasting, induction of diabetes or treatment using peroxisomal/mitochondrial proliferating agents.[6] Palou et al.[10] 2008 showed increasing of *CPT1a* levels after 8 h fasting and greater after 24 h. The objective of this research was to explore the role of CD36 in *CPT1a* expression in the fasted state. We used $CD36^{-/-}$ and wild-type (WT) mice as a control to explore the crucial role of CD36 in the liver when nutrient supply were limited.

Methods

This research was conducted at Gunma University Japan in 2015. Ten to twelve-week old male mice with a homozygous null mutation in CD36 ($CD36^{-/-}$ mice) were generated as previously described,[11,12] and control male wild-type C57BL6j mice were purchased from Japan SLC, Inc. with body weights 22 to 27 grams. Mice were housed in a temperature-controlled room (22°C), exposed to a 12-h light/12-h dark cycle and given ad libitum access to water and standard chow (CE-2, Clea Japan, Inc.).[13] For the fasting experiments, mice were individually housed and the food was withdrawn for 0, 24 h and 48 h; water was provided ad libitum, as previously described.[1] Samples of liver were snapped frozen in liquid nitrogen and conserved at −80°C until further use. The study protocol was approved by The Institutional Animal Care and Use Committee (Gunma University Graduate School of Medicine, Japan) number: 15-024.

Total RNA was isolated from liver using the RNAiso Plus reagent (Takara, Japan). Semi-quantitative RT-PCR was performed with RT-PCR kit (Takara, Japan) according to manufacturer's protocol. RNA was prepared by reverse transcription using oligo-dT and dNTP, and each sample was processed with the RT-PCR kit (TAKARA, Japan). Quantitative real time-PCR was performed using the SYBR Green PCR Master Mix (Applied Biosystems, CA, USA) according to the manufacturer's instructions, and then evaluated using the LightCycler 480 Real-Time PCR system (Roche, CA, USA). The expression level of the target gene was normalized to the glyceraldehyde-3-phosphatase dehydrogenase (GAPDH) mRNA level. The sequences of primers for quantitative real-time PCR used in this study are listed in Table.

Statistical analysis was performed using one-way ANOVA with Bonferroni's post-hoc multiple comparison. A p value <0.05 was considered statistically significant. The data are presented as the means ± S.E. Statistical analysis of the data was performed with IBM SPSS (version 20.0 for Windows, IBM, NY, USA).

Table Primers for Quantitative Real-Time PCR

	Forward	Reverse
mCPT1a	CCATGAAGCCCTCAAACAGATC	ATCACACCCACCACCACGATA
mGAPDH	AGCCCCCAGTCTGTATCCTT	TCCACCACCCTGTTGCTGTA

CPT1a: *carnitinepalmitoyltransferase 1a, GAPDH*: *glyceraldehyde-3-phosphatase dehydrogenase*

Results

We examined the expression levels of the *CPT1a* gene in response to fasting conditions (Figure). Fasting condition increased mRNA level of *CPT1a* in both WT and CD36$^{-/-}$ mice. The dramatically increased of *CPT1a* gene expression seen in 24 h fasted CD36$^{-/-}$ mice. It was nearly two times higher than fed CD36$^{-/-}$ mice or 24 h WT mice. The same pattern also seen in mice with 48 h fasting. After 48 h fasting, the expression of *CPT1a* tends to be higher in CD36$^{-/-}$ mice compared to WT mice.

Discussion

In this study, we reveal an essential role of CD36 to regulate the expression of *CPT1a* in fasting condition. Our previous study underscored the role of FA binding carrier such as fatty acid binding protein 4 and 5 (FABP 4/5) and FA membrane transporter CD 36 in FA voracious organs, example as not just the heart but also the BAT and oxidative skeletal muscle for preserving nutrient homeostasis. When FA uptake is interrupted in these tissues, as a compensating process, there is an increase of glucose uptake and utilization further corrupts the homeostasis of nutrient in the extreme environment such as fasting condition.[1,3,13]

We suggested that in highly restrictive of nutrient availability such as in CD36$^{-/-}$ mice, the liver needs an extra energy to allowing the maintenance of energy homeostasis. Beta-oxidation in liver under low nutrition has two purposes, to fulfill energy demand for glucose product, a process called gluconeogenesis and to produce ketone bodies. Thus, the increased of *CPT1a* gene expression might be a compensatory mechanism to enhance FA influx to mitochondrial membrane. Further experiments, including FA uptake by the liver and TG liver concentration are necessary to confirm our hypothesis.

Conclusion

In conclusion, we demonstrate that CD36 deficiency up regulate *CPT1a* gene expression, indicated that CD36 is essential for the homeostasis of nutrient when requirement for

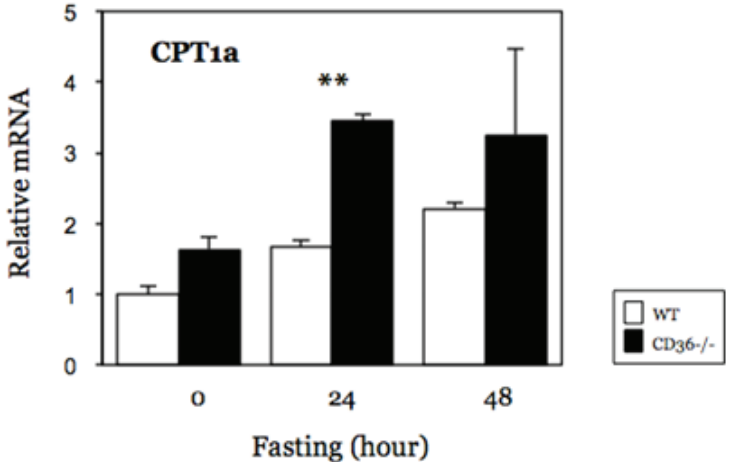

Figure Induction of *CPT1* Gene in Liver of Fasted WT and CD36$^{-/-}$ Mice
The mice were maintained at room temperature for 0, 24 and 48 h fasted state. The total RNA from liver was extracted for quantitative real-time PCR. n=4–5/group, **p<0.01

FA is increased and obtainability of nutrient is restricted.

Conflict of Interest

All the authors have read the manuscript and have agreed to submit it in its current form for consideration for publication in the journal. There are no conflicts of interest to declare.

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