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MITOCHONDRIAL UNCOUPLING PROTEIN 3 (UCP3) IN SKELETAL MUSCLE

Nobuyo Tsuboyama-Kasaoka[1], and Osamu Ezaki

Division of Clinical Nutrition, National Institute of Health and Nutrition, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8636, Japan

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1. ABSTRACT

Mitochondrial uncoupling protein (UCP), mitochondrial transporters, function as a proton channel and increase thermogenesis. UCP1 is expressed in brown adipose tissues (BAT), UCP2 is widely expressed in multiple tissues, while UCP3 is expressed in skeletal muscle. Thus, UCPs, especially UCP3, in skeletal muscles is a good candidates for prevention of obesity and diabetes. However, the role of UCP3 in skeletal muscle for energy expenditure and obesity has been controversial. There is some evidence that the UCP3 is possibly regulated by energy substrate, such as lipid and glucose. These observations suggest that increased energy substrate entry in muscle results in an increase in UCP3 expression which leads to an increase in energy expenditure.

2. INTRODUCTION

Regulation of body weight involves coordination of dietary intake and energy expenditure. Therefore, energy expenditure plays an important role for maintenance of body weight. Basal metabolic rate (BMR), physical exercise, and thermogenesis represent the three components of energy expenditure. In the resting state, BMR represents 70% of the total energy expenditure (1). BMR involves the obligatory energy costs of all cellular functions such as maintenance of ion gradients and protein synthesis. Physical activity varies according to the behavior of the individual. Thermogenesis, defined as energy expenditure above BMR in the resting state, is dependent on food intake, smoking, and exposure to cold. It appears to be very effective to raise the thermogenesis to prevent obesity. Presently, mitochondrial uncoupling protein (UCP) is considered to play pivotal roles in thermogenesis (2). UCP1 is a brown adipose tissue (BAT) specific mitochondrial inner membrane transporter which dissipates the proton gradient, producing heat (2). The thermogenic function of UCP1 *in vivo* was demonstrated by several groups working on transgenic or knock out mice (3, 4). Gene knockout mice lacking UCP1 develop hypothermia when exposed to cold, essentially confirming that UCP1 plays an important role in mediating cold exposure induced nonshivering thermogenesis (4). Furthermore, Kopecky et al. reported that fat mass is reduced in fat-specific UCP1 overexpressed transgenic mice (3). However, in humans, BAT is observed only in neonates, and is poorly developed in adults (5). Since BAT is very scarce in human adults, the role of UCP1 in human energy expenditure has been controversial. Indeed, it has been estimated that skeletal muscle, liver and white adipose tissue (WAT) are the major peripheral tissues that regulate energy homeostasis and thermogenesis in adult humans (6). Recently, three additional isoforms were cloned. In contrast to UCP1, UCP2 (7, 8) is widely expressed in multiple tissues (WAT, BAT, kidney, lung, placenta, lymphocytes, intestine, heart, liver) and UCP3 (9-11) is expressed at high levels in skeletal muscle as well as BAT, and UCP4 is expressed exclusively in the brain (12). Skeletal muscle is an important site of regulated thermogenesis (13) where dissipation of the mitochondrial proton gradient has been reported to contribute up to 50% of the resting metabolic rate (14). Thus, UCPs, especially UCP3, in skeletal muscles is a matter of particular interest in human thermogenesis and obesity. The deduced amino acid sequence of human UCP3 is 57%, 73% and 34%, of UCP1, UCP2, and UCP4, respectively (9, 12). The human UCP3 gene is located on chromosome 11q13, the UCP2 gene is downstream of the UCP3 gene, and the distance between the two genes is 7 kb. UCP3 gene has 7 exons and has two

different transcripts, a long form (UCP3L, 312aa) and a short form (UCP3s, 275 aa), respectively (15). UCP3 contains six transmembrane domains and has a potential purine nucleotide binding region.

At present, many studies of UCP3 including knockout and overexpressed UCP3 mice have been reported. Here, we will review and discuss the regulation of UCP3 expression and its function.

3. FREE FATTY ACID UP-REGULATES UCP3 EXPRESSION

In skeletal muscle, increased amounts of lipids are correlated with reduced skeletal muscle and whole body insulin action (16). Thus, reduction of triglyceride content in skeletal muscles may prevent development of diabetes mellitus. To prevent excessive accumulation of triglyceride in skeletal muscles and obesity, the physiological role and the regulatory expression of UCP3 in skeletal muscle has been extensively studied. Many studies revealed that circulating free fatty acids (FFA) regulated the expression of UCP3 mRNA. Since there is a strong positive correlation between FFA levels in blood and UCP3 expression levels in skeletal muscles, FFA may be the most important regulator of UCP3 in skeletal muscle.

Fasting is known to cause an increase in fatty acid oxidation (17). In skeletal muscle, fasting produces a marked increase in UCP3 mRNA expression in rats (10, 18), mice (19) and human (20). Samec et al. also reported that increased UCP3 mRNA in skeletal muscle in response to fasting and a subsequent decrease in response to refeeding parallel changes in blood FFA levels (21). In 46 h fasted rats, the administration of the antilipolytic agent, nicotinic acid, which completely prevented the fasting-induced increase in FFA, decreased UCP3 mRNA expression in the soleus muscle (22). Kageyama et al. reported that UCP3 mRNA expression in the gastrocnemius muscle was elevated in streptozotocin (STZ)-diabetic rats and plasma concentrations of FFA were significantly increased in STZ-diabetic rats (23). More directly, Weigle et al. showed fatty acid loading by infusion of intralipid with heparin up-regulates UCP3 mRNA in skeletal muscle *in vivo* (24). In humans, the mRNA expression of UCP3 was positively and linearly correlated with circulation FFA (25). The intracellular FFA level appears to reflect the circulating FFA level, because the skeletal muscle rapidly takes in circulating FFA.

There is some evidence that peroxisome proliferator-activated receptors (PPARs), the transcription factor regulated by lipid metabolites, may be involved in up-regulation of UCP3. In muscle cells, FFA and lipid treatment up-regulated UCP3 mRNA expression in C2C12 cells (19) and L6 myotubes (26). BRL49653, a ligand for the nuclear hormone receptor PPAR?, induces expression of UCP3 mRNA in C2C12 cells suggesting that PPAR? may regulate transcription of the UCP3 gene (19). However, Nagase et al. suggested that UCP3 expression in L6 myocytes is activated by the PPARd, because neither WY14643 (PPARa ligand) nor troglitazone (PPAR? ligand) was effective in UCP3 mRNA induction in L6 cells (26). In addition, to examine whether FFA-induced up-regulation of UCP3 was mediated by increased intracellular FFA/acyl-CoA or increased mitochondrial β-oxidation of fatty acids, two studies have been conducted. The addition of a-bromopalmitic acid, an unmetabolizable fatty acid derivative, up-regulated UCP3 in L6 myotubes (26) and refeeding along with administration of methyl palmoxirate (an inhibitor of carnitine: palmitoyl transferase, which that blocks entry of FFA into mitochondria, and thereby increases blood FFA) prevented refeeding induced down-regulation of UCP3 mRNA (19). These findings suggest that accumulation of intracellular fatty acids or acyl-CoA may activate PPARs that lead to up-regulation of UCP3.

4. GLUCOSE METABOLISM AND UCP3 EXPRESSION

As well as fatty acids, it is also conceivable that an increased glucose entry in skeletal muscle results in an increase of UCP3. Several studies have reported findings to support this hypothesis. Exercise training consumes a large amount of ATP in skeletal muscles, in which fatty acids and glucose is utilized for energy supply. We reported that mice exercised for 2 weeks or in a single bout of swimming had 14~18-fold increases of UCP3 mRNA in skeletal muscles 3 h after the last swimming (27). However, 22 h after exercise when the glucose uptake had returned to its basal level, the UCP3 mRNA increases observed in skeletal muscle 3 h after exercise had returned to sedentary levels. GLUT4, the insulin responsive-glucose transporter, mediates the rate-limiting step of glucose metabolism in skeletal muscles and adipose tissues under physiological glucose concentrations (28). The increased expression of GLUT4 in skeletal muscles (29, 30) and adipose tissues (31) results in increased intracellular glucose flux in these tissues and in improvement in whole body glucose tolerance. Expression of the GLUT4 transgene caused an increase in UCP3 mRNA that paralleled the increase in GLUT4 protein in the gastrocnemius muscle (32). Thus, overexpression of GLUT4 in skeletal muscle by transgenic mice up-regulated UCP3 mRNA expression in skeletal muscle. In sciatic nerve-denervated gastrocnemius, expression of UCP3 mRNA decreased by 45% (27). Denervation of skeletal muscle rapidly decreases GLUT4 mRNA (33) and GLUT4 protein (34) which leads to a decrease in glucose uptake. Lin et al. reported that UCP3 and GLUT4 mRNAs in rat skeletal muscles increased two- to three-fold between 6 and 24 h of cold exposure and then decreased to 50% of the control value after 6 days in the cold (35).

There is some clinical evidence to support this hypothesis. In clinical studies, Krook et al. reported that the level of UCP3 mRNA in skeletal muscle of non-insulin-dependent diabetes mellitus (NIDDM) patients was lower than in control subjects (36). They also observed a positive correlation between UCP3 expression in skeletal muscles and whole-body insulin-mediated glucose utilization among NIDDM individuals. Similarly, Willi et al. reported the UCP3 level in skeletal muscle was positively correlated with carbohydrate oxidation and higher insulin-mediated glucose uptake (37). These

observations suggest that increased glucose entry in muscle results in an increase in UCP3 expression which leads to an increase in energy expenditure.

Therefore, the mechanisms of up-regulation of UCP3 by increased glucose uptake need to be clarified. The increased glycolysis due to increased glucose uptake increases the citrate concentration (38). Citrate activates acetyl-CoA carboxylase (ACC) that leads to an increase malonyl-CoA concentration. Since malonyl-CoA is an inhibitor of CPT1, an increase of malonyl-CoA prevents fatty acid β-oxidation in mitochondria (39). Thus, accumulation of fatty acids or fatty acyl-CoA in cytosol may activate PPARs as observed in an increased blood FFA. Alternatively, an increased tricarboxylic acid cycle and ATP production by an increased glycolysis may give a signal to overexpress UCP3. Thus, UCP3 expression may be stimulated when a large amount of glucose or FFA influx occurs in skeletal muscles to prevent excessive production of ATP and to maintain ATP and ADP homeostasis.

In contrast, it is also possible that increased UCP3 or a signal that increases UCP3 expression may result in an increase of glycolysis. Recently, it was shown that decreased ATP and/or phosphocreatine that lead to activation of AMP-kinase, then stimulate glucose uptake by the translocation of GLUT4 from the intracellular site to the plasma membrane (40). In isolated skeletal muscles, conditions that decrease ATP and phosphocreatine, including contraction, hypoxia, 2,4-dinitrophenol (DNP), rotenone, and hyperosmolarity, increased AMP-kinase and glucose uptake (41). Thus, it is possible that an increase in UCP3 may lead to a decrease in ATP, then an increase in glucose uptake. In agreement with this, Fischer et al. showed that overexpression of UCP3 in L6 cells increased 2-deoxyglucose uptake (42). Increased expression of UCP3 in GLUT4 overexpressed mice might be mediated by AMP-kinase activation through unknown mechanism(s).

5. ROLE OF UCP3 IN THERMOGENESIS AND OBESITY

Although many studies of UCP3 expression have been reported, it has not been established whether UCP3 plays an important role in thermogenesis *in vivo*. In obese Zucker fa/fa rats (43) and Wister fatty rats (44) relative to their control lean rats, the UCP3 expression level in skeletal muscle was decreased by 41% and 76%, respectively. In humans, Argyropoulos et al. showed that mutation and polymorphisms of the UCP3 gene were detected in severe obesity and NIDDM patients (45). Furthermore, subjects heterozygous for the splice donor-stop polymorphism had markedly reduced fat oxidation and a high respiratory quotient. Schrauwen et al. showed a negative correlation between skeletal muscle UCP3 expression and BMI, and a positive correlation between UCP3 mRNA levels and RMR in Pima Indians (46). Vidal-Puig et al. reported that stabilization at reduced body weight in humans was associated with a decrease in UCP3 mRNA in skeletal muscle (47). However, no change in UCP3 in skeletal muscle has been reported in obese patients compared with lean patients (25, 48). Thus, the role of UCP3 in skeletal muscle for energy expenditure and obesity has been controversial.

Recently, two studies reported the analyze of UCP3 knockout mice (49, 50). UCP3 knockout mice reduced the uncoupling activity in skeletal muscle, and that its absence led to increased production of reactive oxygen species (ROS). Despite the lack of UCP3, the knockout mice were not obese and showed normal exercise tolerance, fatty acid oxidation, and cold-induced thermogenesis (49) and showed a normal circadian rhythm in body temperature and motor activity and had normal body temperature responses to fasting, stress, thyroid hormone, and cold exposure (50). However, very recently, overproduction of UCP3 in skeletal muscles reduced body weight despite an increased intake of food (51). The mice also exhibited striking reduction of adipose tissue mass and increased glucose clearance rate. Thus, contradictory findings were observed between knockout mice and overexpressed mice. These findings suggested that a novel, unidentified, uncoupling protein may be up-regulated in UCP3 knockout mice and this protein might have function to increase energy expenditure but not prevention of ROS.

Further studies, including analysis of intracellular fatty acid and glucose metabolites in skeletal muscles will be necessary to identify the critical molecule which regulates expression in skeletal muscle. Furthermore, studies including the identification of a novel uncoupling protein in skeletal muscles are required to ascertain the significance and physiological functions of UCP3.

6. REFERENCE

1. Jéquier E: Energy expenditure in obesity. *Clin Endocrinol Metab* 13, 563-580 (1984)
2. Silva J. E & R. Rabelo: Reguration of the uncoupling protein gene expression. *Eur J Endocrinol* 136, 251-264 (1997)
3. Kopecky J, G. Clarke, S. Enerbäck, B. Spiegelnman & L. P. Kozak: Expression of the mitochondrial uncoupling protein gene from the aP2 gene promoter prevents genetic obesity. *J Clin Invest* 96, 2914-2923 (1995)
4. Enerbäck S, A. Jacobsson, E. M. Simpson, C. Guerra, H. Yamashita, M.-E. Harper & L. P. Kozak: Mice lacking mitochondrial uncoupling protein are cold-sensitive but not bese. *Nature* 387, 90-94 (1997)
5. Muzzin P, O. Boss & J.-P. Giacobino: Uncoupling protein 3: Its possible biological role and mode of regulation in rodents and humans. *J Bioenerg Biomembr* 31, 467-473 (1999)
6. Brand M. D, L.-F. Chien, E. K. Ainscow, D. F. S. Rolfe & R. K. Porter: The causes and functions of mitochondrial proton leak. *Biochim Biophys Acta* 1187, 132-139 (1994)
7. Fleury C, M. Neverova, S. Collins, S. Raimbault, O. Champigny, C. Levi-Meyrueis, F. Bouillaud, M. F. Seldin, R. S. Surwit, D. Ricquier & C. H. Warden: Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. *Nat genet* 15, 269-272 (1997)
8. Gimeno R. E, M. Dembski, X. Weng, N. Deng, A. W. Shyjan, C. J. Gimeno, F. Iris, S. J. Ellis, E. A. Woolf & L. A. Tartaglia: Cloning and characterization of an uncoupling protein homolog. *Diabetes* 46, 900-906 (1997)

9. Boss O, S. Samec, A. Paoloni-Giacobino, C. Rossier, A. Dulloo, J. Seydoux, P. Muzzin & J.-P. Giacobino: Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. *FEBS Lett* 408, 39-42 (1997)
10. Gong D.-W, Y. He, M. Karas & M. Reitman: Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone,ß3-adrenergic agonists, and leptin. *J Biol Chem* 272, 24129-24132 (1997)
11. Vidal-Puig A, G. Solanes, D. Grujic, J. S. Flier & B. B. Lowell: UCP3: An uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and brown adipose tissue. *Biochem Biophys Res Commun* 235, 79-82 (1997)
12. Mao W, X. X. Yu, A. Zhong, W. Li, J. Brush, S. W. Sherwood, S. H. Adams & G. Pan: UCP4, a novel brain-specific mitochondrial protein that reduces membrane potential in mammalian cells. *FEBS Lett* 443, 326-330 (1999)
13. Astrup A, J. Bülow, J. Madsen & N. J. Christensen: Contribution of BAT and skeletal muscle to thermogenesis induced by ephedrine in man. *Am J Physiol* 248, E507-E515 (1985)
14. Rolfe D. F. S & M. D. Brand: Contribution of mitochondrial proton leak to skeletal muscle respiration and standard metabolic rate. *Am J Physiol* 271, C1380-C1389 (1996)
15. Solanes G, A. Vidal-Puig, D. Grujic, J. S. Flier & B. B. Lowell: The human uncoupling protein-3 gene. *J Biol Chem* 272, 25433-25436 (1997)
16. Pan D. A, S. Lillioja, A. D. Kriketos, M. R. Milner, L. A. Baur, C. Bogardus, A. B. Jenkins & L. H. Storlien: Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes* 46, 983-988 (1997)
17. Owen O. E, G. A. Reichard Jr, M. S. Patel & G. Boden: Energy metabolism in feasting and fasting. *Adv Exp Med Biol* 111, 169-188 (1979)
18. Boss O, S. Samec, A. Dulloo, J. Seydoux, P. Muzzin & J.-P. Giacobino: Tissue-dependent upregulation of rat uncoupling protein-2 expression in response to fasting or cold. *FEBS Lett* 412, 111-114 (1997)
19. Hwang C.-S & M.D. Lane: Up-regulation of uncoupling protein-3 by fatty acid in C2C12 myotubes. *Biochem Biophys Res Commun* 258, 464-469 (1999)
20. Millet L, H. Vidal, F. Andreelli, D. Larrouy, J.-P. Riou, D. Ricquier, M. Laville & D. Langin: Increased uncoupling protein-2 and -3 mRNA expression during fasting in obese and lean humans. *J Clin Invest* 100, 2665-2670 (1997)
21. Samec S, J. Seydoux & A. G. Dulloo: Post-starvation gene expression of skeletal muscle uncoupling protein 2 and uncoupling protein 3 in response to dietary fat levels and fatty acid composition. *Diabetes* 48, 436-441 (1999)
22. Samec S, J. Seydoux & A. G. Dulloo: Interorgan signaling between adipose tissue metabolism and skeletal muscle uncoupling protein homologs. *Diabetes* 47, 1693-1698 (1998)
23. Kageyama H, A. Suga, M. Kashiba, J. Oka, T. Osaka, T. Kashiwa, T. Hirano, K. Nemoto, Y. Namba, D. Ricquier, J.-P. Giacobino & S. Inoue: Icreased uncoupling protein-2 and –3 gene expression in skeletal muscle of STZ-induced diabetic rats. *FEBS Lett* 440, 450-453 (1998)
24. Weigle D. S, L. E. Selfridge, M. W. Schwartz, R. J. Seeley, D. E. Cummings, P. J. Havel, J. L. Kuijper & H. BeltrandelRio: Elevated free fatty acids induce uncoupling protein 3 expression in muscle. *Diabetes* 47, 298-302 (1998)
25. Boss O, E. Bobbioni-Harsch, F. Assimacopoulos-Jeannet, P. Muzzin, R. Munger, J.-P. Giacobino & A. Golay: Uncoupling protein-3 expression in skeletal muscle and free fatty acids in obesity. *Lancet* 351, 1933 (1998)
26. Nagase I, S. Yoshida, X. Canas, Y. Irie, K. Kimura, T. Yoshida & M. Saito: Up-regulation of uncoupling protein 3 by thyroid hormone, peroxisome proliferator-activated receptor ligands and 9-cis retinoic acid in L6 myotubes. *FEBS Lett* 461, 319-322 (1999)
27. Tsuboyama-Kasaoka N, N. Tsunoda, K. Maruyama, M. Takahashi, H. Kim, S. Ikemoto & O. Ezaki: Up-regulation of uncoupling protein 3 (UCP3) mRNA by exercise training and down-regulation of UCP3 by denervation in skeletal muscles. *Biochem Biophys Res Commun* 247, 498-503 (1998)
28. Lombardi A. M, D. Moller, M. Loizeau, J. Girard & A. Leturque: Phenotype of transgenic mice overexpressing GLUT4 and hexokinase II in muscle. *FASEB J* 11, 1137-1144 (1997)
29. Tsao T.-S, R. Burcelin, E. B. Katz, L. Huang & M. J. Charron: Enhanced insulin action due to targeted GLUT4 overexpression exclusively in muscle. *Diabetes* 45, 28-36 (1996)
30. Leturque A, M. Loizeau, S. Vaulont, M. Salminen & J. Girard: Improvement of insulin action in diabetic transgenic mice selectively overexpressing GLUT4 in skeletal muscle. *Diabetes* 45, 23-27 (1996)
31. Shepherd P. R, L. Gnudi, E. Tozzo, H. Yang, F. Leach & B. B. Kahn: Adipose cell hyperplasia and enhanced glucose disposal in transgenic mice overexpressing GLUT4 selectively in adipose tissue. *J Biol Chem* 268, 22243-22246 (1993)
32. Tsuboyama-Kasaoka N, N. Tsunoda, K. Maruyama, M. Takahashi, H. Kim, D. W. Cooke, M. D. Lane & O. Ezaki: Overexpression of GLUT4 in mice causes up-regulation of UCP3 mRNA in skeletal muscle. *Biochem Biophys Res Commun* 258, 187-193 (1999)
33. Block N. E, D. R. Menick, K. A. Robinson & M. G. Buse: Effect of denervation on the expression of two glucose transporter isoforms in rat hindlimb muscle. *J Clin Invest* 88, 1546-1552 (1991)
34. Henriksen E. J, K. J. Rodnick, C. E. Mondon, D. E. James & J. O. Holloszy: Effect of denervation or unweighting on GLUT-4 protein in rat soleus muscle. *J Appl Physiol* 70, 2322-2327 (1991)
35. Lin B, S. Coughlin & P. F. Pilch: Bidirectional regulation of uncoupling protein-3 and GLUT-4 mRNA in skeletal muscle by cold. *Am J Physiol* 275, E386-E391 (1998)
36. Krook A, J. Digby, S. O'Rahilly, J. R. Zierath & H. Wallberg-Henriksson: Uncoupling protein 3 is reduced in skeletal muscle of NIDDM patients. *Diabetes* 47, 1528-1531 (1998)
37. Willi S. M, L. Maianu, A. Kennedy, B. Wojciechowski, S. Bao, P. Wallace, E. Ganaway & W. T. Garvey: The role of uncoupling proteins in metabolic fuel partitioning and human obesity. *ADA* A13 (1998)
38. Ruderman N. B, A. K. Saha, D. Vavvas & L. A. Witters: Malonyl-CoA, fuel sensing, and insulin resistance. *Am J Physiol* 276, E1-E18 (1999)
39. McGarry J. D, G. P. Mannaerts & D. W. Foster: A possible role for malonyl-CoA in the regulation of hepatic fatty acid oxidation and ketogenesis. *J Clin Invest* 60, 265-270 (1977)
40. Kurth-Kraczek E. J, M. F. Hirshman, L. J. Goodyear & W. W. Winder: 5' AMP-activated protein kinase activation causes

GLUT4 translocation in skeletal muscle. *Diabetes* 48, 1667-1671 (1999)
41. Hayashi T, M. F. Hirshman, N. Fujii, S. A. Habinowski, L. A. Witters & L. J. Goodyear: Metabolic stress and altered glucose transport: activation of AMP-activated protein kinase as a unifying coupling mechanism. *Diabetes* 49, 527-531 (2000)
42. Fischer B. M, C. Huppertz, A. Vidal-Puig, Y.-B. Kim, L. J. Slieker, K. W. Sloop, B. B. Lowell & B. B. Kahn: Uncoupling protein 3 (UCP3) increases glucose uptake in muscle cells through a PI3Kinase (PI3K) dependent mechanism. *ADA* A51 (2000)
43. Boss O, S. Samec, F. Kuhne, P. Bijlenga, F. Assimacopoulos-Jeannet, J. Seydoux, J. P. Giacobino & P. Muzzin: Uncoupling protein-3 expression in rodent skeletal muscle is modulated by food intake but not by changes in environmental temperature. *J Biol Chem* 273, 5-8 (1998)
44. Matsuda J. K Hosoda, H. Itoh, C. Son, K. Doi, I. Hanaoka, G. Inoue, H. Nishimura, Y. Yoshimasa, Y. Yamori, H. Odaka & K. Nakao: Increased adipose expression of the uncoupling protein-3 gene by thiazolidinediones in wistar fatty rats and in cultured adipocytes. *Diabetes* 47, 1809-1814 (1998)
45. Argyropoulos G, A. M. Brown, S. M. Willi, J. Zhu, Y. He, M. Reitman, S. M. Gevao, I. Spruill & W. T. Garvey: Effects of mutations in the human uncoupling protein 3 gene on the respiratory quotient and fat oxidation in severe obesity and type 2 diabetes. *J Clin Invest* 102, 1345-1351 (1998)
46. Schrauwen P, J. Xia, C. Bogardus, R. E. Pratley & E. Ravussin: Skeletal muscle uncoupling protein 3 expression is a determinant of energy expenditure in pima indians. *Diabetes* 48, 146-149 (1999)
47. Vidal-Puig A, M. Rosenbaum, R. C. Considine, R. L. Leibel, G. L. Dohm & B. B. Lowell: Effects of obesity and stable weight reduction on UCP2 and UCP3 gene expression in humans. *Obes Res* 7, 133-140 (1999)
48. Nordfors L, J. Hoffstedt, B. Nyberg, A. Thorne, P. Arner, M. Schalling & F. Lonnqvist: Reduced gene expression of UCP2 but not UCP3 in skeletal muscle of human obese subjects. *Diabetologia* 41, 935-939 (1998)
49. Vidal-Puig A. J, D. Grujic, C. Y. Zhang, T. Hagen, O. Boss, Y. Ido, A. Szczepanik, J. Wade, V. Mootha, R. Cortright, D. M. Muoio & B. B. Lowell: Energy metabolism in uncoupling protein 3 gene knockout mice. *J Biol Chem* 275, 16258-16266 (2000)
50. Gong D. W, S. Monemdjou, O. Gavrilova, L. R. Leon, B. Marcus-samuels, C. J. Chou, C. Everett, L. P. Kozak, C. Li, C. Deng, M. E. Harper & M. L. Reitman: Lack of obesity and normal response to fasting and thyroid hormone in mice lacking uncoupling protein-3. *J Biol Chem* 275, 16251-16257 (2000)
51. Clapham J. C, J. R. S. Arch, H. Chapman, A. Haynes, C. Lister, G. B. T. Moore, V. Piercy, S. A. Carter, I. Lehner, S. A. Smith, L. J. Beeley, R. J. Godden, N. Herrity, M. Skehel, K. K. Changani, P. D. Hockings, D. G. Reid, S. M. Squires, J. Hatcher, B. Trail, J. Latcham, S. Rastan, A. J. Harper, S. Cadenas, J. A. Buckingham, M. D. Brand & A. Abuin: Mice overexpressing human uncoupling protein-3 in skeletal muscle are hyperphagic and lean. *Nature* 406, 415-418 (2000)

Abbreviations: BMR, basal metabolic rate; UCP, uncoupling protein; GLUT4, muscle/adipocyte insulin-responsive glucose transporter; WAT, white adipose tissue; BAT, brown adipose tissue; FFA, fatty acid; STZ, streptozotocin; PPAR, peroxisome proliferator-activated receptor; NIDDM, non-insulin-dependent diabetes mellitus; ATP, adenosine 5'-triphosphate; AMP, adenosine 5'-monophosphate; BMI, body mass index; RMR, resting metabolic rate ; ROS, reactive oxygen species.

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Send Correspondence to: Dr Nobuyo Tsuboyama-Kasaoka, Division of Clinical Nutrition, National Institute of Health and Nutrition, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8636, Japan, Tel: 81-3-3203-5725, Fax: 81-3-3207-3520, E-mail: ntsubo@nih.go.jp