J Bioenerg Biomembr (2010) 42:255–259
DOI 10.1007/s10863-010-9292-9

Short Report: Tissue-specific Expression Profiles of the Uncoupling Protein Family in Normal Control Mice and Genetically ob/ob Mice

Chun-Mei Zhang · Min Zhang · Jin-Gai Zhu · Chen-Bo Ji · Chun Zhu · Chun-Zhao Kou · Da-Ni Qin · Mei-Ling Tong · Xi-Rong Guo

Received: 2 February 2010 / Accepted: 29 April 2010 / Published online: 20 May 2010

Abstract Uncoupling proteins (UCPs) located in the inner mitochondrial membrane are involved in the regulation of energy balance. Thus far, 5 UCP isoforms have been identified, but controversies exist in the research focused on the function of the UCPs (except UCP1) in the pathogenesis of obesity. Because of the known cross-reactivity of the antibodies presently available for the detection of UCP proteins, this study systematically analyzed the differential tissue expression profiles of the 5 UCP isoforms in lean control mice and ob/ob mice by using real-time polymerase chain reaction (PCR) analysis. The results show that the tissue-specific expression patterns of individual isoforms in normal and ob/ob mice are considerably different; this will provide new insights into the functions of UCPs in the pathogenesis of genetic obesity.

Keywords Uncoupling proteins · Obesity · Tissue distribution · ob/ob mice

Chun-Mei Zhang and Min Zhang contributed equally to this work

C.-M. Zhang · M. Zhang · J.-G. Zhu · C.-B. Ji · C. Zhu · C.-Z. Kou · D.-N. Qin · M.-L. Tong (✉) · X.-R. Guo
Department of Pediatrics, Nanjing Maternal and Child Health Hospital of Nanjing Medical University,
Nanjing, Jiangsu Province, China
e-mail: kt99cn@yahoo.com.cn

C.-M. Zhang · J.-G. Zhu · C.-B. Ji · C. Zhu · C.-Z. Kou · D.-N. Qin · X.-R. Guo (✉)
Institute of Pediatrics, Nanjing Medical University,
No.140 Hanzhong Road,
Nanjing, Jiangsu Province, China
e-mail: xrguo@njmu.edu.cn

Introduction

Currently, obesity is rapidly increasing worldwide and is regarded as one of the most important health threats (Jeffery and Sherwood 2008; Visscher and Seidell 2001). Obesity is a complex syndrome that results from an imbalanced intake of energy substrates and energy utilization (Rosenbaum et al. 1997). While the discovery of the adipocyte-secreted hormone leptin and its receptors have greatly enhanced our understanding of the mechanisms that regulate food intake (Friedman and Halaas 1998), the molecular mechanisms involved in the regulation of energy expenditure are not well characterized. Recent studies have suggested that the uncoupling protein (UCP) family located in the inner mitochondrial membrane is involved in the regulation of energy balance (Flier and Lowell 1997; Boss et al. 1998). UCPs uncouple mitochondrial respiration from oxidative phosphorylation, increasing thermogenesis while reducing the efficiency of ATP synthesis (Flier and Lowell 1997; Boss et al. 1998).

To date, 5 homologous UCP isoforms (UCP1-UCP5) have been identified in mammals (Yu et al. 2000). The first UCP to be described is UCP1, which is expressed exclusively in the brown adipose tissue (BAT); it plays an important role in thermogenesis and energy expenditure and is implicated in the pathogenesis of obesity and metabolic disorders in humans (Jia et al. 2010). The current literatures show that UCP2 and UCP3 are ubiquitously expressed throughout the body, UCP4 is predominantly expressed in the brain, and UCP5 is present in the brain and liver (Alán et al. 2009). However, 10 years after their identification, the role of UCP homologues (except UCP1) in energy expenditure and as the cause of obesity remains unclear.

In order to investigate the putative roles of UCPs in obesity, it seems important to determine which UCP isoforms are expressed in obesity and obtain precise quantitative information on their relative abundance. Genetically ob/ob mouse, which has a non-sense mutation in the coding region of the leptin gene that prevents leptin production, is a commonly used animal model for exploring the pathophysiology of obesity. In the present study, we detected the expression characteristics of individual UCP messenger RNAs (mRNAs) in tissues associated with energy metabolism (brain, adipose tissue, liver, and skeletal muscle) between lean and ob/ob mice using quantitative real-time polymerase chain reaction (PCR). The results show that the tissue-specific expression patterns of individual UCP isoforms in normal and ob/ob mice are considerably different; this will provide some new clues for exploring the roles of UCPs in the pathogenesis of genetic obesity.

Materials and methods

Animals

Twelve-week-old male ob/ob mice (body weight, 47.3± 5.4 g; n=12) and male lean control C57BL/6J mice (body weight, 18.4±3.0 g; n=12) were obtained from Model Animal Research Center (MARC) of Nanjing University. The animals were raised for 1 day and subsequently killed by exsanguination under sodium pentobarbital anesthesia. Soleus hind limb muscle, brain, liver, subcutaneous fat, retroperitoneal fat, and scapular BAT were obtained from the animals for the detection of UCP mRNA.

RNA extraction

Total RNA was isolated from the inguinal fat pads of ob/ob mice using Trizol reagent (Invitrogen, Carlsbad, CA) in accordance with the manufacturer's protocol. Each tissue sample (100 mg) was homogenized by adding 1 ml of Trizol reagent. The samples were centrifuged at 12,000× g for 15 min at 4°C, and the aqueous phase was removed and mixed with 0.5 ml of isopropyl alcohol. This was again centrifuged at 12,000× g for 10 min at 4°C, and the pellet was washed with 1 ml of 75% ethanol and then dissolved in 12 μl of RNase-free water. Of the 12 μl, 1 μl was used as an integrity check and for quantification by using the DU-600 ultraviolet spectrophotometer (Beckman Coulter, Inc., Fullerton, CA).

Reverse transcription and real-time PCR

Using an AMV Reverse Transcriptase kit (Promega A3500; Promega, Madison, WI, USA), we performed reverse transcription of 1 μg of total RNA in a 20 μl reaction according to the manufacturer's protocol. Real-time PCR was performed with 1 μl of cDNA using the SYBR Green PCR Master Mix (Applied Biosystems; Foster City, CA, USA) on an Applied Biosystems 7300 Sequence Detection System (ABI 7300 SDS; Foster City, CA, USA). The real-time PCR conditions were as follows: 2 min at 50°C, 10 min at 95°C, and then 40 cycles of 15 s at 95°C and 1 min at 60°C. Melt curve analysis demonstrated that each primer set described in Table 1 amplified a single predominant product, and the size of the PCR products was confirmed by running the products on a 1.2% agarose gel. Mouse β-actin gene, an endogenous housekeeping gene, was used as an internal control.

Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using paired Student's t-test by using the statistical package for social sciences (SPSS) 11.0 software (SPSS; Chicago, IL, USA). The threshold of significance was defined as $P<0.05$.

Results

Tissue-distribution patterns of UCP1

Consistent with previous reports, our findings indicated that UCP1 was expressed exclusively in the BAT. UCP1 mRNA expression was not detected in other selected tissues. The

Table 1 Primer sequences used for quantitative real-time PCR

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
UCP1	CGACTCAGTCCAAGAGTACTTCTCTTC	CCACTTCATCTTACCATTTATTATCGC
UCP2	CAG CCA GCG CCC AGT ACC	CAA TGC GGA CGG AGG CAA AGC
UCP3	CCTACGACATCATCAAGGAGAAGTT	TCCAAAGGCAGAGACAAAGTGA
UCP4	AAGGCTTCCTAAAGCTGTGGC	GACCATCCGACCTCCAGAGT
UCP5	TCCCAACTGCTCAGCGTG	GGTGCTTCTTGGTAATATCATAAACG
β-actin	CCTGAGGCTCTTTTCCAGCC	TAGAGGTCTTTACGGATGTCAACGT

level of UCP1 mRNA in the BAT of the ob/ob mice was decreased to the two-thirds of that in the BAT of the lean control mice (Fig. 1).

Tissue-distribution patterns of UCP2

We confirmed the presence of UCP2 mRNA in all the selected tissues studied (Fig. 2a). In the lean control mice, the transcript levels of UCP2 mRNA decreased in the order of subcutaneous fat > skeletal muscle > brain > liver > retroperitoneal fat > BAT. Compared to the UCP2 mRNA levels in the control mice, those in the ob/ob mice were increased by 2.5-fold in the liver ($P<0.001$), 3.3-fold in retroperitoneal fat ($P<0.001$), and 4-fold in the brain (Fig. 2) and showed a slight decrease in the skeletal muscle, which was not statistically significant ($P>0.05$).

Tissue-distribution patterns of UCP3

As expected, UCP3 was preferentially expressed in the skeletal muscle and BAT. The UCP3 expression decreased by 57% in the muscle, 50% in the liver, and 60% in the BAT of the ob/ob mice compared with that in the control group. No significant differences were observed in the UCP3 transcript levels in the adipose tissues and brain between the 2 groups (Fig. 2b).

Tissue-distribution patterns of UCP4

The UCP4 mRNA expression was detected in all the tested tissues, except BAT. The UCP4 mRNA level in the brain was the highest in the controls, while it was the lowest in the ob/ob mice. No significant differences were observed between the 2 groups in terms of the UCP4 mRNA levels in the skeletal muscle, liver, retroperitoneal fat, and subcutaneous fat (Fig. 2c).

Tissue-distribution patterns of UCP5

In the lean control mice, the highest levels of UCP5 expression were detected in the brain, followed by the liver, muscle, retroperitoneal fat, and subcutaneous fat; UCP5 mRNA expression was undetectable in the BAT. The amount of UCP5 mRNA in the ob/ob mice was down-regulated by 40% in the brain and 50% in the liver compared with that in the control mice. The UCP5 mRNA levels in the muscle, retroperitoneal fat, and subcutaneous fat were equivalent between the 2 groups (Fig. 2d).

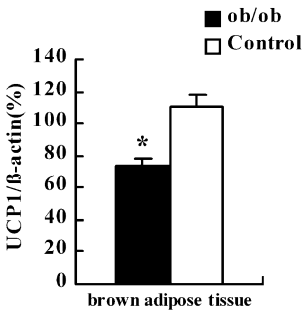

Fig. 1 Differential expression of uncoupling protein 1 (UCP1) in the brown adipose tissue between ob/ob mice and normal control mice. (*$p<0.05$, $n=12$)

Discussion

Obesity is presently the most significant contributor to ill health in the populations across the world (Jeffery and Sherwood 2008). Recently, uncoupling proteins (UCPs), which are members of a family of proton carriers located in the inner mitochondrial membrane, have attracted considerable interest of researchers for their role in energy metabolism and obesity (Boss et al. 1998; Ricquier and Bouillaud 2000). Thus far, 5 UCP isoforms have been identified, but information about their distribution and changes in their expression levels in obese individuals remains incomplete. This is the first study that systematically compared the tissue-specific distribution of UCPs (UCP1–UCP5) in normal and genetically obese ob/ob mice.

Because of the known cross-reactivity of the antibodies presently available for the detection the UCP proteins, western blot is not the preferred method for investigating the UCP expression patterns. Therefore, most pertinent studies have used methods to quantify UCP mRNA levels, such as northern blotting and reverse transcription-polymerase chain reaction (RT-PCR) (Pecqueur et al. 2001). Real-time PCR is a powerful and very sensitive technique that provides a reliable approach to quantitatively assess the expression level of various genes. In this study, we adopted the real-time PCR method to quantify the mRNA levels of the 5 known UCP isoforms in the tissues associated with energy metabolism.

UCP1 is specifically expressed in brown adipocytes to generate heat by uncoupling mitochondrial proton transport from the production of ATP. It has been shown that the upregulation of UCP1 by genetic manipulations can reduce obesity and improve insulin sensitivity (Kopecky et al. 1995). Commins et al showed that Leptin administration reduced white adipose tissue depots in mice via a UCP1-dependent mechanism in brown adipose tissue (Commins et al. 2001).In 1977, Trayhurn et al. (Trayhurn et al. 1977) revealed that in pre-obese ob/ob mice, Leptin deficiency suppressed UCP1 expression and precluded the activation of brown fat thermogenesis. In this study, we reconfirmed that UCP1 mRNA level in the BAT was significantly downregulated in ob/ob mice. The result suggested that decreased UCP1 expression in BAT leading to reduced diet-induced thermogenesis might play an important role in the development of obesity of Leptin-deficiency mice. This

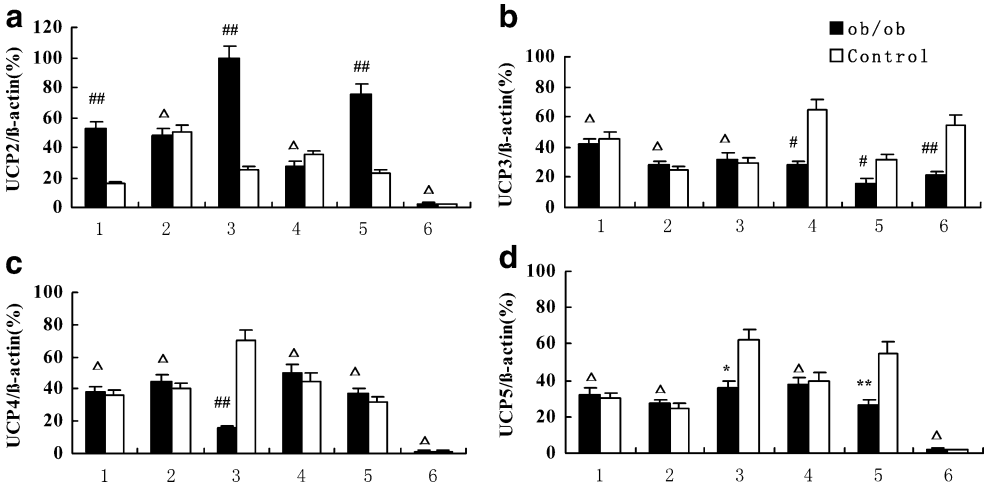

Fig. 2 Differential distribution of uncoupling protein 2 to uncoupling protein 5 (UCP2–UCP5) in the selected tissues of ob/ob mice and normal control mice. **a** Tissue distribution of UCP2. **b** Tissue distribution of UCP3. **c** Tissue distribution of UCP4. **d** Tissue distribution of UCP5. 1, retroperitoneal adipose tissue; 2, subcutaneous adipose tissue; 3, brain; 4, muscle; 5, liver; 6, brown adipose tissue. ($^{\#}p<0.005$; $^{\#\#}p<0.001$; $^{*}p<0.05$; $^{**}p<0.01$; $^{\triangle}p>0.05$, $n=12$)

concept was supported by the studies of Feldmann et al who have revealed that UCP1 ablation could induce obesity in mice fed on a controlled diet, which occurred mainly due to the lack of diet-induced thermogenesis (Feldmann et al. 2009).

UCP2 has been proposed to play a prominent role in the regulation of energy balance. Because the net energy expenditure is reduced in obese subjects, UCP2 expression or activity is predicted to be decreased. However, our experimental evidence revealed that UCP2 mRNA expression was upregulated in the liver, brain, and retroperitoneal fat and was not altered in the skeletal muscle of ob/ob mice. A similar phenomenon has been reported in humans, where UCP2 mRNA levels in white fat were shown to be positively correlated with the body mass index (Millet et al. 1997). Because UCP2 expression is increased in ob/ob mice, it raises the possibility that this enhanced expression could be a compensatory response to the underlying metabolic disturbances in ob/ob mice, such as to diminish the excess reactive oxygen species (ROS). This hypothesis is supported by findings that overexpression of UCP2 could attenuate cellular ROS generation and that knockout of this gene resulted in higher levels of ROS (Arsenijevic et al. 2000; Lee et al. 2009; Teshima et al. 2003). Taken together, the possible mechanisms involved in the upregulation of UCP2 mRNA expression in ob/ob mice need to be systematically analyzed.

UCP3 is predominantly expressed in the skeletal muscles, suggesting that this gene would be important in the regulation of energy expenditure in this organ. A role for UCP3 in the skeletal muscle is supported by the fact that its expression is severely decreased in the denervated muscle, leading to the possible accumulation of excess energy as fat in the muscle and a decreased metabolic capacity (Kontani et al. 2002). Furthermore, Schrauwen et al. observed that in the Pima Indians, the expression of UCP3 in the skeletal muscle was inversely correlated with the body mass index (Schrauwen et al. 1999). Our data also showed that the UCP3 mRNA level in the skeletal muscle of the ob/ob mice was obviously decreased compared with that of the control mice. However, controversial results suggesting no significant correlation between UCP3 content and BMI have been obtained in other populations (Schrauwen et al. 2001; Sbraccia et al. 2002); additionally, the UCP3-knockout mice failed to show any important role for UCP3 in the regulation of whole body weight and energy metabolism (Gong et al. 2000; Vidal-Puig et al. 2000). In conclusion, the exact physiological function of UCP3 is unknown, and our research might offer some new clues for exploring the exact function of UCP3 in energy metabolism.

UCP4 and UCP5 have been described to display relatively high expression in the brain. Researchers have proposed that these genes might be involved in the regulation of ROS production and regional thermogenesis in the central nervous system (CNS) [Mao et al. 1999; Sanchis et al. 1998; Yu et al. 2000]. In our study, we detected UCP4 and UCP5 mRNA expression in all the tested tissues of mice, except BAT. We also found that the level of UCP4 and UCP5 mRNA in the brain was significantly decreased in the ob/ob mice, suggesting that this downregulation might be associated with the thermogenic mechanisms for the regulation of body fat in genetically obese mouse. Further studies are warranted to investigate the underlying mechanism.

In summary, in the present study, we showed that the distribution of the UCP isoform mRNAs in the tissues associated with energy metabolism is considerably different between ob/ob mice and normal mice. The downregulation of UCP1, UCP3, UCP4 and UCP5 in the corresponding tissues is associated with the development of genetic obesity, while the marked upregulation of UCP2 in the brain, liver and adipose tissue in genetically obese mice might be a compensatory response during obesity. The

further gene knockout and/or transgenic experiments are needed to elucidate the role of different expression levels of UCPs in genetic obesity and our results will provide some valuable clues for these researches.

Acknowledgements This study was supported by grants from the National Natural Science Foundation of China (No 30772364, 30801256), the Natural Science Foundation of Jiangsu Province, China (No BK2007230, BK2008078), and Jiangsu Province's Outstanding Medical Academic Leader program (No LJ200624).

References

Alán L, Smolková K, Kronusová E, Šantorová J, Ježek P (2009) J Bioenerg Biomembr 41(1):71–78

Arsenijevic D, Onuma H, Pecqueur C, Raimbault S, Manning BS, Miroux B, Couplan E, Alves-Guerra MC, Goubern M, Surwit R, Bouillaud F, Richard D, Collins S, Ricquier D (2000) Nat Genet 26(4):435–439

Boss O, Muzzin P, Giacobino JP (1998) Eur J Endocrinol 139(1):1–9

Commins SP, Watson PM, Frampton IC, Gettys TW (2001) Am J Physiol Endocrinol Metab 280(2):E372–E377

Feldmann HM, Golozoubova V, Cannon B, Nedergaard J (2009) Cell Metab 9(2):203–209

Flier JS, Lowell BB (1997) Nat Genet 15(3):223–224

Friedman JM, Halaas JL (1998) Nature 395:763–770

Gong DW, Monemdjou S, Gavrilova O, Leon LR, Marcus-Samuels B, Chou CJ, Everett C, Kozak LP, Li C, Deng C, Harper ME, Reitman ML (2000) J Biol Chem 275(21):16251–16257

Jeffery RW, Sherwood NE (2008) BMJ 336:244–246

Jia JJ, Tian YB, Cao ZH, Tao LL, Zhang X, Gao SZ, Ge CR, Lin QY, Jois M (2010) Mol Biol Rep 37(3):1513–1522

Kontani Y, Wang Z, Furuyama T, Sato Y, Mori N, Yamashita H (2002) J Biochem 132(2):309–315

Kopecky J, Clarke G, Enerback S, Spiegelman B, Kozak LP (1995) J Clin Invest 96:2914–2923

Lee SC, Robson-Doucette CA, Wheeler MB (2009) J Endocrinol 203 (1):33–43

Mao W, Yu XX, Zhong A, Li W, Brush J, Sherwood SW, Adams SH, Pan G (1999) FEBS Lett 443(3):326–330

Millet L, Vidal H, Andreelli F, Larrouy D, Riou JP, Ricquier D, Laville M, Langin D (1997) J Clin Invest 100(11):2665–2670

Pecqueur C, Alves-Guerra MC, Gelly C, Levi-Meyrueis C, Couplan E, Collins S, Ricquier D, Bouillaud F, Miroux B (2001) J Biol Chem 276(12):8705–8712

Ricquier D, Bouillaud F (2000) Biochem J 345:161–179

Rosenbaum M, Leibel RL, Hirsch J (1997) N Engl J Med 337(6):396–407

Sanchis D, Fleury C, Chomiki N, Goubern M, Huang Q, Neverova M, Grégoire F, Easlick J, Raimbault S, Lévi-Meyrueis C, Miroux B, Collins S, Seldin M, Richard D, Warden C, Bouillaud F, Ricquier D (1998) J Biol Chem 273(51):36411–36415

Sbraccia P, D'Adamo M, Leonetti F, Buongiorno A, Silecchia G, Basso MS, Tamburrano G, Lauro D, Federici M, Di Daniele N, Lauro R (2002) Clin Endocrinol (Oxf) 57(2):199–207

Schrauwen P, Xia J, Walder K, Snitker S, Ravussin E (1999) Int J Obes Relat Metab Disord 23(12):1242–1245

Schrauwen P, Hesselink MK, Borghouts BEE, LB SG, Saris WH, Keizer HA (2001) Diabetes 50(12):2870–2873

Teshima Y, Akao M, Jones SP, Marbán E (2003) Circ Res 93(3):192–200

Trayhurn P, Thurlby PL, James WP (1977) Nature 266:60–62

Vidal-Puig AJ, Grujic D, Zhang CY, Hagen T, Boss O, Ido Y, Szczepanik A, Wade J, Mootha V, Cortright R, Muoio DM, Lowell BB (2000) J Biol Chem 275(21):16258–16266

Visscher TL, Seidell JC (2001) Annu Rev Public Health 22:355–375

Yu XX, Mao W, Zhong A, Schow P, Brush J, Sherwood SW, Adams SH, Pan G (2000) FASEB J 14:16711–16718