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Cloning of rat uncoupling protein-3 and uncoupling protein-2 cDNAs: their gene expression in rats fed high-fat diet

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Abstract In order to elucidate energy balance in the skeletal muscle, we cloned cDNA of a homologue of uncoupling protein (UCP) from rat skeletal muscle. We also cloned rat UCP-2 cDNA from rat brown adipose tissue (BAT). The UCP cloned from rat skeletal muscle showed 57% and 72% identity with rat UCP-1 and UCP-2. The mRNA was expressed abundantly in the skeletal muscle, moderately in the BAT, and slightly in the white adipose tissue (WAT) with a major band at 2.5 kb and a minor band at 2.8 kb, while the UCP-2 gene expression was widely detected in the whole body with substantial levels in the WAT and with slight levels in the skeletal muscle and BAT. The rat UCP cloned in the present study showed 86% identity with the recently cloned human UCP-3, which was also expressed abundantly in the skeletal muscle with a signal of 2.4 kb. Therefore, the rat UCP was considered to be rat UCP-3. In rats fed high-fat diet the UCP-3 gene expression was augmented 2-fold in the skeletal muscle while UCP-2 mRNA levels were increased significantly (1.6-fold) in the epididymal WAT. Augmented expression of UCPs may provide defense against high-fat induced obesity and impairment of glucose metabolism.

Key words: Uncoupling protein; Skeletal muscle; Energy expenditure; High-fat diet; Obesity; Brown adipose tissue

1. Introduction

The regulation of energy metabolism involves food intake and energy expenditure [1]. In contrast to recent significant advances in the understanding of central regulatory circuits of food intake [2], much less is known about the molecular basis of energy expenditure. A major component of energy expenditure is nonshivering thermogenesis [3]. Nonshivering thermogenesis has been implicated in the regulation of body temperature, body weight and metabolism. Mitochondrial proteins called uncoupling proteins (UCPs) induce nonshivering thermogenesis by creating a pathway that allows dissipation of the proton electrochemical gradient across the inner mitochondrial membrane [4]. UCP-1 is expressed exclusively in the brown adipose tissue (BAT) [5–7], an important site of energy expenditure in rodents. The significance of UCP-1 in adult humans, however, is questionable because of low amount of BAT. Therefore, the existence of other member of UCPs in tissues other than BAT has been postulated. Recently two groups reported the cloning of UCP-2 cDNA in mice and humans, which is expressed widely in the whole body [8,9]. However, UCP-2 mRNA levels were low in the skeletal muscle [8,9], which has been implicated in a substantial portion of nonshivering thermogenesis [10–15]. Therefore, it is postulated that other member of UCP may exist in the skeletal muscle. In the present study, we isolated and sequenced cDNA of a homologue of UCP highly expressed in rat skeletal muscle. In addition, since the cloning of rat UCP-2 cDNA has not been reported yet, in order to examine the possibility that our cloned UCP would be rat UCP-2, we isolated and sequenced rat UCP-2 cDNA. We compared our cloned UCP to the rat UCP-2 and the recently cloned human UCP-3 [16,17].

The pathophysiological significance of UCPs in tissues other than the BAT remains to be elucidated. Mice lacking in UCP-1 have been cold-sensitive but not obese [18]. Postulated up-regulation of UCPs other than UCP-1 may explain the prevention of obesity in mice lacking in UCP-1. The effects of uncoupling of electron transport and ATP synthesis have been investigated by the incubation of cultured muscle cells, L6 cells, and cultured adipocytes, 3T3-L1 cells with chemical uncouplers such as 2,4-dinitrophenol (DNP) [19,20]. The uncoupling causes the increase of the rate of electron transport in the mitochondria to maintain the ratio of ATP:ADP, which leads to the increase of rate of glycolysis and fatty acid break down. Glucose uptake was also increased by chemical uncouplers in L6 cells and 3T3-L1 cells. These findings suggest that UCP may regulate metabolism of carbohydrate and lipid as well as body corpulency. Recently the UCP-2 gene expression has been reported to be increased in the WAT in response to high-fat feeding [8]. Rats fed high-fat diet are accompanied by obesity and impairment of glucose metabolism [21]. In the present study, in order to elucidate pathophysiological significance of our cloned UCP and UCP-2 in obesity and impairment of glucose metabolism, we examined the expression of these genes in the skeletal muscle, BAT and WAT from rats fed high-fat diet.

2. Materials and methods

2.1. Isolation of cDNA of UCP expressed in rat skeletal muscle

Based on the coding sequence of the mouse UCP-2 cDNA [8,9], sense and antisense primers (sense: 5′-TAC GAC TCT GTC AAA CAG TTC TAY AC-3′; antisense: 5′-ATA GGT GAC AAA CAT CAC TAC RTT CCA-3′) were prepared. Reverse transcription-PCR

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(RT-PCR) was performed using Superscript (Gibco, Grand Island, NY) and oligo (dT)-primers with 10 μg of total RNA from rat gastrocnemius muscle as described [22]. The PCR products were subcloned for sequencing. A 0.6 kb PCR product encoding protein which showed 69% and 54% identity with partial sequences of mouse UCP-2 and rat UCP-1, respectively, was obtained. The 5′-rapid amplification of cDNA ends (RACE) and 3′-RACE of the cDNA were performed using 1 μg of poly (A) RNA from rat gastrocnemius muscle, and gene-specific primers (5′-RACE: 5′-GCC AAG TCC CTT TCC ACA GTC CCC TGA C-3′; 3′-RACE: 5′-CTG TGG AAA GGG ACT TGG CCC AAC ATC-3′) with Marathon cDNA Amplification Kit (Clontech Laboratories, Palo Alto, CA). PCR was carried out using LA-Taq Polymerase (Takara, Otsu, Japan) and the following conditions: 20 s at 94°C and 3 min at 68°C for 30 cycles. PCR products were sequenced after subcloning into the pGEMT Easy vector (Promega, Madison, WI). To exclude nucleotide misincorporations during PCR, products of more than two independent PCRs were sequenced.

2.2. Isolation of rat UCP-2 cDNA

Based on the noncoding sequence of mouse UCP-2 cDNA [8,9], sense and antisense primers (sense: 5′-TCT CCA ATT TCT CTC CAT CTT CTG G-3′; antisense: 5′-TCC AGG TCA GCA TGG AGA GCC-3′) were prepared. RT-PCR of rat UCP-2 cDNA was performed using these primers and 1 μg of poly (A) RNA from the BAT as described above. PCR was carried out using LA-Taq Polymerase and the following conditions: 20 s at 94°C, 10 s at 55°C and 1 min and 20 s at 68°C for 32 cycles. To exclude nucleotide misincorporations during PCR, products of more than two independent PCRs were sequenced.

2.3. Tissue preparation, total RNA extraction and Northern blot analysis

Twelve week-old male Sprague-Dawley (SD) rats were killed by decapitation after a blow to the head. Tissues were immediately removed from rats, frozen in liquid nitrogen and stored at −80°C until use. Total RNA was extracted using Trizol reagent (Gibco) [23]. Northern blot analysis was performed as described previously [21]. The probe of the rat UCP cloned in the present study was a 0.6 kb fragment containing amino-acid residues #105–281. The rat UCP-2 probe was a 1.0 kb fragment spanning the entire open reading frame. The rat UCP-1 probe was prepared by RT-PCR using primers (sense: 5′-TAT TCA TTG GGC AGC CCA CAG AGG-3′; antisense: 5′-ACA CAA ACA TGA TGA CGT TCC AGG-3′) and 10 μg of total RNA [22]. The specific activities of these probes were all $1.7–1.9\times10^9$ cpm/μg DNA.

For the study of tissue distribution of gene expression, Rat Multiple Tissue Northern Blots (#7764-1) containing 2 μg of poly (A) RNA per lane (Clontech Laboratories), and the filters containing 10 μg of total RNA from subcutaneous WAT, epididymal WAT, interscapular BAT and gastrocnemius muscle were used. The density of 18 S rRNA stained with ethidium bromide was used to monitor the amount of total RNA in each sample.

2.4. High-fat diet experiment

Eight week-old male SD rats were used. Rats were housed in a temperature-, humidity-, and light-controlled room with free access to water and food. These animals were fed standard diet (n=4), or high-fat diet (n=4) for 4 weeks as described below. The high-fat diet contained 7.5% carbohydrate (wheat flour and bran), 24.5% protein (mainly casein) and 60% fat (mainly lard), whereas the control diet was a standard rat chow containing 61% carbohydrate (wheat flour), 27% protein (bran and corn) and 12% fat (corn oil) [24]. A 0.2 ml blood was sampled from the tail vein after overnight fasting, and the plasma glucose, total cholesterol, and insulin levels were measured. Plasma total cholesterol and triglyceride levels were measured using the blood sampled without fasting on the previous day of the sacrifice. Gastrocnemius muscle, interscapular BAT and epididymal WAT were sampled at the end of the experiment, and RNA extraction was performed as described above.

Filters containing 10 μg of total RNA from the gastrocnemius muscle, interscapular BAT and epididymal WAT were prepared. The density of 18 S rRNA stained with ethidium bromide was used to monitor the amount of total RNA in each sample. Northern blot hybridization was performed as described above. The mRNA levels of the rat UCP cloned in the present study and UCP-2 (arbitrary units) were expressed relative to those in the gastrocnemius muscle from control rats fed standard diet (the mRNA levels in 10 μg of total RNA of the gastrocnemius muscle from control rats fed standard diet were defined as 100 units).

2.5. Statistical analysis

Data were expressed as the mean ± S.E.M. The statistical significance of differences between groups was assessed by Student's unpaired t-test.

3. Results and discussion

Using degenerate primers based on the coding sequence of the mouse UCP-2 cDNA, RT-PCR was performed with total RNA from rat gastrocnemius muscle. The PCR product encoding protein which showed 69% and 54% identity with partial sequences of mouse UCP-2 and rat UCP-1, respectively, was isolated. Using 5′-RACE and 3′-RACE, cDNA containing the entire open reading frame was obtained. The protein encoded by the entire open reading frame of the cDNA showed 73% identity with mouse UCP-2. The relatively low identity with mouse UCP-2 suggests that the protein is a member of UCP but not rat UCP-2.

The cloning of rat UCP-2 cDNA has not been reported yet. In order to further exclude the possibility that the rat UCP cloned in the present study would correspond to rat UCP-2, we cloned and sequenced rat UCP-2 cDNA using RT-PCR with primers of noncoding sequence of the mouse UCP-2 cDNA. The rat UCP-2 cDNA encodes a 309 amino-acid protein showing 99% and 95% identity with the mouse and human UCP-2, indicating that rat UCP-2 is highly homologous to mouse and human UCP-2. The rat UCP cloned in the present study showed 57% and 72% identity with rat UCP-1 and rat UCP-2, respectively (Fig. 1). Therefore, the rat UCP is a member of UCPs but distinct from rat UCP-2.

In order to further characterize the UCP cloned from rat skeletal muscle, the tissue distribution of the gene expression in rats was examined by Northern blot analyses. The cDNA probe hybridized two bands, a major band at 2.5 kb and a minor band at 2.8 kb in rat tissues (Fig. 2). The mRNA was expressed at the highest level in the skeletal muscle, and moderately in the interscapular BAT. Only weak signals were detected in the epididymal WAT and the heart. Slight signals were observed in the subcutaneous WAT and the spleen with longer exposure time for autoradiography (data not shown). The ratio of intensity of the major band to that of the minor band did not vary among the samples. These results demonstrate that the rat UCP is a member of UCP family highly expressed in the skeletal muscle.

During the preparation of this manuscript, recently two groups reported the cloning of human UCP-3 cDNA, and that human UCP-3 mRNA was detected abundantly in human skeletal muscle with a signal of 2.4 kb [16,17]. Human UCP-3 encodes a 312 amino-acid protein showing 86% identity with rat UCP cloned in the present study which consists of 308 amino acids. Although the numbers of amino acids of the rat UCP cloned in the present study and human UCP-3 are different, the abundant gene expression of the rat UCP in the skeletal muscle with a major band at similar size to human UCP-3 mRNA indicates that the rat UCP corresponds to a rat counterpart of human UCP-3, namely, rat UCP-3. The rat UCP is called rat UCP-3 in the remaining part of the paper.

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rUCP-1 MVSSTTSEVQPTMGVKIFSAGVSACLADIITFPLDTAKVRLQIQGEGQA----SSTIRYKGVLGTITTLAKTEGLPKLYSGLPAGIQRQI  86
rUCP-2 MVGFKATDVPPTATVKFLGAGTAACIADLITFPLDTAKVRLQIQGESQGLARTAASAQYRGVLGTILTMVRTEGPRSLYNGLVAGLQRQM  90
rUCP-3 MVGLQPSEVPPTTVVKFLGAGTAACFADLLTFPLDTAKVRLQIQGENPGVQ----SVQYRGVLGTILTMVRTEGPRSPYSGLVAGLHRQM  86
hUCP-3 MVGLKPSDVPPTMAVKFLGAGTAACFADLVTFPLDTAKVRLQIQGENQAVQ-TARLVQYRGVLGTILTMVRTEGPCSPYNGLVAGLQRQM  89
                       I                                                                    II

rUCP-1 SFASLRIGLYDTVQEYFSSGRETPASLGSKISAGLMTGGVAVFIGQPTEVVKVRMQAQSHLHG--IKPRYTGTYNAYRVIATTESLSTLW 174
rUCP-2 SFASVRIGLIDSVKQFYTKGSE-HAGIGSRLLAGSTTGALAVAVAQPTDVVKVRFQAQARAG---GGRRYQSTVEAYKTIAREEGIRGLW 176
rUCP-3 SFASIRIGLIDSVKQFYTPKGTDHSSVAIRILAGCTTGAMAVTCAQPTDVVKVRFQAMIRLG-TGGERKYRGTMDAYRTIAREEGVRGLW 175
hUCP-3 SFASIRIGLIDSVKQVYTPKGADNSSLTTRILAGCTTGAMAVTCAQPTDVVKVRFQASIHLGPSRSDRKYSGTMDAYRTIAREEGVRGLW 179
                                                III

rUCP-1 KGTTPNLMRNVIINCTELVTYDLMKGALVNHHILADDVPCHLLSALVAGFCTTLLASPVDVVKTRFINSLPGQYPSVPSCAMTMYTKEGP 264
rUCP-2 KGTSPNVARNAIVNCTELVTYDLIKDTLLKANLMTDDLPCHFTSAFGAGFCTTVIASPVDVVKTRYMNSALGQYHSAGHCALTMLRKEGP 266
rUCP-3 KGTWPNITRNAIVNCAEMVTYDIIKEKLLDSHLFTDNFPCHFVSAFGAGFCATVVASPVDVVKTRYMNAPPGRYRSPLHCMLRMVAQEGP 265
hUCP-3 KGTLPNIMRNAIVNCAEVVTYDILKEKLLDYHLLTDNFPCHFVSAFGAGFCATVVASPVDVVKTRYMNSPPGQYFSPLDCMIKMVAQEGP 269
                IV                                V

rUCP-1 AAFFKGFAPSFLRLGSWNVIMFVCFEQLKKELMKSRQTVDCTT 307
rUCP-2 RAFYKGFMPSFLRLGSWNVVMFVTYEQLKRALMAAYESREAPF 309
rUCP-3 TAFYKGFMPSFLRLGSWNVMMFVTYEQLKRALMKVQVLRESPF 308
hUCP-3 TAFYKGFTPSFLRLGSWNVVMFVTYEQLKRALMKVQMLRESPF 312
                VI
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Fig. 1. Comparison of the amino-acid sequences of rat UCP-1, rat UCP-2 (GenBank Accession AB006613), rat UCP-3 (GenBank Accession AB006614) and human UCP-3 (UCP-3_L). Rat UCP-3 was called the rat UCP cloned in the present study in the former part of the text where the establishment of the rat UCP as rat UCP-3 had not been done. The sequences were presented in single letter code. Potential membrane-spanning domains were underlined and numbered in roman numerals. The potential purine nucleotide binding domain was underlined doubly. The three mitochondrial energy-transfer protein signatures were boxed. Identities between sequences were shown in asterisks.

Rat UCP-3 cDNA contains a 5′-untranslated region of approximately 0.2 kb, a 3′-untranslated region of approximately 1.4 kb and an open reading frame encoding a 308 amino-acid protein which has a molecular weight of approximately 34 kD. Hydrophobicity profile of rat UCP-3 demonstrates that it contains six predicted membrane-spanning domains like other mitochondrial carriers (Fig. 1). Potential purine nucleotide binding domain, which is implicated in the control of the coupling efficiency [25], was noted in amino-acid residues #263–271 (Fig. 1). Three mitochondrial energy-transfer protein signatures [26] were identified (Fig. 1). These observations suggest that rat UCP-3 cloned in the present study uncouples mitochondrial respiration. There have been reported to be two forms of UCP-3 cDNA in humans, UCP-3_L and UCP-3_S, which may be due to alternative splicing [16]. UCP-3_L contains six predicted membrane-spanning domains like UCP-1 and UCP-2 while UCP-3_S lacks the sixth membrane-spanning domain. In the 3′-RACE of the present study we cloned only rat UCP-3 corresponding to human UCP-3_L. Northern blot analyses demonstrated that rat UCP-3 mRNA was detected as two bands (Fig. 2). It may be due to alternative polyadenylation. Alternatively, UCP-3_L and UCP-3_S may explain the two bands although human UCP-3_L and UCP-3_S was detected as a single band [16].

We compared the gene expression of UCP-3 with that of UCP-2 in rat tissues (Fig. 2). The UCP-2 cDNA probe hybridized a 1.7 kb band. The UCP-2 gene expression was detected widely in the whole body, especially abundantly in the lung and the spleen. UCP-2 mRNA was also observed in the heart, liver, brain, kidney, testis, WAT, BAT and skeletal muscle. In contrast to the UCP-3 gene expression, the UCP-2 gene expression was expressed at substantial levels in the WAT and only at slight levels in the skeletal muscle and BAT. The Northern blot analyses of UCP-3 and UCP-2 (Fig. 2) were performed with the probes of approximately the same specific activities and the same exposure time for autoradiography, while the probe for UCP-2 was approximately 1.7-fold longer than that for UCP-3. Although accurate comparison between mRNA levels of UCP-3 and UCP-2 is difficult, Fig. 2 indicates that the most abundant UCP expressed in the skeletal muscle is UCP-3 in rats.

The UCP-3 mRNA levels were rather higher in the BAT of rats and mice in previous reports [16,17] as compared with our present finding. That difference may be due to using human UCP-3 cDNA as a probe in the previous reports. Moreover, the UCP-2 mRNA levels in rat BAT in our present finding were almost the same as those in some previous report [9], but were lower than those in other previous reports [8,16,17]. That

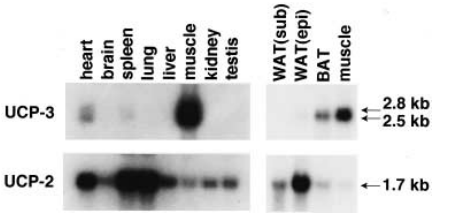

Fig. 2. Tissue distribution of the gene expression of rat UCP-3 and UCP-2 analysed by Northern blots. Rat UCP-3 was called the rat UCP cloned in the present study in the former part of the text where the establishment of the rat UCP as rat UCP-3 had not been done. Results of UCP-3 and UCP-2 mRNA levels were shown in upper and lower portions, respectively. Two μg of poly (A) RNA and 10 μg of total RNA were used in left and right panels, respectively. Muscle, skeletal muscle; BAT, interscapular brown adipose tissue; WAT (sub), subcutaneous white adipose tissue; WAT (epi), epididymal WAT.

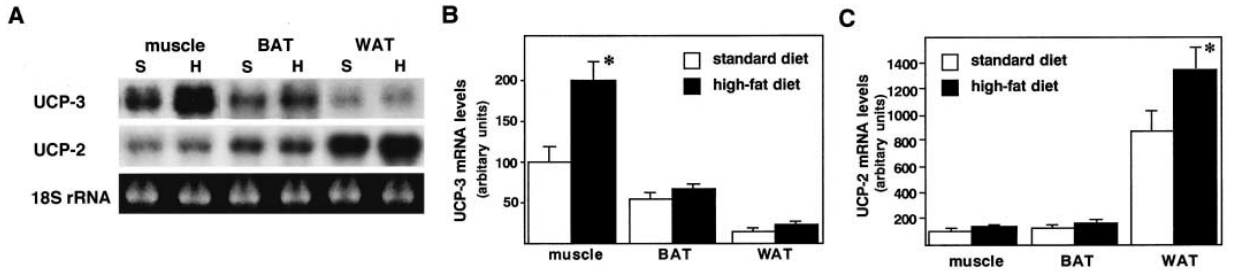

Fig. 3. Northern blot analyses of rat UCP-3 and UCP-2 mRNA in the gastrocnemius muscle, interscapular BAT and epididymal WAT from rats fed standard diet (S) and those fed high-fat diet (H). Total RNA (10 μg/lane) was analysed. A: Representative blots. B: The rat UCP-3 mRNA levels ($n=4$). C: The rat UCP-2 mRNA levels ($n=4$). *$P<0.05$ compared with rats fed standard diet. Muscle, gastrocnemius muscle; BAT, interscapular brown adipose tissue; WAT, epididymal white adipose tissue.

variation may be explained by the difference of probes used. When we compared the UCP-2 mRNA levels in the skeletal muscle of rats and mice of our finding and other reports with those of humans in previous reports, the UCP-2 mRNA levels in some samples from human skeletal muscle were rather higher, while those in other samples were approximately the same levels [8,9,16,17]. In order to elucidate this discrepancy, further studies using more number of samples from human skeletal muscle are necessary since the number of the samples in the previous reports appeared very few.

Next we focused on the pathophysiologic significance of UCP-3 in the skeletal muscles. Skeletal muscle is one of the major tissues responsible for glucose uptake. Bashan et al. and Tsakiridis et al. reported that glucose uptake was elevated in L6 muscle cells and 3T3-L1 adipocytes by treatment of chemical uncouplers such as 2,4-DNP, and that synthesis and translocation of glucose transporters were augmented by the treatment of the chemical uncouplers [19,20]. Together with these findings, it is tempting to speculate that UCP-3 abundantly expressed in the skeletal muscle may contribute to regulation of glucose uptake, and that the regulation of the UCP-3 gene expression can affect glucose metabolism in the skeletal muscle.

Recently Fleury et al. reported the up-regulation of the UCP-2 mRNA levels in the epididymal WAT in response to high-fat diet feeding [8]. We investigated the gene expression of UCP-3 in the gastrocnemius muscle, interscapular BAT and epididymal WAT from rats fed standard diet and those fed high-fat diet. Table 1 summarizes the brief profile of rats fed standard diet and those fed high-fat diet. Rats fed high-fat diet weighed 114% more than control rats fed standard diet, and developed hyperglycemia and hyperinsulinemia (Table 1). The weight of the epididymal WAT increased 2.7-fold as compared with those from rats fed standard diet. The UCP-3 gene expression was augmented 2-fold in the gastrocnemius muscle from rats fed high-fat diet ($P<0.05$) (Fig. 3). The UCP-3 mRNA levels remained unchanged in the interscapular BAT, and epididymal WAT. The ratio of intensity of the major band to that of the minor band did not change. The levels of the UCP-2 gene expression were augmented significantly in the epididymal WAT (1.6-fold) ($P<0.05$), while no significant increase was observed in the gastrocnemius muscle and interscapular BAT (Fig. 3). The UCP-1 mRNA levels remained unaffected in the BAT (data not shown) as previously reported [27]. Thus, the UCP-3 gene expression was preferentially augmented in the skeletal muscle in response to high-fat feeding, while the UCP-2 mRNA levels were preferentially increased in the WAT. Transgenic expression of UCP-1 in the WAT has been reported to reduce genetic obesity in mice [28]. As discussed above, the modulation of oxidative chain by chemical uncouplers increased glucose uptake, synthesis and translocation of glucose transporters [19,20]. It is tempting to speculate that the increased expression of the UCP-3 gene in the skeletal muscle and the UCP-2 gene in the WAT can provide defense against high-fat induced obesity and impairment of glucose metabolism. The mechanisms of up-regulation of the gene expression in response to high-fat diet require further investigation.

It has been reported that mice lacking in UCP-1 were cold-sensitive but not obese, and that the mRNA levels of UCP-2 were up-regulated in the BAT [18]. Furthermore, mice lacking in dopamine β-hydroxylase, which showed decreased basal UCP-1 mRNA levels and unaffected UCP-2 mRNA levels as compared with those in heterozygous mice, did not become obese [29]. In these mice UCP-3 and UCP-2 may contribute to prevention of obesity by their augmented expression.

In the present study, we cloned and sequenced cDNAs of rat UCP-3 and UCP-2. The present study also demonstrates that the rat UCP-3 gene expression was augmented preferentially in the skeletal muscle from rats fed high-fat diet while the UCP-2 mRNA levels were increased in the epididymal WAT from these mice. The elucidation of the pathophysio-

Table 1
Profiles of rats fed standard diet and those fed high-fat diet

	Standard diet	High-fat diet
Body weight (g)	389 ± 15	443 ± 5*
Glucose (mg/dl)	96 ± 3	139 ± 2**
Total cholesterol (mg/dl)	61 ± 3	63 ± 3
Triglyceride (mg/dl)	121 ± 19	238 ± 61
Insulin (μU/ml)	6.3 ± 1.0	45.4 ± 5.8**
Epididymal fat weight (g)	3.2 ± 0.2	8.6 ± 0.7**

Values are mean ± S.E.M. ($n=4$). *$P<0.05$; **$P<0.01$ vs. standard diet.

logical significance of UCP-3 and UCP-2 awaits analyses using transgenic mice with knockout or overexpression of these genes.

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References

[1] Flier, J.S. and Lowell, B.B. (1997) Nature Genet. 15, 223–224.
[2] Friedman, J.M. (1997) Nature 385, 119–120.
[3] Block, B.A. (1994) Annu. Rev. Physiol. 56, 535–577.
[4] Nicholls, D.G. and Locke, R.M. (1984) Physiol. Rev. 64, 1–64.
[5] Jacobsson, A., Stadler, U., Glotzer, M.A. and Kozak, L.P. (1985) J. Biol. Chem. 260, 16250–16254.
[6] Bouillaud, F., Weissenbach, J. and Ricquier, D. (1986) J. Biol. Chem. 261, 1487–1490.
[7] Rothwell, N.J. and Stock, M.J. (1979) Nature 281, 31–35.
[8] Fleury, C., Neverova, M., Collins, S., Raimbault, S., Champigny, O., Levi-Meyrueis, C., Bouillaud, F., Seldin, M.F., Surwit, R.S., Ricquier, D. and Warden, C.H. (1997) Nature Genet. 15, 269–272.
[9] Gimeno, R.E., Dembski, M., Weng, X., Deng, N., Shyjan, A.W., Gimeno, C.J., Iris, F., Ellis, S.J., Woolf, E.A. and Tartaglia, L.A. (1997) Diabetes 46, 900–906.
[10] Jensen, M.D., Jonson, C.M., Cryer, P.E. and Murray, M.J. (1995) Am. J. Physiol. 268, E433–E438.
[11] Davis, T.R.A. (1963) Am. J. Physiol. 213, 1423–1426.
[12] Astrup, A., Simonsen, L., Bülow, J., Madsen, J. and Christensen, N.J. (1989) Am. J. Physiol. 257, E340–E345.
[13] Simonsen, L., Bülow, J., Madsen, J. and Christensen, N.J. (1992) Am. J. Physiol. 263, E850–E855.
[14] Simonsen, J., Stallknecht, B. and Bülow, J. (1993) Int. J. Obes. Relat. Metab. Disord. 17, S47–S51.
[15] Duchamp, C. and Barre, H. (1993) Am. J. Physiol. 265, R1076–R1083.
[16] Boss, O., Samec, S., Paoloni-Giacobino, A., Rossier, C., Dulloo, A., Seydoux, J., Muzzin, P. and Giacobino, J.P. (1997) FEBS Lett. 408, 39–42.
[17] Vidal-Puig, A., Solanes, G., Grujic, D., Flier, J.S. and Lowell, B.B. (1997) Biochem. Biophys. Res. Commun. 235, 79–82.
[18] Enerbäck, S., Jacobsson, A., Simpson, E.M., Guerra, C., Yamashita, H., Harper, M.E. and Kozak, L.P. (1997) Nature 387, 90–93.
[19] Bashan, N., Burdett, E., Gumà, A., Sargeant, R., Tumiati, L., Liu, Z. and Klip, A. (1993) Am. J. Physiol. 264, C430–C440.
[20] Tsakiridis, T., Vranic, M. and Klip, A. (1995) Biochem. J. 309, 1–5.
[21] Masuzaki, H., Ogawa, Y., Hosoda, K., Kawada, T., Fushiki, T. and Nakao, K. (1995) Biochem. Biophys. Res. Commun. 216, 355–358.
[22] Takaya, K., Ogawa, Y., Isse, N., Okazaki, T., Satoh, N., Masuzaki, H., Mori, K., Tamura, N., Hosoda, K. and Nakao, K. (1996) Biochem. Biophys. Res. Commun. 225, 75–83.
[23] Ogawa, Y., Masuzaki, H., Isse, H., Okazaki, T., Mori, K., Shigemoto, M., Satoh, N., Tamura, N., Hosoda, K., Yoshimasa, Y., Jingami, H., Kawada, T. and Nakao, K. (1995) J. Clin. Invest. 96, 1647–1652.
[24] Iwanishi, M. and Kobayashi, M. (1993) Metabolism 42, 1017–1021.
[25] Bouillaud, F., Arechaga, I., Petit, P.X., Raimbault, S., Levi-Meyrueis, C., Casteilla, L., Laurent, M., Rial, E. and Ricquier, D. (1994) EMBO J. 13, 1990–1997.
[26] Palmieri, F. (1994) FEBS Lett. 346, 48–54.
[27] Collins, S., Daniel, K.W., Petro, A.E. and Surwit, R.S. (1997) Endocrinology 138, 405–413.
[28] Kopecky, J., Clarke, G., Enerback, S., Spiegelman, B. and Kozak, L.P. (1995) J. Clin. Invest. 96, 2914–2923.
[29] Thomas, S.A. and Palmiter, R.D. (1997) Nature 387, 94–97.