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Early postnatal maternal separation causes alterations in the expression of β3-adrenergic receptor in rat adipose tissue suggesting long-term influence on obesity

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ABSTRACT

The effects of early postnatal maternal deprivation on the biological characteristics of the adipose tissue later in life were investigated in the present study. Sprague–Dawley rats were classified as either maternal deprivation (MD) or mother-reared control (MRC) groups. MD was achieved by separating the rat pups from their mothers for 3 h each day during the 10–15 postnatal days. mRNA levels of mitochondrial uncoupling protein 1 (UCP-1), β3-adrenergic receptor (β3-AR), and prohibitin (PHB) in the brown and white adipose tissue were determined using real-time RT-PCR analysis. UCP-1, which is mediated through β3-AR, is closely involved in the energy metabolism and expenditure. PHB is highly expressed in the proliferating tissues/cells. At 10 weeks of age, the body weight of the MRC and MD rats was similar. However, the levels of the key molecules in the adipose tissue were substantially altered. There was a significant increase in the expression of PHB mRNA in the white adipose tissue, while the β3-AR mRNA expression decreased significantly, and the UCP-1 mRNA expression remained unchanged in the brown adipose tissue. Given that these molecules influence the mitochondrial metabolism, our study indicates that early postnatal maternal deprivation can influence the fate of adipose tissue proliferation, presumably leading to obesity later in life.

1. Introduction

Early postnatal maternal separation before weaning is acknowledged to be a stressful experience in mammals [1–3]. Numerous reports of preclinical and clinical studies have demonstrated that such adverse experiences in early life profoundly affect normal brain development, leading to functional disruptions in higher brain functions such as learning, memory, cognition, and social interactions [4–6]. In addition to brain disruptions, a growing body of evidence shows that adverse experiences in early life, including maternal separation, influence body-weight gain later in the life [6–10]. These reports investigated the etiological reasons for increased feeding behavior. However, detailed molecular mechanisms underlying this phenomenon are yet to be completely understood. Our primary hypothesis is that the stress induced by early maternal separation potentially affects the biological characteristics of adipose tissue. Therefore, in this study we evaluated the adipose tissue-associated molecules following maternal separation.

Two types of fat, white adipose tissue (WAT) and brown adipose tissue (BAT), exist in mammals including humans and rodents [11]. WAT stores excess calories, and its excessive accumulation causes obesity. BAT dissipates energy to produce heat through non-shivering thermogenesis for protection against cold environments. Therefore, BAT is potentially recognized as a novel target for anti-obesity treatments. BAT functioning is recruited through BAT-specific mitochondrial uncoupling protein 1 (UCP-1). The action of the UCP-1 is known to be mediated through the β3-adrenergic receptor (β3-AR), which is exclusively expressed in both WAT

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and BAT [12]. Prohibitin (PHB) is known to be a ubiquitous and an evolutionarily conserved protein that is localized in the mitochondria [13]. PHB is also known to be specifically required in tissues that undergo extensive cellular proliferation [13,14]. If fact, the depletion of PHB in *Caenorhabditis elegans* results in distinct germ line defects such as diminished oocyte production with smaller blood size [15]. Similarly, deletion of mouse PHB-2, an isoform of PHB, in embryonic fibroblasts results in severely impaired cellular proliferation [14].

The prevalence of diet-induced weight gain, particularly obesity due to high-fat intake in the general population, is one of the serious health problems in the world. Therefore, it is of interest to examine the characteristics of the adipose tissue resulting from high-fat load in animals that were separated from their mothers early in their life. From previous studies that indicate a relationship between maternal separation and body-weight gain [16,17], it is conceivable that a high-fat intake easily affects the characteristics of the adipose tissue. We hypothesized that the maternally separated animals fed with a high-fat diet show increased body mass and adipose tissue proliferation. To test this hypothesis, we used rats to examine whether the stress induced by early MD affects the biological characteristics of the adipose tissue later in life.

2. Materials and methods

This study was carried out in compliance with the guidelines for experimental use and care of laboratory animals set forth by the Kagawa University Animal Ethics Committee. Six pregnant Sprague–Dawley rats purchased from SLC Japan (Shizuoka, Japan) were used in this study. The day of birth was designated as postnatal day (PND) 0. On PND 2, all pups were placed together and randomly assigned back to the lactating mothers so that each mother received 8 pups (4 male and 4 female). Six sets of litters and their mothers were then randomly assigned to the maternal deprivation (MD) and mother-reared control (MRC) groups (3 sets each group). MD pups were removed from their mother and placed together in a new cage for 3 h per day, between 9 and 12 AM each day from PND 10 to PND 15. After separation, the pups were returned to their mother daily. During this period of separation, the pups were placed in a holding cage under identical conditions as those of the MRC group. The MRC group pups were allowed to remain with their mothers at all times. Animals were weaned on PND 21, and were weighed weekly from PND 21 [3 weeks of age] to 10 weeks of age. After weaning, the animals of both groups were fed with a high-fat diet (18.2% protein, 62.2% fat, and 19.6% carbohydrates) (HFD-60, Oriental Yeast, Tokyo, Japan).

At 10 weeks of age, the female pups of the MD and MRC groups ($n = 9$ each group) were anesthetized with 7% chloral hydrate (0.7 mL/100 g body weight; intraperitoneal injection) and perfused intracardially with medical grade physiological saline. Three female offspring from each set of animals were used. WAT and BAT were immediately collected from the retroperitoneal and interscapular regions, respectively. These fat samples were stored at −80 °C until use.

Homogenization of adipose tissue and extraction of total RNA were performed using QIAshredder and AllPrep DNA/RNA/Protein Mini Kits (Qiagen, Venlo, Netherlands) by following the manufacturer instructions. The concentration and purity of the extracted total RNA were evaluated by optical density measurements at 260 and 280 nm by using NanoDrop 1000 (Thermo Fisher Scientific, Waltham, MA, USA). Then, a QuantiTect Reverse Transcription Kit (Qiagen) was used to synthesize the cDNA. The genomic DNA was removed from 0.5 to 1.0 μg of the isolated RNA. To assess an appropriate internal control, co-amplification of a β-actin mRNA was performed for each sample. We used the following forward (F) and reverse (R) primers: for UCP-1 (The national center for biotechnology information (NCBI) reference sequence; NC_005118.3), F: 5′-CTTCTCAGCCGGCGTTTCTG-3′, R: 5′-GGTGATGGTCCCTAAGACACC-3′; for β3-AR (NCBI reference sequence; NC_005115.3), F: 5′-TTCCCAGCGGAGTTTTCATC-3′, R: 5′-AGCGGGTTGAAGGCAGAGT-3′; for PHB (NCBI reference sequence; NC_005109.3) F: 5′-GCGTGGTG AACTCTGCTCTA-3′, R: 5′-TGTACCCAGGGGATGAGGAA-3′; and for β-actin (NCBI reference sequence; NM_031144) F 5′-TTGCTGACAGGATGCAGAA-3′, R 5′-ACCAATCCACACAGAGTACTT-3′. The mRNAs were amplified using the LightCycler system (Roche Diagnostics, Basel, Switzerland). Reactions were performed in a 20-μL volume containing 2 μL of sample cDNA diluted 10-fold with distilled water, 0.5 μM of each forward and reverse primers, and the LightCycler FastStart DNA MasterPLUS SYBR Green I mix (Roche Diagnostics). After the initial denaturation at 95 °C for 20 s, amplification was performed under the following conditions: 40 cycles at 95 °C for 10 s, 58 °C for 5 s, and 72 °C for 20 s. Detection of the fluorescent tracers was carried out at the end of the 72 °C extension period. To confirm the specificity of the amplification, PCR products from each primer pair were subjected to melting-curve analysis after amplification. For melting-curve analysis, the PCR products were denatured by gradually increasing the temperature from 65 °C in 0.2 °C increments. The expression levels of each gene were determined using the ratio of mRNA levels of specific genes relative to those of the housekeeping gene, β-actin. To exclude genomic contamination, electrophoresis of the PCR products, amplified from cDNA using UCP-1, β3-AR, PHB, and β-actin primers, was carried out on a 2% agarose gel, followed by staining with ethidium bromide. A similar electrophoresis of the amplification products without reverse transcription (RT) was also performed as a negative control. The data were analyzed using the LightCycler analysis software. The PCR assay was carried out twice.

mRNA data for the target genes were expressed as the ratio of mRNA levels to those of the housekeeping gene β-actin. Statistical analysis was conducted by two-way repeated measures analysis of variance (ANOVA) for the body weight data and Student's *t*-test for real-time RT-PCR data using the SigmaPlot 12 software (Systat software, Inc., Chicago, IL, USA). The significance level was set at $P < 0.05$.

3. Results

Body weight (g) of MRC and MD rats between 3 and 10 weeks of age is presented in Table 1. Two-way repeated measures ANOVA revealed a significant main effect of age (df 7,112; $F = 868.119$; $P < 0.001$). However, there were no significant differences between the groups (df 1,112; $F = 10587$; $P = 0.226$) and group × age interaction (df 7,112; $F = 0.480$; $P = 0.848$). The post hoc analysis with Tukey–Kramer's test showed that the body weight of MRC and MD animals was statistically similar at all the time-points examined, although age-dependent body-weight gain was seen in animals of both groups.

Fig. 1 illustrates the image of a representative gel electrophoresis of the PCR products showing a single band, which highlights the specificity of amplification. This provides a strong confirmation that the primers we used specifically amplified the target genes UCP-1, β3-AR, PHB, and β-actin, excluding the genomic DNA contamination.

Table 2 shows the mRNA levels, expressed as mean ± standard error of the mean (SEM), of UCP-1, β3-AR, and PHB in WAT and BAT of MRC and MD animals. Statistical analysis using Student's *t*-test revealed that in the WAT there was no significant difference in β3-AR levels between MRC and MD animals, though PHB mRNA levels in MD rats increased significantly. UCP-1 mRNA was not detected in the WAT of either MRC or animals; BAT β3-AR mRNA

Table 1
Mean ± SEM body weights (g) of MRC and MD rats during various weeks of age.

	Weeks							
	3	4	5	6	7	8	9	10
MRC	50.08 ± 0.77	81.54 ± 0.98	118.14 ± 2.15	149.54 ± 3.24	187.07 ± 2.51	209.34 ± 6.28	235.86 ± 4.35	257.02 ± 5.08
MD	49.40 ± 1.05	81.95 ± 1.18	121.93 ± 1.97	156.44 ± 2.90	190.34 ± 3.86	219.38 ± 4.83	240.49 ± 6.25	258.38 ± 7.18

MRC, mother-reared control.
MD, maternal deprivation.
Number of animals examined was nine ($n = 9$, each group).
The two-way repeated measures analysis. of variance (ANOVA) revealed significant main effect of age. However, no significant group effects and age × group interaction. Tukey–Kramer's post hoc test revealed that there was no significant difference in the body weight between MRC and MD rats at any time points examined, although age-dependent body weight gain was statistically significant in both animals of groups.
Animals were weaned on the third week (=PND 21) and were killed on 10 weeks.

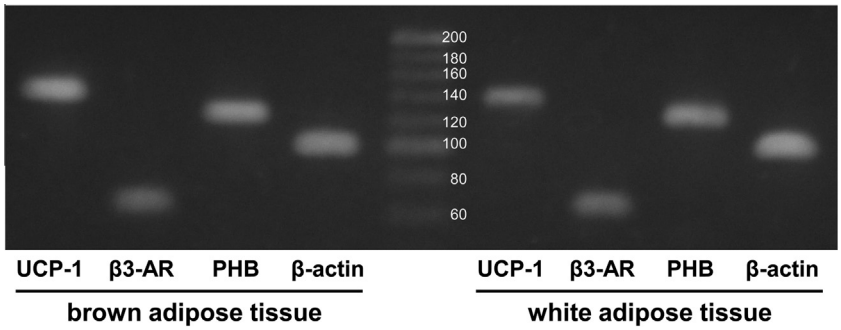

Fig. 1. A representative image of the electrophoresed agarose gel containing PCR products amplified from cDNA with UCP-1, β3-AR, PHB, and β-actin primers, showing a single band. No bands were detected for similar amplification procedure without reverse transcriptase (not shown). Molecular weight (base pair, bp) markers are in the center lane: UCP-1 (139 bp); β3-AR (64 bp); PHB (124 bp); and β-actin (101 bp).

levels decreased significantly in the MD rats. Interestingly, the UCP-1 and PHB mRNA levels in both animal groups were similar. Our result regarding UCP-1 (UCP-1 mRNA was not detected in the WAT) is consisted with earlier reports [11,18,19].

4. Discussion

There is a large body of evidence implying that early postnatal maternal separation causes various functional disruptions of the neuronal, endocrine, physiological, and behavioral systems [3,5,20,21]. In addition to such disruptions, which are seen immediately or after a relatively short time, the current interest has been primarily shifted to the lifelong influence of the perinatal adverse experience/insults [22]. A recent study by Lee and colleagues showed that rats experiencing neonatal maternal separation exhibited high body weight in later life [8–10]. It is widely agreed that such a weight gain is partly due to the increased food intake [8–10,23]. Apart from the influence of increased appetite presumably controlled by the central nervous system, our study indicated that alterations in the biological characteristics of peripheral adipose tissue contribute to weight gain. Our findings revealed novel molecular evidence pointing toward the alteration of mitochondrial metabolism-related molecules in the adipose tissue due to the stress induced during early postnatal maternal separation. Despite the similarity in body weights of MRC and MD rats, the levels of the key molecules in the adipose tissue were substantially altered between the two groups. The PHB mRNA expression was enhanced in WAT and the β3-AR mRNA expression was attenuated in BAT, while the UCP-1 mRNA expression remained unchanged. It is likely that these changes facilitate adipose tissue proliferation in later life.

Table 2
The mRNA expression levels of UCP-1, β3-AR, and PHB in white and brown adipose tissue in MRC and MD rats at 10 weeks of age.

	UCP-1	β3-AR	PHB
White adipose tissue			
MRC	N/D	4.18 ± 0.78	2.59 ± 0.32
MD	N/D	4.77 ± 0.48	3.39 ± 0.16*
Brown adipose tissue			
MRC	1.85 ± 0.25	0.83 ± 0.05	2.31 ± 0.30
MD	1.92 ± 0.26	0.62 ± 0.06*	2.38 ± 0.16

The results are expressed as mean ± SEM value of the relative amounts of mRNA for UCP-1, β3-AR, and PHB to those of the housekeeping gene β-actin.
MRC, mother-reared control.
MD, maternal deprivation.
N/D, not detected.
UCP-1, uncoupling protein-1; β3-AR, β3-adrenergic receptor; PHB, prohibitin.
Number of animals examined was nine ($n = 9$ each group).
* $P < 0.001$ (Student's t-test).

Weight gain/obesity is closely linked to fat proliferation. Therefore, examining the fate of adipose tissue is critical with respect to studying the development of obesity. Many studies have demonstrated that recent increase in the prevalence of human obesity is not only attributed to the genetic causes and leisurely lifestyle, but also to early-life determinants and epigenetic marks that are increasingly recognized as being of great importance [16,24,25]. In particular—given that hypertension, type 2 diabetes mellitus, and hypercholesterolemia are all closely associated with obesity—it is of value to investigate whether the etiological mechanisms underlying obesity development later in life are associated with early postnatal maternal separation stress.

The body-weight gain of MD rats was similar to that of age-matched MRC animals, which contradicted our primary hypothesis. Nevertheless, a high-fat diet was fed to the MD animals. Results of the present study are consistent with some previous reports [26,7,6,27], but are inconsistent with other studies [8–10], although there is a difference in the content of diets that were used. In Lee's experiment, maternally separated rats showed higher body weight than age-matched control animals. The exact reasons for this discrepancy between our and their results are unclear. It is conceivable that the differences in sex or experimental protocol may contribute

to such differences. In fact, in Lee's separation protocol, male rats were separated from their mother for 3 h per day during the first 2 weeks after birth. More recently, the housing method after weaning has been speculated to cause differences in weight gain and chow intake (reviewed by Jahng [10]). Isolated rearing may also promote weight gain and high food intake in MD rats. Food intake and weight gain of group-caged MD animals did not differ from those of the group-caged MRC animals [10].

It is known that the organs/tissues where PHB is highly expressed rely heavily on mitochondrial functions and are particularly vulnerable to mitochondrial dysfunction, which affects the neurons, muscles, heart, liver, renal tubules, adrenal cortex, pancreatic islet cells, and brown adipocytes [28]. Likewise, the PHB proteins are expressed in high levels in mammalian proliferating cells, including neoplastic tissues, and the protein expression declines during cellular senescence [28,29], indicating that PHB plays an essential role in regulating mitochondrial functions [13]. Taking these reports into consideration, the increased expression of PHB in the WAT of MD rats, as seen in this study, strongly suggests that WAT of the MD animals has the potential to influence adipose proliferation later in life, although the body weight of MD rats was similar to MRC animals at 10 weeks. Unfortunately, we have not conducted a time-dependent histological evaluation of rat adipose tissues. Our speculation, however, is proved by the results of a study that showed PHB depletion reduces fat content [30,31]. Further research is needed to clarify the exact mechanism underlying this phenomenon.

Mice deficient in dopamine β-hydroxylase, lacking the ligands (norepinephrine and epinephrine) of β3-AR, show an impaired induction of the UCP-1 gene compared to control animals [32]. More recently, Inokuma's group [33] reported that the administration of β3-AR agonist stopped the "cafeteria" feeding-induced weight gain in wild type mice, but not in UCP-1-knockout mice. Furthermore, they demonstrated that the anti-obesity effect of β3-AR depends on the action of the UCP-1 [33]. This report confirms that the functional recruitment of the UCP-1 is mediated by β3-AR. Turning to the present study, decreased β3-AR mRNA levels accompanied by unchanged UCP-1 mRNA levels observed in the MD rats indicate an attenuated UCP-1 activity in the BAT, since the activity of UCP-1 is initially recruited by β3-AR-mediated signaling events. Therefore, it is predicted that the MD rats have a low ability to utilize UCP-1 mediated thermogenesis. Such depletion of UCP-1 activity may not fully exert its anti-obesity effects on BAT, probably leading to obesity.

We have demonstrated that the rats which experienced early postnatal maternal separation show enhanced PHB levels in WAT, attenuated β3-AR levels, and unchanged UCP-1 levels in BAT. Considering that PHB and UCP-1 are mitochondrial metabolism-related molecules playing a key role in energy metabolism and expenditure, it can be presumed that the alteration of these variables in adipose tissue might contribute in long-term to obesity.

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References

[1] M. Nishi, N. Horii-Hayashi, T. Sasagawa, et al., Effects of early life stress on brain activity: implications from maternal separation model in rodents, Gen. Comp. Endocrinol. 181 (2013) 306–309.
[2] C.B. Faturi, P.A. Tiba, S.E. Kawakami, et al., Disruptions of the mother–infant relationship and stress-related behaviours: altered corticosterone secretion does not explain everything, Neurosci. Biobehav. Rev. 34 (2010) 821–834.
[3] T. Kikusui, Y. Mori, Behavioural and neurochemical consequences of early weaning in rodents, J. Neuroendocrinol. 21 (2009) 427–431.
[4] K.Y. Lee, T. Miki, T. Yokoyama, et al., Neonatal repetitive maternal separation causes long-lasting alterations in various neurotrophic factor expression in the cerebral cortex of rats, Life Sci. 90 (2012) 578–584.
[5] T. Miki, K.Y. Lee, T. Yokoyama et al., Differential effects of neonatal maternal separation on the expression of neurotrophic factors in rat brain. II: Regional differences in the cerebellum versus the cerebral cortex, Okajimas Folia Anat. Jpn. 2013 in press.
[6] M. Kalinichev, K.W. Easterling, P.M. Plotsky, et al., Long-lasting changes in stress-induced corticosterone response and anxiety-like behaviors as a consequence of neonatal maternal separation in Long-Evans rats, Pharmacol Biochem. Behav. 73 (2002) 131–140.
[7] S. Iwasaki, K. Inoue, N. Kiriike, et al., Effect of maternal separation on feeding behavior of rats in later life, Physiol Behav. 70 (2000) 551–556.
[8] V. Ryu, S.B. Yoo, D.W. Kang, et al., Post-weaning isolation promotes food intake and body weight gain in rats that experienced neonatal maternal separation, Brain Res. 1295 (2009) 127–134.
[9] J.H. Lee, H.J. Kim, J.G. Kim, et al., Depressive behaviors and decreased expression of serotonin reuptake transporter in rats that experienced neonatal maternal separation, Neurosci. Res. 58 (2007) 32–39.
[10] J.W. Jahng, An animal model of eating disorders associated with stressful experience in early life, Horm. Behav. 59 (2011) 213–220.
[11] S. Bi, L. Li, Browning of white adipose tissue: role of hypothalamic signaling, Ann N.Y. Acad. Sci. 2013, in press.
[12] J.G. Granneman, M. Burnazi, Z. Zhu, et al., White adipose tissue contributes to UCP1-independent thermogenesis, Am. J. Physiol. Endocrinol. Metab. 285 (2003) E1230–E1236.
[13] M. Artal-Sanz, N. Tavernarakis, Opposing function of mitochondrial prohibitin in aging, Aging (Albany NY) 2 (2010) 1004–1011.
[14] C. Merkwirth, S. Dargazanli, T. Tatsuta, et al., Prohibitins control cell proliferation and apoptosis by regulating OPA1-dependent cristae morphogenesis in mitochondria, Genes Dev. 22 (2008) 476–488.
[15] M. Artal-Sanz, W.Y. Tsang, E.M. Willems, L.A. Grivell, B.D. Lemire, H. van der Spek, L.G. Nijtmans, The mitochondrial prohibitin complex is essential for embryonic viability and germline function in *Caenorhabditis elegans*, J. Biol. Chem. 278 (2003) 32091–32099.
[16] F.R. Cagampang, K.R. Poore, M.A. Hanson, Developmental origins of the metabolic syndrome: body clocks and stress responses, Brain Behav. Immun. 25 (2011) 214–220.
[17] A. Marti, M.A. Martinez-González, J.A. Martinez, Interaction between genes and lifestyle factors on obesity, Proc. Nutr. Soc. 67 (2008) 1–8.
[18] C.J. Narvaez, D. Matthews, E. Broun, et al., Lean phenotype and resistance to diet-induced obesity in vitamin D receptor knockout mice correlates with induction of uncoupling protein-1 in white adipose tissue, Endocrinology 150 (2009) 651–661.
[19] T.J. Schulz, Y.H. Tseng. Brown adipose tissue: development, metabolism and beyond, Biochem J. 2013, in press.
[20] P.M. Plotsky, M.J. Meaney, Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rat, Brain Res. Mol. Brain Res. 18 (1993) 195–200.
[21] P. Feng, D. Vurbic, Z. Wu, et al., Brain orexins and wake regulation in rats exposed to maternal deprivation, Brain Res. 1154 (2007) 163–172.
[22] D.J.P. Barker, Mothers, Babies and Health in Later Life, 2nd ed., Churchill Livingstone, Edinburgh, 1998.
[23] Z. Penke, B. Fernette, C. Nyakas, et al., Neonatal maternal deprivation modifies feeding in response to pharmacological and behavioural factors in adult rat, Neuropharmacology 42 (2002) 421–427.
[24] P. Froguel, P. Boutin, Genetics of pathways regulating body weight in the development of obesity in humans, Exp. Biol. Med. (Maywood) 226 (2001) 991–996.
[25] A. Moleres, T. Rendo-Urteaga, C. Azcona, et al., Il6 gene promoter polymorphism (-174G/C) influences the association between fat mass and cardiovascular risk factors, J. Physiol. Biochem. 65 (2009) 405–413.
[26] J. McIntosh, H. Anisman, Z. Merali, Short- and long-periods of neonatal maternal separation differentially affect anxiety and feeding in adult rats: gender-dependent effects, Brain Res. Dev. Brain Res. 113 (1999) 97–106.
[27] L. Paternain, E. Martisova, F.I. Milagro, et al., Postnatal maternal separation modifies the response to an obesogenic diet in adulthood in rats, Dis. Model Mech. 5 (2012) 691–697.
[28] P.J. Coates, R. Nenutil, A. McGregor, et al., Mammalian prohibitin proteins respond to mitochondrial stress and decrease during cellular senescence, Exp. Cell Res. 265 (2001) 262–273.
[29] A.M. Czarnecka, C. Campanella, G. Zummo, et al., Mitochondrial chaperones in cancer: from molecular biology to clinical diagnostics, Cancer Biol. Ther. 5 (2006) 714–720.
[30] M. Artal-Sanz, N. Tavernarakis, Prohibitin and mitochondrial biology, Trends Endocrinol. Metab. 20 (2009) 394–401.
[31] M. Artal-Sanz, N. Tavernarakis, Prohibitin couples diapause signalling to mitochondrial metabolism during ageing in *C. elegans*, Nature 461 (2009) 793–797.
[32] S.A. Thomas, R.D. Palmiter, Thermoregulatory and metabolic phenotypes of mice lacking noradrenaline and adrenaline, Nature 387 (1997) 94–97.
[33] K. Inokuma, Y. Okamatsu-Ogura, A. Omachi, et al., Indispensable role of mitochondrial UCP1 for antiobesity effect of beta3-adrenergic stimulation, Am. J. Physiol. Endocrinol. Metab. 290 (2006) E1014–E1021.