Changes in UCP Family Expressions in Rat Tissues Due to Diet and Aging

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Summary Regulation of the gene expressions of uncoupling proteins (UCP)-1, 2 and 3 was investigated in the white and brown adipose tissues and skeletal muscle of young and old rats (8-wk- and 8-mo-old, respectively) fed diets containing various fats (hydrogenated fat, corn oil or fish oil) and proteins (casein or soybean) for 1 wk. The mRNA expressions of UCP-1, 2 and 3 were elevated in the white adipose tissues of the young rats fed soybean protein as compared to those fed casein, and that of UCP-2 was also elevated in the brown adipose tissue. The effects of dietary fat type on the expressions were not clear. The UCP-1, 2 and 3 mRNA expressions were markedly reduced in the tissues of the old rats. The UCP-2 expressions were more markedly elevated by dietary soybean protein and reduced by aging than the others, particularly in the white adipose tissue. The expressions of leptin involved in thermogenesis were also reduced by aging. Moreover, in a fasted-refed experiment conducted for the young rats, UCP-2 mRNA induction in the white adipose tissues reached maximal levels at 1 or 2 h and was stimulated by dietary fat or soybean protein. Thus, UCP-2 mRNA expression was markedly affected by diet and aging, particularly in white adipose tissue.

Key Words UCP-1, 2, 3 expressions, soybean protein, fat, aging

Many studies have reported that UCP families are involved in the control of body temperature and regulation of energy balance. UCP-1 is localized in the inner mitochondria membrane, is abundantly expressed in rodent brown adipose tissue and is indispensable for cold tolerance (1, 2). UCP-2 has been cloned recently and sequenced (3). The UCP-2 protein is widely expressed in human and rodent tissues, and has been postulated to play an important role in energy balance, body weight regulation and thermoregulation (3). UCP-2 is highly expressed in white adipose tissue (3–5). More recently, a new UCP-homolog, UCP-3, has been reported as being highly specific to skeletal muscle (6). The gene expression of these UCPs appears to be differentially regulated (7–9). However, the differences in the roles of the three UCPs and the correlation of their gene expressions remain to be elucidated.

Fatty acids have been reported to act as the transcriptional regulators of the expression of lipid-related genes in adipose cells (10). Physiological concentrations of polyunsaturated and monounsaturated fatty acids up-regulated UCP-2 mRNA levels in 3T3-L1 preadipocytes (11). Dietary fatty acids may be a possible physiological regulator of UCP-2 expression. Although there have been numerous publications describing the regulation of UCP-2 or UCP-3 mRNA expression, the results are not always consistent (12, 13). Therefore, the physiological significance and regulation of UCP-2 and UCP-3 expressions remain to be further elucidated. In the present study, we investigated the effects of dietary protein and fat types on UCP-1, 2 and 3 mRNA expressions in the tissues of steady-state rats fed synthetic diets and in fasted-refed rats. Moreover, the effects of the dietary nutrients on the UCP expressions in older rats were investigated.

MATERIALS AND METHODS

Materials. [α-32P]dCTP (111 TBq/mmol) was purchased from ICN Pharmaceuticals, Inc. (Costa Mesa, CA, USA). Nylon filter (Hybond N) was purchased from Amersham (Buckinghamshire, UK). Most other reagents were obtained from Wako (Osaka, Japan) and Sigma (St. Louis, MO, USA).

Animals. Experiment 1. Male Wistar rats (Japan SLC Co., Hamamatsu, Japan), 7-wk- or 8-mo-old, fed a commercially available non-purified diet (No. MF, Oriental Shiryou Co., Osaka, Japan) were food-deprived overnight and then fed a 10 g/100 g hydrogenated fat, corn oil or cod oil diet for 1 or 2 wk. Table 1 shows the diet compositions and the fatty acid compositions of the hydrogenated fat, corn oil and cod oil. Sucrose was replaced with hydrogenated fat, corn oil or cod oil by weight. Rats were individually housed in wire-bottomed cages in a temperature-controlled room (24°C) under an automatic lighting schedule (08:00 h to 20:00 h). Each rat had free access to water and was given an equal energy-containing diet per body weight per day. The amount of food consumed by each rat was measured at 17:00 h every day, and based on the average food consumption, the amount of food expected to be consumed was given for the following day.

The rats were sacrificed between 9:00 and 10:00 h to

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measure the mRNA concentrations of UCP-1, 2 and 3, insulin receptors and leptin. The rats were decapitated to measure the mRNA concentrations of UCP-1, 2 and 3, and then sacrificed at different times, 0, 1, 2 or 4h, after refeeding. Brown and white (epididymal) adipose tissues, and skeletal muscle were immediately removed, frozen in liquid nitrogen and stored at -80°C until used to measure the mRNA concentrations. The mean values for rats fed the corn oil/casein diet were shown below (mean±SD, n=8). The average body weights of the young and old rats were (g) 236±16.8 and 566±68.6, respectively. The average weights of the young and old rats were (g) 32.7±0.3 and 56.7±0.5, respectively. The average plasma glucose and insulin concentrations in each age group were not significantly different due to diet. The mean values for rats fed the corn oil/casein diet were shown below (mean±SD, n=8). The average body weights of the young and old rats were (g) 236±16.8 and 566±68.6, respectively. The average weights of the young and old rats were (g/100g body weight) 0.88±0.15 and 1.06±0.32, respectively. The average plasma glucose concentrations of the young and old rats were (mmol/L) 11.1±1.08 and 11.7±1.81, respectively, and the insulin concentrations were (nmol/L) 0.41±0.11 and 1.06±0.32, respectively.

### RESULTS

**Animal profiles (Experiment 1)**

The young and old rats were fed diets containing various fats (hydrogenated fat, corn oil or fish oil) and proteins (casein or soybean) for 1 wk before they were sacrificed at 8-wk- and 8-mo-old, respectively. When the rats were sacrificed, the mean values of body weights, brown and white (epididymal) adipose tissue weights, and plasma glucose and insulin concentrations in each age group were not significantly different due to diet. The mean values for rats fed the corn oil/casein diet were shown below (mean±SD, n=8). The average body weights of the young and old rats were (g) 236±16.8 and 566±68.6, respectively. The average weights of the young and old rats were (g/100g body weight) 0.88±0.15 and 1.06±0.32, respectively. The average plasma glucose concentrations of the young and old rats were (mmol/L) 11.1±1.08 and 11.7±1.81, respectively, and the insulin concentrations were (nmol/L) 0.41±0.11 and 1.06±0.32, respectively.
Effects of dietary protein and fat types on UCP-1, 2 and 3 mRNA concentrations in white and brown adipose tissues and skeletal muscle of young and old rats. UCP-1 mRNA (top), UCP-2 mRNA (middle) and UCP-3 mRNA (bottom) concentrations in the tissues of young and old rats fed diets differing in protein and fat are shown. Young and old rats were fed casein or soybean protein diets containing 10% hydrogenated fat (Hyd. fat), 10% corn or 10% fish oil for 1 wk and then sacrificed. The mRNA concentrations were normalized to those in young rats fed the casein/hydrogenated fat diet. The values are means±SD, n=8. Three-way ANOVA for mRNA concentrations in each panel are shown. A, aging main effect; P, protein main effect; AxP, interactions.

0.65±0.18, respectively. As these mean values were not significantly different due to diet, each mean value of the dietary groups is not shown.

Effects of dietary protein and fat types on UCP-1, 2 and 3 mRNA expressions (Experiment 1)

The young and old rats were fed experimental diets for 1 or 2 wk. The mRNA expressions of UCP-1, 2 and 3 in brown and white adipose tissues and skeletal muscle were not significantly different in rats fed the diets for 1 and 2 wk. Therefore, only the relative mRNA expressions in rats fed experimental diets for 1 wk are shown.

In the white adipose tissues, the mRNA expressions of UCP-1, 2 and 3 were elevated (p<0.05) in rats fed soybean protein as compared to those fed casein (Fig. 1). The UCP-2 mRNA expressions were significantly elevated (p<0.05) in the white adipose tissue of young rats fed corn oil as compared to the other fats in rats fed soybean protein. No effects of dietary fat types on the UCP-1 and 3 expressions were found. In the brown adipose tissues, the UCP-2 mRNA expressions were significantly elevated (p<0.01) in rats fed soybean protein as compared to the young and old rats fed casein (Fig. 1). No effects of dietary fat types on UCP-1, 2 and 3 expression were found in the brown adipose tissue. In the skeletal muscle, no effects of dietary protein and fat types on UCP-2 and UCP-3 mRNA expressions were found. Thus, the soybean protein effects on UCP mRNA expressions were greater in white adipose tissue than in the other tissues. The UCP-2 mRNA expressions in white adipose tissue were more markedly elevated by the dietary soybean protein than the UCP-1 and 3 mRNA expressions.

Effects of aging on UCP-1, 2 and 3 mRNA expressions (Experiment 1)

The mRNA expressions of UCP-1, 2 and 3 were significantly reduced in the brown and white adipose tissues and skeletal muscle of the old rats as compared to those of the young rats. Particularly in the white adipose tissues of the old rats, the mRNA expressions of UCP-1, 2 and 3 were markedly reduced to 20–40% of those of the young rats. In the brown adipose tissues of the old rats, there was a more significant reduction of UCP mRNA expression (p<0.01) as compared to that in the young rats. In the following order: UCP-2>UCP-1>UCP-3. In the muscle of the old rats, the UCP-2 and 3 mRNA expressions were reduced less (70–80%, p<0.01) than in the other tissues. Thus, the mRNA expressions of UCP-2 were more greatly affected by aging and dietary nutrients than those of UCP-1 and 3. The effects of aging on UCP expressions were more marked in the white adipose tissue than in the other tissues. There were no effects of dietary soybean protein and fat types on UCP expressions in the old rats.

Insulin receptor and leptin mRNA expressions

The insulin receptor mRNA expressions in the liver were not significantly affected by dietary fat types but were induced by dietary soybean protein (Fig. 2). The insulin receptor mRNA expressions in the white adipose tissues were not affected by different proteins or fats. However, the expressions in both tissues of the hydrogenated fat groups were significantly elevated (p<0.05.
by t-test) in rats fed soybean protein as compared to those fed casein. The insulin receptor mRNA expressions in both the liver and white adipose tissue were markedly reduced in the old rats as compared to those in the young rats. The leptin mRNA expressions in the white adipose tissues were markedly reduced in the old rats, although they were not significantly affected by the diet (Fig. 3).

UCP-2 mRNA induction after refeeding a fat-free or corn oil diet to food-deprived rats (Experiment 2)

The time courses for UCP-1, 2 and 3 mRNA induction were measured after refeeding a fat-free or corn oil diet to 8-wk-old food-deprived rats. The mRNA induction of the UCPs in the white and brown adipose tissues were already elevated 1 h after refeeding, and reached a maximal level mostly at 1 or 2 h. Figure 4 shows the time courses for UCP-2 mRNA induction in the tissues. The time courses for mRNA induction of UCP-1 and 3 in adipose tissues are not shown because they were not affected by the diets, and only by time after the refeeding. The mRNA induction of UCP-1 and 3 reached a maximal level at 1 or 2 h after refeeding with the fat-free or corn oil diet.

The mRNA induction of UCP-2 was markedly elevated, particularly at the peak, in the white adipose tissues of rats fed the corn oil diet. The mRNA induction of UCP-2 was significantly elevated in the white adipose tissues by dietary soybean protein as
compared to casein. Moreover, the induction of UCP-2 mRNA was earlier and significantly higher at the peak in rats fed the corn oil diet as compared to those fed the fat-free diet. The induction of UCP-2 mRNA in skeletal muscle was not significantly affected by the feeding of soybean protein or corn oil.

**DISCUSSION**

In Experiment 1, the mRNA expressions of UCP-2 were elevated in the white and brown adipose tissues of the rats fed soybean protein as compared to those fed casein, and the expressions of UCP-1 were elevated only in the white adipose tissue. The mRNA expressions of UCP-2 and 3 were not significantly affected by the dietary protein type even in the muscle. We previously found that the conversion rate of triiodothyronine to thyroxine by liver microsomes and the plasma triiodothyronine concentrations were significantly higher in rats fed soybean protein than in those fed casein (26). The plasma and liver triacylglycerol concentrations were lower in soybean protein-fed rats (26). Triiodothyronine-treatment stimulated UCP-1 mRNA expression in cultured fetal brown adipocytes (27), UCP-2 and 3 mRNA expressions in human skeletal muscle (28), and UCP-2 mRNA expression in rat white and brown adipose tissues and skeletal muscle (29). Therefore, it is suggested that dietary soybean protein stimulated the UCP-1, 2 and 3 expressions through an increase in the thyroxine-to-triiodothyronine conversion rate.

We previously found that plasma and liver triacylglycerol concentrations and lipogenic enzyme activities were significantly suppressed by dietary soybean protein (26). Therefore, dietary soybean protein appeared to reduce the triacylglycerol levels by the suppression of fatty acid synthesis and stimulation of energy expenditure.

Baillie et al. (30) reported that the decrease in fat deposition associated with fish oil ingestion was accompanied by a significant increase in the abundance of skeletal muscle UCP-3 mRNA. However, the abundance of skeletal muscle UCP-2 mRNA was unaffected by the type of dietary oil, and the abundance of UCP-2 mRNA in the liver and heart was significantly lower in rats fed fish oil than in those fed corn oil. Chevillotte et al. (31) reported that n-6 polyunsaturated fatty acids induced a 3-fold rise in UCP-2 expression in primary cultures of human muscle cells, whereas n-3 polyunsaturated fatty acids did not. In the present experiment, UCP-2 mRNA expressions were significantly elevated in white adipose tissue by dietary corn oil as compared to fish oil; but expressions in brown adipose tissues and skeletal muscles were not elevated by any dietary fat. The UCP-1 and 3 mRNA expressions in the tissues were not significantly affected by any dietary oil. Thus, the effects of dietary fatty acid type on the stimulation of UCP expression did not always coincide and still remains to be elucidated.

UCP mRNA expressions in the white and brown adipose tissues and skeletal muscle of old rats were markedly reduced by aging in the following order of reduction abundance: UCP-2>UCP-1>UCP-3. The expression of leptin (involved in thermogenesis) in white adipose tissues was also markedly reduced by aging. These reductions could be the cause of the retardation of energy expenditure in the old rats. Kerner et al. (32) reported that there was a 68% reduction in UCP-3 abundance in the skeletal muscle mitochondria of old rats as compared to adult rats. Barazzoni and Nair (33) reported that gastrocnemius muscle UCP-3 expression was lower in old rats but UCP-2 expression was higher, suggesting differential regulation of UCP-2 and UCP-3 in the muscle.

Leptin impaired several metabolic actions of insulin (34), whereas insulin increased the leptin mRNA levels in adipocytes (35, 36) and both leptin secretion and production by rat white adipose tissue (37). These results support the possibility that insulin is an important regulator of leptin gene expression even though leptin down-regulates insulin functions. Insulin actions are controlled by insulin receptor expression and binding capacity to insulin. Insulin directly stimulates UCP-2 and UCP-3 mRNA expression in skeletal muscle in vitro (38). Leptin increased UCP-1 and 3 levels in brown adipose tissues and UCP-2 mRNA levels in epididymal white adipose tissues (39). The leptin, insulin receptor and UCP-1, 2 and 3 expressions were reduced in the old rats as compared to the young rats. The coordinate reduction may lead to thermogenesis reduction in old rats.

In fasted-refed rats (Experiment 2), the UCP-1, 2 and 3 mRNA induction in white adipose tissue reached a peak at 1 or 2 h and then decreased. The induction of UCP mRNA was rapid, probably due to rapid turnover and rapid response of the mRNAFs to dietary protein and fat. Therefore, the UCP mRNA expressions shown in Fig. 1 should reach a steady state in 1 wk after refeeding with the diets in Experiment 1. UCP-2 mRNA induction was more markedly elevated in rats fed the corn oil diet, particularly at the peak, as compared to rats fed the fat-free diet. The induction of UCP-2 mRNA in white adipose tissues was more markedly elevated in rats fed soybean protein than in those fed casein. Thus, dietary soybean protein and corn oil stimulated the UCP-1, 2 and 3 expressions more clearly in fasted-refed rats than in steady-state rats.

UCP mRNA expressions were more markedly induced by dietary soybean protein in the following order of induction, UCP-2>UCP-1>UCP-3, and reduced by aging in the order of reduction (Experiment 1). UCP mRNA induction and reduction were more markedly changed in the order of white adipose tissue>brown adipose tissue>skeletal muscle. Thus, UCP-2 expression, particularly in the white adipose tissue, is markedly affected by dietary nutrients and aging. Moreover, the turnover of UCP-1, 2 and 3 appeared to be rapid, and to be rapidly involved in thermogenesis after nutritional manipulation. The regulation of UCP protein levels should be elucidated in future studies.
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