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The Effect of High-Sucrose and High-Fat Diets on the Expression of Uncoupling Proteins (UCPs) mRNA Levels in Mice

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The objective of this study was to examine diet-induced changes in the expression of UCP2 mRNA in the liver and UCP3 mRNA in the skeletal muscle of mice fed a high-sucrose or high-fat diet. Male ICR mice, aged 4 weeks, were divided into three dietary groups and fed control (N) or modified AIN-76 high-sucrose (HS) or high-fat (HF) diets for 12 weeks. The serum total cholesterol (TC) and LDL-cholesterol concentrations of the HF group were significantly higher than those of the N and HS groups. The hepatic TC and triglyceride contents of the HS and HF groups were also significantly higher than those of the N group. The HS diet group had higher serum leptin and insulin levels compared to those of the HF group. Hepatic UCP2 mRNA expression was significantly higher in the HS group than in the N group, but the level in the HF group did not differ from that of the N group. Muscular UCP3 mRNA level was significantly higher in the HF group and especially in the HS group than in N the group. We observed that two gene (UCP2, 3) levels exhibited a similar tendency. These results suggest that UCPs mRNA levels and energy expenditure may be altered or controlled by various dietary patterns. Further research is needed to elucidate the effects of diet on the regulation of many obesity-related genes.

Key words : High-sucrose and high-fat diet, UCPs, Liver, Skeletal muscle, mRNA expression

INTRODUCTION

Uncoupling proteins (UCPs) are mitochondrial inner membrane proteins. UCPs allow the dissipation of part of the proton electrochemical gradient generated by the electron transfer chain across the mitochondrial inner membrane and can thus increase heat production by uncoupling respiration from ATP synthesis.[1)]

UCP2 mRNA is expressed in numerous types of tissue such as that in the liver, skeletal muscle, heart, and kidney. UCP3 is expressed mainly in the skeletal muscle.[2-5)] The presence of UCP3 mRNA in the skeletal muscle is of great interest because this tissue is an important site of diet-induced thermogenesis and energy homeostasis in animals.[6)] Also, under resting conditions, skeletal muscle is the major determinant of resting metabolic rate.[7)] UCPs are probably significant since the proton leak, in part sustained by UCPs, contributes up to 50% of the basal respiration rate of the skeletal muscle and up to nearly 30% of the standard metabolic rate in rats.[8)] UCPs mRNA expression has been found to be influenced by many factors including diet type,[9,11)] environmental temperature,[9)] hormones, and exercise.[11)] Specifically, UCP genes are upregulated in response to fat consumption [3,12)] and sucrose/carbohydrate consumption.[2,11)] Also, food restriction decreased UCP3 mRNA expression in the skeletal muscle of rats and fasting increased it 5- to 6-fold.[9)] The results indicate that UCPs might be important factors related to obesity. Under these specific conditions, fatty acyl-CoA is inappropriately esterified, leading to triacylglycerol accumulation in the adipose tissue, muscle, liver, and pancreas.[13)] Increased triglyceride (TG) accumulation is positively associated with insulin resistance and hyterlipidemia.[13,14)]

Therefore, in the present study, we investigated the changes in UCP2 and UCP3 mRNA expression in the liver and skeletal muscle in response to a high-sucrose or high-fat diet in mice. Furthermore, serum leptin and insulin levels were measured to evaluate the effects of dietary patterns on lipid metabolism in mice. Furthermore, serum leptin and insulin levels were measured to evaluate the effects of dietary patterns on lipid metabolism in mice.

MATERIALS AND METHODS

1. Materials

DL-methionine, fiber, choline bitartrate, and chloroform were purchased from the Sigma Chemical Co. (St. Louis, USA). Trizol reagent was purchased from the Invitrogen (Carlsbad, CA, USA). An RT-PCR kit was purchased from the Bioneer Co. (Seoul, Korea). AIN-76 vitamin and mineral

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mixes were purchased from Harlan Teklad (Madison, USA). Casein was purchased from Cottee (Gordon, Australia).

2. Animals and Diets

Twenty one male ICR mice (28.3-32.5 g BW), aged 4 weeks, were purchased from Daehan Biolink Inc. (Eumsung, Chungbuk, Korea). The mice were initially fed an AIN-76 control diet for one week and then were divided into dietary groups and fed either the AIN-76 (N) diet or one of two modified AIN-76 diets adjusted to provide either a high-sucrose (HS) or high-fat (HF) diet as previously reported (see Table 1 and 2 for references) for 12 weeks. The mice were individually housed in polycarbonate cages in a temperature and humidity-controlled (23±1 ℃, 53±2%) room. The animals were maintained on a light/dark cycle (12 hr/12 hr light/dark) with free access to diet and water.

Table 1. Composition of experimental diets

Ingredient	Normal diet[1)]	High sucrose diet[2)]	High Fat diet[3)]
Casein	20.0	20.0	20.0
DL-Methionine	0.3	0.3	0.3
Corn starch	15.0	3.0	-
Sucrose	50.0	66.3	50.0
Fiber	5.0	5.0	5.0
Corn oil	5.0	0.7	-
Beef tallow	-	-	20.0
AIN-76 Mineral mix	3.5	3.5	3.5
AIN-76 Vitamin mix	1.0	1.0	1.0
Cholin bitartrate	0.2	0.2	0.2
Total (%)	100.0	100.0	100.0

1) reference 47, 2) reference 48, 3) reference 49

Table 2. Caloric content of experimental diets (% Kcal)

Diet	Carbohydrate	Protein	Fat	Total
Normal diet	67.3	21.0	11.7	100.0
High-sucrose diet	76.0	22.3	1.7	100.0
High-fat diet	43.4	17.6	39.0	100.0

3. Sampling

Food was withheld for 12 hours before the rats were sacrificed. The blood was centrifuged at 1,100 x g for 15 min at 4 ℃, and serum was stored at -20 ℃ until analysis. The liver and skeletal muscle were collected, immediately frozen in liquid nitrogen, and stored at -80 ℃ until analyzed.

4. Analysis of lipids

Serum TG was enzymatically measured using a commercial kit (Asan Pharm. Co., Seoul, Korea) based on the lipase-glycerol phosphate method.[50)] Serum total cholesterol (TC) was also assayed using a commercial kit (Asan Pharm. Co.) based on the cholesterol oxidase method.[15)] Serum LDL-cholesterol (LDL-C) was calculated from the serum TG, TC, and HDL-cholesterol (HDL-C) concentrations using the Friedewald formula.[16)] Liver tissue was minced thoroughly while on ice, and representative aliquots of each were used to determine the concentrations of TG and TC in the liver using the enzymatic method described above in serum. An atherogenic index (AI) was calculated as: AI = [TC]-[HDL-C]/ [HDL-C].[51)]

5. Analysis of serum leptin and insulin

Serum leptin and insulin levels were analyzed using a mouse/rat leptin radioimmunoassay kit (Mediagnost, Aspenhaustr, Germany) and insulin radioimmunoassay kit (ICN Pharmaceuticals, Inc., Costa Mesa, USA), respectively.

6. Isolation of total RNA

Total RNA from the skeletal muscle and the liver were isolated using the guanidinium thiocyanate-phenol-chloroform extraction method.[17)] Total RNA was quantified by measuring absorption at 260 nm and 280 nm.

7. RT-PCR detection of UCPs mRNA expression

cDNA was made using random hexamer primers as described by the manufacturer (One-step RT-PCR kit from ABgene, USA) (Table 3). The RT-PCR Master Mix contained the thermoprime plus DNA polymerase optimized reaction buffer, dNTP mix, and $MgCl_2$.

Table 3. Primers and reaction conditions used for PCR

Gene	Forward & Reverse primer	AT.[1)]	PS.[2)]
UCP 2-F	5′ GGAGCT TTA GAT GCA GAC CG 3′	55 ℃	1,284
UCP 2-R	5′ GCT CTG GGA TCC TAA ACA GG 3′		
UCP 3-F	5′ GCC CCT ACA CCT GAC CTT GG 3′	50 ℃	1,073
UCP 3-R	5′ CCC CTG GGC AGA GAA GCT TTG TT 3′		

1) AT, annealing temperature (℃); 2) PS, product size (bp)

8. Statistical analysis

Results were expressed as mean ± SD. Differences between means were evaluated using SAS version 8 (SAS Institute, Cary, NC, USA). Significances of differences among the three groups were determined using Duncan's multiple range test and the accepted level of significance was $p < 0.05$.

RESULTS AND DISCUSSION

Earlier studies demonstrated that the mRNA expression of UCPs is affected by dietary composition,[11,18)] fasting,[19)] hyperglycemia[20,21)] and insulin resistance.[22)] These studies demonstrated that UCPs mRNA expression could control energy expenditure. Therefore, we evaluated the effect of diet on different UCPs expressed in different tissue by measuring UCP2 in the liver and UCP3 gene expression in the skeletal

muscle (gastrocnemius) of mice fed normal, HS or HF diets.

Animals on the HF diet ate significantly less food than animals on the control and HS diets. However, there was no difference in energy intake and body weight gain over the 12-week period (Table 4). Several studies have demonstrated that increased carbohydrate and saturated fat may induce obesity in the absence of increased energy intake.[23,24]

Table 4. Body weight gain, feed consumption, energy intake, and feed efficiency ratio of mice

	Normal diet	High-sucrose diet	High-fat diet
Body weight gain (g/12weeks)	10.90±1.09^{NS}	10.54±2.21	11.27±1.32
Feed consumption (g/d)	6.16±0.58^{a}	5.67±0.63^{a}	4.21±0.54^{b}
Energy intake (kcal/d)	24.17±2.24^{NS}	20.60±2.64	20.15±2.71
Feed efficiency ratio	1.95±0.38^{b}	1.71±0.83^{b}	3.12±0.73^{a}

All values are means±SD. Values with different superscripts in the same rows are significantly different ($p<0.05$). Feed efficiency ratio was calculated as (total weight gain/total dietary intake). NS: not significantly different

In this study we demonstrated that high-fat and high-sucrose diets did not affect serum lipid profiles, but did increase TC or TG levels in the liver (Table 5) in the absence of increased energy intake. It is reasonable to assume that the lower dietary intake in the HF diet group may be due to an adaptation to maintain energy balance in the body.

It has been known for several decades that both high-carbohydrate and high-fat diets induce hypertriglyceridemia[25] which typically accompanies insulin resistance.[26] It has been shown that fatty acid oxidation decrease, but esterification of fatty acids and secretion of TG increase in the livers of obese Zucker rats.[27,28] The increased plasma TG levels associated with dietary sucrose might be due to either increased secretion of TG from the liver[24] or decreased TG removal from the plasma.[29] The concentration of circulating TG is determined by delivery into plasma and subsequent removal of TG-rich lipoproteins by the tissues. Plasma TG is derived from the diet and hepatic synthesis and released as VLDL. Increased availability of non-esterified fatty acids and high insulin levels prompt hepatic TG synthesis.[30] In this study, serum TG levels were not significantly different among the groups, but hepatic TG concentrations significantly increased owing to the HF and HS diets. Therefore, it is speculated that HS and HF diets might increase the acyl-CoA pool leading to increased TG storage in the liver, as found in this study (Table 5).

Decreases in insulin-stimulated glucose metabolism have been demonstrated to be associated with decreases in leptin expression and secretion in isolated adipocytes.[31] Enhanced lipolysis has been associated with decreased leptin synthesis in some studies.[32,33] Ainslie *et al.* [34] reported that feeding rats an HF diet for 4 weeks contributed to reductions in leptin secretion in the adipose tissue. In this study, there were significantly higher circulating leptin concentrations in mice fed an HS diet than in those fed an HF diet. The lower leptin secretion in animals on a higher-fat diet than in those fed a high-sucrose diet may be due to an insulin effect, since insulin has been identified as a possible mediator of leptin secretion.[37] Insulin is known to be an important inhibitor of lipolysis.[35] Consumption of an HF diet would be expected to decrease insulin levels leading to less inhibition of lipolysis, which would result in higher free fatty acid availability and increased free fatty acid oxidation. In this study, the HS diet increased serum leptin and insulin levels whereas the HF diet did not (Table 6). It has been reported that the incubation of mice islets for several days in high glucose media induces a dramatic increase in insulin mRNA levels.[36] Our results support, in part, the role of diet in the coordinated regulation of serum leptin and insulin concentrations under our experimental conditions.

To evaluate if dietary patterns change the expression of UCPs mRNA levels, we measured the expression of UCP2

Table 6. Levels of serum leptin and insulin in mice

	Normal diet	High-sucrose	High-fat diet
Leptin (ng/mL)	2.55±1.36^{NS}	3.32±2.14	1.73±0.46
Insulin (μIU/mL)	25.78±2.79^{a}	27.29±4.09^{a}	19.48±4.16^{b}

All values are means ± SD (n=7). Values with different superscripts in the same row are significantly different ($P < 0.05$). NS: not significantly different

Table 5. Lipid profiles of serum and liver in mice

	Normal diet	High-sucrose diet	High-fat diet
Serum			
TG (mg/dl)	78.00±12.83^{NS}	74.85±14.96	70.72±10.61
TC (mg/dl)	180.21±24.72^{NS}	178.27±39.23	204.08±30.17
HDL C (mg/dl)	50.27±9.87^{NS}	46.99±4.66	47.55±7.59
LDL C (mg/dl)	104.62±13.91^{b}	112.57±26.20^{ab}	141.29±18.55^{a}
Atherogenic index	2.58±0.54	3.00±0.48	2.92±0.43
Liver			
TC (mg/g Liver)	5.86±1.53^{c}	16.21±7.30^{a}	11.23±2.37^{b}
TG (mg/g Liver)	32.21±7.50^{b}	53.28±15.33^{a}	58.14±16.20^{a}

All values are means±SD. Values with different superscripts in the same row are significantly different ($P<0.05$). TG, triglyceride; TC, total cholesterol; HDL-C, HDL-cholesterol; LDL-C, LDL cholesterol; Atherogenic index means the ratio of (total cholesterol-HDL-C)/HDL-C. NS: not significantly different

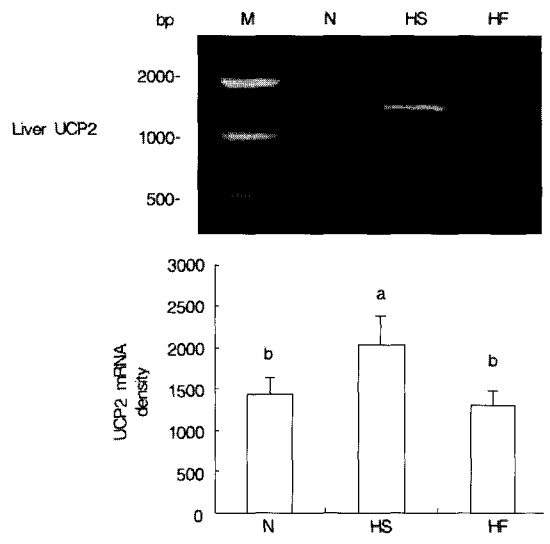

Fig. 1. Expression of UCP2 cDNA in liver by alteration of diet composition.

N, Normal diet group; HS, high sucrose diet group; HF, high fat diet group. The error bars show the standard deviations of the means. Differences were considered significant at $p<0.05$. The expression level in each animal was quantified by densitometry.

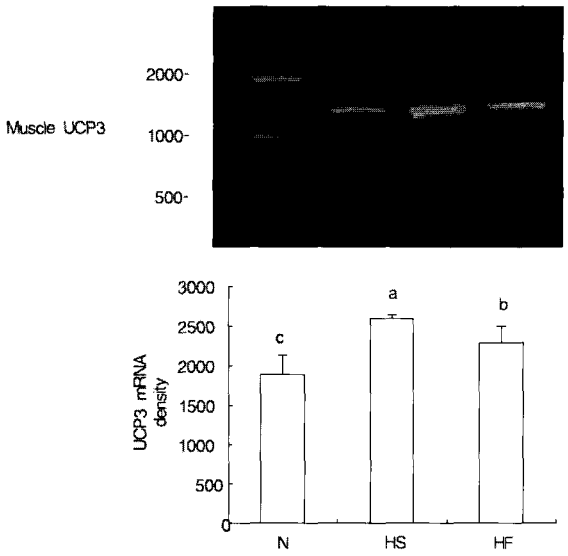

Fig. 2. Expression of UCP3 cDNA in skeletal muscle (gastrocnemius) by alteration of diet composition.

N, Normal diet group; HS, high sucrose diet group; HF, high fat diet group. The error bars show the standard deviations of the means. Differences were considered significant at $p<0.05$. The expression level in each animal was quantified by densitometry.

mRNA in the liver and UCP3 mRNA in the skeletal muscle, as shown in Figures 1 and 2. Hepatic UCP2 mRNA levels in the HS diet group were significantly higher than those in the N group, and muscular UCP3 mRNA levels in both the HS and HF diet groups were higher than those in the N group. However, the high sucrose/carbohydrate diet had a greater effect on the expression of UCPs mRNA in the subjects on the HF diet. These results were the opposite of the results of most earlier investigators.[38,39] Our data suggests that alteration of diet composition affects UCPs mRNA expression. Previous studies reported that an HF diet increased[18,39] or didn't affect[21,40] mRNA expression of UCPs in the skeletal muscle, adipose tissue and liver. Since free fatty acids (FFAs) are thought to increase UCP3 mRNA levels, it has been suggested that they facilitate fatty acid metabolism by UCP2,[40] Fatty acids have been proposed as major regulators of UCP3 mRNA in the skeletal muscle of adult rodents.[42] Perhaps for this reason, an HF diet increased UCPs mRNA expression in the skeletal muscle because it increased the availability of fatty acids as a substrate.[52,53] However, regulation of UCPs mRNA expression by some factors is influenced by muscle type. It would be of interest to draw a comparison between the present findings and the observations of others that UCP gene expression in the soleus muscle is highly dependent on changes in circulating FFAs, and the fact that even under normal feeding conditions, such predominantly slow-twitch muscle is more dependent on circulating lipids as a fuel substrate than fast-twitch muscle (in which glucose is also an important fuel substrate).[21] In another study, HF feeding induced obesity in mice, which was associated with induction of UCP2 expression in white adipose tissue but not with changes in UCP3 expression in the muscle.[21,41] Long-term changes in caloric intake affect the expression of UCP3 mRNA but not of UCP2 mRNA in the skeletal muscle,[38] demonstrating significant differences in how UCP2 and UCP3 are regulated. Other investigators[3,11] have also studied the effects of high sucrose/carbohydrate diets on the expression of UCP mRNA. Levine's study[11] showed that sucrose [10% (w:v) solution] feeding for 2 weeks led to elevated UCP3 gene expression in the muscle and to decreased energy efficiency, suggesting that the effects of sucrose on energy balance may be mediated by UCP3. Furthermore, UCP3 expression positively correlates with whole-body insulin-mediated glucose utilization.[22] In a study by Hidaka et al.,[43] glucose concentrations in streptozotocin-treated rats increased, even though UCP3 mRNA expression in the adipose tissue decreased, and the expression in muscle increased. Perhaps, as the concentration of glucose increases, UCPs' expression increases in parallel.[44] Because it takes less energy to store fat from dietary lipids than to synthesize fat from carbohydrates via de novo lipogenesis, a shift in muscle substrate utilization in favor of glucose during consumption of a low-fat, high-carbohydrate diet would be an energetically more efficient way to deposit fat. This would be in keeping with a role of these UCP homologs in the regulation of lipids as a fuel substrate.[21] In fact, increases in glucose utilization markedly stimulated thermogenesis in association with translocation of glucose transporters to the

plasma membrane.[45] Furthermore, glucose metabolism could be enhanced by overexpression of UCP2,[46] or when muscular UCP3 mRNA expression is upregulated under conditions of energy conservation caused by suppressed thermogenesis.[21] These results suggest that UCPs mRNA levels are altered by changes in diet patterns and provide evidence that energy expenditure can be modulated by diet.

In summary, our results demonstrate that an HS diet increases UCP2 expression in the liver and that both HF and HS diets increase UCP3 in the muscle, suggesting that dietary regulation of mitochondrial UCPs plays an important role in regulating energy metabolism. We also postulate that the utilization of sucrose rather than fat in the liver and skeletal muscle is a more effective way to upregulate the UCPs expression than an HF diet.

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