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Effects of Caloric Restriction on Endocrine Functions and Body Fat Distribution in Overweight Premenopausal Women, Related to their UCP3 (Uncoupling Protein 3) Genotypes*

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ABSTRACT

A mutation in the promoter region of uncoupling protein 3 (UCP3), specifically the −55C→T transition, may influence an individual's energy metabolism and body weight. The objective of this study was to investigate the effect of a weight reduction program on endocrine functions and body fat distribution, related to UCP3 promoter genotype. Ninety overweight pre-menopausal female subjects participated in the weight reduction program at Yonsei University Hospital, and were placed on a calorie-restricted diet (300 kcal less than their daily requirements) for 12 weeks. After 12 weeks, all subjects on the program lost approximately 5% of their initial body weights and had lower Body Mass Index (BMI) values. Among the 90 women, 56 had a normal (without mutation) UCP3 genotype, while 34 women had mutations in the promoter region of UCP3. Despite similar weight reductions in both groups, a significantly higher decrease in abdominal adipose tissue was observed in the normal UCP3 genotype group, compared to the group with mutations. In particular, there was a significant reduction of fat at the lumbar 1 (L1) level in the without-mutation group. Serum levels of total cholesterol, apolipoprotein A1 were significantly decreased in the without-mutation group, by 4.4% and 5.7% respectively. Serum levels of hormones were not significantly changed in both groups after the intervention. However, in the group without the mutations, the leptin level significantly reduced by 23.4% ($p < 0.001$). Serum free fatty acid (FFA) concentration was significantly increased in the group with mutation following the weight reduction program. On the other hand, FFA responses were shown similar increases in both groups. In conclusion, although no difference was found in the magnitude of weight reduction in both groups, there were significant differences in body fat distribution and in endocrine function between the groups.

KEY WORDS: uncoupling protein 3 (UCP3), caloric restriction, pre-menopausal women, fat distribution, endocrine functions.

INTRODUCTION

Obesity, particularly central obesity, is positively associated with many diseases, such as cardiovascular disease, hypertension, lipid disorders and diabetes[1-6]; it is also associated with disrupted metabolic and endocrine functions, such as insulin resistance and alterations of serum hormones.[2][3]

It is reported that as little as 5% reduction of the initial body weight in overweight/obese people can lead to an improvement in weight-related diseases and disorders.[7][8] However, effects of weight reduction on body fat mobilization and endocrine function are diverse. Obesity has multiple causes, and is determined by interactions between environmental and genetic factors.[9][10]

Recently, genetic factors have been shown to influence a predisposition to obesity, by being associated with energy balance.[11] One of these factors is uncoupling protein 3 (UCP3), predominant in skeletal muscles.[11][12] UCP3 may be involved in the reduction of lipids or metabolic substrates, and may lead to heat production rather than energy storage.[11][12] Decreased function or expression of UCP3 could be related to reduced thermogenic capacity, altered energy homeostasis, and a predisposition to obesity.[11][12]

A variation in the promoter region of UCP3 (−55CT), regulating the expression of UCP3, has been reported to have an association with obesity.[11] The 55TT genotype has been associated with increased BMI (Body Mass Index),[12] and with an atherogenic lipid profile.[13] The combination of CT and TT genotypes was associated with increasing waist to hip ratios (WHR) in women of European and Asian origin,[14] even though another study conducted on the Danish population showed no such association.[15] At least in some populations, the UCP3 pro-

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moter polymorphism might be implicated in several phenotypes associated with Type II diabetes and metabolic differences. However, the processes of how this genetic variant influences metabolism are still unknown.

The present research selected 90 premenopausal women who were overweight (BMI > 25 or body fat > 27%), and who were placed on a weight reduction program of caloric restriction (consumption of 300kcal less than the daily energy requirement). The association between UCP3 genotype and the effects of weight reduction on endocrine functions and body fat distribution was studied.

SUBJECTS AND METHODS

1. Subjects

Ninety premenopausal female subjects were recruited from a group of overweight/obese patients who were already recommended by the Yonsei University Hospital to participate in weight reduction programs. Subject selection criteria were a BMI greater than 25 kg/m^2 and body fat greater than 27%. Patients who had diabetes mellitus (DM) or coronary artery disease (CAD) were excluded from the study. The mean age of subjects was 31.2 ± 1.14 years, and their mean BMI was 25.5 ± 0.31 kg/m^2.

2. Weight reduction program

Ninety subjects participated in the weight reduction program for 12 weeks, and they were treated with a low calorie diet (300 kcal/day less than their daily requirements). The subjects were instructed on their calorie-restricted diets by a dietitian, and they selected their own food during the study period. All subjects were given written and verbal instructions on how to complete 3-day (2 weekdays and 1 weekend) dietary records and physical activities per week throughout the whole study period. For their dietary records, subjects were instructed to weigh and record the amounts of foods before meals, as well as the amount of the uneaten food. In order to encourage compliance, the dietitian interviewed participants on a weekly basis throughout the study period, to monitor dietary intake and subjects' weight changes. All participants were encouraged to maintain their usual lifestyle and dietary pattern.

The energy and nutrient contents of daily dietary intakes were calculated by the Computer-Aided Nutritional analysis program (CAN-pro version 1.0) of the Korean Nutrition Society.

3. Anthropometric measurements and determination of fat and muscle

Body weight and height were measured in the morning, unclothed without shoes. The BMI was calculated as {body weight (kg) / height (m^2)}. Waist and hip circumferences were measured and waist-to-hip ratios (WHR) were computed as an indication of the index of body fat distribution. Percentage of body fat was measured with the bioelectrical impedance (Tanita, Japan) method.

Body fat distribution and muscle areas were measured by computerized tomography (CT) scanning, using a General Electric (GE) High Speed Advantage 9800 scanner (Milwaukee, WI). Four cross-sectional images were made for each subject: abdomen at the levels of lumbar 1 (L1) and lumbar 4 (L4), thigh (midway between patella and pubis), and calf (at the most protruding area).

4. Blood collection

Venous blood specimens were collected in EDTA-treated and in untreated tubes following a 12-hour fast at the beginning and the end of the study. The tubes were immediately covered with aluminum foil and were placed on ice in the dark until they were taken to the laboratory (within 1 - 3 hours), and were stored at $-70℃$ until analysis.

5. Oral glucose tolerance test

All subjects received a glucose tolerance test (OGTT) after a 12-hour overnight fast. Blood was collected prior to the test and, following a 75g oral dose of glucose, at 30, 60 and 120 minutes to determine serum concentrations and response profiles of glucose, insulin, C-peptide and free fatty acids (FFA). The diagnosis of diabetes in subjects was undertaken by using a newly developed and modified method by the National Diabetes Data Group (NDDG), and by using the method employed by the World Health Organization (WHO) Expert Committee on diabetes mellitus. The following criteria were used for the presence of diabetes: a casual plasma glucose > 200 mg/dl or fasting glucose > 126 mg/dl, and > 200 mg/dl glucose level 2 hours after the oral glucose load of 75 g.[40] Glucose was measured by the glucose oxidase method using the Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA). Insulin was measured using the immunoradiometric assay of Dainabot (Tokyo, Japan), and C-peptide was measured by radioimmuno-assays with commercial kits from Immuno Nucleo Corporation (Stillwater, MN). FFA was analyzed with a Hitachi 7150 Autoan-

alyzer (Hitachi Ltd. Tokyo, Japan). Each response to oral glucose of glucose, insulin, C-peptide and FFA was calculated by measuring the area under each response curve.

6. Serum lipid profile, apolipoproteins A1 and B, and hormones

The total cholesterol, HDL-cholesterol, and triglyceride levels of the serum were measured enzymatically with commercially available kits on a Hitachi Autoanalyzer 7150 (Hitachi Ltd. Tokyo, Japan). LDL cholesterol was estimated indirectly using the Friedewald formula for subjects with serum triglyceride levels greater than 4.52 mmol/l. Serum apolipoprotein A1 and B were determined turbidimetrically after precipitation by using a specific anti-serum agent (Roche, Switzerland) at 340 nm.

Serum sex hormone binding globulin (SHBG), luteinzing hormone (LH) and estradiol were also measured by immunoradiometric assays (IRMA). Serum total testosterone and insulin-like growth factor-1 (IGF-1) were determined by radioimmuno assays (RIA).

7. Genetic analysis of the uncoupling protein 3 gene

Genomic DNA was extracted from whole blood using a commercially available DNA isolation kit (WIZARDR^{R} Genomic DNA purification kit, Promega Corp., Madison, WI, USA), according to the manufacturer's protocol.

The ABI PRISM^{R} SNaPshot^{TM} ddNTP Primer Extension Kit (PE Biosystems, Foster City, CA, USA) was used to detect single nucleotide polymorphisms (SNPs) by interrogating individual loci in amplification products.

Samples were electrophoresed by using the ABI PRISM 3700 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) and data were analyzed with GeneScan Analysis Software version 3.1 (Applied Biosystems, Foster City, CA, USA).

8. Statistical analysis

For the statistical analysis, the computer software Win SPSS version 10.0 (Statistical Package for the Social Science, SPSS Inc. Chicago, IL) was used. Comparison of the differences in values before and after caloric restriction, related to UCP3 genotype, was made by a paired t-test or Wilcoxon's signed rank test.

RESULTS

1. Clinical characteristics of the subjects according to UCP3 promoter genotypes before and after caloric restriction

The characteristics of the 90 subjects, such as age, weight, BMI, WHR, and % body fat, are shown in Table 1, as averages for the 56 who had the normal UCP3 (CC) genotype and for the 34 who had mutations in the UCP3 (CT/TT) genotype. Both groups showed similar reductions in body weight, BMI and % body fat after the intervention.

At the end of 12 weeks on the weight reduction program, no one had newly onset diabetes, which was determined with the oral glucose tolerance test (OGTT), and the proportion of subjects with hypertension was not significantly different between the two groups.

Regarding smoking, no one in the normal genotype group smoked, while 11.8% of the group with UCP3 mutations was currently smoking.

2. Lipid profiles and serum hormone levels before and after caloric restriction

Table 2 shows the lipid profiles of the subjects before and after the caloric restriction, according to UCP3 promoter genotypes. Significant reduction in the atherogenic index was shown in both groups after weight reduction, while total cholesterol and apolipoprotein A1 were significantly decreased only in the group without the mutations ($p < 0.05$).

Serum levels of hormones were not significantly changed in both groups after the intervention. However, in the group without the mutations, the leptin level was significantly reduced by 23.4% ($p < 0.001$)(Table 3).

Table 1. Clinical characteristics according to UCP3 promoter genotypes in healthy premenopausal women, before and after caloric restriction (n = 90)

	Without-mutation (n = 56)		With-mutation (n = 34)	
	Before	After	Before	After
Age	31.6 ± 1.59		30.5 ± 1.53	
Weight (kg)	64.3 ± 0.93	61.0 ± 0.90***	66.3 ± 1.71	63.1 ± 1.67***
Body mass index (kg/m^2)	25.3 ± 0.31	24.0 ± 0.31***	25.9 ± 0.66	24.7 ± 0.64***
Waist hip ratio (WHR)	0.83 ± 0.01	0.83 ± 0.01	0.83 ± 0.01	0.82 ± 0.01
Body fat (%)	33.6 ± 0.58	32.2 ± 0.55***	35.7 ± 1.11	34.4 ± 1.09***

Mean ± SE

$\dagger p < 0.1$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with initial value by paired t-test

3. Serum concentration, and responses of glucose, insulin, C-peptide and free fatty acids

Table 4 shows initial (fasting) levels and responses of glucose, insulin, c-peptide and free fatty acid (FFA) in the serum, after 75 g of oral glucose administration, before and after the weight reduction program, according to UCP3 promoter genotypes.

The fasting levels of insulin and C-peptide, and the insulin glucose ratio, were not significantly different after 12 weeks on the weight reduction program. On the on the hand, the without-mutation group tended to have increased FFA and insulin levels, and an increased insulin glucose ratio, even though they were not statistically significant ($p < 0.1$). Fasting levels of FFA significantly increased in the with-mutation group; this could mean that the degree of FFA uptake into muscle in the with-

Table 2. Lipids and apolipoprotein levels according to UCP3 promoter genotypes in healthy premenopausal women, before and after caloric restriction (n = 90)

	Without-mutation (n = 56)		With-mutation (n = 34)	
	Before	After	Before	After
Triglyceride (mg/dl)	126.6 ± 11.6	114.1 ± 10.0	110.6 ± 9.09	100.3 ± 9.09
Total cholesterol (mg/dl)	212.0 ± 7.83	202.7 ± 5.96*	199.1 ± 7.07	195.2 ± 6.86
HDL cholesterol (mg/dl)	54.7 ± 2.09	55.3 ± 1.79	53.2 ± 1.66	55.6 ± 2.38
LDL cholesterol (mg/dl)	132.1 ± 7.29	124.6 ± 6.10ƒ	123.8 ± 5.92	119.6 ± 6.19
Athrogenic index	3.02 ± 0.18	2.78 ± 0.16*	2.80 ± 0.16	2.62 ± 0.17*
Total cholesterol/HDL	4.02 ± 0.18	3.78 ± 0.16*	3.80 ± 0.16	3.62 ± 0.17*
LDL cholesterol/HDL	2.52 ± 0.15	2.37 ± 0.15ƒ	2.37 ± 0.13	2.25 ± 0.14
Apolipoprotein AI (mg/dl)	135.2 ± 3.28	127.5 ± 2.99*	130.9 ± 3.97	132.1 ± 3.47
Apolipoprotein B (mg/dl)	81.2 ± 4.18	78.3 ± 3.24	81.4 ± 4.95	77.5 ± 3.10

Mean ± SE ƒ $p < 0.1$, *$p < 0.05$ compared with initial value by paired t-test or by wilcoxon signed rank test
1: Atherogenic index = (Total cholesterol − HDL cholesterol)/HDL cholesterol

Table 3. Hormone levels according to UCP3 promoter genotypes in healthy premenopausal women, before and after caloric restriction (n = 90)

	Without-mutation (n = 56)		With-mutation (n = 34)	
	Before	After	Before	After
SHBG[1] (nmol/L)	42.7 ± 3.93	48.9 ± 4.13ƒ	49.0 ± 7.22	47.8 ± 6.28
Testosterone (ng/ml)	0.38 ± 0.03	0.42 ± 0.03ƒ	0.42 ± 0.04	0.43 ± 0.04
FAI[2]	3.66 ± 0.29	3.63 ± 0.32	4.49 ± 0.75	4.66 ± 0.86
Estradiol (pg/ml)	89.6 ± 10.2	80.8 ± 13.0	90.4 ± 15.4	102.6 ± 15.1
LH[3] (mIU/ml)	7.83 ± 3.75	6.07 ± 1.23	3.57 ± 0.60	3.62 ± 0.78
IGF-1[4] (ng/ml)	415.0 ± 21.7	391.5 ± 25.5	339.4 ± 17.0	339.3 ± 18.2
Leptin (ng/ml)	12.13 ± 0.67	9.29 ± 0.70***	12.4 ± 0.95	11.3 ± 0.92

Mean ± SE ƒ $p < 0.1$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with initial value by paired t-test or by Wilcoxon signed rank test
1: Sex Hormone-binding globulin 2: Free androgen index = {testosterone (ng/ml) × 3.467} / SHBG (nmol/l) × 100
3: Leuteinizing hormone 4: Insulin like growth factor 1

Table 4. Glucose, insulin, C-peptide, and free fatty acid responses to 75 g oral glucose tolerance tests according to UCP3 promoter genotypes in healthy premenopausal women, before and after caloric restriction (n = 90)

	Without-mutation (n = 56)		With-mutation (n = 34)	
	Before	After	Before	After
Fasting level				
Glucose (mg/dl)	94.3 ± 1.83	90.2 ± 3.48	89.1 ± 2.05	85.1 ± 2.12ƒ
Free fatty acid (μEq/L)	525.9 ± 36.4	623.7 ± 51.7ƒ	467.1 ± 54.4	634.5 ± 62.1*
Insulin (μU/ml)	12.4 ± 2.00	9.47 ± 1.04ƒ	10.5 ± 1.57	9.54 ± 2.54
C-peptide (ng/ml)	1.69 ± 0.13	1.59 ± 0.14	1.70 ± 0.06	1.20 ± 0.25
Insulin glucose ratio1	16.9 ± 2.38	13.9 ± 1.50ƒ	15.4 ± 2.24	15.3 ± 4.44
Response area				
Glucose (mg/dl × hr)	221.7 ± 6.68	224.9 ± 9.71	245.9 ± 10.5	230.2 ± 7.81ƒ
Free fatty acid (μEq/L × hr)	523.4 ± 46.2	851.5 ± 68.7***	557.5 ± 57.4	797.3 ± 77.3*
Insulin (μU/ml × hr)	69.8 ± 11.1	70.2 ± 7.25	95.9 ± 15.6	85.7 ± 14.2
C-peptide (ng/ml × hr)	9.67 ± 0.88	12.4 ± 1.34*	10.1 ± 0.32	14.2 ± 0.97*

Mean ± SE ƒ $p < 0.1$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with initial value by paired t-test or by wilcoxon signed rank test
1: Insulin glucose ratio = insulin (pmol/L)/glucose (nmol/L)

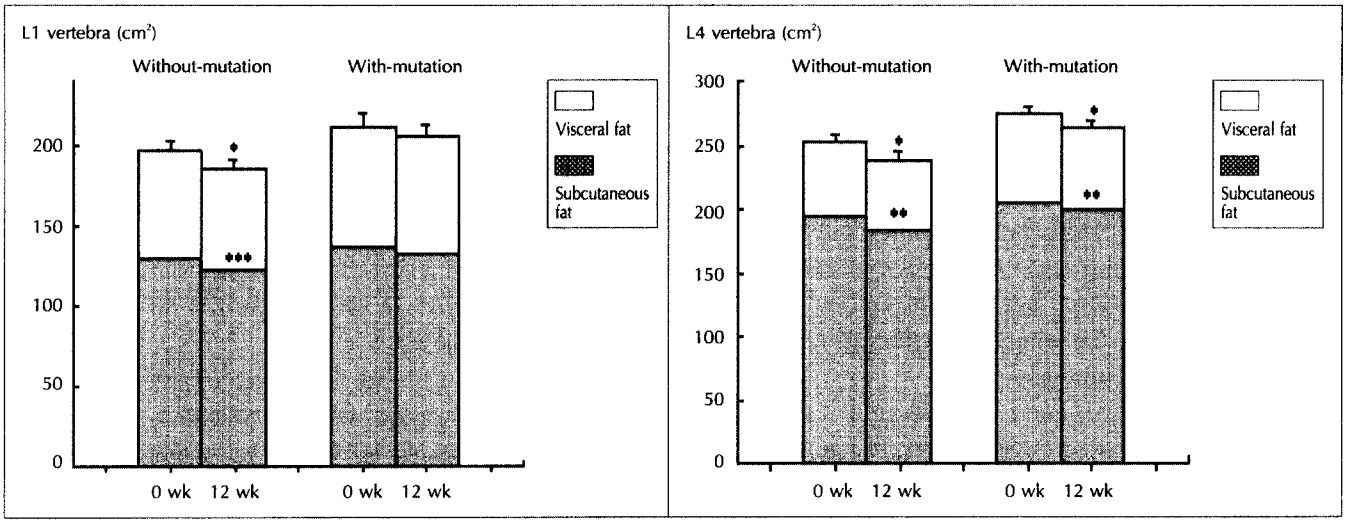

Fig. 1. Fat distribution at L1 and L4 levels according to UCP3 promoter genotypes in healthy premenopausal women, before and after caloric restrction. *p < 0.05, **p < 0.01, ***p < 0.001 valued by paired t-test.

Table 5. Fat and muscle areas at various body sites, according to UCP3 promoter genotypes in healthy premenopausal women, before and after caloric restriction (n = 90)

	Without-mutation (n = 56)		With-mutation (n = 34)	
	Before	After	Before	After
1st lumbar (L1) vertebra				
Total fat (cm²)	196.7 ± 9.11	185.4 ± 9.01***	211.8 ± 14.2	205.5 ± 15.0
4th lumbar (L4) vertebra				
Total fat (cm²)	253.7 ± 7.62	239.1 ± 8.32**	274.1 ± 13.0	263.4 ± 13.6*
				Calf
Fat (cm²)	27.3 ± 0.96	25.8 ± 0.78**	30.6 ± 1.76	28.3 ± 1.63***
Muscle (cm²)	65.0 ± 1.77	63.3 ± 1.41	66.4 ± 2.27	63.9 ± 2.36
Mid thigh				
Fat (cm²)	80.3 ± 2.51	80.0 ± 2.32	86.9 ± 2.91	84.6 ± 2.99**
Muscle (cm²)	110.8 ± 1.57	107.6 ± 1.68**	110.6 ± 1.85	105.3 ± 1.85***

Mean ± SE $\dagger$p < 0.1, *p < 0.05, **p < 0.01, ***p < 0.001 compared with initial value by paired t-test or by Wilcoxon signed rank test

mutation group was lower than in the group without the mutations. Regarding the response areas, both normal and mutation groups showed significant increases in FFA and c-peptide levels.

4. Fat distribution of various body sites

Differences in body fat distribution according to the UCP3 promoter genotype, before and after the weight reduction program, are presented in Figure 1 and Table 5. The without-mutation group showed a significant decrease in fat area at both L1 and L4 levels, while the with-mutation group had the decrease at L4 level, but not at L1 level. Calf fat was significantly decreased in the without-mutation group and the with-mutation group by 7.6% and 5.5%, respectively. On the other hand, the fat area in the mid-thigh was decreased only in the with-mutation group (2.6%, $p < 0.01$).

DISCUSSION AND CONCLUSION

In the present study, overweight premenopausal female subjects, who were placed on a restricted calorie diet (300 kcal/day less than daily energy requirements) for 12 weeks, lost about 5% of their initial body weights and achieved lower BMIs; these results held true for both groups of subjects: those with and without mutations in the promoter region of UCP3.

Despite a similar rate of weight reduction in both groups, decreases in abdominal adipose tissue were more evident in the without-mutation group. In particular, the reduction of fat at Lumbar 1 level was significant only in the without-mutation group. UCP3 structure might be linked with central obesity; in South Indian and British female subjects with diabetes and obesity, the CT and

TT genotypes were associated with an increased waist to hip ratio.[14] However, other studies do not support this link[12]: the −55 C/T UCP3 polymorphism was not associated with obesity, alteration of BMI and WHR, or fasting serum lipids in Danish Caucasian subjects.[15]

It is reported that TT carriers had higher serum levels of total cholesterol, LDL-cholesterol and apolipoprotein B.[13] The present study also showed a significant reduction in total cholesterol, and a tendency for decreased LDL cholesterol, in the without-mutation group, following the weight reduction program.

Except for decreased serum leptin level in without-mutation group, there were no significant changes in serum hormone concentrations in both genotype groups after the weight reduction program. Leptin, an adipose-derived hormone, is reported to play an important role in body fat mass regulation; weight reduction brings about a decreased leptin level, thereby reducing glucose uptake in adipocytes and elevating FFA level by lipolysis in body fat.[16] The present study showed a similar pattern regardless of UCP3 genotype. However, in the with-mutation group, the leptin level did not decrease despite the weight reduction. This result is in accordance with the theory that leptin could affect UCP3 mRNA expression.[17]

Fasting serum levels of FFA significantly increased in the with-mutation group, not in the without-mutation group. It might mean that the degree of FFA uptake into muscle in the with-mutation group was lower than in the without-mutation group. The FFA levels in the circulation, elevated by the increased lipolysis in adipose tissue, seemed to be taken up into skeletal muscle to a greater extent in the without mutation group. UCP3, predominant in skeletal muscle is thought to be a regulator of lipids as a fuel substrate[18] and therefore may be associated with the use of free fatty acid as a fuel; this may result in an increased muscle intracellular FFA level and induce UCP3 expression in skeletal muscle.[19] On the other hand, both with- and without-mutation groups showed a similar increase in FFA response after oral glucose administration. Oral glucose ingestion apparently suppresses the rate of lipolysis in adipose tissue, but not in skeletal muscle; muscle lipolysis seemed to be an important endogenous energy source in this case.[20]

In conclusion, this study showed that caloric restriction (−300 kcal/day) for 12 weeks induced the reduction of body weight. Although no difference was found in the magnitude of weight reduction in both groups, there were significant differences in body fat distribution and in endocrine function between the groups.

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