Effect of Tailor-Made Diet on Weight Loss in Obese Japanese Patients of Type 2 Diabetes Mellitus with Single Nucleotide Polymorphisms in β 3-Adrenergic Receptor, Uncoupling Protein 1 or β 2-Adrenergic Receptor Genes

J Rehabili Health Sci 2008 ; **6** : 31-35

Rapid Communication

Effect of Tailor-Made Diet on Weight Loss in Obese Japanese Patients of Type 2 Diabetes Mellitus with Single Nucleotide Polymorphisms in β3-Adrenergic Receptor, Uncoupling Protein 1 or β2-Adrenergic Receptor Genes

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Received February 18, 2008 ; accepted March 24, 2008

Key words : tailor-made diet; type 2 diabetes mellitus; obese; β3-adrenergic receptor; uncoupling protein 1; β2- adrenergic receptor

1 Introduction

Weight loss is associated with improved glycemic control or other metabolic disorders in type 2 diabetes mellitus (T2DM). However, correlations between BMI and energy intake in patients have been insufficiently studied.[1] The relationship between energy intake and obesity in patients with T2DM is influenced by genetic background on the pathophysiology of the disease. Obesity and metabolic disorders have been associated with mutations called the single nucleotide polymorphism (SNPs) in the β3-adrenergic receptor gene (β3AR; Trp64Arg).[2] The condition is brought on by larger visceral fat mass accompanied by insulin resistance. It has been suggested that this is associated with an increased tendency to gain weight and a low basal metabolic rate which increases serum total cholesterol, triglyceride and decreases HDL cholesterol in obese subjects and as a result causes T2DM or hyperlipidemia.[3,4] One of brown adipose tissue's specific inner-mitochondrial components is uncoupling protein 1 (UCP1) which varies respiration coupling and dissipates oxidation energy as heat.[5] An association between the polymorphic *Bcl* I site (A-G polymorphism at position -3826 bp in the 5' flanking domain; -3826 A/G) of the UCP1 gene and fat gain has been reported.[6] Its dysfunction also leads to obesity, insulin resistance, and T2DM. There are several reports in which the frequency of the UCP1 SNPs (-3826 A/G) in obese people, especially Japanese women, was found to be approximately two times higher than levels found in Finns or Canadians.[7-11] β2-adrenergic receptor gene (β2AR; Arg16Gly) were also reported to relate to cardiovascular function[12,13] or modulate insulin sensitivity and secretion.[14] Visceral fat obesity is more strongly associated with metabolic and cardiovascular diseases than subcutaneous fat obesity. It is well known that people with both obesity and diabetes have an increased risk of cardiovascular disease. Therefore, it is as important for obese T2DM to lose weight as it is for glycemic control, although the T2DM who carry the SNPs of β3AR, UCP1 or β2AR often gain weight easily but find it difficult to lose weight. In this study, we investigated the effect of tailor-made diet on weight loss in obese Japanese T2DM with the SNPs of β3AR (Trp64Arg), UCP1 (-3826 A/G) or β2AR (Arg16Gly).

2 Subject and methods

2.1 Subjects

Patients diagnosed with T2DM who fulfilled the WHO criteria for diabetes were recruited from outpatients visiting the Kajiyama Clinic between 2004 and 2007. The inclusion criteria were adults with obesity (BMI 25 ≦). Patients were excluded if they had any significant diseases that were likely to affect the outcome or compliance with this study. The exclusion criteria were as follows: heart failure, hepatic dysfunction, renal failure, or serious physical or mental conditions. A total of 21 obese adults with T2DM, male / female: 3/18, age 53.6 ± 11.9 years, duration of T2DM 6.6 ± 6.3 years, body weight 80.7 ± 14.8 kg, BMI 33.0 ± 5.5 kg/m^2, waist circumference 102.9 ± 11.8 cm (means ± SD), were assigned to the study group. The study protocol was approved by the Ethics Committee of the School of Comprehensive Rehabilitation at Osaka Prefecture University and all subjects gave their written informed consent before the enrollment.

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2.2 Methods

Body weight (BW) and height were measured and BMI was calculated as weight in kilograms divided by the square of the height in meters (kg/m^2) every 4 weeks. DNA was prepared from the mouth mucous membrane and detected using PCR amplification and Luminex ™ typing application methods (Hitachi Soft, Tokyo, Japan) described elsewhere.[15] Fasting blood samples were collected in the morning after an overnight fast from all participants every 4 weeks and examined by auto analyzer. HbA_{1c} levels were determined by a latex cohesion method (JCA-BM2250, KYOWA MEDEX, Co., Ltd., Tokyo, Japan). Fasting blood glucose (FBG) levels were examined by the hexokinase method (JCA-BM12, Shino Test, Co., Ltd., Tokyo, Japan). Total cholesterol (T-C) and triglyceride (TG) levels were determined by enzyme assay. HDL cholesterol (HDL-C) levels by a direct method (Labospect 008K, Bio Majesty JCA-BM 8060, JEOL, Ltd., Tokyo, Japan) and LDL cholesterol (LDL-C) levels by an enzymatic method (Bio Majesty JCA-BM 8060, JEOL, Ltd., Tokyo, Japan).

All participants received dietary counseling every 4 weeks by the dietitians according to their SNPs of β3AR, UCP1 or β2AR. The goals for the patients assigned to the intensive lifestyle intervention were to achieve and maintain a modest weight loss of 5% from their initial weight, following recommended dietary intake according to each gene type, and undertake physical activity, mainly walking. Patients who carried β3AR SNPs were counseled to reduced caloric intake to 200kcal below their usual total energy expenditure and prepare meals containing 50-55% of calories from carbohydrate, primarily unrefined carbohydrate containing high dietary fiber such as brown rice, grains and vegetables. The patients who carried the SNPs of β2AR were advised to keep their postprandial blood glucose levels low by choosing low glycemic index foods such as unrefined carbohydrate and vegetables. Patients who carried UCP1 were counseled to restrict fat to less than 25% of calories (saturated/ monounsaturated/ polyunsaturated fatty acid ratio = 3:4:3, n-6 fatty acid/ n-3 fatty acid =3～4:1) and reduce calorie intake to 100kcal below their usual total energy expenditure. Protein and fat from soy beans or fish were recommended over meat. If subjects carried mutations of both β3AR and UCP1, they were advised to reduce calorie intake to 300 kcal below their total energy expenditure. All educational dietary efforts, individualized exercise programs, and realistic dietary goals, particularly relating to the severe restriction of calories or fat intake, were included in a written plan. The plan and goals were decided from the perspective of the participants and the goals were presented in a manner that was concise and easy to achieve. At each follow-up session every four weeks, an assessment was carried out to determine if these goals were being met. Dietary intake was assessed by food records collected over seven days at both baseline and after intervention and calculated by using the software Eiyokun (Kenpakusya, Tokyo, Japan).

2.3 Statistical analysis

The characteristics of the study participants were calculated and expressed as means ± SD. Statistical analyses were performed using SPSS version 14.0 (SPSS Inc., Chicago, IL, USA). Paired t tests were used to determine differences between the baseline and after every 4 weeks. A p-value of less than 0.05 was considered statistically significant.

3 Results

The percentage of patients with three SNPs with obese T2DM is shown in Table 1. Patients carried high rates of these SNPs, especially, UCP1 or β2AR SNPs were found in about eighty percent of subjects. The majority of subjects had more than two SNPs (Fig. 1). Nine (43%) of the subjects carried both UCP1 and β 2AR, and five (24%) carried all three SNPs. Table 2

Table 1 Percentage of Patients who carry β3AR, UCP1 or β2AR SNPs

	Homozygous (%)	Heterozygous (%)	Wild (%)
β3AR (Trp64Arg)	10	29	61
UCP1 (-3826 A/G)	14	72	14
β2AR (Arg16Gly)	29	47	24

Table 2 Anthropometric measurements and blood pressure before and after intervention

	before	4wks	8wks	12wks	16wks
Weight (kg)	80.7 ± 14.8	79.5 ± 14.8*	77.5 ± 14.5*	76.3 ± 14.4**	78.1 ± 15.5*
BMI (kg/m^2)	33 ± 5.4	32.1 ± 5.6	31.9 ± 5.7*	31.7 ± 5.6**	31.9 ± 5.7*
Waist circumference (cm)	102.9 ± 11.8	101.5 ± 10.2	103.7 ± 12.8	101 ± 10.1	94.2 ± 8.0
Systolic BP (mmHg)	128 ± 18	125 ± 12	127 ± 14	126 ± 11	126 ± 9
Diastolic BP (mmHg)	75 ± 14	72 ± 9	73 ± 11	75 ± 11	78 ± 11

Data are means ± SD. Significant difference between baseline vs after intervention; * $p < 0.05$, ** $p < 0.01$.

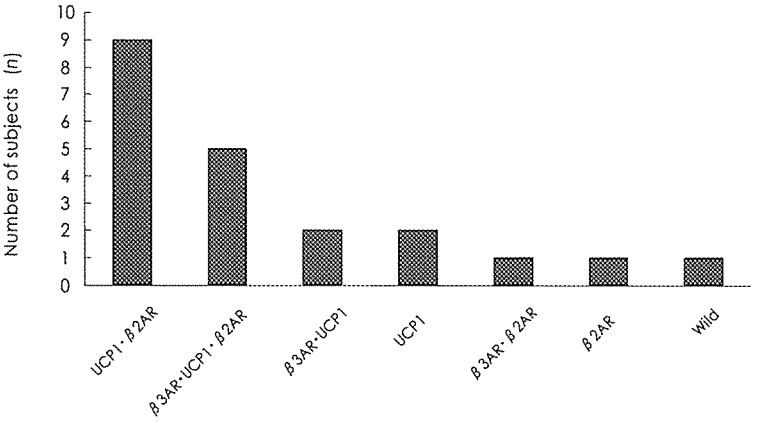

Fig. 1 Number of subjects who carry SNPs.

Table 3 Changes of laboratory data during the study period

	before	4wks	8wks	12wks	16wks
FBG (mg/dl)	142 ± 38	142 ± 30	143 ± 35	132 ± 27	139 ± 38
HbA_{1c} (%)	7.0 ± 1.1	7.0 ± 1.2	6.8 ± 1.3	6.8 ± 1.3	6.8 ± 1.3
Triglyceride (mg/dl)	170 ± 90	139 ± 40	139 ± 52	152 ± 90	143 ± 84
Total-cholesterol (mg/dl)	224 ± 36	217 ± 35	216 ± 32	225 ± 38	220 ± 43
HDL-cholesterol (mg/dl)	58 ± 15	62 ± 27	63 ± 26	60 ± 17	59 ± 13
LDL-cholesterol (mg/dl)	140 ± 39	135 ± 45	128 ± 40	139 ± 38	136 ± 45

Data are means ± SD.

Table 4 Dietary intake of the study group before and after intervention

	before intervention	after intervention
Energy (kcal)	1,765 ± 527	1,379 ± 334*
Protein (g)	69 ± 22	60 ± 11
Fat (g)	51 ± 15	42 ± 12
Carbohydrate (g)	250 ± 96	210 ± 94
Calcium (mg)	510 ± 206	441 ± 174
Magnesium (mg)	220 ± 81	224 ± 75
Potassium (mg)	2,031 ± 764	2,175 ± 724
Iron (mg)	7.7 ± 6.0	14.9 ± 21.1
Retinol (μg)	584 ± 230	499 ± 248
Vitamin K (μg)	137 ± 84	213 ± 135
Vitamin B_1 (mg)	1.0 ± 0.6	3.7 ± 9.1
Vitamin B_2 (mg)	1.1 ± 0.6	4.3 ± 12.1
Vitamin B_{12} (μg)	6.9 ± 6.1	6.1 ± 3.1
Folic acid (μg)	258 ± 148	279 ± 124
Vitamin C (mg)	96 ±104	93 ± 45
Dietary fiber (g)	12.0 ± 6.8	12.5 ± 4.9
Sodium (g)	9.0 ± 3.3	7.4 ± 1.6

Data are means ± SD. Significant difference between baseline vs after intervention; * $p < 0.05$.

shows anthropometric measurements and blood pressure before and after intervention. Body weight and BMI decreased significantly after intervention ($p < 0.05$) and waist circumference also decreased 8%. There was no reduction observed in systolic or diastolic blood pressure after intervention. No specific levels of body temperature in the subjects were observed.

Table 3 indicates the change of laboratory data during the study period. Blood glucose, HbA_{1c} and triglyceride decreased, but not significantly. Dietary intake of subjects before and after intervention are shown in Table 4. We observed a significant decrease in total dietary energy intake of 22 % after intervention. Our results are consistent of dietary intervention by gene type

that found lifestyle intervention resulted in a decrease in fat, carbohydrate and sodium and an increase in the intake of iron, vitamin K, vitamins B_1, B_2, and folic acid.

4 Discussion

The relationship between obesity and genetic background in patients with T2DM is complicated. Our study demonstrated that almost all subjects carried the SNPs of β3AR, UCP1 or β2AR with homozygous or heterozygous. The ratio of these SNPs was much higher than previously reported in studies of obese Japanese women,[16] UCP1 gene polymorphisms with G allele were found to be particularly high. The higher BMI of Japanese men[17] and Australian women[18] with the G allele has been reported. Matsushita et al.[19] reported that estrogen deficiency resulted in reduced UCP1 expression in brown adipose tissue associated with weight gain. In our study, high rates of the SNPs of UCP1 and the fact that all the female patients were postmenopausal may have influenced obesity in the subjects.

Dietary intake of energy, fat and carbohydrate decreased and intake of minerals and vitamins increased after intervention. This was the result of patients being able to improve their diet by decreasing intake of white rice, sweets and fatty foods, while increasing their intake of vegetables. T2DM patients, especially those with obesity, had greater difficulty decreasing the amount of food because they had stronger appetites than people without diabetes and found it hard to increase physical activity because of the problems with their legs or backs. The average weight loss of approximately 3.2%, although not large, led to some improvement in metabolic control. These results indicate a successful outcome that was enhanced by individual education delivered with a subject-centered approach taking into account gene type and lifestyle. The dietary goals were achieved in part due to the subjects being able to understand their own SNPs type and to foster collaboration between patients and educators. All these components of dietary goals and plans were necessary to express individuality in every session. In the present study, most patients succeeded in losing weight because the process was guided by knowing their gene type and a willingness to change lifestyle. In addition, food records complemented the changes in behavior and resulted in a significant reduction in energy intake. However, a few patients did not succeed in reducing weight by 4%. One of the reasons was their heavy weight (86.0 $\pm$ 3.4 kg) and BMI (35.2 $\pm$ 1.3 kg/m^2) in the baseline, compared to the subject group who succeeded in over 4% weight loss (weight: 69.5$\pm$ 4.0 kg, BMI: 29.1$\pm$1.3 kg/m^2). It was also very difficult for severe obese patients to increase daily physical expenditure because of pain in their legs or back. Participants who had little or no family support or who lacked the confidence to achieve and sustain their goals did not achieve a 4 % weight reduction.

A large scale study should be undertaken to reveal the association with T2DM or obesity and to investigate other SNPs, such as ATP-binding cassette transporter A1, fatty acid binding protein 2, LDL receptor defect C complementing and aldolase B, related to regulation of pancreatic β cell function, insulin resistant, lipid metabolism, or energy expenditure[18]. However, the roles of the genes that link them to T2DM or obesity are not clear. The genotypes that account for the association of the genotype with T2DM or obesity should be clarified in the future.

Our study showed that tailor-made diet taking into account each genotype can be an effective method of losing weight and preventing complications of DM, especially in cardiovascular diseases, when carried out with patients guided by knowledge of their own gene type and a willingness to alter their behavior.

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