Different Response of Body Weight Change According to Ketonuria after Fasting in the Healthy Obese

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INTRODUCTION

For the last few decades, the global population of obese people has been increasing exponentially. According to 2008 data from the World Health Organization (WHO), the number of overweight adults exceeds 1.5 billion and obese adults are estimated to exceed 500 million. Obesity is the main cause of several chronic diseases such as diabetes, hypertension, hyperlipidemia, and coronary artery disease (1). Obesity may cause such chronic diseases because of leptin resistance (2, 3), reduced adiponectin secretion (4-6), chronic inflammation (7), insulin resistance (8), and mitochondrial dysfunction (9).

The relationship between obesity and ketone is unknown. When the body lacks carbohydrates or protein, energy comes from lipolysis. Ketone is formed during lipolysis, which can then cause ketonemia or ketonuria. By this mechanism, a very low-calorie diet elevates the serum ketone level (10, 11). A study of overweight adults showed that a ketogenic low-carbohydrate diet can produce greater weight loss than low-fat diet, and that a ketogenic low-carbohydrate diet promotes a greater degree of decreased triglyceride level, increased high-density lipoprotein (HDL) cholesterol level than a low-fat diet (12). Another study reported that a ketogenic diet promotes a non-atherogenic lipid profile as well as weight loss, lower blood pressure, and diminished resistance to insulin with an improvement in blood levels of glucose and insulin. Also it has anti-neoplastic benefits (13).

In a previous study, an obese group of individuals displayed more resistance in formation of ketones than normal weight group. In the study, which was conducted during the Muslim fasting period, ketosis was induced in the normal weight group after 3-6 days, whereas ketosis in the obese group was not induced after 20 days (14). Another study showed that, after a ketogenic diet, a normal weight group had increased blood ketone level after 2-3 days, whereas an obese group showed little or no difference after 10 days (15). In another study, even though ketonuria was observed during several weeks, ketonemia-induced symptoms such as headache, loss of appetite, and nausea were not found in obese subjects (16).

We conducted a retrospective observational study to evaluate whether the body weight reduction response was different by the presence of ketonuria after fasting in the healthy obese.

MATERIALS AND METHODS

Study data

In this retrospective observational study, medical records were
reviewed for the healthy obese patients (body mass index, BMI, ≥ 25 kg/m²) who had participated in a 3-month conventional low-calorie diet program on an out-patient basis, after a routine health check-up conducted from January-December, 2008, at a health promotion center in one university hospital in Suwon, Gyeonggi-do, Republic of Korea. Forty two healthy obese subjects aged in their 20s to their 50s who initially had non-ketonuria at the routine health check-up were selected. In the out-patient clinic, one week later, the presence of urinary ketone was retested after three subsequent fasts. Subjects’ data were excluded any medical history such as hypertension, type 2 diabetes, coronary artery diseases, cerebral artery diseases, and any cancer.

Anthropometry and laboratory measurements
We reviewed medical record such as laboratory data and self-administered questionnaire of study subjects. Among the initial measurement data of routine health check-up, we selected the anthropometry data and several metabolic parameters such as weight, waist circumference, BMI, and body composition parameters such as lean body mass and fat mass using Bio-impedance analysis, blood pressure, fasting blood sugar, triglycerides, HDL cholesterol, and total cholesterol using venous blood and urine analysis, blood pressure, fasting blood sugar, triglycerides, HDL cholesterol, and total cholesterol using venous blood and urine drawn following an 8-hr overnight fast. As well, subjects completed a self-administered questionnaire soliciting information on their history of drinking and smoking. The height and weight were measured with a test gown on using an automatic height-meter; results were 0.1 cm and 0.1 kg, respectively. BMI were measured with a test gown on using an automatic height-meter; results were 0.1 cm and 0.1 kg, respectively.

Measurement of ketonuria
Since all those patients showed no ketonuria in routine health check-ups, we educated patient to fast three subsequent meals the day before urinalysis (one week after the initial check-up) to evaluate the urinary ketonuria (Fig. 1). All patients tolerated the subsequent three meals fasts well with no serious side effects, except for mild general weakness associated with the fasts were observed. The presence of ketonuria was determined using US-3100R urine test paper (Eiken Chemical, Tokyo, Japan). Ketonuria was categorized into four classes: 0, 1+ (10 mg/dL), 2+ (30 mg/dL), 3+ (80 mg/dL). For this study, the results are shown only as qualitative positive state, not blood ketone level.

Statistical analysis
The data was classified into two groups depending on the change of ketonuria state: ketonuria group and non-ketonuria group. The general characteristics were expressed as mean ± standard deviation, and as variable distribution was observed to be normal, the difference between two groups was compared using parametric methods of independent t test. For analyzing the result, SPSS ver.11.5 (SPSS Ltd., Chicago, IL, USA) was used. All significant P values were < 0.05.

**Fig. 1.** Evaluation of ketonuria after initial health check-ups.
Ethics statement
The institutional review board of Ajou University Hospital approved this study and waived informed consent (AJIRB-MED-OBS-09-147).

RESULTS
Comparison of clinical characteristic between two groups according to the change of ketonuria after one week
The 42 subjects were divided into two groups according to the presence of ketonuria after one week: a ketonuria group (n = 18, average age 38.1 ± 13.7 yr), which progressed from non-ketonuria to ketonuria during that time, and a non-ketonuria group (n = 24, average age 35.2 ± 9.6 yr), which displayed persistent non-ketonuria. Compared to the clinical characteristics of the patients at baseline, the two groups showed no statistically significant differences in sex, weight, BMI, waist circumference, and laboratory data. In case of free fatty acid (FFA) and insulin concentration, the decrease showed no difference between both groups (-2.95 ± 3.12 μIU vs -2.94 ± 3.17 μIU, P = 0.994). Unfortunately, laboratory follow-up data were not obtained in all subjects such as fasting blood glucose and lipid concentration, therefore, we could not compare the simple metabolic parameters between the two groups. However, the changes of all laboratory data we reviewed were favorable in the ketonuria group compared to the non-ketonuria group, even though limited laboratory data.

Comparison of anthropometric changes between the groups after three-month obesity control program
After three months, comparisons of the ketonuria group with the non-ketonuria group demonstrated significantly greater reductions in body weight (-8.6 ± 3.6 kg vs -1.1 ± 2.2 kg, P < 0.001), waist circumference (-6.92 ± 1.22 cm vs -2.32 ± 1.01 cm, P < 0.001), fat mass (-2.99 ± 2.17 kg vs 0.35 ± 2.70 kg, P < 0.001), and BMI (-3.16 ± 1.25 kg/m² vs -0.43 ± 0.86 kg/m², P < 0.001), and lean body mass (-3.06 ± 2.76 kg vs 0.02 ± 1.77 kg, P < 0.001) (Table 2). The increase of serum FFA concentration was remarkable in the ketonuria group than in the non-ketonuria group (576.9 ± 390.0 μEq/L vs 13.6 ± 172.3 μEq/L, P < 0.001). In case of serum insulin concentration, the decrease showed no difference between both groups (-2.95 ± 3.12 μIU vs -2.94 ± 3.17 μIU, P = 0.994). Unfortunately, laboratory follow-up data were not obtained in all subjects such as fasting blood glucose and lipid concentration, therefore, we could not compare the simple metabolic parameters between the two groups. However, the changes of all laboratory data we reviewed were favorable in the ketonuria group compared to the non-ketonuria group, even though limited laboratory data.

DISCUSSION
We previously reported that individuals displaying ketonuria after fasting have metabolic superiority over those without ketonuria (18). In that study, the ketonuria group had a lower proportion of obesity, central obesity, or metabolic syndrome; lower weight, waist circumference, fasting blood glucose, triglycerides, blood pressure and insulin; and higher HDL cholesterol. But, the study was limited by its cross-sectional nature, which made difficulty of causality. To overcome this limitation, we presently analyzed the clinical data conducted in an out-patient clinic during a 3-month obesity intervention program by retrospective observation. Especially, we targeted healthy obese subjects without ketonuria at baseline, comparing the changes of body weight and body composition after the 3-month program according to the presence of ketonuria. Body weight, body fat, and waist circumference were all reduced more in the ketonuria

Table 1. Baseline characteristics of the study subjects

| Variables                  | Urine-ketone (-) (n = 18) | Urine-ketone (+) (n = 24) | P value |
|----------------------------|---------------------------|---------------------------|---------|
| Sex (female, %)            | 89                        | 87                        | 0.639   |
| Age (yr)                   | 38.1 ± 13.7               | 35.2 ± 9.6                | 0.438   |
| Body weight (kg)           | 77.5 ± 9.3                | 82.1 ± 13.1               | 0.193   |
| BMI (kg/m²)                | 29.6 ± 3.0                | 30.5 ± 3.3                | 0.338   |
| WC (cm)                    | 88.7 ± 1.5                | 87.9 ± 1.9                | 0.446   |
| SBP (mmHg)                 | 121.2 ± 11.3              | 121.3 ± 8.3               | 0.975   |
| DBP (mmHg)                 | 79.2 ± 8.4                | 79.1 ± 4.9                | 0.963   |
| HR (bpm)                   | 73.6 ± 5.5                | 76.3 ± 8.9                | 0.366   |
| Fat mass (kg)              | 35.3 ± 4.7                | 36.2 ± 3.4                | 0.474   |
| LBM (kg)                   | 49.6 ± 6.9                | 52.1 ± 8.3                | 0.312   |
| FBS (mg/dL)                | 90.5 ± 9.0                | 91.6 ± 11.4               | 0.743   |
| HDL-C (mg/dL)              | 49.5 ± 8.7                | 50.5 ± 10.3               | 0.766   |
| TG (mg/dL)                 | 125.0 ± 77.3              | 140.1 ± 64.6              | 0.540   |
| TC (mg/dL)                 | 196.3 ± 39.2              | 202.0 ± 42.6              | 0.696   |
| FFA (µEq/L)                | 596.1 ± 200.2             | 546.0 ± 194.3             | 0.428   |
| Insulin (µIU)              | 11.8 ± 4.6                | 7.3 ± 3.7                 | 0.002   |

P value was calculated by independent t test. Urine Ketone (-), ketone (-) at initial urine test and ketone (-) at retest 1 week later; Urine Ketone (+), ketone (+) at initial urine test and ketone (+) at retest 1 week later; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; bpm, beat per minute; LBM, lean body mass; FBS, fasting blood sugar; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; TC, total cholesterol; FFA, free fatty acid.
group than in the non-ketonuria group after three subsequent fasting periods, although no ketonuria was evident at baseline in any subject. In addition, the increase of serum free fatty acid concentration in the ketonuria group after intervention, which indicated increased fat oxidation indirectly, was more remarkable than in the non-ketonuria group. Serum insulin concentration was lower in the ketonuria group than in the non-ketonuria group at baseline. However, the reduction after intervention showed no difference between the two groups.

Ketone body is a general term for three substances (acetoacetate, β-hydroxybutyric acid, and acetone) produced by decarboxylation. Ketone bodies are produced in the liver by oxidation of fatty acids and are transported to extra-hepatic tissues in the blood to provide energy. Ketone bodies can be measured in the blood and urine. Increased concentration can occur in circumstances including starvation, long-term strenuous exercise, or uncontrolled diabetes. Yet, virtually nothing is known of the relationship between ketone and obesity.

To date, many studies have investigated the influence of ketogenic diets on weight loss and metabolism. A meta-analysis of 447 patients indicated that a low-carbohydrate diet produced a statistically significant loss of weight compared to a low-fat diet (19). When weight change in 311 overweight/obese premenopausal women after 12 months was compared according to diet type, the greatest weight loss was associated with a low-carbohydrate high-fat diet (20). In another study involving five patients with non-alcoholic fatty liver who consumed a low-carbohydrate, ketogenic diet, four displayed improved histological fat degeneration, inflammation, and degree of fibrosis after 3 months (21). In another study, a low-carbohydrate diet produced a more favorable effect in controlling lipid and blood glucose level than a low-fat diet (22). A plethora of other studies have yielded consistent findings, yet how ketone bodies induce weight loss and a beneficial metabolic effect remain unknown. Considering that ketone is not produced as well in obese subjects than in normal-weight subjects (14, 15), the role of fat oxidation should be suspected in obese individuals. Furthermore, ketone formation after fasting would be expected to reflect a metabolically superior state, in which oxidation and decomposition of fat are achieved more easily. However, a 2008 study involving 16,523 patients who received a physical examination at one center in the year reported that only 8.8% displayed ketone formation after fasting. Considering this result, it is difficult to generally consider that the metabolic state of the body is a normal reaction to ketone formation after fasting.

Insulin can stimulate the storage of free fatty acid into fat tissue via lipoprotein lipase by positive energy balance. Therefore, serum insulin and free fatty acid concentration have a negative correlation and the same pattern was also observed in our study. Lower concentration of serum insulin in the ketonuria group at baseline may indicate reduced fatty acid synthesis and storage in the ketonuria group. Reduction of serum insulin concentration after intervention, which was not different between the two groups, in addition to the increased serum fatty acid concentration in the ketonuria group can explain more increase of fat oxidation than in the non-ketonuria group. Although serum ketone body formation, which we did not measure, and the presence of ketonuria after fasting may induce weight loss, we noticed that the presence of ketonuria after fasting in the healthy obese subjects may be an independent factor for the response of more reduction of body weight. For the control of body weight, the reduction of body fat, not muscle mass, is essential. Therefore, most studies have focused on the reduction of body fat and the increase of fat oxidation may be a core of the issues. To increase fat oxidation, they believe that their efforts are vital to burn more fats, many obese subjects are trying to modulate their diet, especially ketogenic diet, and increase daily physical activity and do regular exercise. Following our study results, in the healthy obese subjects with ketonuria after several subsequent meals fasting, fat oxidation can be easily obtained and their body weight can be reduced more efficiently. Therefore, in the clinical field, the evaluation of ketonuria in the healthy obese subjects is an essential check-up point and may be a good indicator of good response of body weight reduction. Furthermore, regular assessment of the presence of ketonuria in the middle of body weight control program to evaluate fat oxidation indirectly can be a useful tool for the enhancement of fat oxidation in the body weight reduction program.

Our retrospective study has some limitations. The weakest point is the lack of direct fat oxidation measurement. And also we did not measure respiratory quotient (RQ) and ketone was measured through a urine test and the result was consequently confirmed only as a qualitative measure. Quantitative testing of blood test would have been able to elaborate on the correlations much better. Another is the small sample size, mainly female, which makes it hard to generalize the results and the study was only 3 months; a short-term study can show different results from long-term studies. Although all the subjects were educated to fast three subsequent meals prior to the urine test, adherence was self-verified, and so the accuracy of the fasting state might have been compromised. However, despite of these limitations, our study has the strength of a retrospective observation design, and the results of the body weight reduction according to changes of ketonuria after fasting in the healthy obese are valuable.

In conclusion, different body weight changes are evident in subjects with ketonuria after fasting in the healthy obese subjects, displaying more body weight reduction than non-ketonuria subjects.

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