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Plasma klotho levels decrease in both anorexia nervosa and obesity

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ABSTRACT

Objective: The aim of this study was to examine the associations of klotho with body mass index (BMI) in patients with restricting-type anorexia nervosa (r-AN) and obesity.
Method: We examined plasma klotho as well as adiponectin and its isoform levels in comparison in 11 obese patients, 12 r-AN patients, and 11 control participants.
Results: Plasma klotho levels were markedly lower in the obesity and r-AN groups than in the control group. Moreover, plasma klotho levels increased significantly after the recovery of BMI in r-AN patients. Total and high-molecular-weight adiponectin levels were significantly decreased only in obesity. There was no relationship between klotho and total adiponectin levels or klotho and respective adiponectin isoform levels in the entire study population.
Conclusions: These results suggest that klotho may reflect normal nutritional state, and that the decrease of klotho in r-AN and obesity may underlie the deteriorating processes of these disorders.

Introduction

The klotho gene, which encodes a single-pass transmembrane protein expressed primarily in renal tubes, has been identified as a systemic anti-aging hormone [1–3]. It may function as an aging-suppressor gene that extends the life span when it is overexpressed and, conversely, accelerates aging when disrupted [4].

Aging is associated with an increased risk for metabolic disorders and with becoming overweight and obese. Caloric reduction is the only non-pharmacologic intervention that has protective effects against aging. Aging and morbidity are thus associated with the body mass index (BMI). These findings suggest a possible association between klotho function and BMI. However, the relationship between klotho and BMI is still unknown.

Klotho plays a role in adipocyte maturation and systemic glucose metabolism [5,6], and also increases adipocyte differentiation in vitro [7]. These findings suggest that klotho influences energy metabolism. Adiponectin is a protein hormone produced almost exclusively in adipose tissue [8]. We recently reported the relationship between the adiponectin isoforms and anorexia nervosa (AN) [9]. Because adiponectin has a strong association with metabolic dysfunction and is a biomarker of metabolic syndrome [10], we hypothesized that there would be a relationship between klotho and adiponectin levels.

To verify the role of klotho in human metabolism in this study, we therefore examined the association between plasma klotho levels and BMI, and we compared the levels of adiponectin and its isoforms in participants on the opposite ends of the body weight continuum: AN and obesity.

Materials and methods

Participants

Thirty-four women participated in this study: 11 obese patients, 12 patients with restricting-type anorexia nervosa (r-AN), and 11 age-matched controls

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(Table 1). The obese patients had a BMI > 25 kg/m^2, with no other diseases identified as the cause of their obesity.

All the r-AN patients were diagnosed with amenorrhea according to the criteria of Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSMIV) [11].

For comparison with the r-AN and obese patients, age-matched healthy volunteers were recruited from the local community, according to the following criteria: within ± 10% of their ideal body weight (BW), normal caloric intake, and no history of psychiatric illness or metabolic disease. In accordance with the principles of the Declaration of Helsinki, all participants gave informed, written consent to participate. The study was approved by the Institutional Committee of Kagoshima University.

Experimental design

Blood samples were collected in the morning after overnight fasting. Aliquots of serum and plasma were immediately obtained and stored at −80°C. Soluble α-klotho levels were measured in EDTA-plasma using a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) (Immuno-Biological Laboratories, Takasaki, Japan) [12]. The designation α-klotho is used to describe the original klotho gene and its product. Throughout this study, the term *klotho* refers to α-klotho. The concentration of total, high-molecular-weight (HMW), medium-molecular-weight (MMW), and low-molecular-weight (LMW) adiponectin was measured using an ELISA kit (Sekisui Medical Chemical Co, Tokyo, Japan) [13]. The percentage of each adiponectin form to total adiponectin was calculated.

Of the 12 r-AN patients, 6 recovered from the underweight state and regained approximately normal BMI (BMI > 17 kg/m^2). The same measurements were performed in these 6 r-AN patients after recovery. Recovery was defined as a stable BMI > 17 kg/m^2 for at least 1 y.

Statistical analysis

Statistical analyses were performed using SPSS software (version 19.0) (SPSS, Inc., Chicago, IL, USA). An analysis of variance (ANOVA) and the post-hoc Sidak test were used to compare clinical data and klotho levels among the groups. A paired *t* test was used to compare the data from the r-AN patients before and after weight recovery. The relationships among different parameters were examined by Pearson's test. *P*-values <5% were considered significant.

Table 1
Clinical characteristics of the participants and the r-AN patients before and after weight recovery

	Control (n = 11)	r-AN (n = 12)	Obesity (n = 11)
Age (y)	21.00 ± 1.29	21.00 ± 1.65	21.27 ± 0.95
Height (cm)	159.33 ± 1.25	157.55 ± 1.45	159.62 ± 1.22
Weight (kg)	55.63 ± 1.54	32.73 ± 1.12**	90.68 ± 7.48***###
BMI (kg/m^2)	21.84 ± 0.36	13.12 ± 0.26**	35.72 ± 3.17***###
Klotho (pg/mL)	1391.62 ± 144.96	764.64 ± 65.43**	847.09 ± 111.31**
Total adiponectin (μg/mL)	9.32 ± 1.03	7.52 ± 1.18	4.90 ± 0.52*
HMW adiponectin (μg/mL)	4.99 ± 0.80	3.67 ± 0.87	1.56 ± 0.30**
MMW adiponectin (μg/mL)	1.67 ± 0.24	1.38 ± 0.25	1.19 ± 0.14
LMW adiponectin (μg/mL)	2.49 ± 0.27	2.47 ± 0.39	2.14 ± 0.22

	r-AN before weight recovery (n = 6)	r-AN after weight recovery (n = 6)	*P*
Age (y)	18.00 ± 1.37	21.50 ± 1.38	<0.01
Weight (kg)	31.13 ± 1.20	49.15 ± 2.22	<0.001
BMI (kg/m^2)	12.89 ± 0.32	20.35 ± 1.01	<0.01
Klotho (pg/mL)	742.37 ± 96.46	1004.96 ± 103.36	<0.05
Total adiponectin (μg/mL)	6.59 ± 1.95	5.71 ± 1.03	NS
HMW adiponectin (μg/mL)	3.50 ± 1.45	2.48 ± 0.77	NS
MMW adiponectin (μg/mL)	1.41 ± 0.41	1.23 ± 0.12	NS
LMW adiponectin (μg/mL)	1.69 ± 0.55	2.00 ± 0.16	NS

ANOVA, analysis of variance; BMI, body mass index; HMW, high-molecular-weight; LMW, low-molecular-weight; MMW, middle-molecular-weight; r-AN, restricting-type anorexia nervosa
The data represent the mean ± SE. The statistical analysis was performed with an ANOVA and post-hoc Sidak test
* $P < 0.05$ vs. control subjects.
** $P < 0.01$ vs. control subjects.
*** $P < 0.001$ vs. control subjects.
$P < 0.001$ vs. r-AN.

Results

Demographics and clinical characteristics

The clinical characteristics of the groups are presented in Table 1. The fasting plasma concentrations of klotho are shown in Figure 1A. Compared with control participants, the r-AN group ($P < 0.01$) and the obesity group ($P < 0.01$) had significantly lower plasma klotho levels. The fasting plasma concentrations of total and each adiponectin are shown in Figure 1B. Plasma total adiponectin ($P < 0.05$) and HMW adiponectin ($P < 0.01$) levels in the obesity group showed a significant decrease compared with the control group. There was no significant difference in total and each adiponectin level between the control and the r-AN groups.

The changes of clinical characteristics in r-AN after weight recovery

The clinical characteristics of the r-AN group before and after recovery are presented in Table 1. The average duration of the first and second assessment was 3.39 ± 0.56 y. Figure 2 shows the changes in plasma klotho levels after weight recovery. Plasma klotho levels improved and were significantly higher in the r-AN patients after weight recovery ($P = 0.026$). In weight-recovered r-AN patients, we also examined the correlation between the percentage increase in BW and the klotho levels. Plasma klotho levels showed no correlation with the percentage increase in BW ($r = 0.52$; $P = 0.08$). On the other hand, after weight recovery, there was no significant change in the total adiponectin level or in any of the adiponectin isoform levels, and no significant change in the percentage of each adiponectin isoform to total adiponectin (Table 1).

Relationship between klotho and adiponectin in the entire study population

We also examined the correlation of total adiponectin and respective adiponectin isoform levels with klotho levels for the entire study population (i.e., the r-AN [n = 12], the control [n = 11], and the obesity [n = 11] groups combined). Plasma klotho levels showed no correlation with total adiponectin and respective adiponectin isoform levels in the entire study population (total adiponectin: $r = 0.18$; $P = 0.32$; HMW adiponectin: $r = 0.13$; $P = 0.45$; MMW adiponectin: $r = 0.10$; $P = 0.59$; LMW adiponectin: $r = 0.21$; $P = 0.23$).

Discussion

The present study shows that plasma klotho levels decreased both in the underweight and overweight state. Moreover, plasma klotho levels increased significantly with the recovery of BMI in r-AN patients. To our knowledge, this is the first study to describe the relationship between klotho levels and BMI.

Our finding may be consistent with the results reported in previous studies regarding the relationship between BMI and mortality. In those studies, a comparison of the relative risk for mortality with BMI resulted in a U-shaped curve, with the minimum mortality close to BMI 25 kg/m^2; mortality increases when BMI increases >25 kg/m^2 and when BMI decreases below 25 kg/m^2 [14]. Many studies have determined that being overweight is associated with an increased risk for total mortality compared with being normal weight [14–17].

r-AN is characterized by an inordinately strong desire to be thin, severe caloric reduction, weight loss, amenorrhea,