Ghrelin activation and neuropeptide Y elevation in response to medium chain triglyceride administration in anorexia nervosa patients

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SUMMARY

Background & aims: Ghrelin, a peptide found in the stomach, increases appetite and fat-free mass while suppressing energy expenditure. Ghrelin requires modification by medium-chain triglycerides (MCTs) to exert its physiological effects. In this study, we investigated ghrelin activation and the resulting physiological changes following MCT administration.

Methods: Thirty participants were selected from among inpatients diagnosed with anorexia nervosa (AN). The patients were randomly divided into three groups by the MCT content of their nutritional supplement: (1) ‘MCT high’ (>6 g/day), (2) ‘MCT moderate’ (1–6 g/day), and (3) ‘MCT low’ (<1 g/day).

Physical factors such as body weight and composition, as well as levels of nutrition-related serum factors such as acylated (active form) and desacyl (inactive form) ghrelin, leptin, growth hormone, insulin-like growth factor, and neuropeptide Y (NPY) were measured at weeks 0, 2, 4, and 6 of the treatment protocol.

Results: Significantly higher ghrelin activation was found in the ‘MCT high’ than in the ‘MCT low’ group (P < 0.05). The amount of consumed MCT had a curvilinear relationship with the active ghrelin level (P = 0.00). NPY levels in the ‘MCT high’ group were significantly more elevated than in the ‘MCT low’ group (P < 0.05). MCT administration did not significantly affect the remaining factors.

Conclusions: This study clearly demonstrated that MCT activates ghrelin and increases NPY, suggesting that nutritional supplementation with MCT may be effective for the treatment of AN patients in an emaciated state.

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1. Introduction

Ghrelin is an endogenous ligand of the growth hormone secretagogue receptor family [1]. This peptide originates in the stomach and increases appetite through the stimulation of neuropeptide Y (NPY) neurons in the arcuate nucleus, leading to the augmentation of fat-free mass (FFM) and suppression of energy expenditure [2–5]. Ghrelin peptides exist mainly in two forms: active (acylated ghrelin) and inactive (desacyl ghrelin).

The side chain of the third serine residue of ghrelin must be modified by medium-chain triglycerides (MCTs) to exert its physiological effects [1]. MCT is a saturated fatty acid in which the aliphatic tail comprises of 6–12 carbon atoms; it is found in milk, coconut oil (15%), and Makuton Oil® (85%).

Anorexia nervosa (AN) is an eating disorder that results in extreme weight loss due to an intense fear of gaining weight, without the presence of any basal organic disease. Total ghrelin

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blood levels in AN patients is higher than that of healthy subjects because of refeeding to the inhibitory influence of food intake [6]. Moreover, most of the ghrelin in untreated AN patients is inactive (desacyl) ghrelin [7]; this phenomenon reflects a chronic starvation state.

The activation of ghrelin might be effective for treating diseases that result in emaciation. In a pilot study, ghrelin infusion into AN patients resulted in increased daily energy intake compared to the pre-treatment period; there were no serious adverse events [8]. However, ghrelin products are unstable and expensive to produce; therefore, we explored the notion of activating ghrelin via MCT. Previous data showed that MCT ingestion increased plasma ghrelin concentrations in lactating dairy cows [9]. Moreover, ingestion of MCTs increased stomach acylated ghrelin levels in mice, although the amount of total ghrelin remained unchanged [10]. In this study, we investigated ghrelin activation in AN patients receiving nutritional treatment with high MCT supplementation (PemPal®). Additionally, we investigated factors that may be involved in ghrelin activation, such as growth hormone (GH) levels, NPY levels, and body composition.

2. Methods

2.1. Participants

The study group was composed of 30 new AN inpatients who were treated at the Department of Psychosomatic Medicine of Kyushu University Hospital between 2012 and 2013. A diagnosis of AN was based on the eating disorders section of the Structured Clinical Interview for DSM (Diagnostic and Statistical Manual)-IV Axis I Disorders [11]. The enrollment criteria were as follows: 1) Admission to the hospital for enrollment in our “Cognitive Behavioral Therapy” treatment program, and 2) sustained enrollment in the treatment program for at least four months.

2.2. Nutrition program

Behavioral observation was performed during the first two weeks of admission. During this period, electrolyte abnormalities, including dehydration or edema, were almost normalized. After the observation period, the cognitive behavior treatment program commenced and continued until a patient achieved the target body weight, which was set at 60–80% of the standard body weight based on consultation between the patient and the medical staff; data were collected during this period [12]. After observation, all participating patients underwent combined oral and nasal tube feeding. Calorie consumption started at 30–40 kcal/kg/day and increased by 200 kcal/day every two weeks; patients pledged to consume each meal in its entirety. Fig. 1 shows the experimental protocol. The calories administered by nasogastric tube feeding were gradually shifted to oral intake in the latter half of the program. Self-induced vomiting and over-exercising were restricted as factors related to ghrelin activation were measured at 2, 4, and 6 weeks after the start of the protocol; these factors were analyzed and compared among each of the MCT intake categories. Furthermore, the correlation of activated ghrelin and total MCT was assessed between weeks 2 and 6.

2.3. Experimental protocol

Anorexia nervosa patients were randomly assigned to nutritional supplementation groups that included rich amounts of MCT (2.25 g/100 mL) (PemPal®, Nestle Health Science); medium amounts (0.84 g/100 mL) (Isocal RTU®, Nestle Health Science), or none (0 g/125 mL) (Ensure Liquid®). For analysis, the patients were divided into the following categories: ‘MCT-high’ (>6 g/day), ‘MCT-moderate’ (1–6 g/day), and ‘MCT-low’ (0 g/day) according to the average daily amount of MCT included in their nasal feeding nutritional supplements. The last category was referred to as ‘MCT-low’ to account for the small quantities contained in regular food (<0.3 g/day).

Body mass index (BMI), heart rate, body temperature, systolic and diastolic blood pressure, energy intake, appetite, and serum nutrition-related factors (active ghrelin, inactive ghrelin, growth hormone [GH], insulin-like growth factor-1 [IGF-1], and NPY) were measured at 0, 2, 4, and 6 weeks after the start of the protocol; these factors were analyzed and compared among each of the MCT intake categories. Furthermore, the correlation of activated ghrelin and total MCT was assessed between weeks 2 and 6.

2.4. Anthropometry

Food intake was assessed by visual estimation of the patients’ main and side dishes separately by two skilled nurses. Subjects’ heights were measured without shoes using a stadiometer to the nearest 0.1 cm on the day of admission. BMI was calculated as the ratio of body weight (kg) to height (m) squared.

Blood pressure, pulse, and temperature were measured every morning at 6:00. Weight, FFM, and bone mineral density (BMD) were determined for the whole body using dual-energy X-ray absorptionmetry with a HOLOGIC QDR-1000 densitometer (2000 to March 2002) or a HOLOGIC QDR-4500 densitometer (April 2002 to 2006) (Hologic, Inc. Waltham, MA, USA). Both FFM and BMD were measured one week and three-to-four months after admission. All measurements were performed by an experienced technician.

2.5. Laboratory analysis methods

Acylated ghrelin levels were measured using the Active Ghrelin ELISA Kit (Mitsubishi Kagaku Iatron Tokyo, Japan) with interassay and intraassay coefficients of variation less than 8.3% and 8.1%, respectively. Desacyl ghrelin levels were measured using the Desacyl-Ghrelin ELISA Kit (Mitsubishi Kagaku Iatron, Tokyo, Japan) with interassay and intraassay coefficients of variation less than 10.3% and 12.7%, respectively. Serum IGF-1 level was measured using an immunoradiometric assay kit (TFB Inc., Tokyo, Japan) with an interassay coefficient of variation of 1.1–2.4% and a sensitivity of 10 ng/mL. Serum GH level was measured using a chemiluminescent enzyme immunoassay (Beckman–Coulter Inc., CA, USA) with an interassay coefficient of variation of 3.7–8.44% and a sensitivity of 0.003 ng/mL. Serum NPY levels were measured via ELISA (EMD...
Values are expressed as mean ± standard deviation or number of patients. *Unpaired Student’s t test.

MCT: medium-chain triglyceride, AN-R: anorexia nervosa, restricting type, AN-BP: anorexia nervosa, binge-purging type, BMI: body mass index, n.s.: not significant.

Table 1
Clinical characteristics of anorexia nervosa patients by MCT consumption.

<table>
<thead>
<tr>
<th></th>
<th>‘MCT high’ group N = 10</th>
<th>‘MCT moderate’ group N = 10</th>
<th>‘MCT low’ group N = 10</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI at admission (kg/m²)</td>
<td>13.0 ± 1.4</td>
<td>12.6 ± 1.6</td>
<td>12.4 ± 2.4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Duration (years)</td>
<td>7.1 ± 9.9</td>
<td>2.2 ± 2.0</td>
<td>5.3 ± 8.6</td>
<td>n.s.</td>
</tr>
<tr>
<td>Age (years)</td>
<td>26.4 ± 10.8</td>
<td>28.5 ± 12.1</td>
<td>26.2 ± 13.4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Subtype (AN-R/AN-BP)</td>
<td>6/4</td>
<td>5/5</td>
<td>7/3</td>
<td>n.s.</td>
</tr>
<tr>
<td>Length of hospitalization (days)</td>
<td>171.6 ± 63.6</td>
<td>141.1 ± 60.3</td>
<td>148.8 ± 72.2</td>
<td>n.s.</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>36.4 ± 0.19</td>
<td>36.4 ± 0.34</td>
<td>36.55 ± 0.34</td>
<td>n.s.</td>
</tr>
<tr>
<td>Heart rate</td>
<td>63.4 ± 13.3</td>
<td>69 ± 17.3</td>
<td>64.9 ± 10.9</td>
<td>n.s.</td>
</tr>
<tr>
<td>Blood pressure (systolic/diastric)</td>
<td>85.7 ± 11.7/57.1 ± 9.5</td>
<td>83.3 ± 8.7/50.6 ± 9.9</td>
<td>86.7 ± 10.5/54.6 ± 9.3</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

2.6. Statistical analysis

All analyses were performed using SPSS for Windows version 14.0j. Results are presented as means ± standard deviation. Comparisons among groups at weeks 0, 2, 4, and 6 were performed using one-way ANOVA, after which a post-hoc test was used for group comparisons. NPY was compared in terms of the rate of increase from week 0 because of the large difference in absolute values between the first and second assays at that time point. Pearson’s single correlation analysis was performed to compare the amount of ingested MCT to activated ghrelin levels in weeks 2–4 of the treatment protocol. The study was approved by the Ethics Committee of Kyushu University (No. 22-56). All participants provided written informed consent.

3. Results

3.1. Clinical characteristics

There were no significant differences in the clinical characteristics (BMI, age, duration of disease, heart rate, body temperature, and systolic and diastolic blood pressure) of the three groups two weeks after admission (Table 1).

3.2. Basal hormone level

There were no significant differences between active ghrelin, desacyl ghrelin, GH, IGF-1, and NPY levels among the three groups (Table 2).

3.3. Clinical course after MCT administration

Fig. 2 shows that the daily amounts of MCT administered to the ‘MCT-high’ group were significantly higher than those administered to the ‘MCT-low’ group at weeks 2, 4, and 6 (7.07 ± 2.74 vs. 0.55 ± 1.17, P = 0.03; 9.00 ± 2.62 vs. 2.49 ± 2.49, P = 0.00; and 6.48 ± 4.87 vs. 0.11 ± 0.36, P = 0.01; respectively). The average daily amount of MCT administered to the ‘MCT-moderate’ group was higher than that administered to the ‘MCT-low’ group at weeks 2, 4, and 6, but without significance. No significant differences in BMI or energy intake were found among the three groups at 0, 2, 4, or 6 weeks after MCT administration. Moreover, differences in heart rate, body temperature, systolic blood pressure, and diastolic blood pressure between the groups were not significant (data not shown).

3.4. Change of ghrelin concentration after MCT administration

Fig. 3 shows that acylated ghrelin was significantly higher in the ‘MCT high’ group than in the ‘MCT-low’ group at week 4 (2.88 ± 0.83 vs. 1.28 ± 0.13, respectively; P < 0.05; Fig. 3). GH and IGF-1 were not significantly different between the three groups (data not shown). The incremental changes in FFM and BMC from admission to discharge were not significant between the three groups (FFM increments: ‘MCT-low’: 3546 ± 606.4, ‘MCT-moderate’: 4283.0 ± 724.8, ‘MCT-high’: 4546.7 ± 707.8 g; BMC increments: ‘MCT-low’: −1.25 ± 0.54; ‘MCT-moderate’: −0.76 ± 0.54, and ‘MCT-high’: −1.65 ± 0.77).

4. Discussion

MCT nutritional supplementation significantly increased acylated ghrelin in AN patients in a dose-dependent manner. Furthermore, NPY, a peptide that functions downstream of ghrelin, was elevated after MCT administration [15]. Thus, MCT nutritional supplementation may be effective for improving the nutritional status of AN patients who are in a prolonged state of malnutrition and in whom desacyl ghrelin levels are elevated.

Normal fat intake in healthy persons is approximately 50 g/day, with the quantity of MCT estimated to be approximately 0.3 g/day. However, 20 times that amount (i.e., 6 g/day) is necessary to produce a biological effect. MCT is hydrolyzed in the intestinal tract by lipase and carried to the liver directly through the portal vein. It is metabolized faster than long-chain fatty acids during parenteral nutrition [13]. As such, MCT might have contributed to the management of energy metabolism, but not to the activation of ghrelin, in the early days of this treatment protocol. No significant difference was found in desacyl ghrelin levels, possibly because its blood levels are approximately 10-fold higher than those of activated ghrelin [8]. This suggests that the quantity of desacyl ghrelin is not affected by MCT administration but by the secretion capacity of ghrelin itself.

The effects of ghrelin extend beyond appetite improvement, and include the enhancement of cardiac activity, amelioration of cancer
anorexia/cachexia syndrome, prevention of osteoporosis, and preservation of muscle or lean body mass [3,4,14–16]. The cardiovascular effects of ghrelin include improvement of left ventricular contractility and cardiac output, as well as the reduction of arterial pressure and systemic vascular resistance in healthy subjects [3,15]. Additionally, ghrelin increases osteoblastic activity in cell cultures, suggesting bone anabolic effects [16]. In our study, other factors such as body temperature, blood pressure, heart rate, BMI, energy intake, BMD, and GH and IGF-1 levels were not significantly changed among the three groups based on the amount of MCT administration. While the average incremental increase in FFM correlated with the amount of MCT, the change was also not significant.

Our findings may be affected by (1) the food intake during MCT administration being determined by the treatment protocol, particularly as meal quantity increases were incremental, and (2) the period of MCT administration not being long enough to change the body composition. In fact, the body composition was measured for approximately five months before and after hospitalization, while MCT was administered for two to three weeks during this period. Therefore, it is possible that the recovery of nutritional status and volume of exercise were insufficient. Of note, it has been reported that GH responses to ghrelin infusion were blunted in AN patients by the effect of prolonged exposure to hyperghrelinemia or the hyperactivity of the hypothalamo-pituitary-adrenal axis [17]. Hence, the clinical effects of the administration of 6 g/day of MCT may only manifest after a prolonged period in such cases. Future studies ought to focus on the amount of MCT and the length of the period of administration necessary to provide the greatest benefit to the patient.

An important finding of this study was that ghrelin and NPY increased owing to MCT administration; the level of increase was consistent with that observed following conventional administration of the traditional Japanese medicine Rikkunshito, which is

Table 2
Clinical characteristics of anorexia nervosa patients by MCT consumption.

<table>
<thead>
<tr>
<th></th>
<th>‘MCT high’ group N = 10</th>
<th>‘MCT moderate’ group N = 10</th>
<th>‘MCT low’ group N = 10</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acylated ghrelin (fmol/mL)</td>
<td>23.6 ± 15.9</td>
<td>28.5 ± 21.3</td>
<td>24.6 ± 12.6</td>
<td>n.s.</td>
</tr>
<tr>
<td>Desacyl ghrelin (fmol/mL)</td>
<td>210.4 ± 129.0</td>
<td>223.8 ± 176.0</td>
<td>269.2 ± 163.2</td>
<td>n.s.</td>
</tr>
<tr>
<td>GH (ng/mL)</td>
<td>2.63 ± 1.78</td>
<td>8.73 ± 15.4</td>
<td>10.12 ± 25.1</td>
<td>n.s.</td>
</tr>
<tr>
<td>IGF-1 (ng/mL)</td>
<td>180.9 ± 75.9</td>
<td>180.4 ± 108.4</td>
<td>193.9 ± 119.1</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation. *Unpaired Student’s t test.

Fig. 2. Change in daily dose of MCT, energy intake, and BMI after the start of the protocol. BMI: Body mass index, MCT: medium-chain triglyceride.

Fig. 3. Change of acylated ghrelin, desacyl ghrelin, and NPY concentration after MCT administration. MCT: medium-chain triglyceride, NPY: neuropeptide Y.

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generally regarded as effective for improving the human appetite [18]. It has been reported that Rikkunshito increases the plasma level of ghrelin in healthy humans and normal mice [1]. Recently, a flavonoid that is a component of Rikkunshito was shown to reverse cisplatin-induced suppression of plasma acylated ghrelin levels and cause increased food intake in rats; this was mediated by 5-HT2B/2C receptors [19]. The standard Rikkunshito dosage is 7.5 g/day, at which the quantity of blood activated ghrelin increases approximately 1.5-fold. An equivalent effect was observed when administering 6 g/day of MCT in this study. Importantly, the cost per day for MCT (50 Japanese yen) is cheaper than that of herbal medicinal products (150 Japanese yen).

A limitation of our study was that the amount of calories included in the patients’ daily diet was fixed by our treatment protocol; hence, there was not much latitude for measuring any change in calorie intake. Another limitation was that subjective appetite was not evaluated, even though the intervention of psychological factors peculiar to eating disorders, including the extreme desire for thinness, has a strong effect on clinical sequelae [2,8]. In such cases, any increases in the levels of ghrelin may be insufficient to influence the human body.

In a previously described experimental rat model, ghrelin definitely increased bone mineral density [3,16]. MCT could therefore be a valuable treatment option for patients who suffer from malnutrition, such as the elderly and patients with cancer or respiratory failure. We also have high expectations for the successful clinical application of MCT treatment in patients with cachexia or chronic respiratory deficiency.

In conclusion, MCT nutritional supplementation increased the amount of activated ghrelin and NPY in AN patients. Our data suggest that MCT treatment may constitute a promising strategy for undernourished patients.

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Statement of authorship

Keisuke Kawai designed the research, was involved in writing the paper, and has final responsibility for its content. Megumi Nakajima and Sakiko Yamashita analyzed the data and performed the statistical analyses. Masayasu Kojima conducted the research. Shu Takakura assisted with the study. Nobuyuki Sudo and Chiharu Kubo participated in the design of the study and helped draft the manuscript. All authors have read and approved the final manuscript.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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