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GHRELIN ACTIVATION BY INGESTION OF MEDIUM-CHAIN TRIGLYCERIDES IN HEALTHY ADULTS: A PILOT TRIAL

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Abstract: *Objective:* To investigate the efficacy of dietary supplementation of medium-chain triglycerides (MCTs) and its effects on ghrelin activation in healthy adults. *Methods:* The present study examined two protocols with six healthy volunteers: 1) 12-hour profiles of the plasma levels of acylated and desacyl ghrelin without MCT ingestion, and 2) changes in serum ghrelin levels after oral ingestion of 45 g/day of MCTs for 1 week. *Results:* At baseline, serum acylated and desacyl ghrelin levels were 18.2±10.3 and 77.1±23.4 fmol/mL, respectively. The ratio of acylated/desacyl ghrelin was 19%. There were no significant differences in the 12-hour profiles of acylated and desacyl ghrelin. Significant increases were observed in all sampling times of serum acylated ghrelin after 1-week MCTs ingestion. The ratio of acylated/desacyl ghrelin increased to 37.7%. *Conclusions:* Oral ingestion of MCTs increased serum acylated ghrelin levels in healthy adults, suggesting that MCTs administration stimulates food intake.

Key words: Ghrelin, acylation, medium-chain triglycerides (MCT), sarcopenia, cachexia.

Introduction

"Anorexia of aging" is defined as loss of appetite and reduced food intake in old age, and may be associated with decline in skeletal muscle mass, energy expenditure, and physical activity that occur in later years (1, 2). Decreased skeletal muscle mass is related to malnutrition, length of hospital stay, morbidity, and mortality (3, 4); malnutrition is frequent in populations with high morbidity and burden of care. Appetite loss is the major cause of malnutrition in older adults and sometimes difficult to control under established nutritional management settings, especially in clinical settings.

Ghrelin is a novel growth hormone (GH)-releasing peptide widely distributed throughout the gastric mucosa and is made up of 28 amino acids; it was first described in 1999 (5). Ghrelin exhibits orexigenic effects and complex metabolic activities through the GH-independent mechanism, leading to the augmentation of skeletal muscle mass and suppression of energy expenditure and inflammation (6, 7). Therefore, there is growing interest regarding the identification of ghrelin as a potentially valid and well-tolerated anabolic and anti-catabolic treatment for sarcopenia, cachexia, and other wasting disorders.

Ghrelin mainly exist in two forms: active (acylated ghrelin) and inactive (desacyl ghrelin). The third amino acid residue of ghrelin, serine, is modified by octanoic acids, C8:0 medium-chain triglycerides (MCTs); acylation is essential for the biological activity of ghrelin. While desacyl ghrelin is suggested to have limited effects on GH-receptor under physiological conditions (8, 9), acylated ghrelin corresponds to approximately 20% of the total circulating ghrelin, and is responsible for the biological effects of ghrelin (10), indicating that acylation is a vital step for the biological activity of ghrelin.

Therefore, it is hypothesized that ghrelin is acylated via ingestion of MCTs, and this could be a promising treatment option for malnutrition in older adults; however, evidence regarding its efficacy is very limited. In the current study, we investigate the efficacy of dietary supplementation of MCTs and its effects on ghrelin activation in healthy adults.

Materials and Methods

Participants

The current study was approved by the Institutional Research Ethics Committee of Kumamoto Rehabilitation

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Hospital (Kumamoto, Japan), and was performed in accordance with the ethical standards established in the 1964 Declaration of Helsinki and later amendments. All participants provided written informed consent in advance. We recruited six healthy volunteers who were working at the hospital in 2017, comprising 3 women and 3 men with mean age of 38 ± 8 years and mean body mass index (BMI) of 22.0±1.3 kg/m2. At enrollment, the following criteria were applied: 1) normal body weight with BMI of 20–25 kg/m2. The following exclusion criteria were adopted: 1) presence of diseases requiring treatment physically or psychologically, including dyslipidemia and diabetes mellitus; 2) taking any supplements or drugs; and 3) presence of appetite alteration. The participants' body composition were analyzed using bioelectrical impedance analysis (InBody S10; InBody, Tokyo, Japan).

Study protocol

The present study examined two protocols. First, 12-hour profiles of the serum levels of acylated and desacyl ghrelin without MCT ingestion were investigated. Second, changes in serum ghrelin levels after oral ingestion of 45 g/day of MCTs for 1 week were measured. Therefore, the study period of the current trial was designed to be 2 weeks [Fig. 1]. At baseline, the participants were evaluated and advised strictly on their daily lifestyle, including food intake, physical activity, and sleeping time, all of which were reported to potentially alter plasma ghrelin levels (11, 12). Each participant received a standardized meal protocol to control energy intake, set as daily energy intake of 30–35 kcal/kg (body weight), supervised by a registered dietitian throughout the study period. Physical activity level (working during daytime) and sleeping time (6–7 hours at night) were standardized and supervised by a medical doctor throughout the study period.

To examine whether ingestion of MCTs affects serum ghrelin activation, serum ghrelin levels were measured after participants orally ingested 45 g/day of MCTs for 1 week. Participants were instructed to take one tablespoon (15 ml) of MCT oil added to each meal (breakfast, lunch, and dinner) every day, which accounts for 45 g (45 ml) of MCT ingestion a day. The MCTs used in this study were 100% pure MCT oil (Nissin MCT Oil HC, The Nisshin OilliO Group, Ltd., Tokyo, Japan), and per 10 ml of the oil consisted of 8.9 kcal energy, 0 g protein, 10 g lipid, 0 g carbohydrate, and 10 g MCT.

Blood sampling and assay

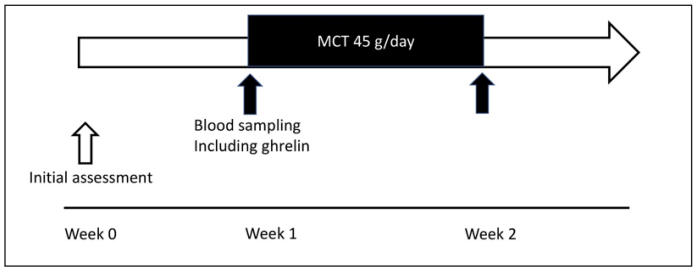
Blood samples for the measurement of serum ghrelin were taken from the antecubital vein after 30 minutes of resting, and were drawn at 07:00, 10:00, 14:00, 17:00, and 19:00 hours to identify 12-hour profiles of serum ghrelin levels. Blood sampling was performed at baseline and after 1 week of MCT ingestion to measure the changes in serum ghrelin levels, complete blood counts and laboratory data. Serum acylated and desacyl ghrelin levels were measured by enzyme-linked immunosorbent assay (SRL, Inc., Tokyo, Japan).

Statistical analysis

All analyses were performed using SPSS version 21 for Windows. Results are presented as mean ± standard deviation (SD). Comparisons were performed using a paired t-test before and after 1 week of daily oral ingestion of MCTs and one-way repeated measures ANOVA for changes in ghrelin 12-hour profiles, after which a post-hoc analysis was performed for before and after comparisons of the effects of MCT ingestion. P values of <0.05 were considered statistically significant.

Figure 1

Study protocol. The present study examined two protocols. First, 24-hour profiles of the plasma levels of acylated and desacyl ghrelin without MCT ingestion were investigated. Second, changes in plasma ghrelin levels after oral ingestion of 45 g/day of MCTs for 1 week were measured. The study period of the current trial was designed to be 2 weeks. MCT: medium-chain triglyceride



Results

Clinical characteristics

Table 1 presented the clinical characteristics of all the participants, showing that the study participants were neither lean nor obese, and that they were a homogenous group of healthy adults.

Baseline plasma ghrelin level

At baseline, in the morning, serum acylated and desacyl ghrelin levels were 18.2±10.3 fmol/mL and 77.1±23.4 fmol/mL, respectively. The ratio of acylated ghrelin to desacyl ghrelin was 19%. There were no significant differences in the 12-hour profiles of acylated and desacyl ghrelin, although acylated ghrelin tended to increase before meals and decreased after meals, without statistical significance [Fig. 2].

Table 1
Clinical characteristics of study participants and changes in parameters before and after 1-week MCT ingestion

		Baseline	Post MCT intake	p
Age	year	38±8	-	-
Sex, male	n	3	-	-
female	n	3		
Height	cm	164.2±3.4		
Weight	kg	59.3±4.1	-	-
Body mass index	kg/m^2	22.0±1.3	-	-
Lean body mass	kg	46.6±5.1	-	-
Skeletal muscle mass	kg	19.4±2.2	-	-
Skeletal muscle mass index	kg/m2	7.1±0.6	-	-
Fat mass	kg	13.1±2.1	-	-
Fat mass	%	21.5±4.1	-	-
Acylated ghrelin*	fmol/ml	18.2±10.3	38.3±23.8	0.016
Desacyl ghrelin*	fmol/ml	77.7±23.4	63.3±21.1	0.089
WBC	count	7033±900	6900±1000	0.563
Hemoglobin	g/dl	14.4±0.8	14.4±0.9	0.918
Total protein	g/dl	7.5±0.1	7.5±0.1	0.341
Albumin	g/dl	4.2±0.1	4.2±0.1	0.175
Total cholesterol	mg/dl	199±25.1	200±26	0.883
Triglyceride	mg/dl	101±9.1	103±8.9	0.412
Fasting glucose	mg/dl	93.5±5.5	94.5±6.0	0.690
CRP	mg/dl	0.07±0.07	0.04±0.02	0.264

CRP, C-reactive protein; MCT, medium-chain triglycerides; WBC, white blood cell; *Description and analysis of ghrelin data were adopted each blood sample of 07:00 o'clock.

Effect of MCT ingestion on plasma ghrelin and serum data

Significant increases were observed in all sampling times of the 12-hour profiles of serum acylated ghrelin after 1-week oral administration of 45 g/day of MCTs [Fig. 2], while significant decreases were observed in 4 out of 5 sampling times for serum desacyl ghrelin. The ratio of acylated ghrelin to desacyl ghrelin increased to 37.7% in the morning after completion of 1 week of MCT ingestion. The increase in serum acylated ghrelin peaked early in the morning but not after meals. We observed that 1 week of MCT administration did not alter total cholesterol, triglyceride, or fasting glucose levels.

Adverse effects

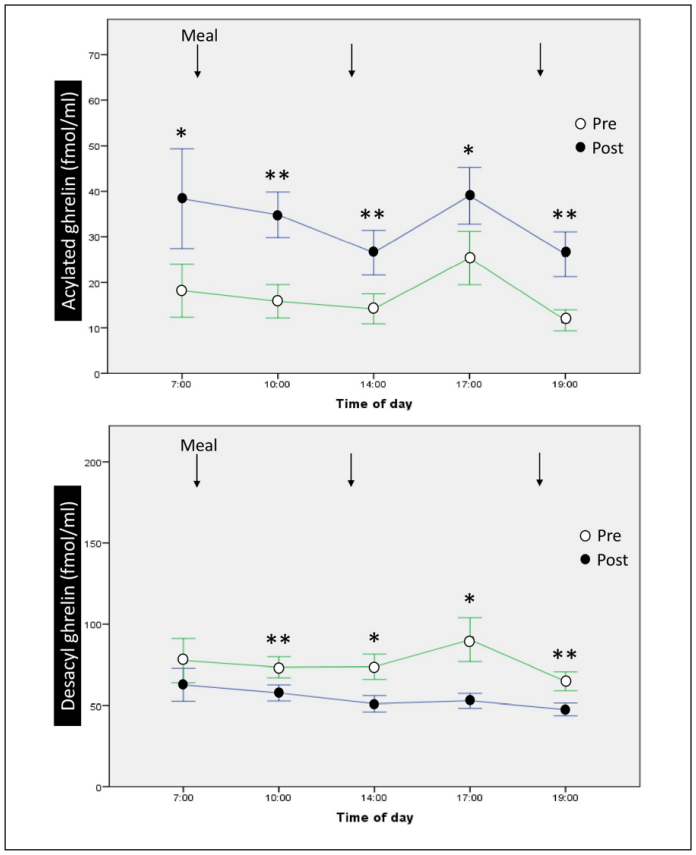
There were no adverse effects reported throughout the study, including digestive symptoms, serum lipid profiles, and fasting glucose levels.

Discussion

Here we report the results of our study examining the effect of oral MCT ingestion on the acylation of serum ghrelin levels in healthy adults. We demonstrate that ghrelin was acylated after MCT ingestion and that the ratio of acylated/desacyl ghrelin level increased. This finding suggests that MCT consumption may trigger acylation (activation) of ghrelin, leading to increased appetite, positive energy balance, and improved nutritional status in malnourished older adults or those at the risk of malnutrition.

Some studies demonstrated that MCT was a good substrate for the conversion of ghrelin to active ghrelin under normal physiological conditions, without affecting total ghrelin concentrations (13-16); however, the samples examined in those studies were animals (e.g., mice, rats, lactating dairy cows, and chickens). For example, Lemarié et al. conducted a study using rats and reported no significant increase in acylated ghrelin levels, despite MCT administration; however, a significant increase was observed in the acylated/total ghrelin ratio (16).

Figure 2
12-hour profiles and changes of acylated ghrelin and desacyl ghrelin before and after 1-week ingestion of MCTs. MCT: medium-chain triglyceride. *, p<0.05; **, p<0.001 for ghrelin level before vs. after MCTs administration



Human studies regarding this subject are limited. There are only two studies available in the literature targeting patients with disease-related nutritional disorders, on the associations of MCT administration and serum acylated ghrelin levels. Ashitani et al. (17) reported that MCTs supplemented via a combination of oral and nasogastric tube administration increased serum acylated ghrelin levels among inpatients diagnosed with anorexia nervosa (AN). In addition, they observed a dose-dependent relationship between MCT administration and serum acylated ghrelin levels in AN patients. Kawai et al. (18) reported the positive effects of orally ingested MCTs on serum acylated ghrelin levels in patients with chronic respiratory diseases. We believe that the above patient groups may have decreased appetites due to such diseases, with increased reactivity of serum ghrelin levels due to MCT administration.

Exogenous ghrelin administration is an effective appetite stimulant, confirmed by several studies published since 2000. Infusion and bolus of acylated ghrelin have consistently been shown to increase appetite and/or food intake in healthy adults (19, 20), obese adults (21), patients with chronic pulmonary diseases (22), cancer (23, 24), heart failure (25), gastric dysfunction (26), and chronic renal failure receiving maintenance dialysis (27). Although there is strong evidence regarding the effects of exogenous ghrelin administration on appetite, circulating GH, adrenocorticotropic hormone, cortisol, glucose, and prolactin in diverse patient cohorts, there is limited evidence demonstrating the positive effects of ghrelin on body composition, pulmonary function, cardiac function, and bone metabolism. Furthermore, some adverse effects due to ghrelin administration were reported in 20% of the study participants, including flushing, gastric rumbles, thirst, somnolence, vertigo, fatigue, and change in mood (28, 29). Therefore, activation of ghrelin via dietary supplementation of MCTs instead of exogenous ghrelin administration may be an effective and safe treatment option for diverse populations with appetite alteration.

To the best of our knowledge, this is the first study to demonstrate that ghrelin is activated via orally ingested MCTs in healthy adults. This finding suggests that acylation (activation) of ghrelin via oral ingestion of MCTs stimulates food intake; induces positive energy balance; and aids in the treatment of older patients with malnutrition, cachexia, and sarcopenia, where little evidence is currently available (30).

Our study had some limitations. First, this study included small samples, 3 women and 3 men, without discussing the sex difference. This might limit the generalizability of the results. Second, another control group, such as the long-chain triglycerides (LCTs) administered group, was lacking. Thus, the single effect of MCTs could not be clarified in the current study design. Therefore, further large-scale and high-quality studies are needed to address these limitations.

In conclusion, oral ingestion of MCTs increased serum acylated ghrelin levels in healthy adults. The present result suggests that treatment with MCTs may be a promising treatment option for patients with malnutrition, as well as sarcopenia and cachexia, while further large-scale and high-quality studies are needed.

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Conflict of interest: No conflict of interest.

Author contributions: YY: supervising, concept, data collecting, interpretation, manuscript drafting. SS, AS: concept, interpretation, manuscript drafting. FN, ST, TH, MK, YY, SN, TB: concept, interpretation, data collecting, manuscript drafting.

Ethical standards: We conducted the study in accordance with the Declaration of Helsinki, and the study was approved by the ethics committee of Kumamoto Rehabilitation Hospital. We obtained written informed consent from all participants.

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