Ghrelin, a 28-amino-acid peptide with N-octanoylation indispensable for binding to the growth hormone secretagogue receptor (GHS-R), was originally discovered in the human and rat stomach [1]. Ghrelin molecules are present as two major endogenous forms, an acylated form (ghrelin) and a des-acylated form (des-acyl ghrelin). Recent studies indicated that aerobic exercise did not change plasma total ghrelin levels, however, dynamics of circulating ghrelin and des-acyl ghrelin during aerobic exercise remains unclear. The purpose of this study is to examine the effects of moderate intensity exercise on plasma ghrelin and des-acyl ghrelin concentrations, and to investigate the relationship between ghrelin molecules and other hormonal and metabolic parameters during exercise. Nine healthy males (25.2 ± 0.5 years) exercised for 60 min at 50% of their maximal oxygen consumptions. We measured the plasma concentrations of ghrelin, des-acyl ghrelin, GH, norepinephrine (NE), epinephrine (E), dopamine (DA), insulin, and glucose. Plasma ghrelin level significantly decreased during exercise, whereas plasma des-acyl ghrelin and total ghrelin levels did not change. Plasma NE, E, DA and GH levels were significantly increased during exercise. Plasma insulin level significantly decreased during exercise, and plasma glucose levels remained steady during exercise. NE, E, DA, and GH were correlated negatively with plasma ghrelin levels. These findings suggest that acute moderate exercise may suppress ghrelin release from the stomach, decrease ghrelin O-acyltransferase activity, and/or activate ghrelin utilization in peripheral tissues and that exercise-induced ghrelin suppression may be mediated by activated adrenergic system.

Key words: Ghrelin, Des-acyl ghrelin, Growth hormone, Adrenergic system, Exercise

Significant lowering of plasma ghrelin but not des-acyl ghrelin in response to acute exercise in men

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Abstract. Ghrelin, an acylated peptide produced predominantly in the stomach, stimulates feeding and growth hormone (GH) secretion via interaction with the GH secretagogue receptor. Ghrelin molecules are present in two major endogenous forms, an acylated form (ghrelin) and a des-acylated form (des-acyl ghrelin). Recent studies indicated that aerobic exercise did not change plasma total ghrelin levels, however, dynamics of circulating ghrelin and des-acyl ghrelin during aerobic exercise remains unclear. The purpose of this study is to examine the effects of moderate intensity exercise on plasma ghrelin and des-acyl ghrelin concentrations, and to investigate the relationship between ghrelin molecules and other hormonal and metabolic parameters during exercise. Nine healthy males (25.2 ± 0.5 years) exercised for 60 min at 50% of their maximal oxygen consumptions. We measured the plasma concentrations of ghrelin, des-acyl ghrelin, GH, norepinephrine (NE), epinephrine (E), dopamine (DA), insulin, and glucose. Plasma ghrelin level significantly decreased during exercise, whereas plasma des-acyl ghrelin and total ghrelin levels did not change. Plasma NE, E, DA and GH levels were significantly increased during exercise. Plasma insulin level significantly decreased during exercise, and plasma glucose levels remained steady during exercise. NE, E, DA, and GH were correlated negatively with plasma ghrelin levels. These findings suggest that acute moderate exercise may suppress ghrelin release from the stomach, decrease ghrelin O-acyltransferase activity, and/or activate ghrelin utilization in peripheral tissues and that exercise-induced ghrelin suppression may be mediated by activated adrenergic system.

Key words: Ghrelin, Des-acyl ghrelin, Growth hormone, Adrenergic system, Exercise
ing ghrelin’s signals for starvation and GH secretion to the brain [13]. As exercise stress induces a variety of catabolic processes involving glycolysis, lipolysis, and protein degradation [14], we assume that release and action of ghrelin may change in response to the alternation of the autonomic activity and/or catabolic state during an acute exercise. While long-term exercise resulting weight loss increases plasma ghrelin level [15, 16], short-term running, cycling, or rowing exercise does not alter plasma total ghrelin [17-23]. A few papers, however, reported that plasma total ghrelin level decreased [24-26] or increased [27-29] during short-term exercise. Schmidt et al. [22] reported that there were no correlations between total ghrelin levels and either of treadmill exercise at 50%, 70%, or 90% maximum oxygen consumption (V\textsubscript{O\textsubscript{2max}}). Kraemer et al. showed that acute resistance exercise reduced circulating total ghrelin levels [30].

The effects of physical exercise on ghrelin and des-acyl ghrelin have yet to be investigated. We here examined the effects of acute exercise on the plasma levels of ghrelin and des-acyl ghrelin in healthy men and studied the relationships between two ghrelin forms and hormonal or metabolic parameters.

Materials and Methods

Subjects
We examined nine healthy men (mean age, 25.2 ± 0.5 years; average body mass index (BMI), 22.6 ± 0.4 kg/m\textsuperscript{2}, and V\textsubscript{O\textsubscript{2max}}, 44.8 ± 1.4 mL/min/kg) who had not engaged in any regular exercise for at least two years. All subjects were healthy, non-smokers, and non-obese and were not currently taking any medications. Prior to beginning the study, the purpose and risks of the study were explained to them to obtain their written informed consent. The study, approved by the local Ethics Committees of the University of Miyazaki and Fukuoka University, was conducted in accordance with the Helsinki Declaration.

Measurement of V\textsubscript{O\textsubscript{2max}}
To measure physical fitness, all of the subjects performed a V\textsubscript{O\textsubscript{2max}} test on a mechanically braked ergometer (Electric Bicycle Ergometer; Lode’s Instrumenten, Groningen, Netherlands). The work rate was initially set at 15 W and thereafter was increased every 1 min by 15 W. The test was continued until the subjects felt exhausted. VO\textsubscript{2} was measured from the mixed expired gas collected in neoprene bags. The volume of the expired gas was quantified using a twin-drum-type respirometer (Fukuda Irika CR-20, Tokyo, Japan), and both the O\textsubscript{2} and CO\textsubscript{2} fractions were analyzed using a mass spectrometer (ARCO-1000; ARCO System, Chiba, Japan). Electrocardiogram and heart rates were monitored throughout the exercise test using a three-lead electrocardiograph.

Exercise test protocol
The subjects were asked to refrain from alcoholic beverages and strenuous physical exercise for at least 3 days prior to each exercise test. After an overnight fast, the subjects performed cycle exercise for 60 min at 50% of V\textsubscript{O\textsubscript{2max}}. Exercise consisted of 3 min warm up and cool down. Exercise intensity was calculated based on the results of V\textsubscript{O\textsubscript{2max}} test. Blood samples were taken from the antecubital vein before exercise, at 15, 30, 45, 60 min during exercise, and at 90 min in the recovery periods.

Ghrelin and des-acyl ghrelin assay
Blood samples were immediately transferred to chilled polypropylene tubes containing EDTA-2Na (1 mg/mL) and aprotinin (500 U/mL), and centrifuged at 4°C. Hydrochloric acid (1N) was added to the samples at 10% volume of plasma volume immediately after the separation of plasma and stored the plasma at –30°C until assayed [32]. The levels of ghrelin and des-acyl ghrelin were measured using two commercially available ELISA kits, respectively (Mitsubishi Chemical Medience Corporation, Tokyo, Japan) [33]. The minimal detection limits of ghrelin and des-acyl ghrelin in this assay were 2.5 and 12.5 fmol/mL, respectively. The intra- and inter assay coefficients of variation were 6.5% and 9.8%, respectively, for ghrelin and 3.7% and 8.1%, respectively, for des-acyl ghrelin.

Other biochemical assay
Plasma GH concentrations were analyzed using a commercially available GH ELISA kit (Biocode SA, Liege, Belgium). Plasma epinephrine (E), norepinephrine (NE) and dopamine (DA) were assayed using high-performance liquid chromatography (SRL Inc, Tokyo, Japan). Plasma glucose was measured using the glucose oxidase methods and plasma insulin using EIA (SRL Inc, Tokyo, Japan).
Lowering of plasma ghrelin in exercise

Significant (Fig. 1B, C). The ghrelin to total ghrelin ratio significantly reduced during exercise (30–60 min) (Fig. 1D). Plasma GH, NE, E, and DA concentrations increased significantly during exercise and decreased at 90 min (Fig. 2A–D). Plasma glucose concentrations gradually decreased during exercise and became significantly lower at 90 min than pre-exercise level (Fig. 3A). Plasma insulin level significantly decreased during exercise (30–60 min) and recovered at 90 min (Fig. 3B). Spearman rank correlation test showed significant negative linear relationships between plasma ghrelin levels and plasma GH, NE, E, or DA levels (each Spearman’s rho = –0.943, \( P = 0.0048 \)) (Fig. 4A–D).

**Discussion**

We here reported significant suppression of plasma ghrelin level but not des-acyl ghrelin level during acute moderate exercise. Plasma ghrelin level was negatively correlated with plasma catecholamines and GH levels during exercise. We thus concluded that acute exercise lowered plasma ghrelin concentration: this was likely
mediated by elevated sympathetic tone, and unrelated to alteration of GH secretion upon exercise.

Plasma ghrelin level decreased during acute moderate exercise in this study. Recently significant suppression or no change of plasma ghrelin level during 60–90 min running or brisk walking were reported, but plasma des-acyl ghrelin and catecholamine concentrations were not examined in these reports [33-35]. Although the physiological significance of ghrelin suppression by exercise is unclear, a number of potential mechanisms may be proposed. In humans, plasma ghrelin concentration increased through the activation of the vagal efferent pathway using sham feeding and by a cholinergic agonist such as pyridostigmine [11, 36]. On the other hand, plasma ghrelin level was decreased by cholinergic antagonists such as pirenzepine and atropine [36, 37]. During acute exercise, vagal efferent activity was attenuated and sympathetic nerve activity increased. Plasma NE, E, and DA levels rose, in fact, during exercise in this study. These findings suggest that the significant suppression of ghrelin level during exercise might be induced by the activation of sympathetic nerve activity and/or relative inactivation of the vagus. Ghrelin, which enhances appetite and increases

Fig. 2: Plasma levels of GH (A), epinephrine (B), norepinephrine (C) and dopamine (D) at rest, during exercise, and during post exercise recovery. Data are mean ± SE. *, $P < 0.05$; **, $P < 0.01$ in comparison with basal levels.

Fig. 3: Plasma levels of glucose (A) and insulin (B) at rest, during exercise, and during post exercise recovery. Data are mean ± SE. *, $P < 0.05$; **, $P < 0.01$ in comparison with basal levels.
food intake in animals and humans, exhibits a diurnal pattern with preprandial increases and postprandial decreases [3, 5]. Ghrelin has been postulated as a hunger signal and meal initiator [38, 39]. Appetite is suppressed during and after intense exercise, however, this suppression does not last long [40]. We suspect that ghrelin suppression by exercise may reflect the diminution of hunger sensations experienced during and after vigorous exercise. Exercise, especially intense exercise, can lead to a variety of gastrointestinal abnormalities, such as abdominal pain or discomfort, nausea, vomiting, and diarrhea [41, 42]. Exercise induces the development of reduced blood flow in the gastric mucosa in an exercise intensity dependent manner [41, 42]. We hypothesize that this exercise-induced ghrelin suppression may be related, at least in part, to gastric mucosal ischemia. Gastric mucosal ischemia also may decrease the activity of ghrelin O-acyltransferase [43], which acylates ghrelin with n-octanoic acid in the gastric mucosa. As these mechanisms are speculative theories, further studies will be necessary to clarify the fundamental mechanisms of exercise-induced ghrelin suppression.

Although some studies have examined the relationships between acute exercise and ghrelin, plasma total ghrelin level did not change [19-22], decreased [24-26] or increased [27-29] during acute exercise. The reason for the discrepancy with our results is unclear. We speculate that this discrepancy is due to combined factors including sample collection, storage conditions, and assay systems used. Hosoda et al. reported that stabilization and acidification are essential for measuring ghrelin level [31]. In the present study, since ghrelin is rapidly converted to des-acyl ghrelin, blood samples were immediately collected in tubes containing EDTA-protBIN (a protease inhibitor) and centrifuged as soon as possible. Hydrochloric acid was immediately added to the plasma samples obtained to stabilize n-octanoyl modification. Moreover, in this study, we used novel sensitive ELISA kits that can separately detect acylated and des-acyl ghrelin up to 2.5 and 12.5 fmol/mL, respectively [32]. Since assay systems for total ghrelin in previous reports recognized all of ghrelin, des-acyl ghrelin, and fragmented ghrelin, they could not detect the change of only ghrelin. Taken together, these optimum sample treatments and the novel sensitive assay systems may probably allow us to detect the specific alteration of ghrelin during exercise.
N-octanoyl modification is essential for the activity of ghrelin. In the present study, no significant changes were found in plasma des-acyl ghrelin concentrations, although we showed the significant suppression of ghrelin and ghrelin/total ghrelin ratio during exercise. These results suggest that ghrelin disappears from the circulation more rapidly than des-acyl ghrelin during acute moderate exercise. In general, the alternative factors of plasma ghrelin level are secretion from the stomach, clearance from the kidneys [44], and utilization in peripheral tissues. Since all the subjects in this study have normal renal function, the suppression of plasma ghrelin level during acute exercise might be due to decreased synthesis and/or increased utilization in peripheral tissues. However, further studies are required to elucidate the mechanisms regulating ghrelin synthesis, acylation and clearance during exercise.

Similar to the previous findings, plasma GH significantly increased during exercise. In addition, negative correlation was found between ghrelin and GH. Exercise is well known to stimulate GH release [45]. GH response to exercise is dependent on the duration/intensity of exercise and the fitness levels of the subjects [46]. Previous studies suggest that exercise stimulates GH secretion without an increase in plasma total ghrelin concentration [17-22, 24-26]. Thus, previous and our findings suggest that ghrelin is less likely to be involved in exercise-induced GH secretion and that decreased ghrelin during exercise may be affected by negative feedback of GH [47].

In conclusion, the present findings indicated that ghrelin but not des-acyl ghrelin levels were suppressed by 60 min moderate cycling exercise. Furthermore, there were negative correlations between ghrelin and catecholamines. These results suggest that exercise-induced ghrelin suppression might be, in part, mediated by the adrenergic system. Further studies will be necessary to clarify the physiological significance of individual ghrelin form in physical exercise.

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