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Plasma ghrelin concentration is decreased by short chain fatty acids in wethers

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Abstract

To examine the effects of short chain fatty acids (SCFAs) on plasma ghrelin concentration, 4 wethers were injected intravenously with SCFA solutions [acetate (ACE), propionate (PRO), and butyrate (BUT) (0.8 mmol/kg BW)] and saline. The experiment was conducted after a 4 × 4 Latin square design. Each solution was injected into the jugular vein catheter with blood samples taken at −10, 0, 5, 10, 15, 20, 25, 30, 40, 50, and 60 min relative to the injection time also from this catheter. Plasma ghrelin concentrations decreased after injection with ACE, PRO, and BUT. Although plasma glucose concentrations increased after injection with PRO and BUT ($P < 0.05$), the increment areas were greater with BUT than with PRO. Plasma insulin concentrations increased after injection with PRO and BUT ($P < 0.05$). The decrement areas in plasma ghrelin concentrations were equal in ACE, PRO, and BUT. These data suggest that SCFAs inhibit ghrelin secretion in wethers and not through increased circulating glucose and insulin as previously proposed.

Keywords: Ghrelin; Short chain fatty acids; Wethers

1. Introduction

Ghrelin can stimulate GH secretion [1] and is a hormone mainly secreted by the abomasum in ruminants [2]. Plasma ghrelin concentration is related to the feeding and nutritional status of sheep [3]. The phenomenon showing increases in plasma ghrelin concentration before meals and decreases after meals [4] is well known. However, the mechanism of ghrelin secretion is not well known in ruminants. In some reports, a negative energy balance increases periprandial ghrelin concentration in dairy and beef cattle [5,6]. Sugino et al [7] demonstrated that cholinergic neurons are involved in the ghrelin secretory response to feeding in sheep. However, metabolites and other hormones can also affect plasma ghrelin concentrations in nonruminants. Nonruminants are observed to have postprandial increases of plasma glucose and insulin concentrations. Circulating insulin can suppress plasma ghrelin concentration in rats [8] and humans [9], and both oral and intravenous doses of glucose decreased plasma ghrelin concentration in humans [10]. However, ruminants absorb feed-derived carbohydrates as short chain fatty

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acids (SCFAs) from the rumen, and there are virtually no postprandial increases of plasma glucose concentration. Thus, it is probable that the inhibiting factor for ghrelin secretion may be different between nonruminants and ruminants. In ruminants, plasma SCFA concentrations increased immediately after feeding and have been theorized to be related to postprandial changes in plasma metabolic hormone (insulin and GH) concentrations [11]. Thus, SCFAs may suppress plasma ghrelin concentration. However, because some SCFAs in circulating plasma increase plasma glucose and insulin concentrations in ruminants, the effects of SCFAs on plasma ghrelin concentration may include the effects of glucose and insulin. The objective of this study was to investigate how some major SCFAs [acetate (ACE), propionate (PRO), and butyrate (BUT)] affect plasma ghrelin concentration and to consider the relationship between glucose, insulin, and ghrelin in wethers.

2. Materials and methods

The procedures used in the present study were performed in accordance with the principles and guidelines for animal use set by Hiroshima University which were formulated to comply with regulations of the Japanese Ministry of Education, Culture, Sports and Technology. All experiments were approved by the Animal Care and Use Committee of Hiroshima University.

2.1. Animals

Four Suffolk wethers (1 year old; 58.2 ± 3.7 kg BW) had catheters (Argyle 14 G CV catheter kit; Nippon Sherwood Medical Industries Ltd, Tokyo, Japan) inserted into the jugular vein ≥1 d before the first treatment. The catheter was used for the injection of solution and for blood sampling. Wethers were fed a sufficient quantity of alfalfa hay cubes and barley (7:3) to meet dietary maintenance requirements on the basis of the Japanese Feeding Standards [12] and had free access to water and trace mineralized salt block. Diets were offered twice daily at 9:00 AM and 5:00 PM.

2.2. Treatments and blood sampling

The experimental design was a 4 × 4 Latin square that consisted of 4 intravenous injection treatments with saline (CON), ACE, PRO, or BUT. ACE, PRO, and BUT solutions were made by dissolving sodium acetate, sodium propionate, and sodium butyrate (Nacarai Tesque, Inc, Kyoto, Japan), respectively, with saline and adjusted to pH 7.6. Each SCFA solution was injected at the rate of 0.8 mmol/kg BW. The injection rate was determined by following Sano et al [13], and the injection amounts of ACE, PRO, and BUT were equal to approximately 0.31%, 0.47%, and 0.71% of daily ME intake, respectively. Each treatment was performed with ≥1 d apart to avoid measurement values being affected by the previous treatment. At 4 h after morning feeding (1:00 PM), each SCFA solution or saline was injected singly into the jugular vein catheter. After injection, 10 mL of saline was injected to flush the catheter. Blood samples (5 mL) were taken at −10, 0, 5, 10, 15, 20, 25, 30, 40, 50, and 60 min relative to the injection time and collected into heparinized tubes with aprotinin (500 KIE/mL blood; Sigma-Aldrich, Inc, Tokyo, Japan). Catheters were flushed with heparinized saline (3 mL) to avoid blood clotting. The extracted blood samples were centrifuged at 2,330*g* for 15 min at 4°C. Harvested plasma was stored at −80°C before assay.

2.3. Chemical analyses

Plasma ghrelin and insulin concentrations were measured with time-resolved fluoro immunoassay. Assay for bioactive ghrelin was conducted as described previously by Sugino et al [3]. The ghrelin concentration was measured by competitive solid-phase immunoassay with the use of europium-labeled synthetic bovine ghrelin and polystyrene microtiter strips (Nalge Nunc Int, Tokyo, Japan) coated with anti-rabbit γ-globulin. Intra- and inter-assay CVs were 6.9T and 5.5%, respectively. Least detectable dose and IC_{50} in this assay system were 0.025 and 0.831 ng/mL, respectively. Insulin assay was conducted as described previously by Takahashi et al [14]. The insulin concentration was measured by competitive solid-phase immunoassay with the use of europium-labeled synthetic bovine insulin and polystyrene microtiter strips coated with anti-guinea pig γ-globulin. Intra- and inter-assay CVs variation were 2.2% and 1.8%, respectively. Least detectable dose and IC_{50} in this assay system were 0.016 and 1.073 ng/mL, respectively.

Plasma glucose concentration was determined with a glucose analyzer (GA-1151; Arkray, Inc, Kyoto, Japan). Plasma acetate concentrations were determined with a commercially available kit (F kit acetate; R-Biopharm AG, Darmstadt, Germany). Plasma β-hydroxy butyrate (BHBA) concentration was determined with an automated biochemistry analyzer (Beckman Coulter K.K., Tokyo, Japan).

2.4. Calculations and statistics

The values of plasma hormones and metabolites were expressed as least squares means of 4 wethers with SE or SEM. The decrement area was calculated for ghrelin, and the incremental areas were calculated for glucose and insulin, with the values expressed as least squares means of 4 wethers with SE as well. Data were analyzed as a 4 × 4 Latin square with a mixed linear model that used restricted maximum likelihood of the JMP program package (Version 5.01 for Windows computer system; SAS Institute Inc, Cary, NC, USA). For the statistical analysis of the differences in the values before injection (mean from −10 and 0 min), the decrement for ghrelin or increment for glucose and insulin areas, the models included the treatment as the fixed effect, and wethers and period as the random effects. The comparisons among treatments were evaluated by Student *t* test. For statistical analysis of the values of the concentrations after injections (5 to 60 min), time and the interaction between treatment × time were added to the fixed effects. Factorial contrasts tested the effect of SCFA injections (CON vs ACE, CON vs PRO, and CON vs BUN). A P value < 0.05 was considered significant.

3. Results

At pre-injection time (−10 and 0 min), no differences were observed among treatments in plasma ghrelin, glucose, insulin, ACE, and BHBA concentrations (Fig. 1 and Fig. 2).

In all SCFA treatments, plasma ghrelin concentrations after injection were lower compared with CON ($P < 0.05$; Fig. 1A). The decrement areas of plasma ghrelin were greater with all SCFA treatments than with CON ($P < 0.05$; Table 1) but not different among ACE, PRO, and BUT.

After injection, plasma glucose concentrations did not differ between CON and ACE, but in PRO and BUT plasma glucose concentrations were greater than with CON ($P < 0.05$; Fig. 1B). The increment area of plasma glucose was largest in BUT, followed by PRO, whereas the increment areas in CON and ACE were smaller than with PRO and BUT ($P < 0.05$; Table 1).

After injection, plasma insulin concentration did not differ in CON and ACE, but in PRO and BUT plasma insulin concentrations were higher than with CON ($P < 0.05$; Fig. 1C). The increment area of plasma insulin in ACE was smaller than with PRO and BUT, although it was greater than with CON ($P < 0.05$; Table 1). The increment areas of plasma insulin were similar in PRO and BUT.

Plasma acetate concentration in ACE was higher than with CON after injection ($P < 0.05$; Fig. 2A). Plasma BHBA concentration in BUT was higher than with CON after injection ($P < 0.05$; Fig. 2B).

4. Discussion

The aim of our study was to determine whether SCFAs affect plasma ghrelin concentrations. We determined plasma ACE concentration by sodium acetate injection in ACE to ascertain the increase and confirmed that plasma ACE concentrations increased clearly after ACE injection. Plasma BHBA concentration increased with BUT; however, this indicated that injected BUT was rapidly converted to BHBA. Similarly, the increase of plasma glucose concentration in PRO indicated that injected PRO was converted to glucose.

Our results showed that intravenous injections of ACE, PRO, and BUT reduced plasma ghrelin concentrations. Some reports have suggested that glucose or insulin or both could decrease plasma ghrelin concentration in nonruminants [8–10,15,16]. We observed increases of plasma glucose concentrations in PRO and BUT. Injected PRO was converted to glucose by itself, and BUT enhanced glucagon that eventually induced a plasma glucose increase [17]. We also observed increases of plasma insulin concentrations in PRO and BUT. These findings suggest that increased plasma concentrations of glucose and insulin might mediate depressions of plasma ghrelin concentrations by PRO and BUT. However, Sugino et al [18] demonstrated neither glucose nor insulin changed plasma ghrelin concentration in sheep with the use of the hyperglycemic clamp test. In our study, the increment areas of plasma glucose and insulin were different among treatments. No changes were observed in the decrement areas of plasma ghrelin concentration. Therefore, we assumed that plasma ghrelin concentrations might have decreased as direct responses to SCFAs. This was because the decrement areas of plasma ghrelin concentrations after SCFA injection were not different between ACE, PRO, and BUT, although their energy contents and metabolism were different. Presumably, the ghrelin response to SCFAs might be a molar-dependent action generated directly via their chemoreceptors. Ghrelin secretion is regulated by autonomic nerves in rats [19]. Sugino et al [3] demonstrated that the intravenous infusion of cholinergic blockers inhibited a meal-induced decrease in plasma ghrelin concentration, suggesting that cholinergic activity sup-

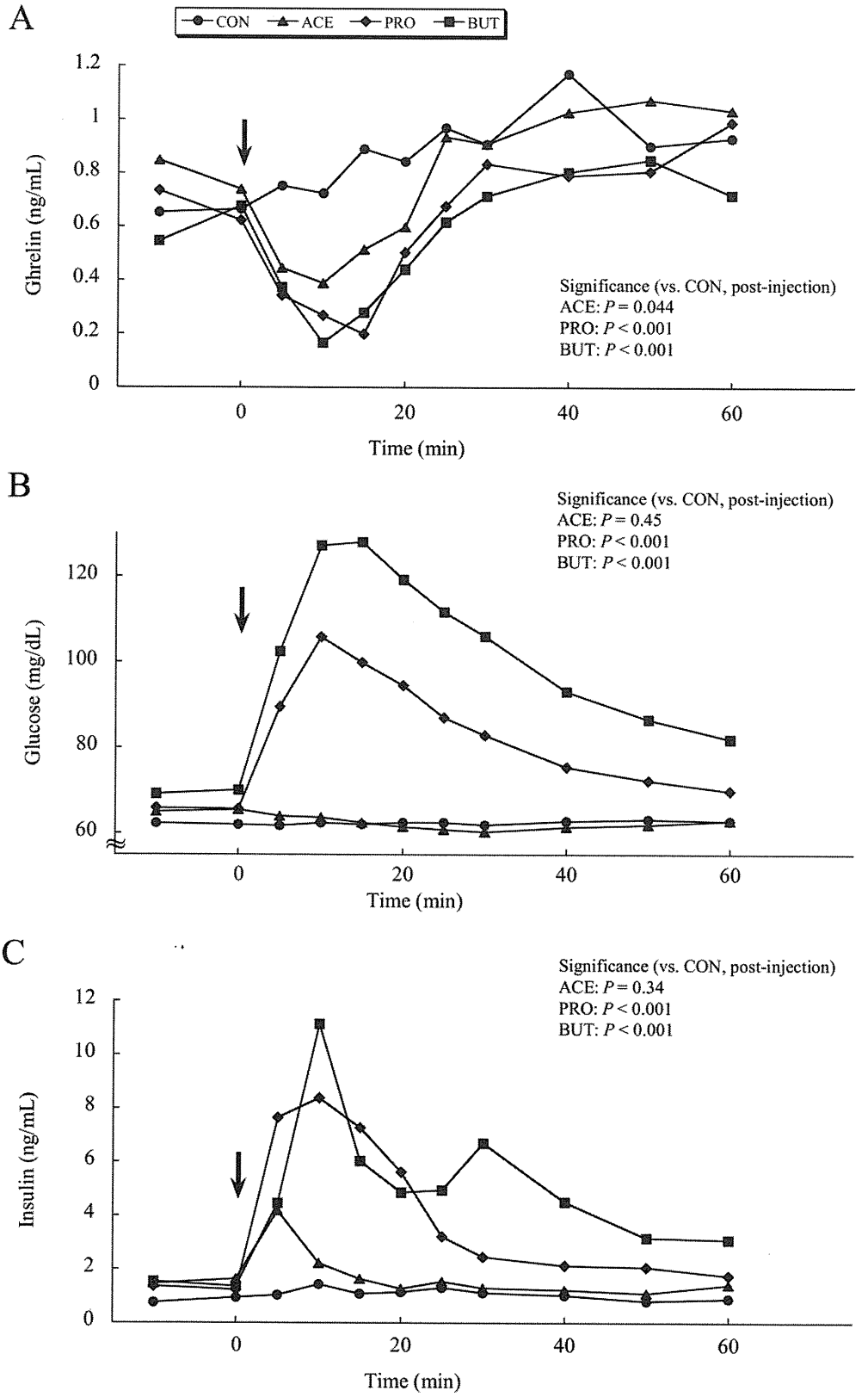

Fig. 1. Plasma ghrelin (A), glucose (B), and insulin (C) concentrations in CON ([circf]), ACE ([trif]), PRO ([diaf]) and BUT ([squlf]). Values are expressed as least squares means (n = 4). Their pooled SEM was 0.037 ng/mL for ghrelin, 3.00 mg/dL for glucose, and 0.36 ng/mL for insulin. The arrow shows the injection time. The pre-injection values of (mean from −10 and 0 min) plasma ghrelin, glucose, and insulin were not different among treatments. Statistical contrasts (figure inset) refer to decrement (A) or increment (B,C) areas.

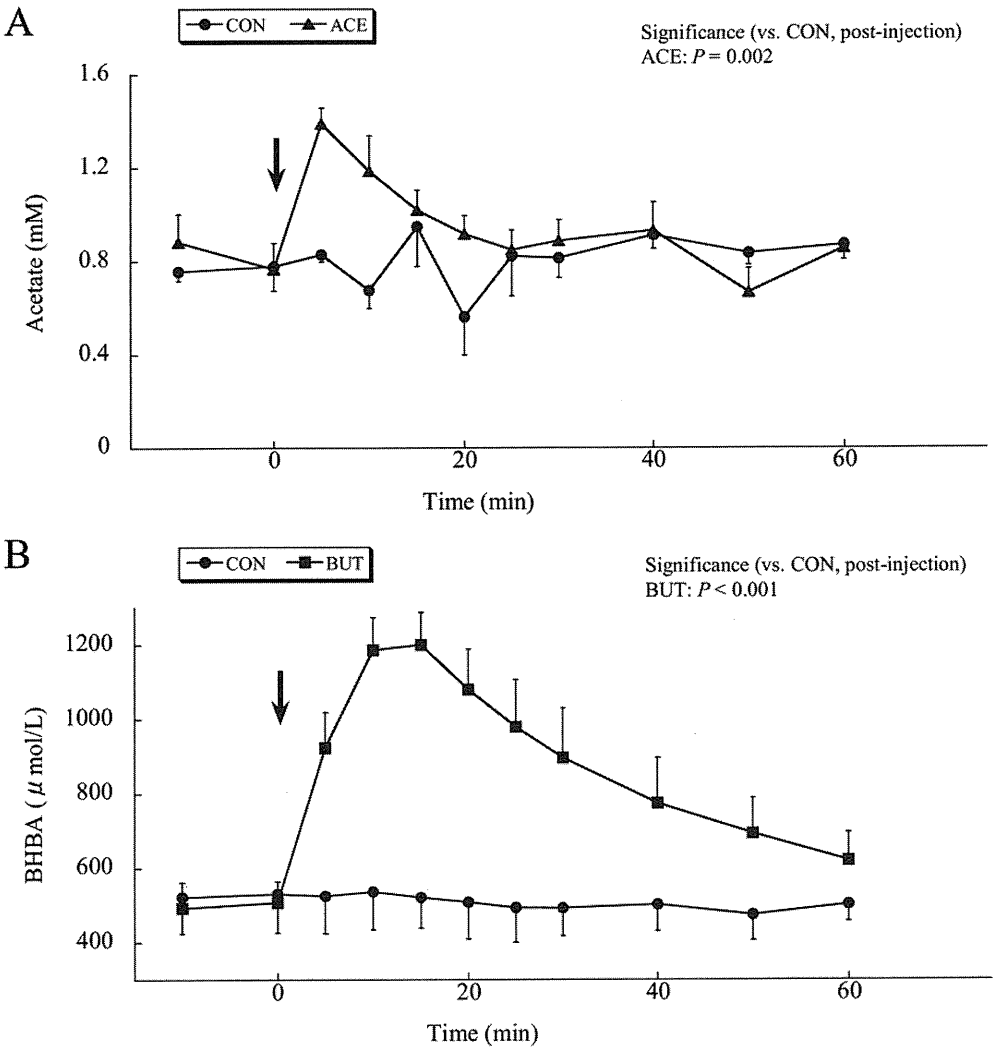

Fig. 2. Plasma acetate (A) and BHBA (B) concentrations in CON (]circf]), ACE ([trif]), and BUT ([squlf]). Values are expressed as least squares means ± SE (vertical bar; n = 4). The arrow shows the injection time. At pre-injection values (mean from −10 and 0 min) of plasma acetate and BHBA were not different among treatment. Statistical contrasts (figure inset) refer to increment areas.

pressed ghrelin secretion. Thus, we suggest here that activation of vagal afferents sensitive to SCFAs might be involved in the depression of plasma ghrelin concentration, although we could not identify any SCFA-stimulated sites because of intravenous injections of SCFAs. Although we could not elucidate the mechanisms by which SCFAs decreased plasma ghrelin concentration, we supposed that other factors aside from glucose and insulin might be at work in this study.

In conclusion, this study documents the effects of SCFAs on ghrelin secretion in ruminants for the first

Table 1
Decrement area for plasma ghrelin and increment areas for plasma glucose and insulin in wethers.

Item	CON, mean	ACE, mean	PRO, mean	BUT, mean	SEM	*P* value for treatment
Ghrelin: decrement area (ng.min/mL)	−0.024[b]	−0.282[a]	−0.279[a]	−0.230[a]	0.053	0.022
Glucose: increment area (mg.min/dL)	0.908[c]	0.106[c]	21.1[b]	32.4[a]	2.45	0.028
Insulin: increment area (ng.min/mL)	0.310[c]	0.864[b]	2.69[a]	3.53[a]	0.658	0.020

Abbreviations: CON, saline; ACE, saline with sodium acetate; PRO, saline with sodium propionate; BUT, saline with sodium butyrate.
Values with different superscripts (a,b,c) are different between treatments ($P < 0.05$).

time. We showed that ACE, PRO, and BUT equally decreased plasma ghrelin concentration.

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Review

Therapeutic applications of ghrelin to cachexia utilizing its appetite-stimulating effect

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ABSTRACT

Ghrelin, which is a natural ligand for the growth hormone (GH)-secretagogue receptor (GHS-R), stimulates food intake in both animals and humans. Ghrelin is the only circulating hormone known to stimulate appetite in humans. Ghrelin also stimulates GH secretion and inhibits the production of anorectic proinflammatory cytokines. As GH is an anabolic hormone, protein stores are spared at the expense of fat during conditions of caloric restriction. Thus, ghrelin exhibits anti-cachectic actions via both GH-dependent and -independent mechanisms. Several studies are evaluating the efficacy of ghrelin in the treatment of cachexia caused by a variety of diseases, including congestive heart failure, chronic obstructive pulmonary disease, cancer, and end-stage renal disease. These studies will hopefully lead to the development of novel therapeutic applications for ghrelin in the future. This review summarizes the recent advances in this area of research.

Contents

1. Introduction

Ghrelin, which is a natural ligand for the growth hormone (GH)-secretagogue receptor (GHS-R) [49], plays a critical role in a variety of physiological processes, including the stimulation of growth hormone secretion, and regulation of energy homeostasis by stimulating food intake and promoting adiposity via a GH-independent

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mechanism [50,52,85]. GH, which regulates insulin-like growth factor (IGF)-I levels, is an anabolic hormone that spares protein stores at the expense of fat utilization during conditions of caloric restriction. GH and IGF-1 are the major mediators of metabolism involved in the regulation of energy balance. Ghrelin inhibits the production of anorectic proinflammatory cytokines, including interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α [23,25]. The combination of these actions suggests this peptide has benefits for the treatment of cachexia.

Cachexia is defined as a complex metabolic syndrome associated with underlying illness and characterized by loss of muscle with or without loss of fat mass [29]. The prominent clinical feature of cachexia is weight loss in adults or growth failure in children. Anorexia, inflammation, insulin resistance and increased muscle protein breakdown are frequently associated with cachexia. Cachexia is distinct from starvation, age-related loss of muscle mass, primary depression, malabsorption and hyperthyroidism and is associated with increased morbidity. Although there is real need for pharmacological treatment for cachexia, the track record of pharmacological interventions is limited. They include anabolic/androgenic steroids, appetite stimulants, protein anabolic agents and cytokine modulators [28,36]. Recently, several trials attempting to treat cachexia of different etiologies with ghrelin have been expanded [4]. This review summarizes the recent advances in this area of research.

2. Actions of ghrelin

2.1. Orexigenic action

Ghrelin has a well-established role in stimulating appetite and increasing food intake [17,90]; peripheral administration of ghrelin stimulates GH secretion and food intake in both animals and humans [91,93]. Ghrelin is the only hormone known to stimulate appetite after peripheral administration. Ghrelin, which increases c-fos expression in the arcuate nucleus, also activates hypothalamic neuropeptide Y (NPY)/Y1 receptors and agouti-related peptide (AgRP) pathways [16,46,74]. In addition, ghrelin induces food intake via the orexin pathway [82]. These functions are mediated at least in-part by vagal nerve pathways [19]. Repeated administration of ghrelin resulted in significant weight gain in rats [92] and patients with chronic obstructive pulmonary disease (COPD) [60]. Increases in adiposity were associated with body weight gain in animal experiments [27,84,92], while adiposity decreased in patients after total gastrectomy [1,3,98], esophagectomy [1,3,98] with gastric tube reconstruction and total hip replacement for osteoarthritis [1,3,98]. (Gastrectomized and esophagectomized patients were cancer patients and, in the study of gastrectomized patients, the placebo group also showed a decline in fat mass.) This discrepancy may result from the differences in the doses and frequencies of ghrelin administration. Long-term, twice-weekly injection with low-dose ghrelin (40 μg/kg) significantly decreased fat mass in aged mice [7]. While weekly food intake did not increase under these conditions, ghrelin-induced GH secretion may have contributed to low adiposity. In contrast, lean body mass increased in rodents [7,20] and humans [1,3,60,98] following ghrelin administration. Also, lean body mass increases following ghrelin mimetic administration [66,78]. These effects, which reflect increases in muscle mass, are promising for cachexia treatment, as losses in body weight and sarcopenia are characteristic features of cachexia.

2.2. Stimulation of GH secretion

Ghrelin strongly stimulates GH secretion in humans [5,8,40,80], several-fold more potently than GHRH under similar circumstances. Furthermore, ghrelin and GHRH synergistically increases GH release [40]. Ghrelin might also play a role on GH release in a non-acute setting [65]. GH regulates IGF-I levels, promotes anabolism, and increases muscle strength [35,86]. While GH enhances lipolysis, IGF-1 stimulates protein synthesis, myoblast differentiation, and muscle growth. Recombinant GH is currently approved by the U.S. Food and Drug Administration for use in HIV/AIDS wasting, parenteral nutrition-dependent short bowel syndrome, pediatric chronic kidney disease, and adult and pediatric GH-deficiency states [38]. Pharmacological doses of this agent, however, cause problematic side effects, such as dose-related arthralgias, carpal-tunnel syndrome, paresthesias, insulin resistance, sodium retention, and peripheral edema. In contrast, stimulation of GH production to supraphysiological levels following ghrelin administration has a paucity of severe side effects [3].

Ghrelin's ability to increase circulating IGF-1 levels has been demonstrated in human studies of congestive heart failure (CHF) and COPD, in which 3-week ghrelin injections tended to increase IGF-1 levels [60,63]. This effect is less evident as that seen for GHS, such as MK-677 and anamorelin (RC-1291). Long-term treatment (6 months) with MK-677 in patients with hip fractures increased IGF-1 levels by 84% in comparison to 17% after placebo [12]. Anamorelin treatment induced impressive increases in food intake in a 12-week trial of cancer cachexia; post-treatment IGF-1 levels were 36.5 ng/mL after anamorelin treatment in comparison to 5.95 ng/mL after placebo. In a 6-day trial of healthy volunteers, post-treatment IGF-1 levels increased to greater than 60 ng/mL after anamorelin treatment in comparison to <0 ng/mL after placebo [32,33]. In our study of patients undergoing total hip replacement for osteoarthritis, serum IGF levels changed significantly following eight daily ghrelin injections; the changes in post-treatment IGF-1 levels were 30.0 ng/mL for ghrelin in comparison to 5.6 ng/mL for placebo. These changes were not observed after 21 daily treatments. In males, however, serum IGF-1 levels after ghrelin treatment remained elevated in comparison to the placebo group. Thus, the timing and duration of ghrelin injection and the subjects receiving treatment (e.g., disease features and sex) may influence the effect of ghrelin on IGF-1 levels. In addition, serum levels of IGF-1, which is primarily produced by the liver, reflect the systemic effects of IGF-1. GH also induces the synthesis of IGF-I in non-hepatic tissues. The local (autocrine/paracrine) effects of IGF-1 may play distinct roles in various tissues, including muscle mass regulation [54,86]. For instance, local muscle-restricted IGF-1 transgene expression accelerates the regeneration of injured skeletal muscle in mice, modulating inflammatory responses and limiting fibrosis [69].

2.3. Anti-inflammatory action

Evidence that ghrelin exerts anti-inflammatory actions has been accumulating. Ghrelin suppresses the production of proinflammatory cytokines, including IL-1β, IL-6, and TNF-α both *in vitro* [25,55] and *in vivo* [37,81,94]. In clinical trials, daily administration of ghrelin for 3 weeks decreased inflammatory cytokine levels and neutrophil density in sputum from patients with chronic respiratory infections [48]. In contrast, ghrelin induces the anti-inflammatory cytokine IL-10 [37,88].

Ghrelin inhibits the activation of NF-κB, a transcription factor known to control the production of multiple proinflammatory cytokines during inflammatory insults [55,88,94]. Although the molecular mechanisms and cellular targets mediating ghrelin inhibition of NF-κB activation remain to be determined, the vagus nerve may play an important role in the ghrelin-mediated inhibition of proinflammatory cytokine release [83,94]. Cachexia and muscular wasting occur via protein degradation by the ubiquitin–proteasome pathway [44]. Two muscle-specific

ubiquitin ligases, muscle RING-finger protein-1 (MuRF1) and atrogin-1/muscle atrophy F-box (MAFbx), are up-regulated under catabolic conditions. NF-κB activation may regulate skeletal muscle proteasome expression and protein degradation. The elevations in MuRF1 and MAFbx expression seen in skeletal muscle after thermal injury, arthritis, and dexamethasone administration were normalized, attenuated, and prevented, respectively, by ghrelin or GHS administration [13,72,97]. IGF-1 prevents the expression of MuRF1 and MAFbx by inhibiting Forkhead box O (FOXO) transcription factors via stimulation of the phosphatidylinositol-3-kinase (PI3K)/Akt pathway. The IGF-1 receptor triggers activation of several intracellular kinases, including phosphatidylinositol-3-kinase (PI3K) [76]. Thus, the effects of ghrelin on NF-κB activation and IGF-1 synthesis are favorable for minimizing inflammatory responses and sarcopenia in patients with cachexia.

In addition, ghrelin promotes thymopoiesis during aging, suggesting an important biological role of ghrelin in generation of naive T cells and age-associated thymic involution [26]. This also suggests a possible therapeutic benefit of harnessing ghrelin signaling pathway in the reconstitution of thymic function in immunocompromised subjects such as cachectic patients. Moreover, ghrelin may be a key player in coupling metabolism to immunity [24], considering that reduction in ghrelin levels is associated with increased inflammation during obesity [89] and that increased ghrelin by calorie restriction is associated with decreased inflammation [99].

2.4. Other actions

The role of ghrelin in stimulating gastric emptying and acid secretion is well-established [68]. This effect may ameliorate gastrointestinal symptoms in patients with anorexia–cachexia syndrome. Ghrelin also increases endogenous nitric oxide (NO) release [75,96], which may influence the orexigenic and anti-inflammatory actions of ghrelin [51,59]. These qualities may be important in the treatment of cachexia.

Ghrelin influences insulin secretion and glucose metabolism [22,85]. Ghrelin may have obesogenic/diabetogenic properties. These properties may be direct effects of ghrelin on pancreatic islet function and/or indirect effects through GH secretion modulation. Also, ghrelin is part of a mechanism that integrates the physiological response to fasting [100]. This is very likely of physiological relevance to protection against food scarcity by building energy reserves. Moreover, this action may be beneficial for catabolic states such as cachexia. At least, no harmful effects of ghrelin on glucose metabolism or diabetic patients have been observed in our clinical studies of repeated ghrelin administration to patients with cachexia [60,63] or undergoing surgery [3].

3. Plasma ghrelin levels in cachexia

Plasma ghrelin levels are elevated in cachectic conditions caused by a variety of underlying disorders [31,43,47,64,73]. Although this phenomenon has been called "ghrelin resistance", these elevations may be a compensatory response reflecting the negative energy balance state. While there is usually an inverse relationship between plasma ghrelin levels and BMI, no significant difference in ghrelin levels between normal subjects and cachectic patients after matching for BMI. Thus, difference in ghrelin levels between them is less apparent after controlling for BMI. In patients with ESRD, conflicting results (i.e., increases [11,70,71], decreases [42], or no change [42,79]) for circulating ghrelin concentrations have been reported [57]. Aygen et al. recently reported elevations in both ghrelin and des-acyl ghrelin in ESRD patients undergoing hemodialysis (HD) in comparison to age-matched healthy controls [11]. Iglesias et al. indicated that patients undergoing HD possessed similar ghrelin concentrations to the control group; only peritoneal dialysis (PD) patients exhibited significantly lower ghrelin concentrations at baseline than those found in patients on conservative management [42]. These conflicting results are due, at least in-part, to cross-sectional studies using different ghrelin assays that compared patients with different residual renal functions, ages, genders, and nutritional status. Residual renal function may affect the metabolism and clearance of ghrelin. Longitudinal studies following patients with renal disease using ghrelin assays measuring both ghrelin and des-ghrelin are required to determine the pathophysiologic role of ghrelin in cachexia associated with ESRD. Post-hemodialysis serum ghrelin levels are significantly lower than pre-hemodialysis ghrelin levels, supporting the view that ghrelin is cleared by hemodialysis [11,45,57].

4. Clinical studies

Trials seeking to apply the effects of ghrelin to the treatment of cachexia have been expanding. These studies have sought to evaluate ghrelin as a treatment for patients with the cachexia associated with CHF, COPD, cancer, ERSD, etc. Cachexia, which manifests as excessive weight loss in the setting of an underlying chronic disease [58], is typically associated with anorexia as a major cause of weight loss. Weight loss and decreased appetite are the major causes of morbidity and mortality in patients with anorexia-cachexia syndrome. There is an immediate need for effective, well-tolerated treatments to stimulate appetite [18], prompting several trials to explore the application of ghrelin as a treatment for patients with cachexia.

4.1. CHF cachexia

Ghrelin induces a positive energy balance state through both GH-dependent and -independent mechanisms and has protective cardiovascular effects [61]. GH treatment may be especially useful in a subgroup of patients with cardiac cachexia [6]. Ghrelin stimulates food intake, induces adiposity, regulates the central nervous system to decrease sympathetic nerve outflow, and inhibits apoptosis of cardiomyocytes and endothelial cells in a GH-independent manner. Nagaya et al. investigated the effects of ghrelin on cardiac cachexia in ten patients with CHF [63] (Table 1). Daily administration of ghrelin for 3 weeks increased both food intake and body weight. This study also demonstrated improvements in patient exercise capacity, muscle wasting, and left ventricular function. Ghrelin treatment also resulted in significantly decreased plasma

Table 1
Clinical studies of ghrelin for cachexia treatment.

Diseases	Reference	Year	Study design	Number of patients	Ghrelin administration
CHF	[58]	2004	Open-label pilot study	10	Ghrelin, 2 μg/kg b.i.d. for 3 weeks, i.v.
COPD	[55]	2005	Open-label pilot study	7	Ghrelin, 2 μg/kg b.i.d. for 3 weeks, i.v.
Cancer cachexia	[62]	2004	Acute, randomized, placebo-controlled, cross-over study	7	Ghrelin, 5 pmol/kg/min i.v. for >180 min
Cancer cachexia	[72]	2008	Randomized, placebo-controlled, cross-over study	21	Ghrelin, 2 or 8 μg/kg, i.v. for 4 days, once a day
ESRD	[89]	2005	Acute, randomized, placebo-controlled, cross-over study	9	Ghrelin, 3.6 nmol/kg, s.c.
ESRD	[9]	2009	Randomized, placebo-controlled, cross-over study	12	Ghrelin, 12 μg/kg, s.c. for 1 week, once a day

norepinephrine levels. Although this study was neither randomized nor placebo-controlled, the eight CHF patients who did not receive ghrelin (control group) were followed to rule out any time-course effects during hospitalization. None of the aforementioned parameters changed in patients with CHF who did not receive ghrelin therapy. Further studies will be necessary to identify the pathways involved in this ghrelin effect and to determine the best therapeutic strategies for ghrelin use to combat the wasting process found in cardiac cachexia [6]. Clinical trials are currently attempting to reproduce these data in a double-blind, placebo-controlled fashion.

4.2. COPD cachexia

Patients with COPD often exhibit some degree of cachexia [62], which is an independent risk factor for mortality in COPD; GH treatment increases muscle mass in such patients. COPD and CHF are both associated with multiple pathophysiological disturbances, including anemia and neurohormonal activation [53]. In COPD patients, ghrelin exhibits anti-inflammatory effects. Chronic respiratory infections, characterized by neutrophil-dominant airway inflammation, lead to end-stage cachexia [10]. The cytotoxicity of accumulated neutrophils against bronchial and alveolar epithelial cells induces a deterioration of pulmonary function in COPD, resulting in excess energy expenditure and weight loss in patients. Intravenous ghrelin treatment for 3 weeks reduced both neutrophil counts in sputum samples and the volume of sputum, suggesting suppression by ghrelin of excess neutrophilic influx [48].

An open-label pilot study examined the ability of ghrelin to improve cachexia and functional capacity in patients with COPD; ghrelin was administered intravenously for 3 weeks to seven cachectic patients with COPD [60]. Repeated ghrelin administration significantly increased food intake, body weight, lean body mass, and peripheral and respiratory muscle strength. Ghrelin treatment ameliorated the exaggerated sympathetic nerve activity, as indicated by marked decreases in plasma norepinephrine levels. In cachectic patients with COPD, treatment with ghrelin improved appetite, body composition, muscle wasting, functional capacity, and sympathetic augmentation. Subsequently, another placebo-controlled trial demonstrated that ghrelin increased both appetite and body weight with an apparent dose-dependent trend toward improved physical performance (chair stand score) [34]. A larger clinical trial is currently being conducted to confirm these data in a double-blind, placebo-controlled fashion. Comparisons of this treatment to current standard medications will be required [53].

4.3. Cancer cachexia

Anorexia, frequently encountered in cancer patients, is one of the major causes of malnutrition and cachexia in this patient population. Ghrelin administration resulted in significant increases in weight and food intake in rodent models of cancer cachexia [21,39,87]. In all studies, ghrelin improved both food intake and weight gain in rodent models. DeBoer et al. determined that weight gain resulted from a reversal in the loss of lean body mass, a critical component of cachexia [21].

Several randomized, double-blind placebo-controlled trials have demonstrated the efficacy and safety of ghrelin or GHS in patients with cancer-associated cachexia [32,67,77]. Nearry et al. performed a randomized, placebo-controlled, cross-over clinical trial to determine if ghrelin could stimulate appetite in seven cancer patients with severe anorexia [67]. Ghrelin infusion resulted in a marked increase in energy intake in comparison to saline-treated controls; all patients in the study demonstrated increased food consumption. The meal appreciation score was also higher in ghrelin-treated individuals. Strasser et al. detailed a randomized, double-cross-over, phase 1/2 study in 21 patients with advanced cancer [77]. They infused a low or high dose of ghrelin or placebo before lunch daily for 4 days in each course. Nutritional intake or eating-related symptoms did not differ between the ghrelin- and placebo-treated groups. More patients, however, preferred ghrelin to placebo at the middle and end of study, although this finding was not dose-dependent. In contrast to the results of Neary et al., this study did not demonstrate any increases in food intake. As the patient characteristics and study designs were very different in the two studies, further investigation will be required.

An important concern regarding the use of ghrelin in cancer cachexia is that ghrelin may increase growth factors, such as GH and IGF-1, to stimulate tumor growth. Additionally, ghrelin itself may have mitogenic potential. As far as we know, no *in vivo* data have examined the differences in tumor growth after ghrelin or GHS treatment. Long-term, large-scale clinical trials are required to determine if ghrelin treatment promotes tumor growth.

4.4. End-stage renal disease (ESRD)

ESRD is a chronic condition frequently associated with nutritional dysfunction [15]. This type of malnutrition is highly resistant to intervention and a major predictor of morbidity and mortality for patients on either peritoneal dialysis (PD) or hemodialysis. Wynne et al. sought to determine if a single injection of ghrelin could enhance food intake in patients with evidence of malnutrition receiving maintenance peritoneal dialysis [95]. Nine PD patients exhibiting mild to moderate malnutrition administered either ghrelin or a saline placebo subcutaneously were examined in a randomized, double-blind, cross-over protocol. Ghrelin administration significantly increased mean absolute energy intake during the study meals and maintained nonsignificant increases observed in energy intake over the first 24 h without a subsequent rebound. This research group has subsequently sought to analyze the efficacy of repeated ghrelin administrations in malnourished dialysis patients [9]. They performed a double-blind randomized cross-over study of a week of daily subcutaneous ghrelin injections in a group of 12 malnourished dialysis patients. Ghrelin administration significantly increased appetite, with increases in energy intake noted at the first study meal. Persistence of this effect throughout the week was confirmed by food diaries and final study meals, indicating that daily ghrelin treatment achieved a sustained positive change in energy balance in malnourished dialysis patients. In support of these data, an animal study using a nephrectomized rat model of renal cachexia demonstrated that daily treatment for 2 weeks with ghrelin or two GHS agents (BIM-28125 and BIM-28131) resulted in increased food intake, improved lean body mass accrual, and decreased circulating inflammatory cytokines [20]. Long-term studies are needed to demonstrate the efficacy in improving appetite, weight gain, lean body mass, and quality of life.

4.5. Others

Ghrelin treatment has also been applied to other causes of anorexia, sarcopenia, and emaciation, including anorexia nervosa (AN) [41], functional dyspepsia [2], aging [3], postgastrectomy anorexia [1], esophagectomy [98], chemotherapy [30,56], and thermal injury [14]. In addition, the effects of ghrelin mimetics were examined in the age-dependent sarcopenia [3,66]. These applications certainly provide additional insight into the successful treatment of cachexia, a wasting syndrome developing in the setting of a variety of chronic illnesses.

5. Conclusion

Ghrelin exhibits anti-cachectic effects in a number of animal and human studies. Ghrelin treatment is safe and well-tolerated.

Several larger-scale clinical trials are currently attempting to reproduce these effects for the treatment of cachexia, including that associated with CHF, cancer, COPD, and ESRD. Long-term, large-scale trials are eagerly awaited to determine if ghrelin is an effective therapy for cachexia.

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Additional Information: Information on the NCRR is available at http://www.ncrr.nih.gov/. Information on the Reengineering the Clinical Research Enterprise can be obtained from http://nihroadmap.nih.gov.

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COMMENTS AND OPINIONS

Considering Selection Bias When Developing a Search Strategy

We read with great interest the article by Sciarretta et al[1] on antihypertensive treatment and development of heart failure in hypertension. They performed the largest network meta-analysis in essential hypertension, to our knowledge, and showed that the use of diuretics and renin-angiotensin system inhibitors are the most effective first-line antihypertensive drug for preventing heart failure. In this meta-analysis, however, the search strategy and study selection are somewhat unclear.

The Candesartan Antihypertensive Survival Evaluation in Japan (CASE-J) trial, published in 2008,[2] assessed cardiovascular outcomes in high-risk hypertensive patients receiving either candesartan or amlodipine. The primary end point of the CASE-J trial was a composite of cardiovascular morbidity and mortality, including heart failure. We think that this trial meets the inclusion criteria in the

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meta-analysis by Sciarretta et al,[1] but it was not included despite a careful search using 2 databases by 2 investigators. Also, neither the KYOTO HEART study[3] nor the Heart Institute of Japan Candesartan Randomized Trial for Evaluation in Coronary Artery Disease (HIJ-CREATE)[4] was included. The authors also checked in the references of a previous meta-analysis by Verdecchia et al.[5] This could not be enough to identify all randomized controlled trials to evaluate a wide range of antihypertensive drugs because this previous meta-analysis by Verdecchia et al[5] aimed to compare old antihypertensive drugs (diuretics and β-blockers) or placebo with new drugs (renin-angiotensin system inhibitors or calcium channel blockers). We are afraid that other important clinical trials are not included in the meta-analysis by Sciarretta et al.[1]

Although a meta-analysis can provide more precise estimates of interventions, it always has a potential for selection bias. Therefore, we would like to know in more detail the search strategy used in this meta-analysis based on the Preferred Reporting Items for Systematic Reviews and Meta-analysis statement.

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Network Meta-analysis of Heart Failure Prevention by Antihypertensive Drugs

We agree with the conclusions of the "network meta-analysis" of heart failure prevention by antihypertensive drugs, which used a Bayesian technique and 26 clinical trials.[1] We have reported similar results supporting the superiority of a diuretic using both the network meta-analytic technique of Lumley,[2] and a Bayesian technique, which included data from all 47 or 53 of the then published hypertension clinical trials.[3,4] We noted the great preponderance of evidence favoring chlorthalidone, which accounts for more than 90% of their heart failure cases. Although a network meta-analysis in 2004 suggested no significant difference between chlorthalidone vs another diuretic as initial therapy to prevent heart failure,[5] data from all 61 hypertension trials involving 355 225 subjects (from the first Veterans Administration trial[6] to the very recent Valsartan Amlodipine Randomized Trial[7]), using the therapeutic categories of Sciarretta et al[1] suggests a major difference in precision among diuretics (**Figure**). "Other diuretics" has the smallest number of heart failure cases of any treatment, nearly 4-times smaller than that for chlorthalidone. The *P* value for chlorthalidone is nearly 5 orders of magnitude smaller than that of "other diuretics." Our results were robust to many sensitivity analyses, such as when combining "conventional therapy" and "β-blocker," including only studies that randomized subjects to initial therapy, omitting data from studies done in nonhypertensive patients, and omitting data from the Antihypertensive and Lipid-Lowering to prevent Heart Attack Trial (ALLHAT). We add to the conclusions of Sciaretta et al[1] that the clinical trial evidence is strongest, by far, for chlorthalidone as the most effective antihypertensive agent to prevent heart failure.

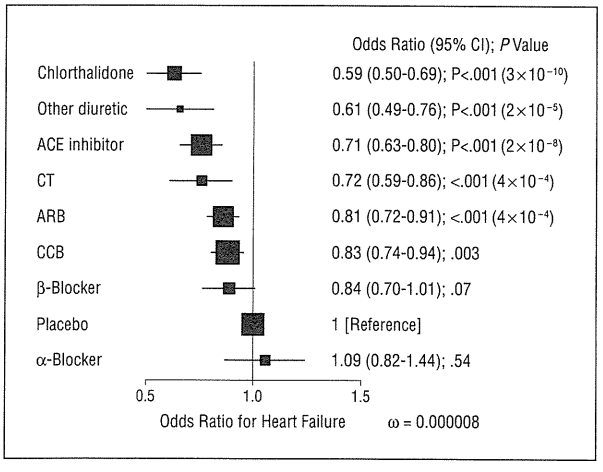

Figure. Odds ratios (95% confidence intervals [CIs]) for incident heart failure associated with different types of antihypertensive drugs in clinical trials, determined by the network meta-analytic technique of Lumley.[2] ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; CT, conventional therapy (diuretic and/or β-blocker); ω, incoherence.

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In reply

We thank Nakao and colleagues for their letter. A selection bias in meta-analyses is mainly due to errors in the search strategy to screen the trials to be considered. These errors are related to an arbitrary selection of the trials or to the choice of factious search and eligibility criteria to select them. As a result, some studies are excluded from the analysis although they should be considered.

To avoid a selection bias, in our article[1] we predefined and clearly reported the process for study selection, in keeping with the PRISMA check list. Our search keywords were generic and impartial and could not systematically exclude trials with specific characteristics. The meta-analysis by Verdecchia et al,[2] which evaluated our same outcome (ie, heart failure [HF]), was only used as a control.

In a recent comprehensive meta-analysis, Bangalore et al[3] evaluated all the trials assessing an antihypertensive therapy published between 1950 and 2010. All the trials con-

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Correction

Error in Correspondence. In the Letter to the Editor titled "Considering Selection Bias When Developing a Search Strategy" by Nakao et al, published in the March 14, 2011, issue of the *Archives* (2011;171[5]: 471-472), an incorrect e-mail address appeared in the Correspondence section. The correct e-mail address is as follows: kenji.ueshima@at3.ecs.kyoto-u.ac.jp.

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ORIGINAL ARTICLE
Hypertension and Circulatory Control

Influence of Coronary Risk Factors on Coronary Events in Japanese High-Risk Hypertensive Patients

– Primary and Secondary Prevention of Ischemic Heart Disease in a Subanalysis of the Candesartan Antihypertensive Survival Evaluation in Japan (CASE-J) Trial –

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Background: The Candesartan Antihypertensive Survival Evaluation in Japan (CASE-J) trial was conducted to compare the effects of candesartan and amlodipine on cardiovascular events in Japanese high-risk hypertensive patients. The aim of the present subanalysis was to evaluate the influence of coronary risk factors on coronary events in these patients as an observational study irrespective of allocated drugs.

Methods and Results: The adjusted hazard ratios (HRs) of the association of baseline risk factors including gender, age, allocated drugs, body mass index, systolic/diastolic blood pressure (SBP/DBP), diabetes mellitus (DM), hyperlipidemia (HL), smoking, left ventricular hypertrophy, previous ischemic heart disease (IHD), previous cerebrovascular events, and chronic kidney disease (CKD) with coronary events in 4,703 patients who were enrolled in the CASE-J trial, were examined. The coronary events occurred in 83 patients, and were significantly associated with previous IHD, DM, male sex, CKD, and low DBP. Significant predictors were previous IHD (HR, 3.89), DM (HR, 3.10), male sex (HR, 1.81), CKD (HR, 1.60), and low DBP (HR, 1.36), respectively. In 4,107 patients without previous IHD, DM (HR, 4.88), HL (HR, 2.67), and DBP (HR, 1.39) were significantly associated with the risk of coronary events, while male sex (HR, 3.03), CKD (HR, 2.44), and DM (HR, 2.15) were in 596 patients with previous IHD.

Conclusions: DM is the important factor in both primary and secondary prevention of coronary events. Comprehensive risk management including surveillance of DM, CKD and HL is needed for preventing coronary events, in addition to blood pressure control. (*Circ J* 2011; **75:** 2411–2416)

Key Words: Coronary event; Coronary risk factor; Hypertension; Japanese; Prevention

Although the current incidence of acute myocardial infarction in Japan is still lower than that in North America and Europe,[1] a recent report indicated that there has been a steady trend of increasing incidence of acute myocardial infarction during the past 30 years in the Japanese population.[2] We should be deeply concerned about this increase of coronary events in Japan with regard to westernized lifestyle and aging of the population. Hypertension has been one of the major risk factors for cardiovascular (CV) events, and CV risks are well known to cluster in hypertensive patients.[3,4] It is important to consider coronary risk factors in hypertensive patients when we try to reduce the incidence of ischemic heart disease (IHD) in terms of primary and secondary prevention.

Editorial p 2316

The Candesartan Antihypertensive Survival Evaluation in Japan (CASE-J) trial was conducted to compare the effects of the angiotensin II receptor blocker candesartan and the calcium channel blocker amlodipine on the incidence of CV events in Japanese high-risk hypertensive patients.[5,6] The CASE-J trial found that candesartan and amlodipine equally suppressed total CV mortality and morbidity in high-risk hy-

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Table 1. Selected Coronary Risk Factors and Definitions for Baseline Characteristics

Gender
Age
Allocated drugs: candesartan or amlodipine
BMI at baseline
SBP at baseline
DBP at baseline
Smoking (including previous history of smoking)
DM: at least one of the following factors: fasting blood glucose ≥126 mg/dl, causal blood glucose ≥200 mg/dl, HbA_{1c} ≥6.5%, 2-h blood glucose on 75-g OGTT ≥200 mg/dl, or current treatment with hypoglycemic agent
HL: current treatment with anti-lipidemic agent
Cerebrovascular disease: history of cerebral hemorrhage, cerebral infarction, or transient ischemic attack until 6 months prior to the screening
LVH: thickness of the posterior wall of left ventricle or thickness of the wall of interventricular septum ≥12 mm on echocardiography or Sv1+Rv5 ≥35 mm on electrocardiography
IHD: angina pectoris, and a past history (≥6 months before giving informed consent) of myocardial infarction
CKD: proteinuria ≥+1 or renal impairment (estimated glomerular filtration rate <60 $ml \cdot min^{-1} \cdot 1.73 m^{-2}$ by a predictive equation) within 3 months at the time of giving informed consent
Anti-hypertensive medication prior to screening

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; DM, diabetes mellitus; OGTT, oral glucose tolerance test; HL, hyperlipidemia; LVH, left ventricular hypertrophy; IHD, ischemic heart disease; CKD, chronic kidney disease.

Table 2. Baseline Subject Characteristics

	All patients (n=4,703)	Previous IHD (+) (n=596)	Previous IHD (–) (n=4,107)
Gender, male (%)	2,597 (55.2)	397 (66.6)	2,200 (53.6)
Age (years), mean±SD	63.9±10.5	67.2±8.7	63.4±10.7
Allocated drugs, candesartan (%)	2,354 (50.1)	298 (50.0)	2,056 (50.1)
BMI, mean±SD	24.6±3.7	24.3±3.3	24.6±3.7
SBP, mean±SD	162.9±14.2	157.5±12.3	163.6±14.3
DBP, mean±SD	91.7±11.2	86.9±9.1	92.4±11.3
Smoking (no) (%)	3,205 (68.1)	346 (58.1)	2,859 (69.5)
DM (yes) (%)	2,018 (42.9)	203 (34.1)	1,815 (44.2)
HL (yes) (%)	2,078 (67.3)	325 (54.5)	1,753 (42.7)
LVH (yes) (%)	1,612 (34.3)	178 (29.9)	1,434 (34.9)
Previous IHD (yes) (%)	596 (12.7)	596 (100)	0 (0)
Previous stroke (yes) (%)	473 (10.1)	41 (6.9)	432 (10.5)
CKD (yes) (%)	2,571 (54.7)	296 (49.7)	2,275 (55.4)
Previous AHD (yes) (%)	3,165 (67.3)	512 (85.9)	2,653 (64.6)

AHD, anti-hypertensive drug. Other abbreviations see in Table 1.

pertensive patients under strict blood pressure (BP) control.

Although some Japanese cohort studies have been performed to clarify the coronary risk factors, this trial was an observational study irrespective of allocated drugs, and the purpose of the present subanalysis was to clarify the adverse effects of coronary risks in Japanese high-risk hypertensive patients. Although prior cohort studies focused on the general population, the present study focused on those subjects taking anti-hypertensive drugs. Therefore, the influence of risk factors on coronary events under control could be clarified.

Methods

Study Design

The CASE-J trial was a prospective, multicenter, randomized, open-label, active-controlled, 2-arm parallel-group comparison study to evaluate the efficacy of candesartan and amlodipine for reducing the incidence of CV events in high-risk hypertensive patients.[5,6] The rationale and complete design of the CASE-J trial have been previously reported.[5] Briefly, 4,728 patients with high-risk hypertension were randomly assigned to either a candesartan- or amlodipine-based treatment regimen. High risk was defined as the presence of any one of the following: (1) severe hypertension (systolic BP/diastolic BP [SBP/DBP] ≥180/110 mmHg; (2) type 2 diabetes mellitus (DM); (3) history of stroke or transient ischemic attack >6 months prior to screening; (4) left ventricular hypertrophy (LVH; SV1+RV5 ≥3.5 mV on electrocardiography [ECG] and/or LV wall thickness ≥12 mm on echocardiography), angina pectoris, or a history of myocardial infarction >6 months prior to screening; (5) proteinuria or serum creatinine concentration ≥1.3 mg/dl; and (6) arteriosclerotic peripheral artery obstruction.[5] The target BP was determined according to the guideline proposed by the Japanese Society of Hypertension.[7] Finally, 4,703 randomly assigned patients were included in the analysis.

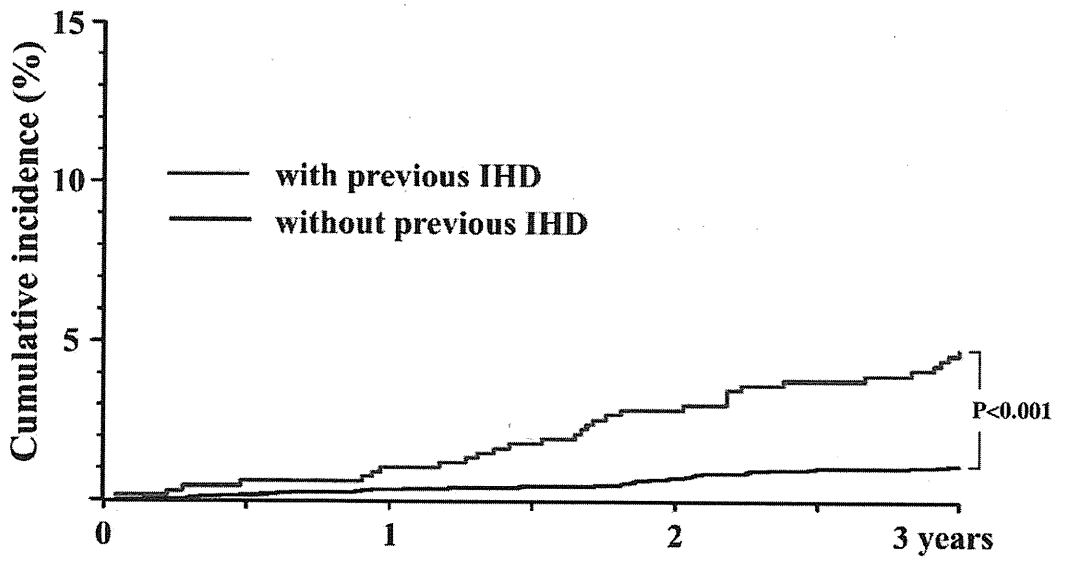

Figure. Cumulative incidence of the primary composite endpoint adjusted for baseline characteristics in patients without ischemic heart disease (IHD) at baseline and with IHD at baseline.

Outcome Measurements

In the CASE-J trial, the primary endpoint was the first fatal/non-fatal CV event (a composite of cardiac events including sudden death, angina pectoris, acute myocardial infarction, or heart failure; cerebrovascular events including stroke or transient ischemic attack; renal events including serum creatinine concentration ≥4.0 mg/dl, doubling of the serum creatinine concentration, or end-stage renal disease; and vascular events including dissecting aortic aneurysm or arteriosclerotic occlusion of a peripheral artery).[6] In the present subanalysis, we focused on the incidence of coronary events. Thus, the endpoint of this subanalysis was coronary events, and they were defined as a composite of sudden death (unexpected death happening within 24 h without external causes), angina pectoris (nitrate-sensitive chest pain and ischemic ECG changes during chest pain or stress testing), and acute myocardial infarction (nitrate-resistant chest pain, elevated myocardial specific enzymes, and typical ECG signs). Event evaluation was performed independently by the Event Evaluation Committee, which was blinded to the assigned treatment groups and adjudicated according to the prespecified protocol criteria as described here.[6]

Baseline Characteristics

Background coronary risk factors such as gender, age, allocated drugs, body mass index (BMI), SBP, DBP, smoking, type 2 DM, hyperlipidemia (HL), LVH, history of previous IHD, history of cerebrovascular disease, chronic kidney disease (CKD), and anti-hypertensive medication prior to the screening were analyzed (Table 1). Baseline characteristics of enrolled patients are listed in Table 2. Moreover, we focused on the presence or absence of previous IHD including angina pectoris, and a past history (≥6 months before giving informed consent) of myocardial infarction, which were 1 of the inclusion criteria in the CASE-J trial. Enrolled patients were divided into 4,107 patients without previous IHD and 596 patients with previous IHD. In the present study, risk factors for the first coronary event were assessed in patients without previous IHD and those for the recurrence of coronary events were done in patients with previous IHD. Their baseline characteristics are also given in Table 2. When we analyzed the data of patients with or without coronary risk factors as an observational study irrespective of allocated drugs, there were statistical differences between these 2 groups. Analyses were then adjusted by baseline characteristics as described in the following section.

Statistical Analysis

Data are expressed as mean±SD or proportions. Risk-adjusted cumulative incidence of coronary events was calculated using the corrected group prognosis method,[8] with adjustment for baseline characteristics, including history of prior anti-hypertensive treatment, allocated drugs, age, sex, BMI, type 2 DM, history of cerebrovascular disease, history of IHD, renal dysfunction, history of vascular disease, SBP and DBP at baseline. The hazard ratio (HR) and 95% confidence intervals (95%CIs) were estimated using Cox regression analysis. We also used multiple Cox regression analysis to examine the association between rate of coronary events and coronary risk factors (gender, age, allocated drugs, BMI, SBP, DBP, smoking, DM, HL, LVH, previous IHD, previous cerebrovascular disease, CKD, and previous anti-hypertensive medication). All statistical tests were 2-sided with an alpha level of 0.05, and were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA).

Results

Changes in BP

Blood pressure was generally controlled to <140/80 mmHg in both groups. Mean SBP/DBP was 162.9±14.2/91.7±11.2 mmHg at baseline and 135.4±11.9/77.0±9.2 mmHg after 3 years. While the mean SBP/DBP in patients without previous IHD was 163.6±14.3/92.4±11.3 mmHg at baseline and

Table 3. Prognostic Value of Coronary Risk Factors in All Enrolled Patients

	HR (95%CI)	P value
Male (vs. female)*	1.81 (1.06–3.10)	0.031
Age: 10-year increase	1.09 (0.83–1.44)	0.525
Amlodipine (vs. candesartan)	1.32 (0.85–2.04)	0.214
BMI: 1-kg/m² increase	1.01 (0.83–1.44)	0.525
SBP (at entry): 10-mmHg increase	1.14 (0.96–1.37)	0.145
DBP (at entry): 10-mmHg decrease*	1.36 (1.09–1.68)	0.006
Smoking: no (vs. yes)	1.02 (0.62–1.69)	0.945
DM: no (vs. yes)*	3.10 (1.90–5.04)	<0.001
HL: no (vs. yes)	1.36 (0.86–2.14)	0.186
LVH: no (vs. yes)	1.26 (0.80–1.99)	0.319
Previous IHD: no (vs. yes)*	3.89 (2.40–6.31)	<0.001
Previous stroke: no (vs. yes)	0.78 (0.34–1.82)	0.569
CKD: no (vs. yes)*	1.60 (1.01–2.54)	0.046
Previous AHD: no (vs. yes)	1.54 (0.85–2.81)	0.159

HR, hazard ratio; CI, confidence interval. Other abbreviations see in Tables 1,2.
*P<0.05.

Table 4. Prognostic Value of Coronary Risk Factors in Patients Without Previous IHD (Primary Prevention)

	HR (95%CI)	P value
Male (vs. female)	1.44 (0.73–2.84)	0.294
Age: 10-year increase	1.18 (0.83–1.64)	0.364
Amlodipine (vs. candesartan)	1.07 (0.61–1.87)	0.811
BMI: 1-kg/m² increase	0.99 (0.91–1.07)	0.810
SBP (at entry): 10-mmHg increase	1.18 (0.94–1.54)	0.160
DBP (at entry): 10-mmHg decrease	1.39 (1.05–2.18)	0.020
Smoking: no (vs. yes)	0.70 (0.35–1.37)	0.296
DM: no (vs. yes)	4.88 (2.35–10.16)	<0.001
HL: no (vs. yes)	2.67 (1.47–4.85)	0.001
LVH: no (vs. yes)	1.21 (0.65–2.24)	0.555
Previous stroke: no (vs. yes)	1.03 (0.40–2.63)	0.957
CKD: no (vs. yes)	1.21 (0.67–2.19)	0.521
Previous AHD: no (vs. yes)	1.19 (0.62–2.28)	0.595

Abbreviations see in Tables 1–3.
*P<0.05.

Table 5. Prognostic Value of Coronary Risk Factors in Patients With Previous IHD (Secondary Prevention)

	HR (95%CI)	P value
Male (vs. female)*	3.05 (1.15–14.63)	0.025
Age: 10-year increase	1.00 (0.63–1.28)	0.992
Amlodipine (vs. candesartan)	1.77 (0.87–3.63)	0.117
BMI: 1-kg/m² increase	1.03 (0.91–1.15)	0.673
SBP (at entry): 10-mmHg increase	1.16 (0.84–1.57)	0.360
DBP (at entry): 10-mmHg decrease	1.22 (0.82–1.81)	0.323
Smoking: no (vs. yes)	1.54 (0.72–3.45)	0.268
DM: no (vs. yes)*	2.15 (1.06–4.38)	0.035
HL: no (vs. yes)	0.54 (0.26–1.12)	0.098
LVH: no (vs. yes)	1.39 (0.69–2.81)	0.360
Previous stroke: no (vs. yes)	0.34 (0.05–2.54)	0.290
CKD: no (vs. yes)*	2.44 (1.16–5.13)	0.018
Previous AHD: no (vs. yes)	5.13 (0.68–38.49)	0.112

Abbreviations see in Tables 1–3.
*P<0.05.

135.7±12.0/77.3±9.3 mmHg after 3 years, the mean SBP/DBP in patients with previous IHD was 157.5±12.3/86.9±9.1 mmHg at baseline and 133.2±11.4/75.1±8.2 mmHg after 3 years. But both SBP and DBP in patients with previous IHD were slightly but significantly lower than in those without previous IHD at baseline and after 3 years (P<0.001, respectively).

Prognostic Value of Coronary Risk Factors for Coronary Event Rate

During 3.2±0.9 years of follow-up (5th–95th percentile interval, 1.0–4.2), coronary events occurred in 50 patients without previous IHD (1.2%; 15 sudden death, 11 angina pectoris, 24 myocardial infarction) at baseline for a rate of 3.7 per 1000 patient-years and in 33 patients with previous IHD (5.5%; 11 sudden death, 11 angina pectoris, 11 myocardial infarction) at baseline for a rate of 16.9 per 1,000 patient-years (adjusted HR, 3.89; 95%CI: 2.40–6.31; P<0.001; **Figure**). We also evaluated the prognostic value of the coronary risk factors in all enrolled patients. As shown in **Table 3**, the onset of coronary events was significantly associated with previous IHD (adjusted HR, 3.89), DM (adjusted HR, 3.10; 95%CI: 1.90–5.04; P<0.001), male sex (adjusted HR, 1.81; 95%CI: 1.06–3.10; P=0.031), CKD (adjusted HR, 1.60; 95%CI: 1.01–2.54; P=0.046), and DBP (10-mmHg decrease, adjusted HR, 1.36; 95%CI: 1.09–1.68; P=0.006).

In addition, the prognostic value of the coronary risk factors for each event category was evaluated in patients without previous IHD (primary prevention) and in patients with previous IHD (secondary prevention), respectively. As shown in **Table 4**, the onset of coronary events in patients without previous IHD was significantly associated with DM (adjusted HR, 4.88; 95%CI: 2.35–10.16; P<0.001), HL (adjusted HR, 2.67; 95%CI: 1.47–4.85; P=0.001), and DBP (10 mmHg decrease, adjusted HR, 1.39; 95%CI: 1.05–2.18; P=0.02). In patients with previous IHD, the incidence of coronary events was significantly associated with male sex (adjusted HR, 3.05; 95%CI: 1.15–14.63; P=0.025), CKD (adjusted HR, 2.44; 95%CI: 1.16–5.13; P=0.018), and DM (adjusted HR, 2.15; 95%CI: 1.06–4.38; P=0.035; **Table 5**). Although a significant difference was observed in the risk factors between primary prevention and secondary prevention, interactions between the coronary risk factors and previous IHD were not significant except for HL. Notably, DM was the common predictor of coronary events both in primary and secondary prevention.

Discussion

Increases in BP loads induce myocardial remodeling, such as cardiac hypertrophy, myocardial fibrosis and coronary endothelial damage. Progression of myocardial remodeling and coronary atherosclerosis leads to coronary artery disease, heart failure, arrhythmia and sudden death. In men, morbidity and mortality rates due to coronary artery disease increase by approximately 15% with a 10-mmHg increase in SBP.[9] Conventional anti-hypertensive drug therapy primarily using diuretics and β-blockers, however, does not markedly reduce the incidence of coronary artery disease, whereas it markedly decreases the incidence of stroke.[10] Coronary risk factors other than hypertension may have a markedly different impact on the occurrence of coronary artery disease.[11] Accordingly, the aim of the present study was to clarify the influence of coronary risk factors on coronary events in Japanese high-risk hypertensive patients as a subanalysis of the CASE-J trial. The follow-up rate and coronary event evaluation are markedly reliable from this cohort study. Furthermore, although some