rhythmias and improved early survival prognosis (10). The mechanism of the action of ghrelin seems to be related to CSNA. For instance, some studies have shown that ghrelin is able to centrally suppress renal sympathetic nerve activity (11, 12), and peripheral treatment also appears to attenuate cardiac sympathetic tone within the first week after MI (13).

Currently, however, there is a paucity of studies describing the function of endogenous ghrelin from within the first few hours of onset to long term after MI. In the present study, we investigated whether ghrelin-knockout mice have increased CSNA, multiple arrhythmias, and high mortality after MI, and whether the underlying mechanism is associated with an impaired suppression of CSNA.

Materials and Methods

Animal models of MI

All experiments were approved by the Animal Ethics Committee of the National Cerebral and Cardiovascular Center Research Institute and were conducted in accordance with the guidelines of the Physiological Society of Japan. Myocardial infarction was induced in male ghrelin-knockout (KO) mice and their wild-type (WT) C57BL/6J littermates (12–14 wk of age). They were anesthetized with isoflurane (induced at 4%, maintained at 1.5%), intubated, and supported by an animal ventilator (stroke volume, 150 μl; respiratory rate, 135 strokes/min). After a left thoracotomy, the left anterior descending coronary artery was ligated 2–3 mm from its origin using a 6–0 Prolene suture. The chest was closed, and the mice were allowed to recover. During the procedure, electrocardiography (ECG) signals were recorded until 30 min after MI. All mice were on a 12-h light, 12-h dark cycle at 25 C, and were provided with food and water *ad libitum*.

Measurement of conscious arterial blood pressure (BP) and heart rate (HR)

HR and systolic and diastolic BP were recorded using an automatic sphygmomanometer tail-cuff pressure transducer (BP-98A; Softron Co. Ltd., Tokyo, Japan). Each value recorded was derived from three consecutive measurements (within 2 min), which were then averaged to give one value representative of each experimental condition.

Histological examination

After being killed 2 wk post-MI, the mice hearts were divided from apex to base into two equal transverse sections. The apex part was fixed with 4% paraformaldehyde and was then embedded in paraffin. Paraffin sections were stained using Masson's trichrome and hematoxylin and eosin staining for measurement of infarct size. Slices were mounted and photographed. We determined the total infarct size by measuring the area of the infarction for each slice, multiplying the area by the slice thickness, and summing the area of all slices. Infarct size was presented as a percentage of the total left ventricular wall.

Catecholamine measurement

In separate groups of animals, we collected blood samples and whole hearts before MI and 15 and 30 min after MI. Plasma concentrations and heart tissue homolytic solution concentrations of epinephrine, norepinephrine, and dopamine were measured by HPLC (BML, Tokyo, Japan).

Ghrelin measurement

Blood samples were obtained from WT mice before MI and 15 min, 30 min, and 1 d after MI. Plasma was separated for ghrelin measurement using RIA system (14).

Electrocardiogram monitoring of arrhythmia and HR variability analysis

Mice were anesthetized with ip injections of combination urethane-chloralose (750 and 35 mg/kg, respectively), with supplemental doses as needed (15). ECG signals were then recorded using a physiological analyzing system (Bio Amp, AD Instruments, CA). After the HR had stabilized, we recorded at baseline for a minimum of 15 min; next, ghrelin (150 μg/kg; Peptide Institute, Inc., Osaka, Japan) was injected sc. Signals were recorded for 15 min thereafter. In cases in which methylatropine bromide (1 mg/kg) was administered, it was injected ip 5 min after the ghrelin injection, and recordings were made for 10 min. We then performed the MI procedure, followed by recording for 30 min. Autonomic nervous function was examined by a power spectral analysis of HR variability (LabChart Pro 7.0, ADInstruments, Sydney, Australia). HR was used to generate a power spectral density curve by using the fast Fourier transform. The range of the low-frequency (LF, 0.4–1.5 Hz) or high-frequency (HF, 1.5–5 Hz) component was chosen on the basis of other previous studies (16). From these, we calculated the parameters of LF, HF, percentage of LF in total power (nLF), percentage of HF in total power (nHF), and ratio of LF to HF power (LF/HF).

Perineural capsaicin treatment

Capsaicin, an afferent neurotoxin, has been used in many nervous blockade experiments (17–19). After the mice were anesthetized with 1.5% isoflurane, the skin was incised, and the vagal nerves were isolated in the neck using blunt dissection. To block the conduction of vagal afferent fibers, Kimwipes pledgets soaked with 1.0% capsaicin in cold-pressed, extra virgin olive oil (Sigma, St. Louis, MO) were placed topically on each nerve for 30 min. A strip of Parafilm (4.0 mm wide) was placed under the vagus nerves to prevent capsaicin from coming into contact with the surrounding tissue. After the capsaicin exposure, the pledgets were removed, the nerves were rinsed with saline, and the skin was sutured. The control group underwent nerve exposure and treatment with the vehicle. The mice were tested 4 d after perineural application of capsaicin or vehicle, using the HR variability protocol described above.

Statistical analysis

All values are expressed as means ± SEM. One-way ANOVA was used to test for differences among the groups of mice. Where statistical significance was reached, *post hoc* analyses were incorporated using the paired or unpaired *t* test with the Dunnet's correction for multiple comparisons. The Kaplan-Meier survival analysis was performed to compare survival curves between KO

and WT mice after MI. A value of $P < 0.05$ was considered statistically significant.

Results

Baseline body weight, HR, and BP

The baseline characteristics of the ghrelin knockout (KO) and wild-type (WT) genotypes are shown in Supplemental Table 1 published on Te Endocrine Society's Journals Online web site at http://endo.endojournals.org. There were no differences in body weight, HR, or systolic and diastolic BP between the groups.

Mortality and cause of death after MI

Postoperative survival was monitored for 2 wk (Fig. 1). Despite the fact that infarct sizes in the surviving animals were similar in the two groups (KO 59.7 ± 12.1% *vs.* WT 60.4 ± 12.7%; Supplemental Fig. 1), the total survival rate was significantly lower among KO mice (26.9%; 12 of 46) than among WT mice (41.5%; 20 of 41; $P < 0.05$). Based on ECG recordings and postmortem findings, the causes of death were classified into three groups: malignant arrhythmia within 30 min of the MI, LV rupture, or heart failure (Table 1). LV rupture was diagnosed from the large amount of blood observed filling the chest cavity, and heart failure was based on obvious pulmonary edema and pleural effusion. The incidence of malignant arrhythmia was significantly higher in KO mice than in WT mice (17.4%, eight of 46 *vs.* 2.4%, one of 41; $P < 0.05$), and death by heart failure was also higher in KO mice (10.9% *vs.* 0%; $P < 0.05$). There were no significant differences in the incidences of LV rupture between the two groups.

Catecholamine and ghrelin measurement

Before MI, the plasma concentrations of epinephrine and norepinephrine did not significantly differ between WT and KO mice, but 15 and 30 min after MI, the plasma concentrations in KO mice dramatically increased over

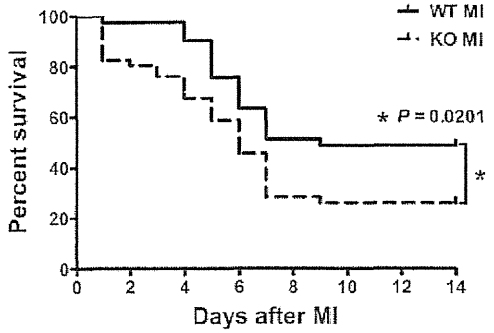

FIG. 1. . Survival curve. The survival rate of ghrelin-KO mice (n = 46) was significantly lower than that of WT mice (n = 41) within 2 wk after MI ($P = 0.0201$). KO, Ghrelin-KO.

that in WT mice, especially at 30 min. At 30 min, epinephrine was approximately 5-fold and norepinephrine approximately 6-fold higher in KO mice than in WT mice ($P < 0.05$). In heart tissue, a significant difference between the two genotypes was observed only in norepinephrine, and this was observed both before and after MI. In WT mice, the content of norepinephrine reached a peak at 15 min, and the concentration reduced from this at 30 min. In KO mice, norepinephrine was higher at 30 min than at 15 min ($P < 0.05$; Fig. 2). Furthermore, there is no apparent change in plasma ghrelin level before MI and 15 min, 30 min, and 1 d after MI in WT mice (Supplemental Fig. 2).

HR variability, arrhythmia, and mortality before and after MI

As shown in Figs. 3 and 4, we next examined the HR variability before and after MI. This technique has been used to examine cardiac autonomic activity separately for the sympathetic and parasympathetic nerves in humans and rodents (16, 20). In anesthetized KO mice, at baseline, the CSNA represented by LF/HF was significantly higher than that in WT mice (2.18 ± 0.43 *vs.* 0.98 ± 0.09; $P < 0.05$), although the parasympathetic nerve activity represented by nHF was not significantly different. We observed a decrease in nHF in the KO mice after MI that was lower than that observed in WT mice ($P < 0.05$), and an outburst of LF/HF that was significantly higher than that in WT mice (25.5 ± 11.8 *vs.* 1.4 ± 0.3; $P < 0.05$). In KO mice, this was accompanied with an increased percentage of arrhythmia beats to total beats in the 30 min after MI and a higher mortality caused by malignant arrhythmia within the 30 min after MI ($P < 0.05$).

Pre-MI ghrelin administration

In anesthetized mice at baseline, 15 min before MI, there was no obvious change in HR or BP after an acute sc administration of ghrelin (150 μg/kg; Supplemental Fig. 3). In KO mice, ghrelin significantly decreased CSNA represented by LF/HF after MI and significantly increased parasympathetic nerve activity represented by nHF, although the CSNA and parasympathetic nerve activity at baseline remained unchanged (Fig. 3). In particular, the surge in the LF/HF ratio in KO mice after MI was significantly suppressed by ghrelin. In conjunction with the CSNA inhibition, the percentage of arrhythmias and the mortality of the KO mice within 30 min after MI were reduced (52.4%, 11 of 21 *vs.* 18.2%, four of 22; $P < 0.05$; Fig. 4).

Blockade of vagal nerves by methylatropine bromide

Administration of the pharmaceutical anticholinergic methylatropine bromide (1 mg/kg) after the ghrelin injec-

TABLE 1. Causes of death within 2 wk after MI

	n	Mortality (% of n)	Causes of death (% of N)		
			Arrhythmias within 30 min	Rupture of the heart	Heart failure
WT	41	21(58.5%)	1(2.4%)	20(48.8%)	0(0%)
KO	46	34(73.9%)[a]	8(17.4%)[a]	21(45.7%)	5(10.9%)[a]

MI was introduced by ligation of left anterior descending coronary artery. The causes of death were classified into three groups: malignant arrhythmia on ECG recordings within 30 min after MI, LV rupture diagnosed from the large amount of blood observed filling the chest cavity, and heart failure based on obvious pulmonary edema and pleural effusion.

[a] $P < 0.05$ compared with the WT mice.

tion increased HR but had no obvious effect on the LF/HF ratio or nHF in KO mice before MI. However, the inhibitory effect of ghrelin on the CSNA after MI was completely blocked by methylatropine bromide in KO mice (Fig. 3). Moreover, compared with only administration of ghrelin, this combined administration increased the incidence of arrhythmias and significantly reduced the survival rate of KO mice after MI (55.6%, 5 of 9 *vs.* 18.2%, 4 of 22; $P < 0.05$; Fig. 4).

Blockade of vagal afferent nerves by perineural capsaicin

A similar result as with methylatropine bromide administration was observed after perineural treatment of both cervical vagal nerves with capsaicin. In KO mice, before MI, there was no detectable difference in the LF/HF ratio or nHF after ghrelin injection between the capsaicin and vehicle groups. However, after MI, the ability of ghrelin to inhibit CSNA was completely suppressed by blockade of the vagal afferent nerves; the LF/HF ratio significantly increased in the capsaicin group, and the nHF decreased ($P < 0.05$). Correspondingly, there was an increased incidence of arrhythmias and mortality in the capsaicin group compared with the vehicle group (53.9%, 7 of 13 *vs.* 16.7%, two of 12; $P < 0.05$; Fig. 5).

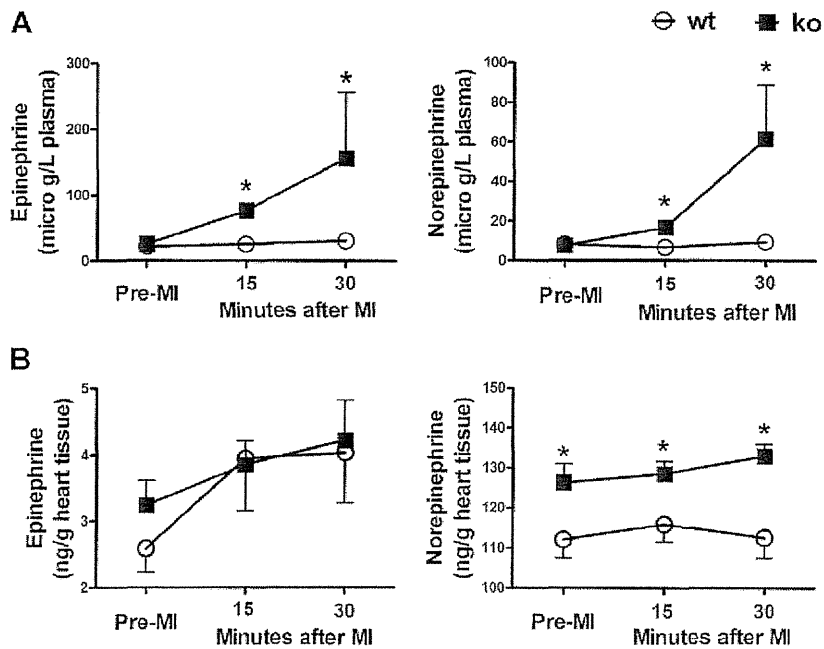

FIG. 2. Catecholamine measurement. The plasma concentrations (A) and the content in heart tissue (B) of epinephrine and norepinephrine of WT and ghrelin-KO mice before MI and 15 and 30 min after MI. Values are the mean ± SEM of eight mice using HPLC. *, $P < 0.05$ *vs.* WT mice. KO, Ghrelin-KO; L, liter.

Discussion

In the present study, using KO mice, we first demonstrated that endogenous ghrelin plays crucial roles in preventing the adverse increase in CSNA, and then reducing arrhythmias after acute MI. Ghrelin also improves the survival prognosis for the 2 wk after MI. It is important to emphasize that within the first 30 min of MI, a significantly greater incidence of malignant arrhythmias was observed in KO mice. These arrhythmias were accompanied with a surge in CSNA, which can be suppressed, and the associated mortality reduced by prior administration of ghrelin. Taken together, the cardioprotective effects of ghrelin, at least in the acute phase, are likely to be mainly mediated by the suppression of CSNA.

In previous studies, it has been shown that MI in anesthetized animal models initiated an increase in CSNA, and a subsequent decrease in parasympathetic nerve activity (21–23). After MI, in conscious rats, a chronic administration of ghrelin decreased the activated LF and LF/HF ratio, reflecting CSNA (13). Moreover, early ghrelin intervention can prevent the adverse increase in CSNA after acute MI, as well as reduce arrhythmias and improve early survival prognosis (10). In this study, the higher incidence of arrhythmias and mortality of KO mice was ver-

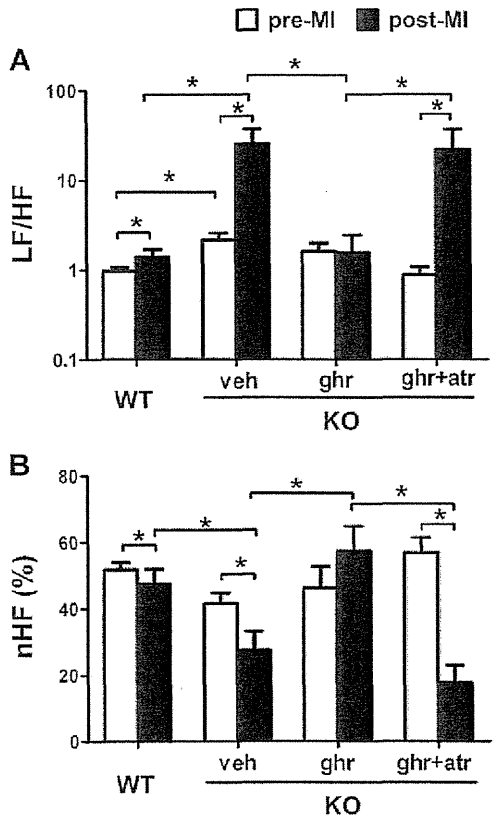

FIG. 3. HR variability analysis. Cardiac sympathetic nerve activity represented by LF/HF (A), and parasympathetic nerve activity represented by nHF (B) of WT and ghrelin-KO mice (with vehicle, ghrelin only, or ghrelin plus methylatropine bromide administration) before or after MI. Values are the mean ± SEM of 17, 13, 10, and nine mice in WT, KO veh, KO ghr, and KO ghr + atr groups, respectively. *, $P < 0.05$. KO, ghrelin-knockout; veh: vehicle administration; ghr: ghrelin (150 μg/kg, sc); atr: methylatropine bromide (1 mg/kg, ip).

ified using two different anesthetics, isoflurane and urethane-chloralose. Isoflurane has potential antiarrhythmic effects (24–26); hence, we used urethane-chloralose when measuring HR variability. Our present data also show that at baseline, the CSNA of the KO mice was significantly stronger than that of the WT mice. The CSNA of both genotypes increased after MI; however, in the ghrelin KO mice, the outburst of CSNA was nearly 20 times that of WT mice, as represented by the LF/HF ratio. Therefore, endogenous ghrelin seems to have an important effect on balancing CSNA and parasympathetic nerve activity, especially in a critical case.

Another evidence of systemic and CSNA comes from the catecholamine measurements. Circulating catecholamines, epinephrine, and norepinephrine originate from two sources. Epinephrine is released by the adrenal medulla upon activation of preganglionic sympathetic nerves. Norepinephrine is also released by the adrenal medulla, but the primary source of circulating norepinephrine is spillover from postganglionic fibers of the sympathetic nerves innervating blood vessels. Before MI, there were no differences between the two genotypes, but in KO mice after MI, catecholamine concentrations dramatically increased to 5–6 times those of the WT mice. These findings reflect stronger systemic sympathetic nerve activation in KO mice after MI. Moreover, in KO mice, the heart tissue content of norepinephrine, mainly produced from cardiac postganglionic sympathetic nerves, was higher than that of WT mice before and after MI, consistent with the results of CSNA using the HR variability analysis. Although the increase in CSNA after MI appears to have immediate benefits (*i.e.* providing inotropic support to the heart to maintain cardiac output), this enhanced sympathetic tone is associated with an increased risk of ventricular arrhythmias (1), a leading cause of heart failure and sudden death (27). Indeed, we noted in our study that ventricular arrhythmias often preceded, or even instigated, sudden cardiac death. In this study, we also showed that ghrelin treatment just before MI prevented the adverse increase in CSNA and reduced the incidence of arrhythmias and mortality in KO mice, within the first 30 min after MI.

There is accumulated evidence that supports an indirect cardioprotective effect of ghrelin through the central modulation of CSNA and parasympathetic nerve activity (11–13, 28, 29). Studies have shown that the receptor for ghrelin is located in the main cardiovascular control centers in neurons of the nucleus tractus solitarius (NTS) (12), and that the central administration of ghrelin directly attenuates renal sympathetic nerve activity (11, 12). However, it has also been shown that ghrelin receptors are synthesized in vagal afferent neurons and transported to the afferent terminals in the stomach, and that ghrelin produced in the stomach stimulates the gastric vagal afferent nerve and influences neuronal activity in the NTS, resulting in an increase in feeding behavior. Thus, blockade of the gastric vagal afferent nerve abolishes ghrelin-induced feeding, GH secretion, and activation of neuropeptide Y-producing and GHRH-producing neurons (17). We have also previously demonstrated the existence of GHS-R in rat myocardium. Costaining with acetylcholine esterase suggests that the GHS-R is localized in the vagal nerve terminals in the heart (13). In this study, using immunofluorescence analysis, it was also shown that GHS-R1a was exclusively detected on, or at least in the very close proximity of, the choline acetyltransferase-positive nervous terminals in mouse heart (Supplemental Fig. 4). In addition, several studies have already demonstrated a cardiocardiac negative feedback reflex that occurs through a vagal afferent inhibition of sympathetic efferent activity (30–32). Therefore, peripheral ghrelin potentially also

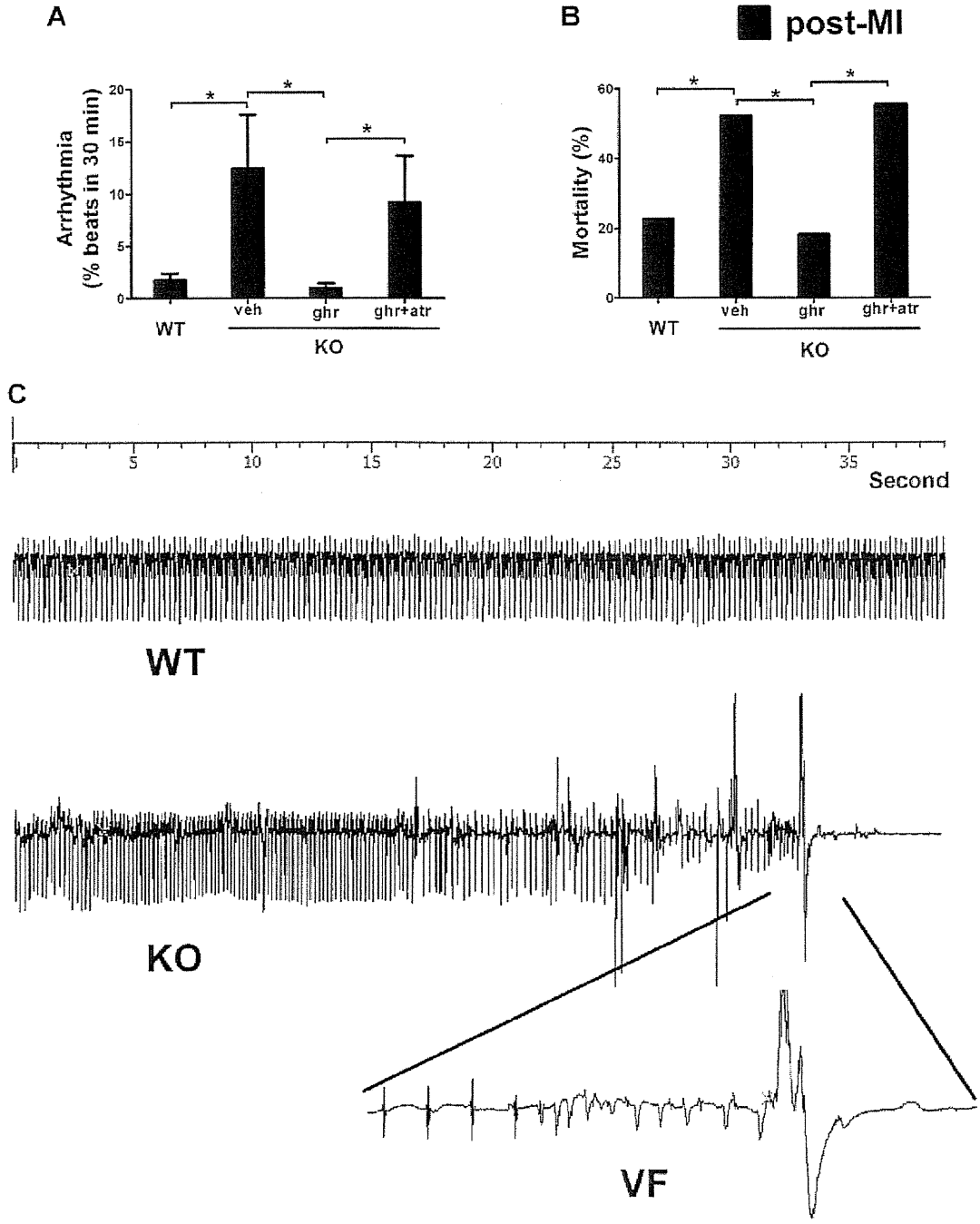

FIG. 4. Arrhythmias and mortality. Arrhythmias as a percentage of total beats 30 min after MI (A) and mortality (B) of WT and ghrelin-KO mice (with vehicle, ghrelin only, or ghrelin plus methylatropine bromide administration) after MI. C, A typical presentation of an electrocardiogram after MI. Values of arrhythmias (A) are the mean ± SEM of 22, 21, 22, and nine mice in WT, KO veh, KO ghr, and KO ghr + atr groups, respectively. Mortality (B) are five of 22 in WT, 11 of 21 in KO veh, four of 22 in KO ghr, and five of nine in KO ghr + atr groups, respectively. *, $P < 0.05$. KO, ghrelin-KO; VF, ventricular fibrillation; veh, vehicle administration; ghr, ghrelin (150 μg/kg, sc); atr, methylatropine bromide (1 mg/kg, ip).

acts on the cardiac vagal afferent nerve, which sends projection to NTS, resulting in a decrease in CSNA of mice with MI (33), although other possibilities cannot be excluded, *i.e.* ghrelin may also act on cardiac myocytes and produce effects. The findings of our present study support the hypothesis. After blockade of the vagal nerves by methylatropine bromide administration or perineural capsaicin treatment, the beneficial effects of ghrelin, such as inhibition of CSNA and arrhythmias and improvement of prognosis, were dramatically abolished. Therefore, the effect of ghrelin on CSNA is likely to be mainly through its actions on the vagal afferent nerves.

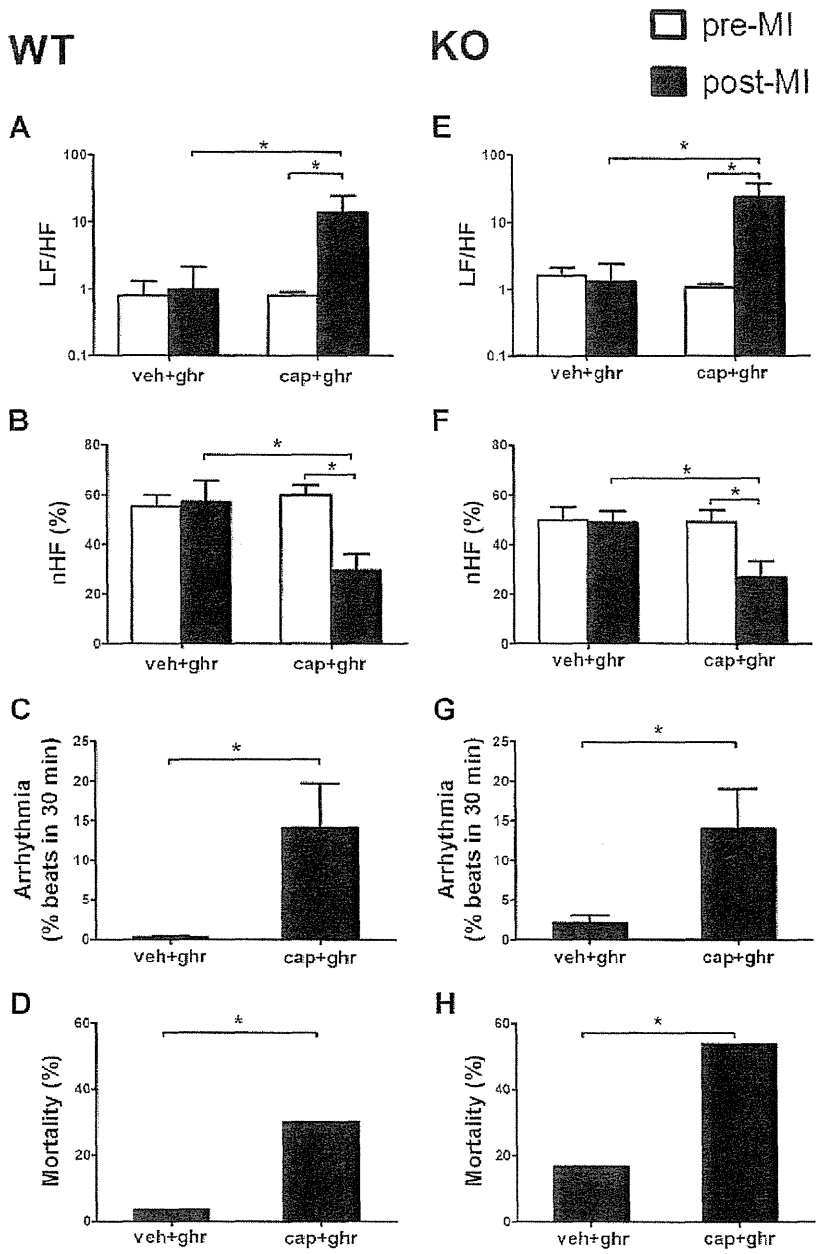

FIG. 5. Vagal afferent nerve blockade with capsaicin. Cardiac sympathetic nerve activity represented by LF/HF (A and E), and parasympathetic nerve activity represented by nHF (B and F) before or after MI. Arrhythmias as a percentage of total beats 30 min after MI (C and G), and mortality (D and H) of WT and ghrelin-KO. Values of A, B, C, E, F, and G are the mean ± SEM of 12, 10, 12, and 13 mice in WT veh + ghr, WT cap + ghr, KO veh + ghr, KO cap + ghr groups, respectively. Deaths (D and H) are one of 28 in WT veh + ghr, three of 10 in WT cap +ghr, two of 12 in KO veh + ghr, and seven of 13 in KO cap + ghr groups, respectively. *, $P < 0.05$. KO, ghrelin-KO; ghr, ghrelin (150 μg/kg, sc); veh, perineural treatment of both cervical vagal nerves with vehicle; cap, perineural treatment of both cervical vagal nerves with 1.0% capsaicin for 30 min.

In our study, we also found that therapeutic doses of ghrelin have little influence on BP, HR, and CSNA at baseline. Previous research in conscious rats after MI has shown that an acute administration of ghrelin decreased the activated LF and LF/HF ratio; however, in sham-operated rats, the LF, LF/HF ratio, and HR were not substantially affected by ghrelin administration. Thus, these findings indicate that ghrelin may have a stronger effect on the activated sympathetic nervous system than on the non-activated system (13). Similar phenomena have also been observed in humans (34). Therefore, ghrelin could be a relatively safe therapeutic agent in the treatment of MI.

Since the discovery of ghrelin about 10 yr ago, several potential physiological functions of exogenous ghrelin are

being demonstrated, including GH secretion, increase of food intake, as well as some beneficial effects on the cardiovascular system as were shown in our previous studies (10, 13). The present study shows that not only exogenous but also endogenous ghrelin suppresses CSNA, prevents the incidence of arrhythmias, and improves prognosis after acute MI. These beneficial effects of ghrelin are likely to be mainly vagally mediated. These data suggest the potential usefulness of ghrelin as a new therapeutic agent in MI.

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Review

Ghrelin and cardiovascular diseases

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Ghrelin;
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Sympathetic nerve

Summary In 1999, a peptide from the stomach called ghrelin was discovered, which exerts potent growth hormone releasing powers. Subsequent studies revealed that it exerts a potent orexigenic action. In addition, the beneficial effects of ghrelin in cardiovascular diseases have been recently suggested. In humans as well as in animals, administration of ghrelin improves cardiac function and remodeling in chronic heart failure. In an animal model for myocardial infarction, ghrelin treatment early after coronary ligation effectively reduces fatal arrhythmia and, consequently, mortality, suggesting the potential therapeutic role of the peptide in acute myocardial infarction. Although how ghrelin may influence the cardiovascular system is not fully understood, the cardiovascular beneficial effects are mediated possibly through a combination of various actions, such as an increase in growth hormone level, an improvement in energy balance, direct actions to the cardiovascular cells, and regulation of the autonomic nervous activity. Of note, current experimental evidence suggests that ghrelin may act centrally to decrease sympathetic nervous system activity through peripheral afferent nerve. Thus, administration of ghrelin might become a unique new therapy for cardiovascular diseases.

Contents

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Introduction

Small synthetic compounds called growth hormone secretagogues (GHS) stimulate the release of growth hormone (GH) from the pituitary [1]. In 1996, a receptor for GHS (GHS-R) was cloned from swine and human pituitary gland, and it was revealed that GHS-R is one of the G-protein coupled receptors with seven membrane spanning domains [2]. Since the discovery of the receptor, the quest for searching the endogenous ligand for GHS-R started by many groups worldwide. Most of them thought that the endogenous ligand should be found in the brain and selected the brain as a starting material for the purification of the ligand, based on the assumption that pituitary hormones are regulated by midbrain-pituitary axis. However, although studied thoroughly and extensively, all purification attempts using the brain as a source ended unsuccessfully. Thus, the endogenous ligand was not found until 1999, when it was finally identified from the stomach, the tissue that almost nobody had expected as a source. Using a reverse pharmacology approach, a 28 amino-acid peptide was isolated from the rat stomach and named ''Ghrelin'' derived both from the word root ''ghre'' in Proto-Indo-European languages meaning ''grow'', and from the abbreviation for ''GH-release,'' a characteristic feature of the peptide [3]. Since the discovery of ghrelin, a number of unique features have been identified. First, the discovery of ghrelin from the stomach indicates that the release of GH from the pituitary is regulated not only by the hypothalamus but also by the digestive tract. Second, ghrelin has a unique structural property that is an acylation in its third residue, usually serine. This is the first peptide hormone with acyl modification. Interestingly, the acylation is essential for the ghrelin's ability for the binding to and the activation of its receptor, GHS-R. Third, subsequent studies revealed that exogenously administered ghrelin potently stimulates appetite in humans and in rodents [4,5]. Fourth, not only in the release of GH and in the stimulation of appetite, the roles of ghrelin have also been implicated in the cardiovascular, bone, gastrointestinal, and immune systems [6]. In the present review, we will discuss some of these characteristic features of ghrelin and its possible therapeutic roles in cardiovascular diseases.

Ghrelin is a potent GH secretagogue

Ghrelin was originally discovered as an endogenous ligand for GHS-R and, in fact, has potent GH releasing activity. Intravenous ghrelin administration markedly increases plasma GH levels in humans and in rats. After a bolus ghrelin injection, the level of GH peaks at 15—20 min and the elevation lasts longer than 60 min thereafter [7]. Since the effect of ghrelin on the release of GH is not observed after resection of the gastric branch of the vagus nerve, the vagal afferent nerve is supposed to mediate the effect [8]. The hypothesis is supported by the fact that GHS-R is synthesized in vagal afferent neurons and transported to the afferent nerve terminals [8,9].

Ghrelin as a gastrointestinal hormone

X/A-like cells are among four types of endocrine cells in the oxyntic mucosa of the stomach, and so named as their function had been undefined until recently and as their morphology is similar to pancreatic alpha cells. In situ analysis revealed that ghrelin and its mRNA are mainly localized in X/A-like cells. In the stomach, the 28 amino acids of mature ghrelin are cleaved off from its precursor preproghrelin which is composed of 117 amino acids in rats or in humans. From the submucosal layer of the stomach, ghrelin is secreted into the blood stream (not into the gastrointestinal tract). From plasma ghrelin levels in patients with gastrectomy or gastric bypass surgery, it is demonstrated that the stomach is a major organ secreting the circulating ghrelin [10]. Although the contents are much less, cells producing ghrelin are also found in the intestines and in specific regions of the brain such as the arcuate nucleus.

Octanoyl modification

The distinguished structural feature of ghrelin is its fatty acid modification at the third residue (serine in most species including humans) [3,11] (Fig. 1). Interestingly, the acylation, particularly n-octanoyl modification, is conserved among many species including mammals, fish, birds, and

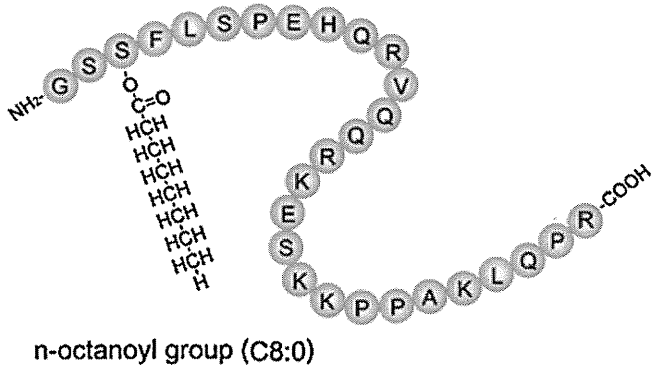

Figure 1 The structure of human ghrelin. Ghrelin is a 28 amino acid peptide discovered from the stomach. A distinguished structural feature of ghrelin is its n-octanoylation at the third serine residue, which is necessary for its receptor binding and function.

amphibians and is necessary for full binding of ghrelin to its receptor, GHS-R, and thus for expressing the biological function. The attachment of octanoate to the third serine residue of ghrelin is catalyzed by a membrane-bound enzyme, named ghrelin O-acyltransferase (GOAT) [12].

Localization of ghrelin receptor, GHS-R

In the brain, gene expression of the receptor for ghrelin, GHS-R, is detected predominantly in the arcuate nuclei, in the ventromedial nuclei, and in the hypocampus [2]. To a lesser extent, it is also detected in pituitary and in detate gyrus. Outside of the brain, various organs including lung, liver, kidney, pancreas, and gastrointestinal tract expressed GHS-R gene. In the cardiovascular system, GHS-R is expressed in the heart and in the aorta. It is also reported that, GHS-R gene can be detected in cultured cardiomyocyte cell line and in human vascular endothelial cells.

Ghrelin as a hunger hormone

Exogenously administered ghrelin has a potent appetite-stimulating effect [4]. Since the orexigenic effect of ghrelin can be observed in GH-deficient dwarf rats, the appetite-promoting effect is independent of GH release. The plasma level of ghrelin and mRNA level in the stomach are increased by fasting and decreased by feeding [10,13]. Oral or intravenous administration of glucose decreases plasma ghrelin level. Since ghrelin has a potent orexigenic action, ghrelin can serve as a ''hunger hormone.'' In addition, fasting plasma ghrelin level is low in obese people [14] and high in lean people and in patients with anorexia nervosa. Since ghrelin induces weight gain by promoting appetite and by reducing fat utilization [15], the nutritional state seems to be a major determinant of release of ghrelin from the stomach.

Multiple actions of ghrelin

Since its discovery, many studies were conducted and it has been demonstrated that ghrelin has multiple biological actions, all of which could affect the cardiovascular system.

Activation of GH/IGF-1 pathway

Ghrelin activates the pathway of GH and its mediator, insulin-like growth factor-1 (IGF-1), both of which are anabolic hormones necessary for skeletal and myocardial growth and for metabolic homeostasis. Since GH/IGF-1 exerts effects on cardiac structure and function, ghrelin can affect the cardiovascular system through the elevation of plasma GH levels.

Stimulation of appetite

Endogenous ghrelin and its receptor are involved in the regulation of food intake and adiposity. Intravenous infusion of ghrelin is reported to increase food intake and body weight in healthy subjects [16–18] and to stimulate appetite and food intake in patients with congestive heart failure [19], chronic obstructive pulmonary disease [20], cancer [21], functional dyspepsia [22], and anorexia nervosa [23]. Recently, in a prospective randomized, placebo-controlled, clinical trial, it was suggested that administration of ghrelin after esophagectomy increased oral food intake, attenuated weight loss, and improved decreased lean body weight after operation [24]. Cachexia, which is a catabolic state characterized by weight loss and muscle wasting, is associated with hormonal changes and cytokine activation in severely sick patients. Since ghrelin causes a positive energy balance through GH-dependent and independent mechanisms, it could improve cachexia due to severe pathological conditions as seen in many end-stage diseases. In fact, in ghrelin-treated cachectic patients with congestive heart failure, increases in body weight, in lean body mass, and in muscle strength are reported [19]. Therefore, it is conceivable that ghrelin administration can be a novel therapeutic approach for cachexia in humans.

Direct cardiovascular action

Ghrelin is demonstrated to dilate human artery [25] and the action is endothelium-independent. In addition, ghrelin inhibits apoptosis of cultured cardiomyocytes and endothelial cells possibly through activation of extracellular signal-regulated kinase-1/2 and Akt serine kinases [26]. Together with the localization of GHS-R in the cardiovascular system, these results suggest that ghrelin may act directly on the cardiovascular system.

Anti-inflammatory action

Ghrelin suppresses the production of proinflammatory cytokines, including IL-1β, IL-6, and TNF-α. In addition, ghrelin inhibits the activation of nuclear factor-κB (NF-κB), a transcriptional factor regulating the gene expression of pro-inflammatory cytokines [27]. In patients with pulmonary infections, it is reported that chronic administration of ghrelin is reported to decrease inflammatory cytokine levels. The inhibition of the release of proinflammatory

cytokines could be mediated at least partly by the ghrelin-induced activation of the vagus nerve. Since cardiovascular diseases are often accompanied by an augmented inflammatory response, ghrelin may exert its protective actions through these anti-inflammatory potentials.

Suppression of sympathetic nerve

As described below, ghrelin potently inhibits sympathetic nerve which is often over-activated in cardiac diseases.

Cardiovascular actions of ghrelin

Since the administration of ghrelin has been demonstrated to decrease blood pressure, reduce cardiac afterload, and increase cardiac output without affecting heart rate in humans and in animals, the therapeutic potentials of ghrelin in cardiac diseases have been speculated. In addition, ghrelin potently stimulates GH release from the pituitary gland, improves energy balance, and modulates the autonomic nervous system, all of which could have beneficial effects on the cardiovascular system. Moreover, the receptor for ghrelin, GHS-R, can be demonstrated in the cardiac ventricles and in the blood vessels, suggesting that ghrelin might have direct cardiovascular actions.

Ghrelin in cardiovascular diseases

Heart failure

In rats with heart failure, chronic ghrelin treatment improved cardiac systolic dysfunction [28]. In addition, in patients with congestive heart failure, intravenous administration of ghrelin (2 μg/kg, twice a day) for three weeks significantly improved left ventricular ejection fraction (from 27% to 31%; $p < 0.05$), and increased peak workload and peak oxygen consumption during exercise, which was accompanied by a dramatic decrease in plasma norepinephrine (from 1132 to 655 pg/mL; $p < 0.001$) [19]. The therapeutic potential of ghrelin is, therefore, suggested in heart failure patients.

Myocardial infarction

Left ventricular remodeling after myocardial infarction is often associated with subsequent heart failure, which could lead to a fatal outcome. In a rat model of experimental myocardial infarction, peripheral ghrelin administration attenuated left ventricular dysfunction and remodeling was examined as described below.

Chronic treatment: subcutaneous administration of ghrelin at a dose of 100 μg/kg twice a day for two weeks significantly improved left ventricular enlargement induced by myocardial infarction. In addition, there was a substantial improvement in cardiac function parameters in ghrelin-treated rats compared with saline-treated controls. Furthermore, ghrelin attenuated an increase in interstitial fibrosis in the non-infarct region. Importantly, the infarction-induced increase of heart rate was suppressed completely in ghrelin-treated animals [29].

Acute treatment: whether one bolus subcutaneous injection of ghrelin 1 min after the coronary ligation leads to a beneficial effect during the acute phase was next examined using a rat model of myocardial infarction [30]. Surprisingly, the high mortality rate after myocardial infarction was significantly reduced by the early bolus of ghrelin administration [61% in saline-treated rats vs 23% in ghrelin-treated rats ($p < 0.05$)]. In addition, mortality due to fatal arrhythmias was also improved by the ghrelin treatment. Furthermore, the ghrelin-treated group had significantly fewer arrhythmic insults by the second to third hour after myocardial infarction [30]. The results show that one bolus of ghrelin treatment early after myocardial infarction improves survival after myocardial infarction by preventing the increase in frequency of ventricular arrhythmias.

Myocardial ischemia/reperfusion injury

It is reported that administration of ghrelin protects the heart against ischemia/reperfusion injury [31]. The cardioprotective effects of ghrelin are independent of GH release and likely involve binding of the peptide to receptors in the heart. The anti-apoptotic effect of ghrelin via the ERK 1/2 and PI3K/Akt-dependent pathway could potentially contribute to the beneficial effect of ghrelin infusion on myocardial ischemia/reperfusion injury.

Pulmonary hypertension

Whether ghrelin would impede pulmonary arterial hypertension during chronic hypoxia has been examined [32]. Conscious male Sprague Dawley rats were housed in a hypoxic chamber (10% oxygen) and received daily subcutaneous injection of ghrelin. While, in saline-treated rats, chronic hypoxia significantly elevated pulmonary arterial pressure and increased wall thickness of peripheral pulmonary arteries, the hypoxia-induced development of pulmonary arterial hypertension (110% increase in control vs 48% increase in ghrelin group), pulmonary vascular remodeling was significantly attenuated in ghrelin-treated animals. Therefore, the therapeutic benefits of ghrelin for pulmonary hypertension are suggested, particularly in subjects prone to chronic hypoxia.

Sympathetic inhibitory action of ghrelin

Recently, the effects of ghrelin on blood pressure, sympathetic nervous system activity, and mental stress responses were investigated in lean and overweight or obese individuals and it was found that stress-induced significant increase in these parameters were significantly reduced by 1 h intravenous infusion of ghrelin irrespective of obese phenotype [33]. In addition, administration of ghrelin significantly suppressed heart rate increase and ghrelin significantly suppressed plasma norepinephrine level in both humans and animals. Furthermore, it has been reported that the intracerebroventricular administration of ghrelin inhibited the sympathetic nerve activity [34].

Using a rat model of myocardial infarction, we investigated the beneficial effect of peripheral subcutaneous

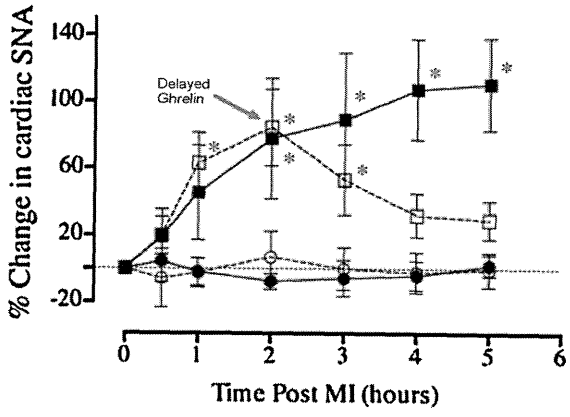

Figure 2 The sympathetic inhibitory effect of ghrelin after myocardial infarction. Transient responses in cardiac sympathetic nervous activity (SNA; percent increase in cardiac SNA of integrated area of the raw nerve signal) in sham rats (closed circle) and three groups of myocardial infarction (MI) rats: untreated (closed square); ghrelin treated immediately after myocardial infarction (open circle); and ghrelin treated 2 h after MI (open square). Either immediately after MI or 2 h after MI, ghrelin treatment effectively reduces the up-regulated SNA. *Significantly different from before MI (time "0") ($p < 0.05$). Adapted from Schwenke et al. [30].

ghrelin administration. Direct recording of cardiac sympathetic nerve activity revealed that ghrelin administration prevents an increase in cardiac sympathetic nerve activity as shown in Fig. 2. Importantly, the effect of ghrelin was accompanied by a reduction in mortality [30]. In acute myocardial infarction, the initial increase in the cardiac sympathetic nervous activity often leads to the fatal ventricular arrhythmia. The results, therefore, suggest that ghrelin-induced attenuation of the early increase in cardiac sympathetic nerve activity could potentially improve cardiac prognosis.

Ghrelin signaling through cardiac vagal afferent pathway

Interestingly, the orexigenic effect of peripherally administered ghrelin was suppressed by ligation of the gastric branch of the vagal nerve [8] or by pre-treatment with capsaicin, a neurotoxin specific for sensory afferent, indicating that vagal sensory afferent mediates the appetite promoting effect of peripherally administered ghrelin [8]. Furthermore, when ghrelin was microinjected into the nucleus of the solitary tract, the brain region important for controlling the autonomic nervous system, there was observed significant decreases in heart rate and mean arterial pressure [35]. In addition, GHS-R is shown to localize on the nerve terminals within the heart [9]. Furthermore, the sympathoinhibitory effect of intravenous administration of ghrelin was abolished in post-gastrectomy vagotomized patients, suggesting the vagus nerve is important for the effects of peripheral ghrelin [36]. Taken together, by acting on the vagal afferent nerve, which sends signals to the vasomotor center of the medulla via the nucleus of the solitary tract, ghrelin might exert its potent sympathetic inhibitory action

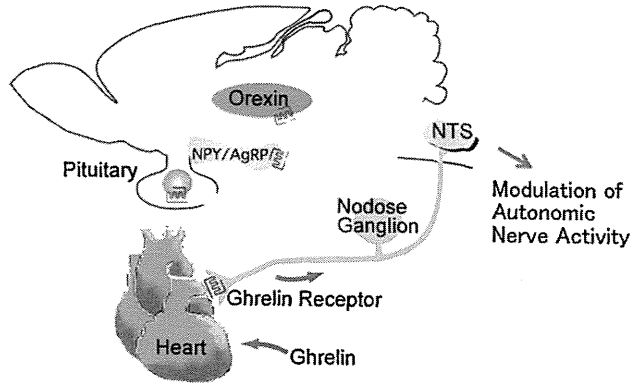

Figure 3 The signaling pathway in autonomic modulatory actions of ghrelin. Ghrelin acts on the cardiac vagal afferent nerve terminals, which send signals to the vasomotor center of the medulla through the nucleus of the solitary tract (NTS), which inhibits the sympathetic nerve activity and protects the heart from excessive damage.
Adapted from Kishimoto et al. [9,37].

resulting in decreases in sympathetic activity and in heart rate elevated after myocardial infarction (Fig. 3).

Conclusion

As described above, ghrelin has potent cardioprotective actions in diseases such as heart failure, myocardial infarction, pulmonary hypertension, and fatal arrhythmias through various mechanisms including GH release, direct actions on cardiovascular cells and inhibition of the sympathetic nervous activity. Since ghrelin is an endogenous hormone, it has advantages over other medications. It is, therefore, suggested that ghrelin can be a promising new treatment for cardiac diseases.

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REVIEW

Traditional risk factor management for stroke: a never-ending challenge for health behaviors of diet and physical activity

Yoshihiro Kokubo

Purpose of review
Recently, many guidelines have given new evidence on the risk factors for stroke. In this review, I refer to the most important guidelines for primary prevention of stroke and hypertension, especially focused on diet and physical activity.

Recent findings
The health behavior recommendations in recent guidelines for the primary prevention of stroke are virtually identical, and the same recommendations appear in the recent guidelines for the management of hypertension, especially with respect to diet and physical activity. The recommended health behaviors consist of weight reduction, reduction of salt intake, increase in fruit and vegetable intake, decrease in saturated and total fat intake (increase in fish intake), physical activity, and moderation of alcohol consumption. Fruits and vegetables have high levels of potassium, antioxidants, phytochemicals, and dietary fiber, and thus are also considered preventive of cardiovascular disease and its risk factors. It was found that individuals with many of these health behaviors have been shown to have a lowered risk of stroke.

Summary
The health behaviors, especially those related to diet and physical activity, appearing in recent guidelines for the management of hypertension are also important for the primary prevention of stroke, and appear in recent stroke guidelines.

Keywords
diet, guidelines, health behavior, physical activity, primary prevention of stroke

INTRODUCTION

Stroke is one of the leading causes of mortality and morbidity worldwide [1]. Figure 1 shows the schema of the progression from lifestyle behaviors to the onset of cardiovascular disease (CVD) as follows:

Environmental factor (lifestyle) → risk factors → cardiovascular disease

Environmental and genetic factors are the key factors for the primary stage of preventive CVD. Environmental factors include diet, physical activity, smoking, drinking, mental condition, and socioeconomic factors. Cardiovascular risk factors, such as hypertension, diabetes, and dyslipidemia, are the key factors for the secondary stage of preventive CVD. It is essential to consider these factors in order to prevent CVD, irrespective of whether lifestyle behaviors and risk factors were improved in the early stage.

Hypertension is the strongest risk factor for CVD worldwide [2,3]. The total population-attributable fractions of high-normal blood pressure and hypertension for CVD were approximately 50% in men and 30% in women [4]. When high blood pressure levels and other risk factors, such as chronic kidney disease [5] or diabetes mellitus [6▪], are combined, the risk of cardiovascular disease becomes much

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KEY POINTS

- Reduced intake of sodium and increased intake of potassium.
- Higher consumption of fruits, vegetables, low-fat dairy products and foods reduced in saturated fat (rich in fish and n-3 polyunsaturated fat).
- Increasing physical activity.
- Lifestyle recommendations for primary prevention of stroke are similar to those for preventing hypertension.

higher. The prevention of hypertension is the best way to prevent primary strokes. According to the 2007 European Society of Cardiology/European Society of Hypertension (ESC/ESH) Guidelines for the management of arterial hypertension [7], the Seventh Report of the Joint National Committee (JNC 7) [8], and the Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2009) [9], the factors that are effective for the prevention of hypertension are weight reduction, reduction of salt intake, increase in fruit and vegetable intake, decrease in saturated and total fat intake, physical activity, and moderation of alcohol consumption (Table 1). These factors are also considered as preventive for stroke (Table 2).

The Guidelines for the Primary Prevention of Stroke from the American Heart Association/American Stroke Association state that several aspects of diet can contribute to elevated blood pressure: excess salt intake, weight, drinking, and low potassium intake [10▪▪]. These guidelines also recommend a DASH (Dietary Approaches to Stop Hypertension)-style diet, which emphasizes consumption of fruits, vegetables, and low-fat dairy products. The European Stroke Organization (ESO) guidelines similarly recommend the following measures in order to reduce the risk of stroke: moderate drinking, regular physical activity, maintenance of appropriate weight, and a diet low in salt and saturated fat and rich in fruits, vegetables, and fiber [11]. However, antioxidant vitamin supplements and hormone replacement therapy are not recommended for the primary prevention of stroke in the ESO guidelines [11].

This review will focus on the prevention of stroke events through dietary modifications and increased physical activity, which are never-ending challenges for health behaviors of diet and physical activity.

DIET

Diet is one of the important lifestyle factors for the prevention of stroke, because we habitually eat three times a day. As mentioned in the Introduction, the following dietary factors have been related to stroke prevention: reduction of salt intake, increase in fruit and vegetable intake, and decrease in saturated and total fat intake.

Reduction of salt intake

In the INTERSALT (International Study of Salt and Blood Pressure) study, it is estimated that with a 100 mmol lower daily sodium intake the average decrease in blood pressure from age 25 to 55 would be by 9.0 mmHg for systolic and 4.5 mmHg for diastolic [12]. In Japan, the incidence of stroke is higher than in other countries, partly because both the salt intake and the frequency of hypertension are higher in Japan than in other countries. In a large Japanese general population, sodium intake was increasing associated with mortality from stroke [hazard ratio 1.6, 95% confidence interval (CI) 1.2–2.0], ischemic stroke (hazard ratio 2.0, 95% CI 1.4–2.9), and CVD (hazard ratio 1.4, 95% CI 1.2–1.7) [13].

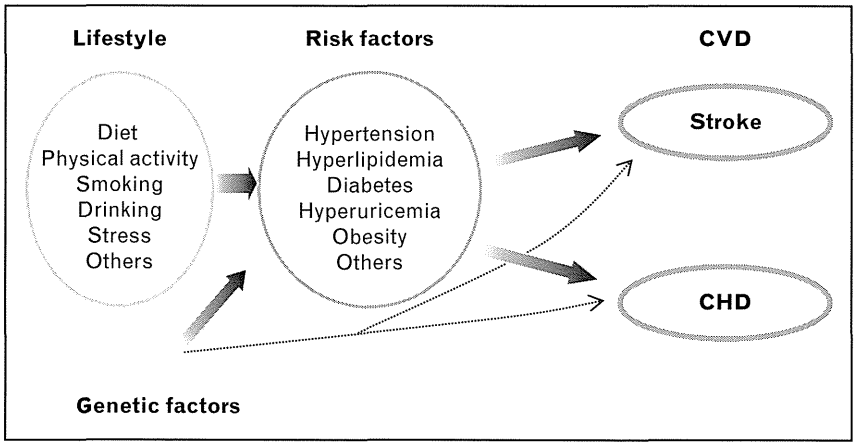

FIGURE 1. Schema of the progression from lifestyle changes to the incidence of cardiovascular disease. CHD, coronary heart disease; CVD, cardiovascular disease.

Table 1. Lifestyle recommendations to prevent hypertension in various guidelines

	JNC 7 [8]	ESC/ESH [7]	JSH 2009 [9]
Weight reduction	Maintain normal body weight	Weight reduction (and weight stabilization)	Maintaining appropriate body weight: BMI <25kg/m^2
Adopt DASH eating plan	Consume a diet rich in fruits, vegetables, and low-fat dairy products with a reduced content of saturated and total fat	Increase in fruit and vegetable intake and decrease in saturated and total fat intake	Increased intake of fruits and vegetables. Reduce intake of cholesterol and saturated fatty acids. Increased intake of fish (fish oil)
Dietary sodium reduction	Reduce dietary sodium intake to no more than 100 mmol per day	Reduction of salt intake	Salt restriction to <6 g/day
Physical activity	Engage in regular aerobic physical activity such as brisk walking (at least 30 mim per day)	Physical exercise	Exercise: In hypertensive patients with no cardiovascular disease, exercise, which is primarily moderate aerobic exercise, should be performed periodically (for 30 min daily if possible)
Moderation of alcohol consumption	Limit consumption to no more than 2 drinks per day in men and to no more than 1 drink per day in women and lighter weight men	Reduction of excessive alcohol intake	Restriction of alcohol intake: 20–30 ml/day in men and 10–20 ml/day in women as ethanol
Stop smoking	For overall cardiovascular risk reduction, stop smoking	Smoking cessation	Quitting smoking

Table 2. Lifestyle recommendations to prevent primary stroke in various guidelines

	Guidelines for the primary prevention of stroke: AHA/ASA Guideline [10▪▪]	The European Stroke Initiative recommendations for stroke management [11]
Weight reduction	Among overweight and obese persons, weight reduction is reasonable as a means of reducing risk of stroke	Individuals with an elevated body mass index are recommended to take a weight-reducing diet
Diet and nutrition	Reduced intake of sodium and increased intake of potassium as indicated in the report Dietary Guidelines for Americans are recommended to lower blood pressure	A diet low in salt is recommended
	A DASH-style diet, which emphasizes consumption of fruits, vegetables, and low-fat dairy products and is reduced in saturated fat, also lowers blood pressure and is recommended	
	A diet that is rich in fruits and vegetables and thereby high in potassium is beneficial and may lower risk of stroke	A diet low in saturated fat, high in fruit and vegetables and rich in fibre is recommended
Physical activity	Increased physical activity is recommended because it is associated with a reduction in risk of stroke	Regular physical activity is recommended
	Adults should engage in at least 150 min (2 h and 30 min) per week of moderate intensity or 75 min (1 h and 15 min) per week of vigorous intensity aerobic physical activity	

DASH, Dietary Approaches to Stop Hypertension.

Increase in fruit and vegetable intake

Cohort studies have shown that high fruit and vegetable intake reduces stroke incidence and mortality. Two meta-analysis studies reported that fruit and vegetable consumption decreases the risk of stroke [14,15]. The risk reductions of stroke from meta-analysis of seven cohorts were 11% (95% CI 7–15%) for each additional portion per day of fruit and 5% (3–8%) for fruit and vegetables [14]. Those of nine cohorts were 26% (21–31%) for more than five servings per day of fruit and vegetables and 11% (3–17%) for three to five servings per day compared to less than three servings per day [15]. In participants in a large Japanese cohort study, the inverse association between fruit intake and risk was observed only among nonsmokers [16]. Smoking, which causes oxidative stress, diminishes the healthy effects of fruits and vegetables. The mechanism by which high intake of fruits and vegetables prevents stroke may involve the high levels of potassium, antioxidants, phytochemicals, and dietary fiber in these foods, since each of these components has been shown to individually prevent cardiovascular risk factors.

Potassium excretion has been negatively correlated with blood pressure in individuals. A 50 mmol/day lower urinary excretion of potassium has been associated with an average blood pressure increase of 3.4 mmHg systolic or 1.9 mmHg diastolic [12]. A meta-analysis of 33 randomized controlled trials of potassium supplementation showed that the supplementation significantly reduced systolic and diastolic blood pressure. The systolic and diastolic blood pressures were decreased by 4.0 and 2.5 mmHg, respectively, in hypertensive patients, whereas they were decreased by 1.8 and 1.0 mmHg, respectively, in normotensive individuals.

In the US general population, higher potassium intake was associated with lower mortality risk (hazard ratio 0.80, 95% CI 0.67–0.94, per 1000 mg/day of potassium) [17▪]. In a pooled meta-analysis of 15 cohorts including 247 510 participants at baseline for 5–19 years of follow-up, a 42 mmol/day higher potassium intake was associated with a 21% lower risk of stroke (hazard ratio 0.79, 95% CI 0.68–0.90) [18▪].

Fruits and vegetables are rich sources of antioxidants that may help prevent CVD, including vitamins C and E, carotenoids, polyphenols, and flavonoids. Cohort participants in the top quartiles of baseline plasma vitamin C concentrations had 41–61% lower risk than did those in the bottom quartiles [19–21]. In a Rotterdam cohort study, compared with participants in the lowest tertile of vitamin C intake, those in the second and third tertiles of vitamin C intake had a significantly lower risk of ischemic stroke (hazard ratios 0.69 and 0.66, 95% CIs 0.49–0.98 and 0.46–0.93, respectively) [22]. In another study, a high serum vitamin A concentration was found to have a beneficial effect on early outcome in ischemic stroke [23]. Dietary supplementation with antioxidants including vitamins C, E, and beta-carotene was tested in a meta-analysis of randomized controlled trials, and no significant association with secondary CVD prevention was observed [24].

Flavonoids are a large group of polyphenolic compounds abundant in vegetables, fruits, tea, and red wine, and may contribute to the protective effect of these foods. There are five subclasses of flavonoids, that is, flavonols, flavones, flavanones, flavan-3-ols and anthocyanidins, which have been estimated to contribute to daily dietary intake and thus potentially have effects on health [25]. In a cohort study, Finnish men in the highest quartile of flavonol and flavan-3-ol intake had a relative risk of 0.55 (95% CI 0.31–0.99) and 0.59 (0.30–1.14) for ischemic stroke, respectively, as compared with the lowest quartile [26]. In a Japanese cohort study, green tea consumption of five or more cups per day was associated with a relative risk of 0.88 (95% CI 0.79–0.98) for ischemic stroke compared with less than one cup per day [27]. Recent cohort studies have shown that coffee consumption is protective of stroke. The relative risks of total stroke in Swedish women were 1.00 (reference), 0.78 (95% CI 0.66–0.91), 0.75 (95% CI 0.64–0.88), and 0.77 (95% CI 0.63–0.92) for coffee consumption of less than 1 cup/day, 1–2 cups/day, 3–4 cups/day, and at least 5 cups/day, respectively [28].

Many studies on the intake of soy, a food rich in isoflavones, have reported an impact on decreasing plasma cholesterol levels [29,30]. However, only a few studies exist on the impact of soy intake on CVD, because the average soy intake in Japanese is 10–70 times higher than that in Western people [31,32]. A prospective study of Dutch women did not support the idea that dietary isoflavones lowered the risk of CVD [32]. The quantity of isoflavones consumed by Dutch women, however, was quite small [32]. A prospective study of Japanese men and women showed that soy intake was weakly and inversely associated with total mortality but not mortality due to CVD [33]. Recently, a Japanese community-based prospective study with 40 462 participants has shown that high consumption of soy and isoflavones was associated with a reduced risk of incidence and mortality of cerebral and myocardial infarction among women, particularly postmenopausal women [34]. This study suggests that the consumption of dietary isoflavones and soy intake may be beneficial to

postmenopausal women for the prevention of ischemic CVD.

Population cohort studies have revealed dietary fiber, consisting of water-soluble and water-insoluble fiber, to be inversely associated with the risks of coronary heart disease (CHD) [35,36▪▪] and of stroke [37]. Water-soluble fiber may result in an improvement of glycemic control and a lowering of triglyceride levels [38], as well as a cholesterol-lowering effect [39], especially on low-density lipoprotein cholesterol. Insoluble fiber may slow the intestinal absorption of foods and reduce the levels of clotting factors [40], fibrinolysis [41], coagulation [42], and inflammatory markers [43]. Recently, a relatively large Japanese population-based prospective study has shown higher dietary intakes of total and insoluble fiber to be associated with reduced risk of total strokes, cerebral infarction and intracerebral hemorrhage in women [44▪]. The inverse associations between dietary total fiber intake and cardiovascular diseases were statistically significant only for nonsmoking men and women, but not for smoking men and women.

Fish and n-3 polyunsaturated fat (decrease in saturated and total fat intake)

In two large Japanese community samples, dietary intake of fish and n-3 polyunsaturated fatty acid was inversely associated with incidence of CHD [45] and mortality of CVD [46]. However, the association between intake of fat and stroke is controversial. A meta-analysis of randomized controlled trials showed no effects of n-3 fatty acids on cardiovascular events [47]. A large cohort study in men in the US did not show an increase in the risk of ischemic stroke according to dietary intakes of total fat, animal fat, saturated fat, and vegetable fat [48]. The estimated level of association between dairy intake and stroke events pooled from seven prospective studies was not statistically significant [49▪]. In a Japanese cohort, a high consumption of animal fat and cholesterol was associated with a reduced risk of cerebral infarction death, whereas those relationships have been diminished in Western counties [50], where the animal product intake is higher than in Japan.

Interestingly, an increased concentration in serum of n-3 polyunsaturated fatty acids, especially docosahexaenoic acid, a marker of fish or fish oil consumption, may protect against atrial fibrillation according to a prospective population-based study [51,52]. Atrial fibrillation is a strong risk factor for cerebral infarction [53]. Therefore, an increased concentration of n-3 polyunsaturated fatty acids may be of benefit for the prevention of cerebral infarction, especially cardio-embolism infarction.

PHYSICAL ACTIVITY

The 2008 Physical Activity Guidelines for Americans include an extensive review of the literature and concludes that individuals engaged in physical activity have a 25–30% lower risk of stroke than inactive individuals [54▪,55]. In another study, moderate activity was associated with a lower risk of stroke compared with inactivity (hazard ratio 0.80, 95% CI 0.74–0.86) [56]. This association was mediated through beneficial effects on body weight, blood pressure, serum total cholesterol levels, and glucose levels. The 2008 Physical Activity Guidelines for Americans recommended that adults should engage in at least 150 min per week of moderate-intensity or 75 min per week of vigorous-intensity aerobic physical activity, or an equivalent combination of moderate and vigorous-intensity aerobic activity [10▪▪,54▪].

According to the Physical Activity Guideline for Older Adults [57▪], to promote and maintain health, older adults should participate in moderate-intensity aerobic activity for at least 30 min on 5 days of the week, or vigorous-intensity aerobic activity for at least 20 min on 3 days of the week [58]. Even in older adults with chronic illnesses or disabilities, significant health benefits can be obtained by daily nonstrenuous physical activity, such as stretching exercises [55].

Physical activity in leisure time (2–5 h per week) has been significantly associated with a reduced severity of ischemic stroke at admission [59]. Japanese individuals who reported the highest level of physical activity (i.e. walking 1 h/day) had lower mortality of stroke than did those in the second lowest physical activity category (i.e. walking 0.5 h/day) (hazard ratio 0.71, 95% CI 0.54–0.94). [60]

COMBINED IMPACT OF HEALTH BEHAVIORS

In the above mentioned guidelines for the primary prevention of stroke, there is a study on health behaviors for the prevention of stroke, with a focus on diet and physical activity [61]. This prospective population study (20 040 men and women aged 40–79 at baseline) reported on the potential combined impact of four health behaviors, that is current nonsmoking, physical inactivity, moderate alcohol intake, and plasma concentration of vitamin C at least 50 μmol/l (indicating fruit and vegetable intake of at least five servings a day), on incidence of stroke. The relative risks for stroke were 1.15 (95% CI 0.89–

1.49) for three health behaviors, 1.58 (1.22–2.05) for two, 2.18 (1.63–2.92) for one, and 2.31 (1.33–4.02) for none ($P < 0.001$ for trend), compared with people with all four health behaviors.

CONCLUSION

The health behavior recommendations in guidelines for the primary prevention of stroke are highly similar, and the same recommendations appear in the guidelines for the management of hypertension, especially with respect to diet and physical activity. The recommended health behaviors consist of weight reduction, reduction of salt intake, increase in fruit and vegetable intake, decrease in saturated and total fat intake, physical activity, and moderation of alcohol consumption. Individuals with many of these health behaviors were found to be at lower risk of stroke.

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Conflicts of interest

There are no conflicts of interest.

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