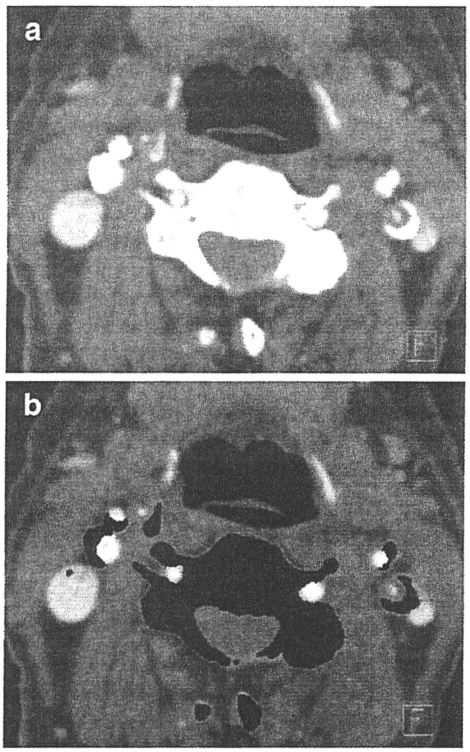

Fig. 2 **a** Combined image generated from two datasets obtained at different tube voltage. **b** Dual-energy CT image of a carotid artery eliminated hard plaques with application of DE hard plaque removal. The pixels detected as bone or calcifications are displayed with a CT number of −1,000 HU on the DE hard plaque removal CT image

DSA examinations were performed using a biplane DSA unit with rotational 3D DSA (INTEGRIS BV3000, Philips Healthcare, Best, Netherlands). Common carotid arteries were selectively catheterized, and anteroposterior, lateral, right anterior oblique, and left anterior oblique images were obtained.

Carotid artery stenosis was quantified according to NASCET criteria [1] on MIP images and on DSA images at the same plane. Carotid artery stenosis was measured independently by two experienced radiologists with 8 and 16 years of experience in vascular imaging. The readers evaluated the grade of stenosis according to the following scale: 0–25%, 25–50%, 50–75%, 75–99%. Interobserver variability was assessed using Cohen's kappa test. Correlation between CTA and DSA was determined by means of cross tabulation, and accuracy for detection and grading of stenosis was calculated.

Results

Evaluation of stenosis was possible for all vessels postprocessed with DE head bone and hard plaque removal software (Fig. 3). In contrast, conventional CTA did not allow the evaluation of stenosis in 13 out of 18 vessels on MIP images because calcifications covered the lumen. Good correlation (r^2=0.9504) was observed between the degree of carotid stenosis measured on CTA images after DE hard plaque removal and on DSA images (Fig. 4). Sensitivity and specificity for detecting hemodynamically relevant (>70%) stenosis was 100% and 92%, respectively.

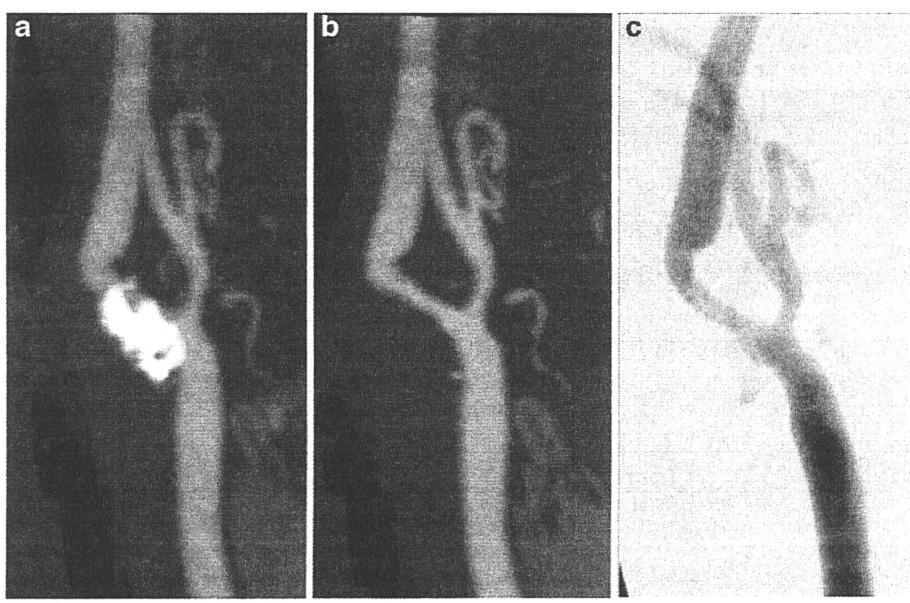

Fig. 3 **a** Conventional CTA image does not allow the visualization of the intravascular lumen due to the dense calcification. **b** CTA image after DE hard plaque removal: the calcification is almost completely removed and a quantification of stenosis is possible. The image quality is comparable with that of DSA (**c**)

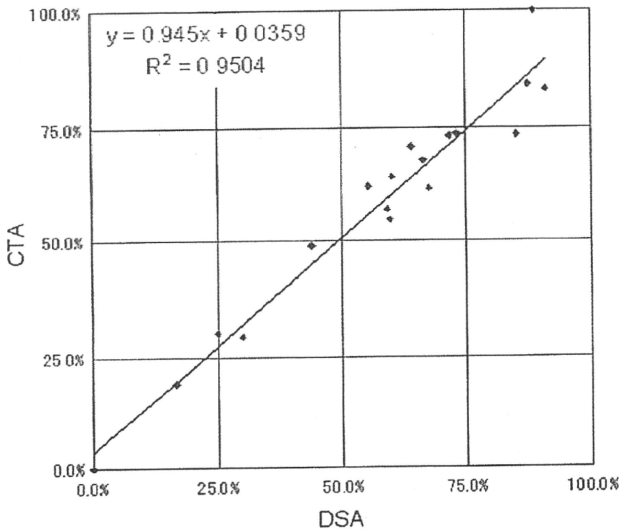

Fig. 4 Correlation between DE hard plaque removal CTA and DSA for the stenosis measurements. Good correlation between the two methods is observed (r^2=0.9504) for the quantification of carotid stenosis

One vessel with severe stenosis (87.4% according to DSA) was overestimated and displayed as a 99% stenosis-like lesion on the DE hard plaque removal CTA images (Fig. 5). Cohen's kappa test revealed a high level of interobserver agreement, with kappa coefficient being 0.91 for CTA and 0.73 for DSA, respectively (Table 1).

Discussion

Symptomatic patients with high grade carotid stenosis will benefit from carotid endarterectomy or stenting as secondary prevention of ischemic stroke [1–3]. The indication of these therapies is decided according to the degree of stenosis in addition to the symptoms experienced by the patient, thus precise carotid stenosis quantification is essential. The accepted gold standard for evaluation of carotid artery stenosis is catheter angiography; however, many reports have suggested that the sensitivity of multislice CTA (MSCTA) in evaluating the degree of carotid artery stenosis has become comparable with that of angiography while being associated with a lower level of risk [4–9].

Bone-subtraction CTA (BSCTA), where a nonenhanced scan is used to create a bone mask which is then subtracted from the contrast-enhanced CTA data, has been proven to be a robust method for the evaluation of intracranial vessels [13–15]. In this method, two volume datasets are matched for subtraction but, regarding neck bone and carotid artery calcification, misregistration errors are inevitable because neck bone and carotid arteries often move during pre- and postcontrast scan due to pulsation or neck movement [15]. In addition, the neck is more difficult to immobilize than the skull.

Our study showed that calcified plaques were almost completely removed from the carotids after applying DE bone removal and hard plaque removal postprocessing to dual-energy CTA images, and high quality DSA-like imaging was achieved. The results were in good correlation

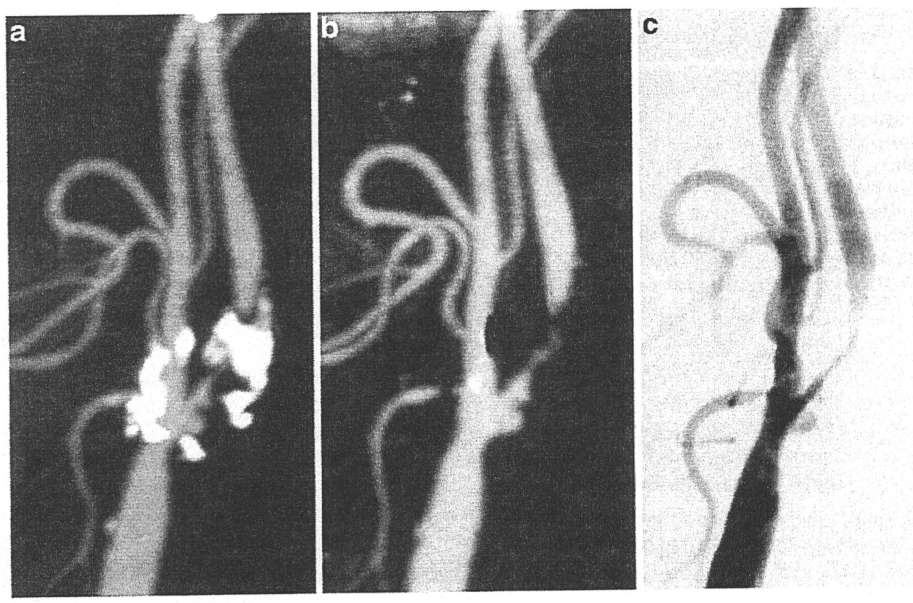

Fig. 5 **a** Conventional CTA image: quantification of stenosis is impossible. **b** CTA image after DE hard plaque removal: the calcified plaque is almost completely removed, yet parts of the lumen are as well, resulting in a display of a 99% stenosis-like lesion. **c** DSA image shows a patent lumen with 87% stenosis

Table 1 Interobserver agreement for assessment of stenosis on DE hard plaque removal CTA and DSA

CTA	0–25%	25–50%	50–75%	75–100%	Reader 2
0–25%	2				2
25–50%		3			3
50–75%			9	1	10
75–100%				3	3
Reader 1	2	3	9	4	
Kappa coefficient=0.91					

DSA	0–25%	25–50%	50–75%	75–100%	Reader 2
0–25%	1	1			2
25–50%		1	1		2
50–75%			9	1	10
75–100%				4	4
Reader 1	1	2	10	5	
Kappa coefficient=0.73					

with DSA in terms of quantification of carotid artery stenosis with dense calcifications. Although axial source image is reliable in grading stenosis, MIP reconstructions can be helpful when horizontal or tortuous course of the vessel or a very short stenosis can render the assessment of the stenosis difficult on axial images [16]. We used MIP images as the first-line method to quantify the degree of carotid stenosis because this study focused on feasibility of DE hard plaque removal. In clinical settings, axial source images were used in grading the degree of carotid artery stenosis in the presence of dense calcification.

Iodine shows a much larger increase in CT value with decreasing X-ray tube voltage than bone and calcification, which is the basis for iodine–bone separation using dual-energy CT. The voxels detected as bone or calcifications were displayed with a CT number of −1,000 HU on the DE hard plaque removal CTA images [11]. We found that the areas where bone or calcifications had been removed were slightly larger than the calcified plaques observed in the original images, meaning that calcifications seemed to be overestimated. This may be due to blooming artifacts or partial volume effects. Although moderate or mild stenosis measurements may be accurate, severe stenosis can be overestimated when the stenotic part runs very close to calcified plaque as was observed in one of our cases. This result can be altered by applying different kernels. Application of a hard kernel might clarify the border between calcification and iodine; however, we applied a relatively soft kernel (D30) to obtain smooth 3D images. According to theoretical considerations, image pixels with a CT value greater than 100 HU in the 140-kV image would be classified either as iodine pixels or bone pixels depending on their CT values [12]. However, in patients with severe stenosis, the contrast enhancement (CT value increase) may be weak in the lumen at the position of maximum stenosis because of the small number of iodine pixels. Also, partial volume effects may lead to an overestimation of plaque pixels resulting in an overestimation of severe stenosis. One solution may be to increase the injection rate of the contrast bolus to obtain higher CT values in the cross sections of maximum stenosis.

DE hard plaque removal offers the advantage that images from one single CT acquisition (albeit with a dual source) can be used for removing hard plaque and estimating calcified carotid stenosis. The unenhanced CT acquisition usually needed for BSCTA as a mask for subtraction thus becomes unnecessary, which reduces radiation dose to the patient and eliminates misregistration due to neck movement or arterial pulsation. The radiation dose of a dual-energy scan is comparable with a normal single-source scan. In fact, the average $CTDI_{vol}$ of our initial five carotid dual-source CTA studies was 11.1 mGy, while that of normal CTA with single-source scan was approximately 10.6 mGy, at 120 kV, 300 mA s (effective).

Conclusion

With DE hard plaque removal CTA, calcified plaques could be removed from carotid CTA images and high quality DSA-like imaging could be achieved. DE hard plaque removal is therefore useful for the evaluation of carotid stenosis with severe calcification.

References

1. North American Symptomatic Carotid Endarterectomy Trial Collaborators (1991) Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. N Engl J Med 325:445–453
2. European Carotid Surgery Trialists' Collaborative Group (1991) MRC European Carotid Surgery Trial: interim results for symptomatic patients with severe (70–99%) or with mild (0–29%) carotid stenosis. Lancet 337:1235–1243
3. Barnett HJ, Taylor DW, Eliasziw M et al (1998) Benefit of carotid endarterectomy in patients with symptomatic moderate or severe stenosis. North American Symptomatic Carotid Endarterectomy Trial Collaborators. N Engl J Med 339:1415–1425
4. Anderson GB, Ashforth R, Steinke DE, Ferdinandy R, Findlay JM (2000) CT angiography for the detection and characterization of carotid artery bifurcation disease. Stroke 31:2168–2174
5. Moll R, Dinkel HP (2001) Value of the CT angiography in the diagnosis of common carotid artery bifurcation disease: CT angiography versus digital subtraction angiography and color flow Doppler. Eur J Radiol 39:155–162
6. Randoux B, Marro B, Koskas F et al (2001) Carotid artery stenosis: prospective comparison of CT, three-dimensional gadolinium-enhanced MR, and conventional angiography. Radiology 220:179–185
7. Josephson SA, Bryant SO, Mak HK, Johnston SC, Dillon WP, Smith WS (2004) Evaluation of carotid stenosis using CT angiography in the initial evaluation of stroke and TIA. Neurology 63:457–460
8. Lell M, Fellner C, Baum U et al (2007) Evaluation of carotid artery stenosis with multisection CT and MR imaging: influence of imaging modality and postprocessing. AJNR Am J Neuroradiol 28:104–110
9. Silvennoinen HM, Ikonen S, Soinne L, Railo M, Valanne L (2007) CT angiographic analysis of carotid artery stenosis: comparison of manual assessment, semiautomatic vessel analysis, and digital subtraction angiography. AJNR Am J Neuroradiol 28:97–103
10. Saba L, Sanfilippo R, Pirisi R, Pascalis L, Montisci R, Mallarini G (2007) Multidetector-row CT angiography in the study of atherosclerotic carotid arteries. Neuroradiology 49:623–637
11. Johnson TR, Krauss B, Sedlmair M et al (2007) Material differentiation by dual energy CT: initial experience. Eur Radiol 17:1510–1517
12. Flohr TG, McCollough CH, Bruder H et al (2006) First performance evaluation of a dual-source CT (DSCT) system. Eur Radiol 16:256–268
13. Venema HW, Hulsmans FJ, den Heeten GJ (2001) CT angiography of the circle of Willis and intracranial internal carotid arteries: maximum intensity projection with matched mask bone elimination-feasibility study. Radiology 218:893–898
14. Tomandl BF, Hammen T, Klotz E, Ditt H, Stemper B, Lell M (2006) Bone-subtraction CT angiography for the evaluation of intracranial aneurysms. AJNR Am J Neuroradiol 27:55–59
15. Lell MM, Ditt H, Panknin C et al (2007) Bone-subtraction CT angiography: evaluation of two different fully automated image-registration procedures for interscan motion compensation. AJNR Am J Neuroradiol 28:1362–1368
16. Silvennoinen HM, Ikonen S, Soinne L, Railo M, Valanne L (2007) CT angiographic analysis of carotid artery stenosis: comparison of manual assessment, semiautomatic vessel analysis, and digital subtraction angiography. AJNR Am J Neuroradiol 28:97–103

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Natriuretic Peptides Enhance the Production of Adiponectin in Human Adipocytes and in Patients With Chronic Heart Failure

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Objectives We investigated the functional relationship between natriuretic peptides and adiponectin by performing both experimental and clinical studies.

Background Natriuretic peptides are promising candidates for the treatment of congestive heart failure (CHF) because of their wide range of beneficial effects on the cardiovascular system. Adiponectin is a cytokine derived from adipose tissue with various cardiovascular-protective effects that has been reported to show a positive association with plasma brain natriuretic peptide (BNP) levels in patients with heart failure.

Methods The expression of adiponectin messenger ribonucleic acid (mRNA) and its secretion were examined after atrial natriuretic peptide (ANP) or BNP was added to primary cultures of human adipocytes in the presence or absence of HS142-1 (a functional type A guanylyl cyclase receptor antagonist). Changes of the plasma adiponectin level were determined in 30 patients with CHF who were randomized to receive intravenous ANP (0.025 μg/kg/min human ANP for 3 days, n = 15) or saline (n = 15).

Results Both ANP and BNP dose-dependently enhanced the expression of adiponectin mRNA and its secretion, whereas such enhancement was inhibited by pre-treatment with HS142-1. The plasma adiponectin level was increased at 4 days after administration of human ANP compared with the baseline value (from 6.56 ± 0.40 μg/ml to 7.34 ± 0.47 μg/ml, $p < 0.05$), whereas there was no change of adiponectin in the saline group (from 6.53 ± 0.57 μg/ml to 6.55 ± 0.56 μg/ml).

Conclusions Natriuretic peptides enhance adiponectin production by human adipocytes in vitro and even in patients with CHF, which might have a beneficial effect on cardiomyocytes in patients receiving recombinant natriuretic peptide therapy for heart failure. (J Am Coll Cardiol 2009;53:2070–7) © 2009 by the American College of Cardiology Foundation

Plasma natriuretic peptide levels are increased in patients with congestive heart failure (CHF), and the measurement of these peptides is used widely to assess the presence, severity, and prognosis of CHF (1,2). Both atrial natriuretic peptide and brain natriuretic peptide (ANP and BNP, respectively) have a beneficial effect in patients with heart failure because of their various biological actions (3–5).

See page 2078

Adiponectin is a circulating cytokine derived from adipose tissue that has attracted considerable interest because of its identification as a risk factor for cardiovascular disease (6,7) and CHF (8). Adiponectin production is down-regulated in patients with coronary risk factors that are associated with the development of heart failure (9,10).

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Recently, adiponectin was reported to have a cardioprotective effect against ischemia-reperfusion injury (11) and hemodynamic stress (12,13) in mice. Interestingly, it has been reported that the level of N-terminal pro-brain natriuretic peptide shows a positive correlation with the plasma adiponectin concentration in patients with chronic heart failure (14).

Given these experimental and clinical observations, we hypothesized that natriuretic peptides might increase adiponectin production in patients with heart failure to protect the cardiovascular system. Accordingly, in the present study, we investigated whether natriuretic peptides could directly increase adiponectin production by these adipocytes (and the cellular mechanisms involved) and confirmed this effect on adiponectin in the clinical setting.

Abbreviations and Acronyms

ANP = atrial natriuretic peptide
BNP = brain natriuretic peptide
CHF = congestive heart failure
GC-A = type A guanylyl cyclase receptor
hANP = human atrial natriuretic peptide
NPR = natriuretic peptide receptor
PKG = protein kinase G

Methods

Agents. Both human ANP and BNP were purchased from Sigma-Aldrich (St. Louis, Missouri). HS142-1, a functional guanylyl cyclase-A type receptor antagonist, was provided by Kyowa Hakko Kogyo Co., Ltd. (Mishima, Japan). A cGMP analog (8-pCPT-cGMP) and a selective cGMP-dependent protein kinase G (PKG) inhibitor (R_p-8-Br-PET-cGMP-S) were obtained from Biolog Life Science Institute (Bremen, Germany). An antibody directed against mouse adiponectin (MAB3608) was purchased from Chemicon International, Inc.

Primary culture and in vitro study of human adipocytes. Subcutaneous adipocytes derived from the adipose tissue of 6 women were obtained commercially together with culture medium from Zen-Bio, Inc. (Research Triangle Park, North Carolina. The donors were nonsmokers with a mean body mass index of 27.0 kg/m^2 (range 25.9 to 29.1 kg/m^2) and an average age of 47 years (range 29 to 63 years). Cells were maintained in adipocytes maintenance medium (i.e., AM-1) containing Dulbecco's modified Eagle medium/Ham's F-12 (1:1, v/v), 3% fetal calf serum, 15 mmol/l HEPES (pH 7.4), biotin, pantothenate, human insulin, 1 μmol/l dexamethasone, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B at 37°C in a humidified atmosphere of 95% air/5% CO_2. The medium was changed every 2 days. Primary cultures of the adipocytes were used to examine the effects of natriuretic peptides (ANP or BNP) on the expression of adiponectin.

Before these experiments, the cells were plated in adipocyte basal medium (i.e., BM-1) containing Dulbecco's modified Eagle medium/Ham's F-12 (1:1, volume/volume), 15 mmol/l 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.4), biotin, and pantothenate for 24 h. Then the indicated concentrations of either natriuretic peptide (from 10^{-11} to 10^{-9} mol/l) were added to the BM-1 medium. After 24 h of incubation, the medium was harvested for Western blotting to measure the secretion of adiponectin, and the cells were also harvested for ribonucleic acid (RNA) analysis. The effect of each natriuretic peptide on adiponectin messenger ribonucleic acid (mRNA) levels was determined by quantitative real-time polymerase chain reaction (PCR).

Measurement of adiponectin. In patients with CHF, the plasma adiponectin concentration was measured by the use of an ELISA kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) according to the manufacturer's protocol. Adiponectin secretion by primary cultured human adipocytes was assessed by Western blotting of the culture medium, as previously described (15), and the immunoreactive bands were quantified by densitometry (Molecular Dynamics, Sunnyvale, California).

Reverse transcriptional-PCR. Total RNA was extracted from adipocytes derived from human white fat with the use of RNA-Bee-RNA Isolation Reagent (Tel-Test, Inc., Gainesville, Florida). Then, 200 ng of total RNA was reversed transcribed and amplified by the use of an Omniscript RT kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The forward primers for type A guanylyl cyclase receptor (GC-A) and natriuretic peptide receptor (NPR)-C were 5′-CCAGTTCCAAGTCTTTGCCAAGACAGCA and 5′-GGAAGACATCGTGCGCAATA, respectively, and the reverse primers for GC-A and NPR-C were 5′-CATTGTGTAGAAACAGCATGCCCTTGACGA and 5′-TGCTCCGGATGGTGTCACT, respectively. As a positive control, we used the samples of human cardiac tissue under the protocol approved by the institutional review board of the National Cardiovascular Center (No. 14-18) (16).

Quantitative real-time PCR analysis. Quantitative real-time PCR was performed as described previously (17). Oligonucleotide primers and TaqMan probes for human adiponectin and glyceraldehyde 3-phosphate dehydrogenase were purchased from Applied Biosystems (Foster City, California).

Subjects and design of the clinical study. We prospectively studied 30 consecutive CHF patients who were admitted to the emergency department of the National Cardiovascular Center between April and July 2006. The exclusion criteria were as follows: age >80 years, cardiogenic shock or hypotension (systolic blood pressure <100 mm Hg), and renal failure (serum creatinine >2.0 mg/dl). This study was approved by the Committee on Human Investigation of the National Cardiovascular Center, and all patients who participated gave informed consent. The 30 patients were randomized to 2 groups, a human atrial natriuretic peptide (hANP) group consisting of 15 patients who received administration of hANP and a control group consisting of 15 patients who were administered saline. In the hANP group, from immediately after the diagnosis of

JACC Vol. 53, No. 22, 2009
June 2, 2009:2070–7

acute exacerbation of CHF, hANP (0.025 μg/kg/min) was infused intravenously for 3 days. The study protocol did not restrict or specify any other diagnostic or therapeutic strategies. Blood for measuring the plasma adiponectin level was sampled before and 1 and 7 days after finishing the administration of hANP or saline (days 1, 4, and 10, respectively) (Fig. 3A).

Statistical analysis. For analysis of differences between the various treatments of adipocytes, analysis of variance was performed, followed by the appropriate post-hoc test. The differences in adiponectin levels between days 1 and 4 in each group were tested with a paired *t* test. The changes in adiponectin levels from day 1 to 4 between ANP group and saline group was tested with an unpaired *t* test. Results are expressed as the mean ± SEM, and p values of <0.05 were considered significant.

Results

Effect of natriuretic peptides on the expression and secretion of adiponectin by primary cultured human adipocytes. First, we checked the expression of GC-A and NPR-C mRNA by using reverse transcriptional-PCR. As shown in Figure 1A, both GC-A and NPR-C mRNA was detectable in primary cultured human adipocytes. To investigate the effects of natriuretic peptides on the regulation of adiponectin production in adipocytes, we incubated primary cultured human adipocytes with recombinant ANP. When ANP was used at a concentration of 10^{-10} mol/l (pathological plasma concentration), it increased adiponectin mRNA expression after 6 h of incubation and reached a maximum after 12 h (Fig. 1B). Next, we incubated human adipocytes with ANP at the concentration of from 10^{-11} mol/l (normal plasma concentration) to 10^{-9} mol/l (pharmacological plasma concentrations) and demonstrated enhanced adiponectin mRNA expression and adiponectin secretion into the medium in a dose-dependent manner, whereas these changes were completely inhibited by pretreatment with HS142-1 (Figs. 1C and 1D). Incubation of adipocytes with BNP also increased the expression of adiponectin mRNA in a dose-dependent manner and this effect was completely blocked by pretreatment with HS142-1 (Figs. 1E and 1F).

Involvement of cGMP/PKG signaling in natriuretic peptide-induced synthesis of adiponectin. Because both ANP and BNP exert their biological effects by promoting cGMP production, to investigate the role of the GC-A/cGMP/PKG signaling pathway in adiponectin production, we measured the changes of cGMP in ANP-treated primary cultured human adipocytes. We found that incubation with ANP increased the cGMP level and that this effect was blunted by co-treatment with HS142-1 (data not shown). Next, we treated human adipocytes with the cGMP analog 8-pCPT-cGMP and the PKG inhibitor (R_p)-8-Br-PET-cGMP-S. The activation of PKG by 8-pCPT-cGMP (50 μmol/l for 12 h) produced an increase of adiponectin mRNA expression similar to that observed after incubation with ANP. The effect of ANP on adiponectin mRNA expression was abolished in the presence of (R_p)-8-Br-PET-cGMP-S (100 nmol/l) (Fig. 2A). Consistent with these findings, adiponectin secretion into the culture medium also was increased by stimulation of the cGMP/PKG-dependent pathway (Fig. 2B). These results suggested that natriuretic peptides promote adiponectin synthesis via the GC-A/cGMP/PKG-dependent pathway.

Increase of plasma adiponectin levels in CHF patients treated with hANP. To confirm the effect of natriuretic peptides on the production of adiponectin, we conducted the clinical study. Thirty consecutive patients who met the inclusion criteria were enrolled in this clinical study. Fifteen patients were randomized to the ANP group, and 15 were assigned to the saline group. Baseline variables and treatments of the 2 groups are shown in Table 1. There were no differences in baseline clinical characteristics, hemodynamics, biochemical data, or medications. There was also no significant difference in the baseline plasma level of adiponectin between the 2 groups. As shown in Figure 3B, the plasma level of adiponectin did not change throughout the study in the saline group. On the other hand, the plasma adiponectin level at 1 day after finishing the administration of hANP (day 4) was significantly increased compared with the baseline value (day 1) in the ANP group, and it returned to baseline by 7 days after the completion of hANP infusion (day 10). These results suggested that hANP infusion led to an increase of the plasma adiponectin level in patients with CHF.

Discussion

In the present study, we demonstrated a novel effect of natriuretic peptides (ANP and BNP) on the production of adiponectin by adipocytes in both experimental and clinical studies. First, we clearly demonstrated that pathophysiological and pharmacological concentrations of either ANP or BNP increased adiponectin synthesis by primary cultured human adipocytes. Second, we showed that administration of recombinant ANP increased the plasma adiponectin level in patients with CHF.

ANP and BNP play an important role in the regulation of cardiovascular homeostasis. Their actions are primarily mediated via GC-A, which is expressed in various tissues and organs, including the kidneys, blood vessels, adrenal glands, and heart (18). Consistent with a previous report (19), we demonstrated that GC-A and NPR-C are expressed by human adipocytes. In the present study, we demonstrated a novel effect of both ANP and BNP on primary cultured human adipocytes, which was that pathophysiological or pharmacological concentrations of both peptides augmented adiponectin production by human adipocytes, with this effect being inhibited by treatment with HS142-1. Furthermore, we demonstrated that natriuretic peptides augment the production of adiponectin via a cGMP-dependent

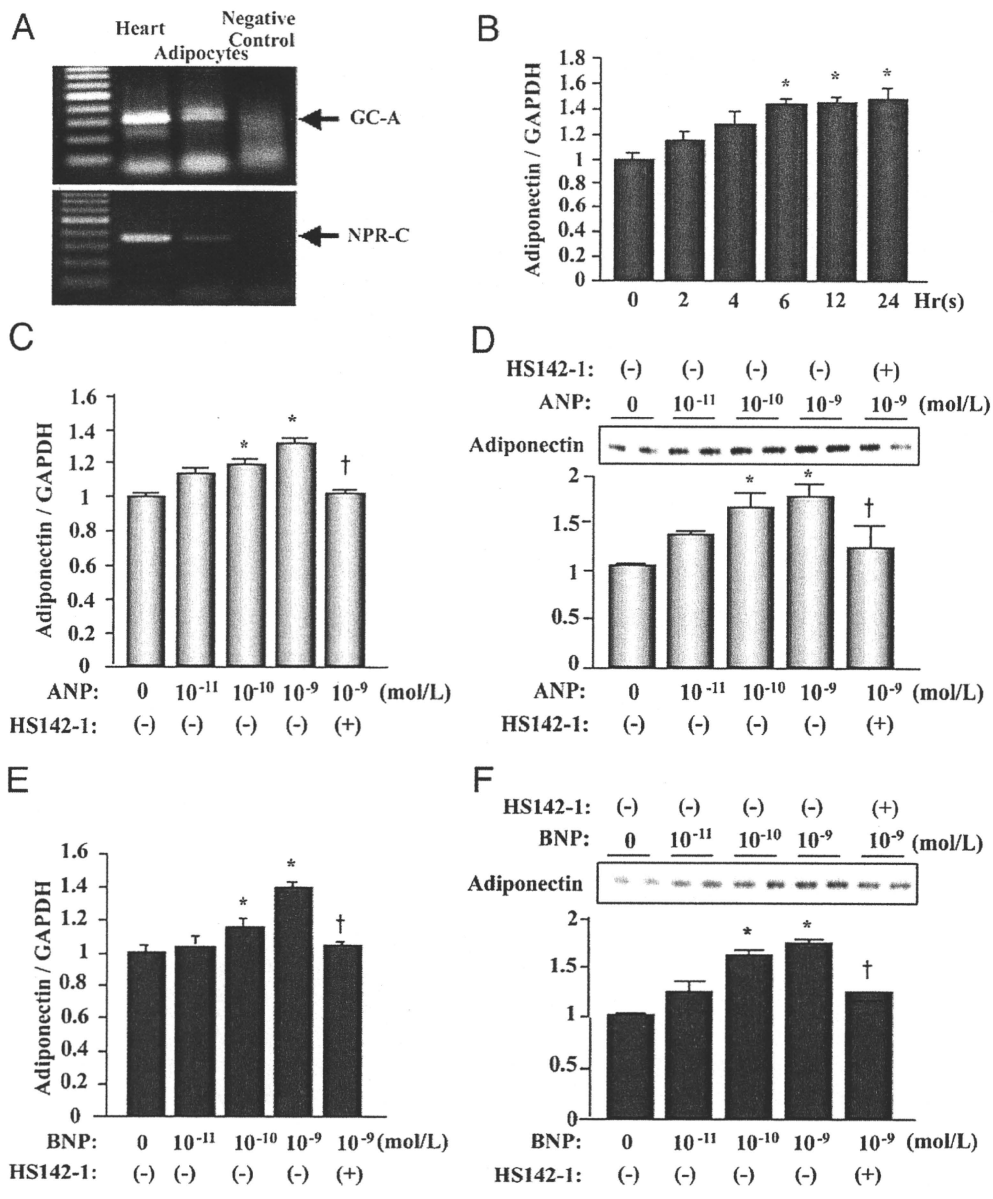

Figure 1 **Effect of Natriuretic Peptides on the Expression and Secretion of Adiponectin by Primary Human Adipocytes**

(A) Expression of GC-A receptors **(top)** and NPR-C **(bottom)** mRNA by primary cultured human adipocytes. Reverse-transcription PCR revealed expression of both GC-A receptors and NPR-C by human adipocytes. **(B)** Effect of ANP (10^{-10} mol/l) on the expression of adiponectin mRNA as determined by quantitative real-time PCR. **(C)** Dose-dependent effect of ANP on adiponectin mRNA expression, as determined by quantitative real-time PCR. Human adipocytes were treated with the indicated concentrations of ANP for 24 h. **(D)** Dose-dependent effect of ANP on adiponectin secretion into the culture medium. **(Top)** A representative Western blot of adiponectin. **(Bottom)** Quantitative analysis of adiponectin by densitometry. Values are normalized to the control. *$p < 0.05$ versus control, †$p < 0.05$ versus ANP 10^{-9} mol/l. **(E)** Dose-dependent effect of BNP on adiponectin mRNA expression, as determined by quantitative real-time PCR. **(F)** Dose-dependent effect of BNP on adiponectin secretion into the culture medium as determined by Western blotting. **(Top)** Representative Western blot of adiponectin. **(Bottom)** Quantitative analysis of adiponectin by densitometry. Values are normalized to the control. *$p < 0.05$ versus control. †$p < 0.05$ versus BNP 10^{-9} mol/l. ANP = atrial natriuretic peptide; BNP = brain natriuretic peptide; GC-A = type A guanylyl cyclase receptor; mRNA = messenger ribonucleic acid; NPR-C = natriuretic peptide receptor C; PCR = polymerase chain reaction.

pathway. These findings are important evidence that ANP and BNP regulate adiponectin production by human adipocytes.

Intravenous infusion of nesiritide (recombinant human BNP) has been reported to have beneficial hemodynamic effects in patients with CHF (4,5). The use of ANP also has been reported to have beneficial effects in patients with acute myocardial infarction (20,21). These beneficial effects have been attributed to the cardiovascular-protective actions of natriuretic peptides, including diuresis, natriuresis, vaso-

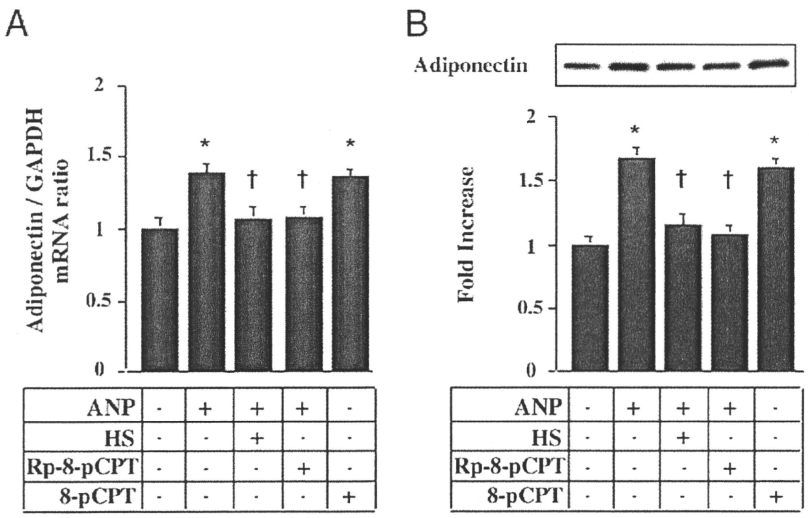

Figure 2 **Involvement of the cGMP/PKG Signaling Pathway in the Induction of Adiponectin Synthesis by ANP**

(A, B) Involvement of PKG was assessed by the treatment of primary cultured human adipocytes with 8-pCPT-cGMP (8-pCPT) and (R_p)-8-Br-PET-cGMP-S (Rp-8-pCPT). Adiponectin mRNA levels were determined by quantitative real-time PCR **(A)** and secretion of adiponectin into the culture medium was determined by Western blotting. Quantitative analysis of adiponectin secretion into the culture medium was done by densitometry **(B)**. Values are normalized to the control. *$p < 0.05$ versus control. †$p < 0.05$ versus ANP. HS = HS142-1; other abbreviations as in Figure 1.

dilation, and reduction of activity of the sympathetic nervous system and the renin-angiotensin-aldosterone system (3–5). In the present study, we administered recombinant ANP to patients with CHF and observed the changes of plasma adiponectin. The plasma adiponectin level of the ANP group was increased at 1 day after the finish of ANP administration compared with that in the control group, and then returned to baseline by 7 days after the completion of administration in patients with CHF.

Importantly, Moro et al. (22) showed that ANP did not affect the secretion of adiponectin in human abdominal adipose tissue from overweight women. This result may appear contradict ours, but we believe that is not the case. First, the concentration of ANP they used (10^{-6} mol/l) in the experiment of cultured adipocytes was greater than our concentration. Second, our data that recombinant ANP increased the plasma adiponectin levels were drawn from patients with heart failure, whereas the data of Moro et al. (22) were from cultured fat tissues of overweight women who underwent plastic surgery. However, they also demonstrated the potential stimulatory effect of ANP on adiponectin production from human adipose tissue in the presence of

Table 1 **Clinical Characteristics of the 2 Groups**

	hANP Group (n = 15)	Saline Group (n = 15)	p Value
Age (yrs)	60 ± 19	59 ± 19	NS
Sex (male/female)	9/6	10/5	NS
Heart rate (beats/min)	62 ± 11	66 ± 7	NS
Body mass index (kg/m^2)	21.4 ± 1.1	21.1 ± 1.7	NS
Systolic blood pressure (mm Hg)	116 ± 9	113 ± 9	NS
Diastolic blood pressure (mm Hg)	76 ± 12	74 ± 6	NS
NYHA functional class (II/III)	14/1	10/5	NS
LVEF by echocardiography (%)	32 ± 2	31 ± 8	NS
Plasma BNP (pg/ml)	506 ± 39	537 ± 33	NS
Other medications n (%)			
Loop diuretics	9 (60)	10 (67)	NS
Spironolactone	5 (33)	8 (53)	NS
ACEI or ARB	12 (80)	11 (80)	NS
Beta-blockers	13 (86)	12 (80)	NS

ACEI = angiotensin-converting enzyme inhibitors; ARB = angiotensin II receptor blockers; BNP = brain natriuretic peptide; hANP = human atrial natriuretic peptide; LVEF = left ventricular ejection fraction; NS = not significant; NYHA = New York Heart Association.

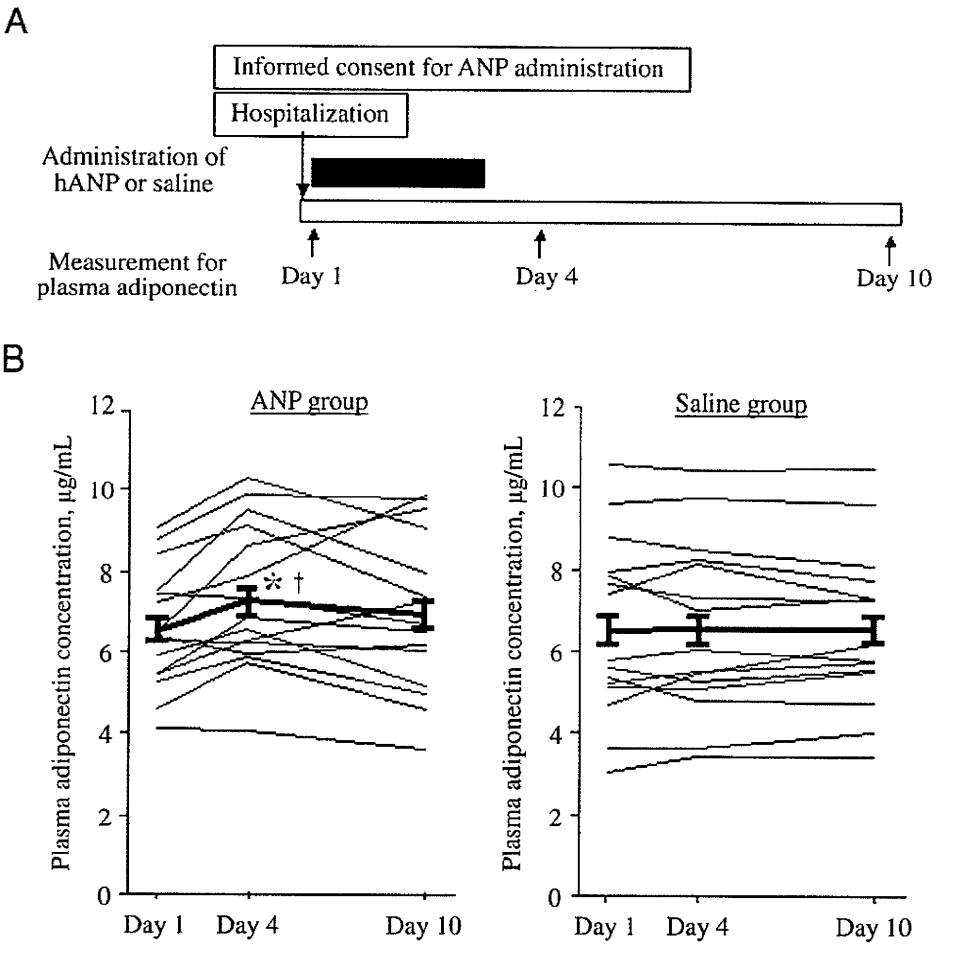

Figure 3 **Increased Plasma Adiponectin Level in Patients With CHF After ANP Treatment**

(A) Outline of the study protocol. hANP or saline was infused continuously for 3 days in the ANP and saline groups, respectively. The **black bar** indicates administration of either hANP (0.025 μg/kg/min) or saline. **(B)** The plasma adiponectin concentration profile after treatment in both groups. *p < 0.05 versus baseline in the ANP group; †p < 0.05 versus at the corresponding time in the saline group. CHF = congestive heart failure; hANP = human atrial natriuretic peptide; other abbreviations as in Figure 1.

hormone-sensitive lipase inhibitor, which inhibits the formation of lipolysis-derived byproducts by ANP-induced lipolysis (22).

Recently, Yu et al. (23) demonstrated the increased ANP-induced lipolysis rates in large adipocytes compared with small adipocytes. Thus, the difference of adipocyte size between patients with CHF and obesity might contribute to the different pattern of adiponectin secretion. Finally, catecholamines also are involved in the control of lipolysis in humans (24). Thus, the prolonged exposure of high plasma level of catecholamines or the treatment with beta-adrenergic receptor blockers in patients with CHF also might affect the distinct pattern of adiponectin secretion from adipocytes. Although precise mechanisms are unknown, the human adipocytes could secrete adiponectin when the certain stress was loaded. However, it remains possible that factors such as tumor necrosis factor-alpha (25) and alpha-adrenergic stimulation (26), both of which are increased in patients with CHF, may influence the expression of adiponectin or that adiponectin levels are affected by medical treatment, so further investigations are needed.

It is not clear whether ANP augments the plasma adiponectin levels in healthy subjects because of the ethical problems. However, we have reported that the plasma adiponectin level increased along with an increase of plasma BNP levels in 1,538 healthy subjects (27). These results suggest that an increase of natriuretic peptides augments the plasma adiponectin levels and exerts a cardioprotective effect in clinical settings.

Under normal conditions the adult heart utilizes predominantly fatty acids to derive the majority of its energy (28). However, metabolic remodeling such as a marked shift in substrate preference away from fatty acids toward glucose is observed in hypertrophic and failing hearts and the decrease

in fatty acid oxidation is not fully compensated for by an increase in glucose oxidation (29). Thus, the failing heart suffers from chronic energy starvation (30). Insulin resistance also is common in patients with heart failure (31). Adiponectin improves both glucose metabolism and insulin resistance via the AMPK signaling pathway (32). Therefore, we believe that the administration of recombinant natriuretic peptide has beneficial effects on cardiac energy metabolism via adiponectin in patients with CHF.

Interestingly, the plasma adiponectin level was reported to be decreased in patients with risk factors for heart failure (9,33–35) and increased along with BNP after the onset of heart failure (14). Although approximately 10% increase in adiponectin levels in the ANP group seems relatively small, this would not be the case because there was about a 20% reduction in plasma adiponectin levels in patients with coronary artery disease compared with those in control subjects (35), which leads us to believe that the 10% increase in adiponectin is important from the viewpoint of pathophysiology of heart diseases. Therefore, we hypothesized that ANP and/or BNP regulates the plasma level of adiponectin in patients with CHF and conducted this study.

Conclusions

We demonstrated that natriuretic peptides increase the production of adiponectin by human adipocytes, as well as in patients with CHF. These findings may help to shed more light on the pathophysiology of heart failure.

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REFERENCES

1. Maisel AS, Krishnaswamy P, Nowak RM, et al. Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. N Engl J Med 2002;347:161–7.
2. Stanek B, Frey B, Hulsmann M, et al. Prognostic evaluation of neurohumoral plasma levels before and during beta-blocker therapy in advanced left ventricular dysfunction. J Am Coll Cardiol 2001;38:436–42.
3. Levin ER, Gardner DG, Samson WK. Natriuretic peptides. N Engl J Med 1998;339:321–8.
4. Mills RM, LeJemtel TH, Horton DP, et al. Sustained hemodynamic effects of an infusion of nesiritide (human b-type natriuretic peptide) in heart failure: a randomized, double-blind, placebo-controlled clinical trial. Natrecor Study Group. J Am Coll Cardiol 1999;34:155–62.
5. Colucci WS, Elkayam U, Horton DP, et al. Intravenous nesiritide, a natriuretic peptide, in the treatment of decompensated congestive heart failure. Nesiritide Study Group. N Engl J Med 2000;343:246–53.
6. Lakka HM, Laaksonen DE, Lakka TA, et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA 2002;288:2709–16.
7. Ninomiya JK, L'Italien G, Criqui MH, Whyte JL, Gamst A, Chen RS. Association of the metabolic syndrome with history of myocardial infarction and stroke in the Third National Health and Nutrition Examination Survey. Circulation 2004;109:42–6.
8. Ingelsson E, Sundstrom J, Arnlov J, Zethelius B, Lind L. Insulin resistance and risk of congestive heart failure. JAMA 2005;294:334–41.
9. Kenchaiah S, Evans JC, Levy D, et al. Obesity and the risk of heart failure. N Engl J Med 2002;347:305–13.
10. Hunt SA, Baker DW, Chin MH, et al. ACC/AHA guidelines for the evaluation and management of chronic heart failure in the adult: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1995 Guidelines for the Evaluation and Management of Heart Failure). J Am Coll Cardiol 2001;38:2101–13.
11. Shibata R, Sato K, Pimentel DR, et al. Adiponectin protects against myocardial ischemia-reperfusion injury through AMPK- and COX-2-dependent mechanisms. Nat Med 2005;11:1096–103.
12. Shibata R, Ouchi N, Ito M, et al. Adiponectin-mediated modulation of hypertrophic signals in the heart. Nat Med 2004;10:1384–9.
13. Liao Y, Takashima S, Maeda N, et al. Exacerbation of heart failure in adiponectin-deficient mice due to impaired regulation of AMPK and glucose metabolism. Cardiovasc Res 2005;67:705–13.
14. Kistorp C, Faber J, Galatius S, et al. Plasma adiponectin, body mass index, and mortality in patients with chronic heart failure. Circulation 2005;112:1756–62.
15. Maeda N, Takahashi M, Funahashi T, et al. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes 2001;50:2094–9.
16. Okada K, Minamino T, Tsukamoto Y, et al. Prolonged endoplasmic reticulum stress in hypertrophic and failing heart after aortic constriction: possible contribution of endoplasmic reticulum stress to cardiac myocyte apoptosis. Circulation 2004;110:705–12.
17. Tsukamoto O, Minamino T, Okada K, et al. Depression of proteasome activities during the progression of cardiac dysfunction in pressure-overloaded heart of mice. Biochem Biophys Res Commun 2006;340:1125–33.
18. Nakao K, Ogawa Y, Suga S, Imura H. Molecular biology and biochemistry of the natriuretic peptide system. II: Natriuretic peptide receptors. J Hypertens 1992;10:1111–4.
19. Sengenes C, Zakaroff-Girard A, Moulin A, et al. Natriuretic peptide-dependent lipolysis in fat cells is a primate specificity. Am J Physiol Regul Integr Comp Physiol 2002;283:R257–65.
20. Kitakaze M, Asakura M, Kim J, et al. Human atrial natriuretic peptide and nicorandil as adjuncts to reperfusion treatment for acute myocardial infarction (J-WIND): two randomised trials. Lancet 2007;370:1483–93.
21. Hayashi M, Tsutamoto T, Wada A, et al. Intravenous atrial natriuretic peptide prevents left ventricular remodeling in patients with first anterior acute myocardial infarction. J Am Coll Cardiol 2001;37:1820–6.
22. Moro C, Klimcakova E, Lolmede K, et al. Atrial natriuretic peptide inhibits the production of adipokines and cytokines linked to inflammation and insulin resistance in human subcutaneous adipose tissue. Diabetologia 2007;50:1038–47.
23. Yu J, Yu HC, Kim KA, et al. Differences in the amount of lipolysis induced by atrial natriuretic peptide in small and large adipocytes. J Pept Sci 2008;14:972–7.
24. Moro C, Galitzky J, Sengenes C, Crampes F, Lafontan M, Berlan M. Functional and pharmacological characterization of the natriuretic peptide-dependent lipolytic pathway in human fat cells. J Pharmacol Exp Ther 2004;308:984–92.
25. Maeda N, Shimomura I, Kishida K, et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat Med 2002;8:731–7.
26. Fasshauer M, Klein J, Neumann S, Eszlinger M, Paschke R. Adiponectin gene expression is inhibited by beta-adrenergic stimulation via protein kinase A in 3T3-L1 adipocytes. FEBS Lett 2001;507:142–6.

27. Ohara T, Kim J, Asakura M, et al. Plasma adiponectin is associated with plasma brain natriuretic peptide and cardiac function in healthy subjects. Hypertens Res 2008;31:825–31.
28. Makinde AO, Gamble J, Lopaschuk GD. Upregulation of 5′-AMP-activated protein kinase is responsible for the increase in myocardial fatty acid oxidation rates following birth in the newborn rabbit. Circ Res 1997;80:482–9.
29. van Bilsen M, Smeets PJ, Gilde AJ, van der Vusse GJ. Metabolic remodelling of the failing heart: the cardiac burn-out syndrome? Cardiovasc Res 2004;61:218–26.
30. Shen W, Asai K, Uechi M, et al. Progressive loss of myocardial ATP due to a loss of total purines during the development of heart failure in dogs: a compensatory role for the parallel loss of creatine. Circulation 1999;100:2113–8.
31. Swan JW, Anker SD, Walton C, et al. Insulin resistance in chronic heart failure: relation to severity and etiology of heart failure. J Am Coll Cardiol 1997;30:527–32.
32. Yamauchi T, Kamon J, Minokoshi Y, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002;8:1288–95.
33. Della Mea P, Lupia M, Bandolin V, et al. Adiponectin, insulin resistance, and left ventricular structure in dipper and nondipper essential hypertensive patients. Am J Hypertens 2005;18:30–5.
34. Hotta K, Funahashi T, Arita Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000;20:1595–9.
35. Kumada M, Kihara S, Sumitsuji S, et al. Association of hypoadiponectinemia with coronary artery disease in men. Arterioscler Thromb Vasc Biol 2003;23:85–9.

Key Words: adiponectin ▪ natriuretic peptides ▪ heart failure ▪ adipose tissue.

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ORIGINAL ARTICLE
Heart Failure

Prevalence and Clinical Implication of Metabolic Syndrome in Chronic Heart Failure

– Report From MetS-CHF Study –

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Background: Metabolic syndrome (MetS) is a pathological condition with a clustering of metabolic components and is a well-known risk and prognostic factor for ischemic heart disease (IHD). However, the prevalence and clinical significance of MetS remain to be fully elucidated in chronic heart failure (CHF), an important clinical syndrome caused by various cardiac abnormalities.

Methods and Results: The present nationwide, large-scale clinical study enrolled 3,603 patients with stage C/D CHF from 6 institutes in Japan. First, the prevalence of MetS in CHF patients was demonstrated to be 45% in males and 19% in females, which is more than double compared with the general population in Japan. The CHF patients with MetS were characterized by younger age, higher prevalence of current smoking and drinking, IHD, and hypertensive heart disease, whereas the prevalence of HF with preserved ejection fraction and MetS was higher in elderly female patients. Next, the contribution of the metabolic components (waist circumference, hypertension, glucose intolerance/diabetes mellitus and dyslipidemia) was found to be comparable between the ischemic and the non-ischemic CHF patients.

Conclusions: The prevalence of MetS in CHF patients is more than double compared with the general population in Japan and suggest that the metabolic components may have a substantial effect on the development of both ischemic and non-ischemic CHF. (*Circ J* 2010; **74:** 2612–2621)

Key Words: Chronic heart failure; Metabolic syndrome; Obesity; Sex

Over the past decades, the prevalence of obesity, lifestyle diseases (eg, diabetes mellitus, dyslipidemia, hypertension, and metabolic syndrome (MetS)) and resultant cardiovascular disease has been rapidly increasing in Japan because of the westernization of lifestyle.[1] MetS is a pathological condition with clustering of metabolic components, including dysglycemia, elevated blood pressure, elevated triglyceride levels, low high-density lipoprotein (HDL) cholesterol levels and obesity.[2] It has been repeatedly demonstrated that MetS is substantially involved in the increased risk of atherosclerotic diseases with resultant poor prognosis after acute coronary syndrome.[3–9] Although recent studies have reported the relationship between MetS and congestive heart failure,[10,11] the prevalence and clinical significance of MetS in chronic heart failure (CHF) remain to be fully elucidated. CHF is a complex clinical syndrome that can result from any structural or functional cardiac disorders, including coronary artery disease, hypertensive heart disease, myocardial disease and valvular heart disease.[12] CHF is a clinical syndrome in which not only heart failure with

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preserved ejection fraction (HFPEF), but also heart failure with reduced ejection fraction (HFREF), is substantially involved.[13,14] Indeed, HFPEF and HFREF respectively account for approximately half of the CHF patients.[15,16]

Editorial p2550

We have been conducting a nationwide clinical study supported by the Japanese government on the current status of CHF in Japan with special reference to MetS (MetS-CHF Study). This is the first report of our study, which addresses the prevalence and clinical significance of MetS in Japanese patients with CHF.

Methods

The ethical committees of each institute approved the study protocol and all patients provided written informed consent.

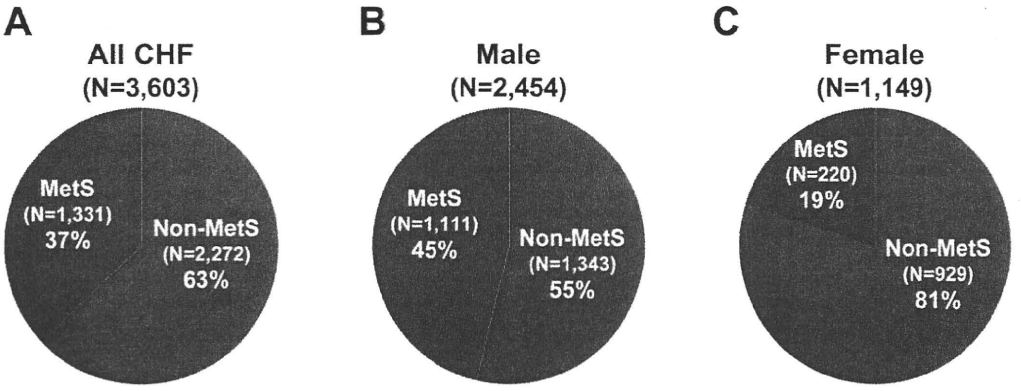
Study Population

Between September 2006 and December 2008 we enrolled 3,603 CHF patients in stages C/D according to the ACC/AHA Guidelines[12] from 6 institutes in Japan. For each patient, we prospectively collected from the participating hospitals

Table 1. Baseline Characteristics of Patients With Symptomatic CHF

	Male (n=2,454)	Female (n=1,149)	P value
Age (years)	67.9±0.2	71.1±0.4	<0.001
Cigarette smoking, n (%)			
Never	811 (48.9%)	856 (79.8%)	<0.001
Former	343 (20.7%)	153 (14.2%)	NS
Current	505 (30.4%)	64 (6.0%)	<0.001
Alcohol intake, n (%)			
Never	722 (30.7%)	760 (80.9%)	<0.001
Former	220 (11.2%)	35 (3.7%)	<0.001
Current	1,027 (52.1%)	144 (15.3%)	<0.001
BMI (kg/m^2)	23.1±0.1	22.1±0.2	<0.001
Waist circumference (cm)	86.7±0.2	81.9±0.4	<0.001
Blood pressure (mmHg)			
Systolic	125.7±0.4	126.3±0.6	NS
Diastolic	72.2±0.3	70.7±0.4	<0.001
Heart rate (beats/min)	71.8±0.3	74.7±0.4	<0.001
NYHA class			
I	490 (20.0%)	133 (11.6%)	<0.001
II	1,683 (68.9%)	814 (70.9%)	NS
III	246 (10.1%)	187 (16.3%)	<0.001
IV	24 (1.0%)	14 (1.2%)	NS
Stage C/D	2,381 (97.4%)/63 (2.6%)	1,113 (97.0%)/35 (3.0%)	NS
LVEF (%)	54.4±0.3	59.5±0.5	<0.001
HFREF (EF<50%)	797 (36.2%)	269 (25.7%)	<0.001
HFPEF (EF≥50%)	1,402 (63.8%)	777 (74.3%)	<0.001
SAS	5.7±0.04	4.6±0.06	<0.001
HT	1,876 (76.4%)	864 (75.2%)	NS
DM or fasting glucose ≥110 mg/dl	1,253 (51.1%)	526 (45.8%)	<0.01
Dyslipidemia	1,754 (71.7%)	816 (71.0%)	NS
IHD	1,264 (51.5%)	352 (30.6%)	<0.001
HHD	230 (9.4%)	126 (11.0%)	NS
CM	508 (20.7%)	216 (18.8%)	NS
VHD	494 (20.1%)	428 (37.2%)	<0.001
CHD	29 (1.2%)	32 (2.8%)	<0.001
Medications			
ACEI/ARB	1,793 (73.1%)	765 (66.6%)	<0.001
β-blocker	1,237 (50.4%)	507 (44.1%)	<0.001
Statin	876 (35.7%)	381 (33.2%)	NS

Values are mean±SEM.
CHF, chronic heart failure; BMI, body mass index; LVEF, left ventricular ejection fraction; HFREF, heart failure with reduced ejection fraction; EF, ejection fraction; HFPEF, heart failure with preserved ejection fraction; SAS, specific activity scale; HT, hypertension; DM, diabetes mellitus; IHD, ischemic heart disease; HHD, hypertensive heart disease; CM, cardiomyopathy; VHD, valvular heart disease; CHD, congenital heart disease; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker.



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	General Population			CHF Patients
	Tanno and Sobetsu Study (Ref. 18)	Hisayama Study (Ref. 19)	Kanazawa Study (Ref. 20)	MetS-CHF Study
Male	18%	21%	16~21%	45%
Female	6%	8%	3~10%	19%

Figure 1. Prevalence of metabolic syndrome (MetS) in patients with chronic heart failure (CHF) was 37% of all patients with CHF (**A**), 46% of male patients (**B**) and 20% of female patients (**C**). Compared with the prevalence in the general population in the Tanno-Sobetsu,[18] Hisayama[19] and Kanazawa[20] studies, the prevalence of MetS was more than double (**D**).

the baseline demographic data, including age, sex, height, weight, waist circumference, coronary risk factors (blood pressure, lipid profile, fasting plasma glucose, smoking status), medications, comorbidities (previous myocardial infarction or stroke, dialysis, and atrial fibrillation) by use of a web data collection system (Tohoku Fujitsu, Sendai, Japan).

Definition of MetS

According to the new definition by the Japanese Committee for the Diagnostic Criteria of MetS in April 2005, we defined MetS as the presence of 2 or more abnormalities in addition to waist circumference (≥85 cm in males and ≥90 cm in females). Other abnormalities examined were dyslipidemia, hypertension, and glucose intolerance/diabetes mellitus. Dyslipidemia was defined as use of lipid-lowering drugs and/or elevated lipid levels (plasma triglycerides ≥150 mg/dl or HDL<40 mg/dl in men or 50 mg/dl in women). Glucose intolerance/diabetes mellitus was defined as use of antidiabetic drugs and/or fasting glucose ≥110 mg/dl. Hypertension was defined as use of antihypertensive drugs and/or systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥80 mmHg.

Definition of CHF

In the present study, we included patients with stages C/D CHF defined by the ACC/AHA 2005 Guidelines (ie, they had developed symptoms of HF, at least NYHA class II).[12] According to the ESC 2007 Guideline, we further divided them into 2 groups: HFPEF (LV ejection fraction (EF) ≥50%, n=2,179) and HFREF (LVEF<50%, n=1,066).[17]

Data Collection

Baseline demographic data (age, sex, height, body weight, and waist), CHF stage, medications, including angiotensin-converting enzyme inhibitors (ACEI), angiotensin-receptor blockers (ARB), β-blockers, and statins, risk factors (hypertension, glucose intolerance/diabetes mellitus and dyslipidemia), blood pressure, pulse rate, blood data (lipid profile and glucose), and comorbidities (ischemic heart disease (IHD), hypertensive heart disease, cardiomyopathy, valvular heart disease, and congenital heart disease) were collected from the medical records. LVEF was measured by echocardiography.

Statistical Analysis

Continuous variables are expressed as mean±SEM. Comparisons between 2 groups were conducted with unpaired t-test for continuous variables and chi-test for categorical variables. Statistical analyses were performed using Prism 4 (GraphPad Software, La Jolla, CA, USA). $P<0.05$ was considered to be statistically significant.

Results

Characteristics of CHF Patients

Among the 3,603 consecutive patients with stage C/D CHF, there were 2,454 men (68%, 68±0.2 years) and 1,149 women (32%, 71±0.4 years) (Table 1). In total, 1,331 patients had MetS (37%) and 2,272 did not (63%) (Figure 1A, Table 2). Of the 2,454 male patients with CHF, 1,111 had MetS (45%) and 1,343 did not (55%) (Figure 1B, Table 3), and of the

Table 2. Comparison of Non-MetS and MetS Patients With Symptomatic CHF

	Total		
	Non-MetS (n=2,272)	MetS (n=1,331)	P value
Sex, n (%)			
Male	1,343 (59.1%)	1,111 (83.5%)	<0.001
Female	929 (40.9%)	220 (16.5%)	<0.001
Age (years)	69.7±0.3	67.6±0.3	<0.001
Cigarette smoking, n (%)			
Never	1,129 (63.6%)	538 (56.2%)	<0.001
Former	323 (18.2%)	173 (18.1%)	NS
Current	323 (18.2%)	246 (25.7%)	<0.001
Alcohol intake, n (%)			
Never	1,008 (55.7%)	474 (43.2%)	<0.001
Former	162 (9.0%)	93 (8.5%)	NS
Current	640 (35.3%)	531 (48.3%)	<0.001
BMI (kg/m^2)	21.2±0.1	25.5±0.2	<0.001
Waist circumference (cm)			
Male	81.2±0.2	92.8±0.2	<0.001
Female	77.9±0.3	97.1±0.5	<0.001
Blood pressure (mmHg)			
Systolic	123.6±0.4	129.8±0.5	<0.001
Diastolic	70.3±0.3	74.2±0.3	<0.001
Heart rate (beats/min)	72.8±0.3	72.6±0.4	NS
NYHA class			
I	354 (15.6%)	269 (20.3%)	<0.001
II	1,576 (69.6%)	921 (69.5%)	NS
III	303 (13.4%)	130 (9.8%)	<0.001
IV	32 (1.4%)	6 (0.4%)	<0.001
Stage C/D	2,193 (96.7%)/75 (3.3%)	1,301 (98.3%)/23 (1.7%)	<0.01
LVEF (%)	55.7±0.4	56.7±0.4	NS
HFREF (EF<50%)	700 (34.0%)	366 (30.8%)	NS
HFPEF (EF≥50%)	1,357 (66.0%)	822 (69.2%)	NS
SAS	5.2±0.05	5.6±0.06	<0.001
HT	1,525 (67.1%)	1,215 (91.3%)	<0.001
DM or fasting glucose≥110 mg/dl	890 (39.2%)	889 (66.8%)	<0.001
Dyslipidemia	1,402 (61.7%)	1,168 (87.8%)	<0.001
IHD	882 (38.8%)	734 (55.1%)	<0.001
HHD	192 (8.5%)	164 (12.3%)	<0.001
CM	477 (21.0%)	247 (18.6%)	NS
VHD	714 (31.4%)	208 (15.6%)	<0.001
CHD	49 (2.2%)	12 (0.9%)	<0.01
Medications			
ACEI/ARB	1,534 (67.5%)	1,024 (76.9%)	<0.001
β-blocker	1,058 (46.6%)	686 (51.5%)	<0.01
Statin	638 (28.1%)	619 (46.6%)	<0.001

Values are mean±SEM.
MetS, metabolic syndrome. Other abbreviations see in Table 1.

1,149 female patients with CHF, 220 had MetS (19%) and 929 did not (81%) (**Figure 1C**, **Table 3**). The prevalence of MetS in the general Japanese population has been previously reported as approximately 20% in men and approximately 10% in women in the Tanno-Sobetsu Study, the Hisayama Study (males 58±11 years, females 59±11 years), and the Kanazawa Study (males 68±8 years, females 66±9 years),[7,18–20] so our results show a prevalence of MetS in Japanese CHF patients as more than double that of the general population (**Figure 1D**).

As shown in **Table 1**, the present stage C/D CHF patients were characterized by a higher prevalence of hypertension and dyslipidemia, followed by glucose intolerance/diabetes mellitus, in both sexes. Furthermore, the male CHF patients

Table 3. Comparison of Non-MetS and MetS Patients With Symptomatic CHF

	Male			Female		
	Non-MetS (n=1,343)	MetS (n=1,111)	P value	Non-MetS (n=929)	MetS (n=220)	P value
Age (years)	68.9±0.3	66.6±0.3	<0.001	70.8±0.4	72.6±0.7	<0.05
Cigarette smoking, n (%)						
Never	441 (48.6%)	370 (49.3%)	NS	688 (79.4%)	168 (81.6%)	NS
Former	195 (21.4%)	148 (19.7%)	NS	128 (14.8%)	25 (12.1%)	NS
Current	272 (30.0%)	233 (31.0%)	NS	51 (5.8%)	13 (6.3%)	NS
Alcohol intake, n (%)						
Never	402 (37.9%)	320 (35.3%)	NS	606 (81.0%)	154 (80.6%)	NS
Former	131 (12.3%)	89 (9.8%)	NS	31 (4.2%)	4 (2.1%)	NS
Current	529 (49.8%)	498 (54.9%)	NS	111 (14.8%)	33 (17.3%)	NS
BMI (kg/m^2)	21.3±0.2	25.3±0.2	<0.001	21.1±0.2	26.5±0.5	<0.001
Blood pressure (mmHg)						
Systolic	122.6±0.6	129.4±0.5	<0.001	125.1±0.7	131.6±1.4	<0.001
Diastolic	70.2±0.3	74.6±0.4	<0.001	70.4±0.04	72.2±0.9	NS
Heart rate (beats/min)	71.5±0.4	72.2±0.4	NS	74.7±0.6	74.7±1.0	NS
NYHA class						
I	240 (18.0%)	250 (22.6%)	<0.01	114 (12.3%)	19 (8.6%)	NS
II	929 (69.5%)	754 (68.2%)	NS	647 (69.7%)	167 (75.9%)	NS
III	149 (11.1%)	97 (8.8%)	NS	154 (16.6%)	33 (15.0%)	NS
IV	19 (1.4%)	5 (0.4%)	<0.001	13 (1.4%)	1 (0.5%)	NS
Stage C/D	1,296 (96.7%)/ 44 (3.7%)	1,085 (98.3%)/ 19 (1.7%)	<0.05	897 (96.7%)/ 31 (3.3%)	216 (98.2%)/ 4 (1.8%)	NS
LVEF (%)	53.3±0.5	55.8±0.5	<0.001	59±0.5	61.4±1.0	NS
HFREF (EF<50%)	468 (38.7%)	329 (33.2%)	<0.01	232 (27.3%)	37 (18.8%)	<0.05
HFPEF (EF≥50%)	740 (61.3%)	662 (66.8%)	<0.01	617 (72.7%)	160 (81.2%)	<0.05
SAS	5.6±0.06	5.8±0.06	<0.05	4.6±0.07	4.5±0.1	<0.001
HT	870 (64.8%)	1,006 (90.5%)	<0.001	655 (70.5%)	209 (95.0%)	<0.001
DM or fasting glucose≥110 mg/dl	506 (37.7%)	747 (67.2%)	<0.001	384 (41.3%)	142 (64.5%)	<0.001
Dyslipidemia	787 (58.6%)	967 (87.0%)	<0.001	615 (66.2%)	201 (87.3%)	<0.001
IHD	632 (47.1%)	632 (56.9%)	<0.001	250 (27.0%)	102 (46.4%)	<0.001
HHD	104 (7.7%)	126 (11.3%)	<0.01	88 (9.5%)	38 (17.3%)	0.001
CM	294 (21.9%)	214 (19.3%)	NS	183 (19.8%)	33 (15.0%)	NS
VHD	332 (24.7%)	162 (14.6%)	<0.001	382 (41.3%)	46 (20.9%)	<0.001
CHD	19 (1.4%)	10 (0.9%)	NS	30 (3.2%)	2 (1%)	NS
Medications						
ACEI/ARB	935 (69.6%)	858 (77.2%)	<0.001	599 (64.5%)	166 (75.5%)	<0.01
β-blocker	666 (49.6%)	571 (51.4%)	NS	392 (42.2%)	115 (52.3%)	<0.01
Statin	364 (27.1%)	512 (46.1%)	<0.001	274 (29.5%)	107 (48.6%)	<0.001

Values are mean±SEM.
Abbreviations see in Tables 1,2.

were characterized by higher prevalence of larger body mass index, glucose intolerance/diabetes mellitus, and IHD, whereas the female patients were in a higher NYHA class, had lower exercise tolerance, and higher prevalence of both preserved LVEF and valvular heart disease.

MetS in CHF

In both male and female patients with CHF, those with MetS were characterized by younger age, higher prevalence of current smoking and drinking, IHD, and hypertensive heart disease, lower NYHA class, better exercise tolerance, and more likelihood of taking medications such as ACEI/ARB, β-blockers or statins (Tables 2,3, Figure 2). The prevalence of HFPEF was significantly higher in the MetS group compared with the non-MetS group (Table 3, Figure 3).

When compared with the patients with HFREF, those with HFPEF were characterized by higher prevalence of elderly and female patients, obesity, hypertensive and valvular heart disease, and less likelihood of taking medications such as ACEI/ARB, β-blockers or statins (Table 4).

Metabolic Components in CHF

In the present study, the contribution of single or combined metabolic components was observed in both the ischemic

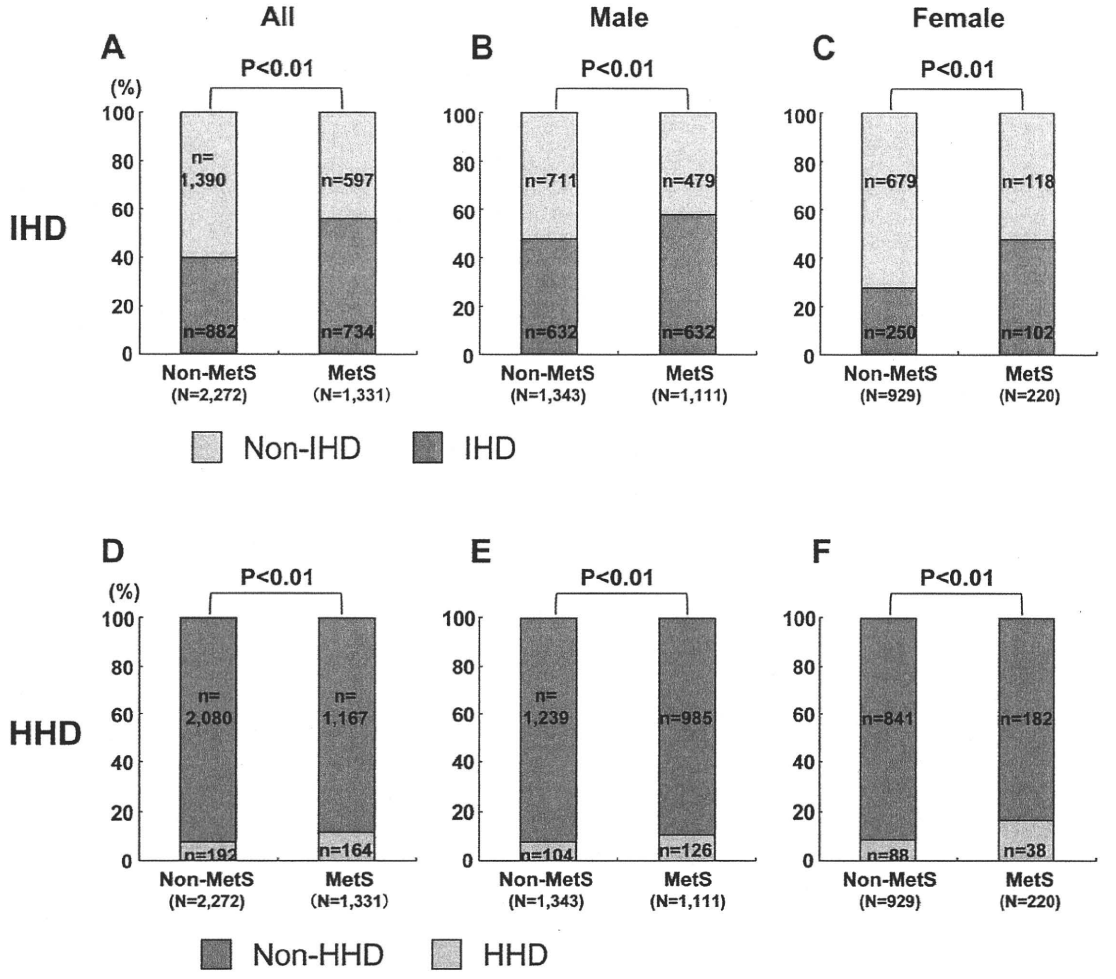

Figure 2. Effect of metabolic syndrome (MetS) on ischemic heart disease (IHD) and hypertensive heart disease (HHD). IHD was significantly more prevalent in CHF patients with MetS than in those without it for all patients (**A**), and in male (**B**) or female patients (**C**). Similarly, for HHD for all patients (**D**), and male (**E**) and female patients (**F**).

and non-ischemic CHF patients (**Figure 4A**). Although the prevalence of ischemic CHF was significantly higher in most of the subgroups with more than 3 metabolic components, the contribution of other single or combined metabolic components was either comparable between the 2 groups or stronger in the non-ischemic CHF group (**Figure 4A**). Although the prevalence of combined metabolic components varied, these components were comparably associated with both HFPEF and HFREF (**Figure 4B**).

Discussion

The novel findings of the present study are that (a) the prevalence of MetS in CHF was more than double that of the general Japanese population, (b) MetS was associated with ischemic or hypertensive heart disease-related heart failure, (c) HFPEF was characterized by a higher prevalence of elderly and female patients with MetS, and (d) the prevalence of the metabolic components was comparable between the ischemic and non-ischemic CHF patients. To the best of our knowledge, this is the first study to provide evidence for a relationship between MetS and CHF.

Prevalence of MetS in CHF

It has been reported that the prevalence of MetS in the general Japanese population is 10–20% in men and 2–8% in women, as defined by the current Japanese criteria.[7,18,19] In contrast, the present study demonstrated a prevalence of MetS (45% in men and 19% in women) that is more than 2-fold that of the general population, suggesting that the presence of MetS is an important therapeutic target of CHF treatment. It is conceivable that the increased prevalence of MetS in CHF patients is both the cause and the result of CHF, as activation of both the sympathetic nervous system and renin–angiotensin system causes the metabolic components.[21] In order to address this important issue, we are now performing a cohort study in which we follow-up MetS patients without CHF to examine the development of CHF in them.

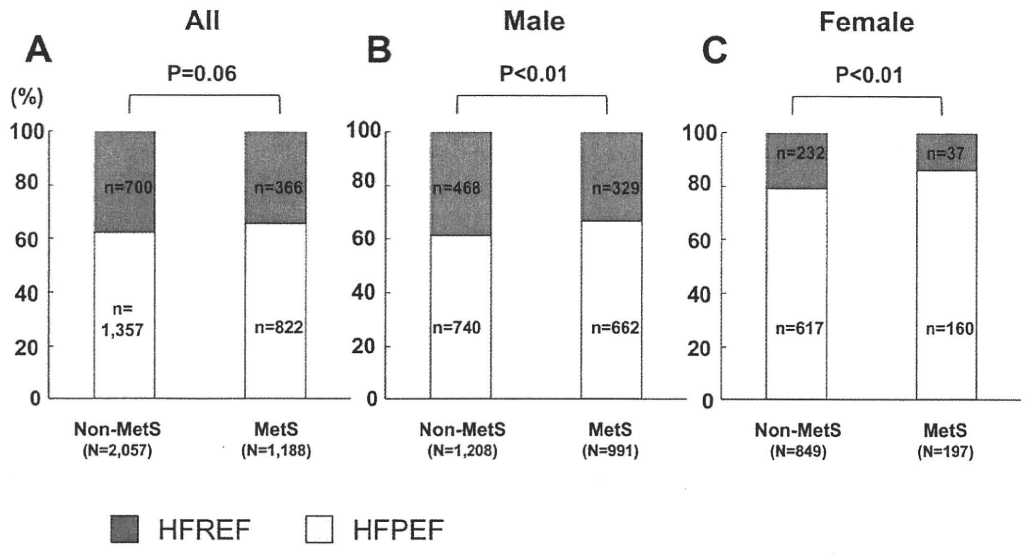

Figure 3. Effect of metabolic syndrome (MetS) on left ventricular ejection fraction. The prevalence of heart failure with preserved ejection fraction (HFPEF) was significantly greater for males (B) and females (C) with MetS than in those without MetS and tended to be so for all patients with chronic heart failure (A). HFREF, heart failure with reduced ejection fraction.

Role of MetS in Ischemic and Hypertensive Heart Disease

MetS has been identified as a risk and prognostic factor for IHD and stroke.[8,22,23] In the present study, MetS was highly associated with IHD in both male and female patients with CHF. Thus, the prevention of IHD is extremely important for preventing the development of CHF, both by life-style modification and the use of anti-atherosclerotic drugs in order to achieve stabilization and regression of systemic atherosclerosis. Furthermore, because hypertension is associated with obesity,[24] it is also important to treat obesity for blood pressure control in order to prevent the development of hypertensive heart disease.

Comparison of HFPEF and HFREF

It has been demonstrated that heart failure can also occur in patients with preserved LVEF, which is often observed in hypertensive heart disease mainly caused by LV diastolic dysfunction.[14] It is now widely accepted that HFPEF is a major cardiovascular disorder with poor prognosis, accounting for approximately 50% of patients with heart failure symptoms,[15,16] and our study demonstrated that 67% of CHF patients had HFPEF (Table 4). The present results also indicate the different clinical characteristics of HFPEF and HFREF patients, and the former were characterized by a higher prevalence of elderly and female patients, obesity, and hypertensive and valvular heart disease. Although it has been previously demonstrated that the major determinants of diastolic dysfunction are enhanced myocardial stiffness and impaired relaxation capacity,[25] further studies are needed to clarify the association between these clinical factors and LV dysfunction.

Metabolic Components in Ischemic and Non-Ischemic CHF

In the present study, among the metabolic components in the CHF patients, the prevalence of both hypertension and dyslipidemia was higher, followed by glucose intolerance/diabetes mellitus, probably because of environmental and genetic factors. In order to prevent the development of CHF, all components of MetS should be controlled (ie, blood pressure by anti-hypertensive drugs, lipid-lowering by HMG-CoA reductase inhibitors, and glucose control by diet therapy, exercise and antidiabetic drugs), which is known to ameliorate vascular function and stabilize atheroma.[26–33] In contrast, smoking and alcohol intake may not be highly related to the development of CHF compared with hypertension or dyslipidemia, so smoking cessation and moderate alcohol intake are recommended in the early stage of CHF.[9]

In the present study, MetS was related to the development of HFPEF (LVEF≥50%) in both male and female patients with CHF. Although the precise mechanisms are unknown, coronary microvascular dysfunction with preserved systolic function might be linked to this phenomenon.[34,35]

The present study also demonstrated that there are single or combined metabolic components in both non-ischemic CHF and ischemic CHF patients, a consistent finding with a previous report regarding the lipid levels and heart failure incidence in Caucasians.[36] Therefore, these metabolic components should be regarded as important therapeutic targets for CHF caused by both ischemic and non-IHD.

Study Limitations

First, although we were able to collect the data for a relatively large number of CHF patients, their prognoses need to be elucidated. As we are currently performing a follow-up study for them, we will report the results separately in the future. Second, we used the 2005 definition of the Japanese Committee for the Diagnostic Criteria of MetS, so we were unable to compare the present data with that of non-Japanese studies. We plan to use other diagnostic criteria, such as the National Cholesterol Education Program-Adult Treatment

Table 4. Comparison of HFPEF and HFREF Patients With Symptomatic CHF

	Total		
	HFPEF (n=2,179)	HFREF (n=1,066)	P value
Sex, n (%)			
Male	1,402 (64.3%)	797 (74.8%)	<0.001
Female	777 (35.7%)	269 (25.2%)	<0.001
Age (years)	69.6±0.3	67.7±0.4	<0.001
Non-MetS	1,357 (62.3%)	700 (65.7%)	0.06
MetS	822 (37.7%)	366 (34.3%)	0.06
Cigarette smoking, n (%)			
Never	453 (41.2%)	470 (60.4%)	<0.01
Former	307 (27.9%)	133 (17.1%)	<0.01
Current	339 (30.8%)	175 (22.5%)	<0.01
Alcohol intake, n (%)			
Never	925 (52.2%)	435 (49.7%)	<0.001
Former	149 (8.4%)	85 (9.7%)	<0.001
Current	699 (39.4%)	356 (40.6%)	<0.001
BMI (kg/m^2)	23.1±0.1	22.4±0.2	<0.01
Waist circumference (cm)			
Male	86.9±0.3	85.9±0.3	<0.001
Female	82.1±0.4	80.9±0.8	<0.001
Blood pressure (mmHg)			
Systolic	128.1±0.4	120.7±0.6	<0.001
Diastolic	72.2±0.3	70.5±0.4	<0.001
Heart rate (beats/min)	72.3±0.3	73.8±0.5	<0.05
NYHA class			
I	429 (19.7%)	134 (12.6%)	<0.001
II	1,510 (69.5%)	743 (69.9%)	NS
III	215 (9.9%)	173 (16.3%)	<0.001
IV	19 (0.9%)	13 (1.2%)	NS
Stage C/D	2,125 (97.7%)/49 (2.3%)	1,027 (96.5%)/37 (3.5%)	<0.05
LVEF (%)	65.3±0.2	37.2±0.3	<0.001
SAS	5.4±0.05	5.0±0.06	<0.001
HT	1,725 (53.2%)	729 (68.4%)	<0.001
DM or fasting glucose≥110 mg/dl	1,042 (47.8%)	554 (52.0%)	<0.05
Dyslipidemia	1,499 (68.8%)	802 (75.2%)	<0.001
IHD	894 (41.0%)	501 (47.0%)	<0.001
HHD	258 (11.8%)	73 (6.8%)	<0.001
CM	319 (14.6%)	365 (34.2%)	<0.001
VHD	695 (31.9%)	169 (15.9%)	<0.001
CHD	48 (2.2%)	6 (0.6%)	<0.01
Medications			
ACEI/ARB	1,483 (68.1%)	835 (78.3%)	<0.001
β-blocker	880 (40.9%)	711 (66.7%)	<0.001
Statin	702 (32.2%)	403 (37.8%)	<0.01

Values are mean±SEM.
Abbreviations see in Tables 1,2.

Panel III (NCEP/ATPIII),[37] American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI),[38] and International Diabetes Federation (IDF),[39] in future analyses. Third, although MetS is the association and clustering of metabolic components, we were unable to exclude CHF patients complicated by severe hypertension, severe dyslipidemia, or severe diabetes mellitus. This issue also remains to be examined in future studies. Last, the present study lacks an appropriate control group in the same population, which why we used the data from the Kanazawa Study of the Japanese general population in 2007 that demonstrated a prevalence of MetS of 16–21% in 50- to 80-year-old males and in females, prevalence of 3% in the 50s, 5% in the 60s, 8% in the 70s, and 10% in the 80s.[20]