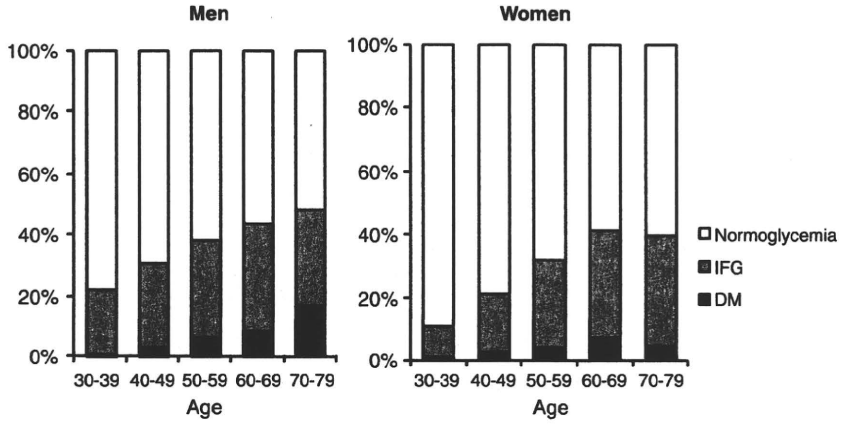

Figure 1 Frequency of type 2 diabetes mellitus according to sex and age.

Table 1 Baseline characteristics of study subjects according to fasting glucose categories at baseline

	Men				*Women*			
	Normoglycemia	*IFG*	*DM*	P-*value*	*Normoglycemia*	*IFG*	*DM*	P-*value*
Number of subjects, *n*	1458	874	154	—	2126	611	98	—
Age, in years	54 ± 14	57 ± 12	60 ± 10	<0.001	52 ± 13	59 ± 11	60 ± 10	<0.001
Body mass index, kg m^{-2}	22.5 ± 2.8	23.3 ± 2.9	23.3 ± 3.2	<0.001	21.8 ± 3.0	23.1 ± 3.4	24.5 ± 4.2	<0.001
Blood pressure category, %[a]				<0.001				<0.001
Optimal blood pressure	37	24	20		49	23	17	
Normal blood pressure	19	19	17		16	16	17	
High-normal blood pressure	16	19	14		13	18	15	
Hypertension	28	39	49		21	43	51	
Hypercholesterolemia, %[b]	26	33	36	<0.001	38	54	59	<0.001
Medication, %								
Hypertension	10	12	18	0.002	8	16	22	<0.001
Diabetes	—	—	36	—	—	—	38	—
Smoking status, %				0.156				0.325
Current	55	51	50		13	10	11	
Quit	25	29	32		3	3	4	
Never	19	20	18		84	87	85	
Drinking status, %				<0.001				0.330
Current	76	77	76		34	32	24	
Quit	2	2	9		1	1	2	
Never	22	20	15		65	67	74	

Abbreviations: DM, diabetes mellitus; DBP, diastolic blood pressure; IFG, impaired fasting glucose; SBP, systolic blood pressure.
Normoglycemia: fasting glucose levels <5.6 mmol l^{-1}; IFG: fasting glucose levels 5.6 to 6.9 mmol l^{-1}; DM: fasting glucose levels ⩾7.0 mmol l^{-1} or medication for diabetes.
[a]Blood pressure category was based on the ESH–ESC 2007 guidelines: optimal (SBP <120 mm Hg and DBP <80 mm Hg), normal blood pressure (SBP 120–129 mm Hg and DBP 80–84 mm Hg), high-normal blood pressure (SBP 130–139 mm Hg and DBP 85–89 mm Hg) and hypertension (SBP ⩾140 mm Hg or DBP ⩾90 mm Hg or antihypertensive drug use).
[b]Hypercholesterolemia: antilipidemic drug user or total cholesterol ⩾5.7 mmol l^{-1} ± values are the means ± s.d.'s.

The significant interaction terms between fasting blood glucose and BP categories were observed in CVD (P=0.046); however, the interaction term was not significant after exclusion of DM subjects.

Using the HRs, we estimated the PAF for CVD to exposure to the combined impact of fasting glucose and BP categories at baseline (Figure 3). The population-attributable risk percentage for CVD incidence was estimated at 3.7% for subjects with normoglycemia and high-normal BP, 5.7% for subjects with IFG and normal or high-normal BP group and 8.2% for subjects with DM and any BP category group, when comparing these groups with the normoglycemic and optimal BP group.

DISCUSSION

In this population cohort study, we found that DM was a risk factor for CVD, stroke and CHD, whereas an IFG of 5.6 to 6.9 mmol l^{-1} was a risk factor for CVD and CHD only. A combined effect of IFG

Table 2 Age- and multivariable-adjusted hazard ratios (95% confidential intervals) for cardiovascular disease according to blood glucose category

	Blood glucose category			
	Normoglycemia	*IFG*	*Diabetes*	*P-value for trend*
Men and women, number	3584	1485	252	
Person-years, in years	42701	16741	2594	
Cardiovascular disease				
Case	184	139	41	
Age and sex-adjusted	1	1.34 (1.07–1.68)	2.45 (1.73–3.45)	<0.001
Multivariable-adjusted	1	1.25 (1.00–1.58)	2.13 (1.50–3.03)	<0.001
Coronary artery disease				
Case	78	70	18	
Age and sex-adjusted	1	1.54 (1.10–2.13)	2.53 (1.51–4.25)	<0.001
Multivariable-adjusted	1	1.46 (1.04–2.04)	2.28 (1.34–3.88)	0.001
Stroke				
Case	106	69	23	
Age and sex-adjusted	1	1.21 (0.89–1.65)	2.51 (1.58–3.96)	<0.001
Multivariable-adjusted	1	1.11 (0.81–1.52)	2.08 (1.29–3.35)	0.016
Men, number	1,458	874	154	
Person-years, years	16,901	9844	1560	
Cardiovascular disease				
Case	107	91	25	
Age-adjusted	1	1.19 (0.90–1.58)	1.93 (1.25–2.99)	0.007
Multivariable-adjusted	1	1.13 (0.85–1.51)	1.75 (1.12–2.73)	0.032
Coronary artery disease				
Case	50	50	11	
Age-adjusted	1	1.39 (0.93–2.06)	1.89 (0.98–3.64)	0.027
Multivariable-adjusted	1	1.31 (0.87–1.96)	1.69 (0.86–3.31)	0.077
Stroke				
Case	57	41	14	
Age-adjusted	1	1.01 (0.68–1.52)	2.00 (1.11–3.61)	0.103
Multivariable-adjusted	1	0.97 (0.64–1.46)	1.78 (1.00–3.12)	0.216
Women, number	2,126	611	98	
Person-years, in years	25,800	6897	1033	
Cardiovascular disease				
Case	77	48	16	
Age-adjusted	1	1.62 (1.12–2.33)	3.70 (2.14–6.40)	<0.001
Multivariable-adjusted	1	1.49 (1.02–2.16)	3.07 (1.73–5.45)	<0.001
Coronary artery disease				
Case	28	20	7	
Age-adjusted	1	1.86 (1.04–3.25)	4.62 (1.99–10.72)	<0.001
Multivariable-adjusted	1	1.83 (1.01–3.32)	4.32 (1.81–10.31)	<0.001
Stroke				
Case	49	28	9	
Age-adjusted	1	1.53 (0.96–2.45)	3.54 (1.71–7.29)	<0.001
Multivariable-adjusted	1	1.36 (0.84–2.19)	2.66 (1.22–5.80)	0.018

Abbreviations: DM, diabetes mellitus; IFG, impaired fasting glucose.
Multivariate analyses were adjusted for age, body mass index, hypertension, hyperlipidemia and smoking and drinking status.
Blood glucose categories: Normal, fasting glucose levels $<5.6\,mmol\,l^{-1}$; IFG, fasting glucose levels $5.6–6.9\,mmol\,l^{-1}$; DM, fasting glucose levels $\geq 7.0\,mmol\,l^{-1}$ or medication for diabetes.

and prehypertension on the incidence of CVD was observed. The high-normal BP subjects in any glucose category and the normal BP subjects with IFG in the Japanese population showed increased risks of CVD. To our knowledge, this study is the first on the combined impact of these borderline risk factors, IFG and prehypertension on the incidence of CVD in a general Asian population.

Previous cohort studies have shown that DM is a risk factor for CVD, stroke[14,15] and CHD.[13] The results of our study are also essentially compatible with the previous cohort studies in Japan. The Hisayama Study demonstrated that glucose intolerance for 2421 participants was a risk factor for increased incidence of stroke and CHD.[15] Iso *et al.*[20] reported that glucose abnormalities were a risk factor for ischemic stroke in a Japanese population by using nonfasting glucose levels. The NIPPON DATA 80 Study indicated that DM, defined by nonfasting blood glucose levels, was a risk factor for CVD mortality.[33] In the Funagata Diabetes Study, IFG was not a risk factor

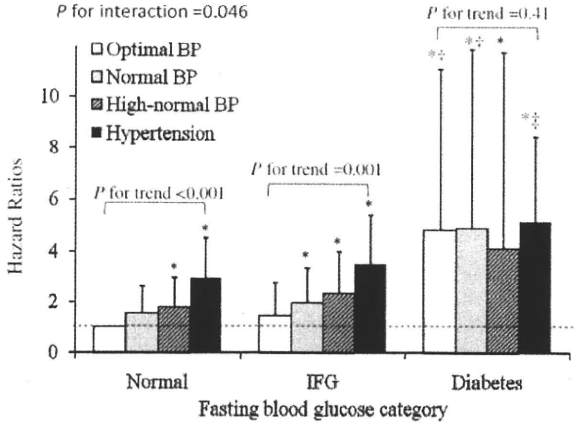

Figure 2 The influence of fasting glucose and BP categories on multivariable-adjusted hazard ratios and 95% confidence intervals for the incidence of cardiovascular disease. $^{*}P<0.05$, compared with normoglycemic subjects with optimal BP. $^{\dagger}P<0.05$, compared with normoglycemic subjects in the same BP category. $^{\ddagger}P<0.05$, compared with normoglycemic subjects with hypertension.

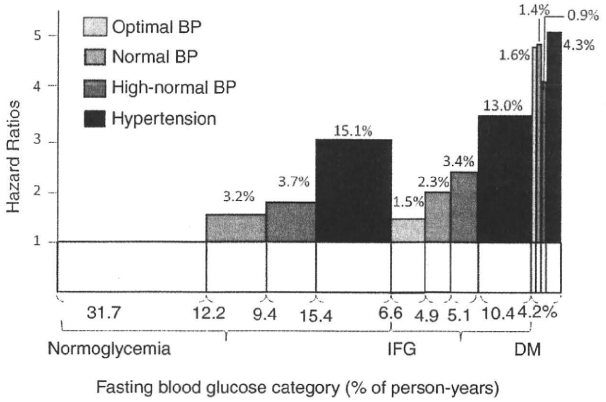

Figure 3 The hazard ratios and population attributable fractions for CVD to exposure to the combined impact of glucose (normoglycemia, impaired fasting glucose and diabetes) and blood pressure categories (optimal, normal, and high-normal blood pressures and hypertension) at baseline were estimated. The gray and black areas represent excessive incidence of CVD in the high blood glucose and high blood pressure categories compared with the subjects with normoglycemia and optimal blood pressure as a reference.

for CVD mortality, although impaired glucose tolerance was a risk factor for CVD.[34]

Compared with previous studies, our study has several methodological strengths. First, our cohort population was relatively large and was selected at random from an urban population in contrast to most other cohort populations in Asia, which were selected from rural populations.[15,20,34] Second, all of our cohort participants were examined at one place and measured using the same autoanalyzer at one laboratory. Finally, our study examined the risk of CVD incidence, not CVD mortality.

In our study, we used the definitions of IFG and CVD/CHD set forth by the 2003 American Diabetes Association recommendations. In the Framingham Heart Study, the 2003 IFG definition was predictive of CHD in women but not in men,[17] a finding which was similar to our results. However, fewer studies have examined the association of the 2003 IFG definitions for CHD and stroke. Kanaya *et al.*[35] showed that the 2003 defination for IFG was not associated with increased risk of CHD or stroke among postmenopausal women with coronary artery disease.Kim *et al.*[36] reported that one-third of the population has IFG according to the 2003 definition. However, many of these individuals do not have increased prevalence of CHD.

Hu *et al.*[19] reported that hypertension and DM increased stroke risk independently and that their combination additively increased stroke risk. In our study, the risks of CVD in the normoglycemic and IFG groups were linearly related to the BP category (*P*-value for trend <0.001). However, the risks of CVD in the DM group did not change with BP category (*P*-value for trend=0.4), which was compatible with a previous result for trends between glucose category and hypertension status.[20] Recently, the ACCORD BP Study has shown that targeting an SBP <120 mm Hg, as opposed to an SBP <140 mm Hg, did not reduce the rate of a composite outcome of fatal and nonfatal major cardiovascular events in patients with type 2 diabetes.[37] Although present studies suggest that decreasing BP may be an effective way to prevent CVD in normoglycemic or IFG subjects, further investigations are required to clarify the interaction between the BP categories of DM subjects at risk for CVD in other large cohorts.

The percentage of the PAF for CVD incidence in normoglycemic subjects with high-normal BP or IFG subjects with normal or high-normal BP (PAF=12.6%) was 1.5 times higher than that in the DM subjects in any BP category (PAF=8.2%). Also, the PAF suggested that 12.6% of CVD cases would be preventable if the borderline glucose and blood pressure levels were controlled to within normoglycemic and optimal BP ranges.

Our results showed that hyperglycemia conferred a slightly higher risk of CVD incidence in women than in men, although men had greater absolute event rates for CVD. Previous studies have shown that the impact of DM on the risk of CVD is significantly greater in women than in men.[13,17,38] Lee *et al.* reported that the HRs of coronary heart disease for DM were 2.6 for women and 1.9 for men. In the Framingham Heart Study, [17] IFG was associated with increased CHD risk only in women (HR=1.7; 95% CI, 1.0–3.0). The reason for these sex differences in the association between DM and CVD remains unclear.

Our study has several limitations. The primary limitation is the regression dilution bias; this study was based on a single day measurement of serum glucose and BP levels.[39] That is, the fasting serum glucose and BP levels might have been misclassified. Second, as we did not perform glucose tolerance tests, we may have missed subjects with impaired glucose tolerance. Finally, we did not examine the combined effect of BP categories and glucose abnormalities after stratification by CVD subtypes, such as stroke and CHD because of the small sample size.

In conclusion, DM is a risk factor for CVD, stroke, and CHD, whereas an IFG of 5.6 to 6.9 mmol l^{-1} is a risk factor for CVD and CHD in women. The risks of CVD in the normoglycemic and IFG groups were linearly related to the BP category. The high-normal BP subjects in any glucose categories and the normal BP subjects with IFG showed increased risks of CVD in this Japanese population. Further investigations of larger cohorts of DM subjects are needed.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Natural Killer T Cells Are Involved in Adipose Tissues Inflammation and Glucose Intolerance in Diet-Induced Obese Mice

Kazue Ohmura, Naoki Ishimori, Yoshinori Ohmura, Satoshi Tokuhara, Atsushi Nozawa, Shunpei Horii, Yasuhiro Andoh, Satoshi Fujii, Kazuya Iwabuchi, Kazunori Onoé and Hiroyuki Tsutsui

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Natural Killer T Cells Are Involved in Adipose Tissues Inflammation and Glucose Intolerance in Diet-Induced Obese Mice

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Background—Macrophage and lymphocyte infiltration in adipose tissue may contribute to the pathogenesis of obesity-mediated metabolic disorders. Natural killer T (NKT) cells, which integrate proinflammatory cytokines, have been demonstrated in the atherosclerotic lesions and in visceral adipose tissue.

Objective—To determine whether NKT cells are involved in glucose intolerance and adipose tissue inflammation in diet-induced obese mice.

Methods and Results—To determine whether NKT cells are involved in the development of glucose intolerance, male β_2-microglobulin knockout (KO) mice lacking NKT cells and C57BL/6J (wild-type) mice were fed with a high-fat diet (HFD) for 13 weeks. Body weight and visceral obesity were comparable between wild-type and KO mice. However, macrophage infiltration was reduced in adipose tissue and glucose intolerance was significantly ameliorated in KO mice. To further confirm that NKT cells are involved in these abnormalities, α-galactosylceramide, 0.1 μg/g body weight, which specifically activates NKT cells, was administered after 13 weeks of HFD feeding. α-Galactosylceramide significantly exacerbated glucose intolerance and macrophage infiltration as well as cytokine gene expression in adipose tissue.

Conclusion—NKT cells play a crucial role in the development of adipose tissue inflammation and glucose intolerance in diet-induced obesity. (***Arterioscler Thromb Vasc Biol.* 2010;30:193-199.**)

Key Words: obesity ■ natural killer T cells ■ macrophages ■ visceral adipose tissues ■ glucose intolerance

Obesity, specifically visceral obesity, increases the risk for metabolic disorders, such as type 2 diabetes mellitus, dyslipidemia, and hypertension as well as atherosclerotic cardiovascular diseases. Previous studies have demonstrated that the accumulation of macrophages within adipose tissue is well documented in obese individuals and that adipose tissue inflammation plays an important role in the pathogenesis of these metabolic disorders.[1,2] Macrophages are attracted by chemokines, such as monocyte chemoattractant protein 1 (MCP-1), and contribute to local inflammation through the release of other inflammatory cytokines, such as tumor necrosis factor (TNF) α. In high-fat diet (HFD)-fed obese mice, it has been shown that infiltration of macrophages into adipose tissue coincides with the occurrence of obesity-mediated metabolic disorders.[2] The important role of adipose tissue macrophages in the pathogenesis of metabolic disorders has further been supported by recent data in C-C motif chemokine receptor 2 (CCR2)–deficient mice.[3] The $CCR2^{-/-}$ mice exhibited a reduction in adipose tissue macrophages in association with an improvement of glucose homeostasis and insulin sensitivity. However, the abolished monocyte and macrophage recruitment into peripheral tissue via interaction with MCP-1 could not completely inhibit HFD-mediated metabolic disorders, suggesting that other inflammatory cells may play a role in this context. Wu et al[4] and Rocha et al[5] demonstrated that CD3-positive T lymphocytes are present in human adipose tissue; and regulated upon activation, normal T cell expressed secreted (RANTES), a T-cell–specific chemokine, and its respective receptor CCR5 are expressed in adipose tissue from obese patients. However, the role of other types of lymphocytes in adipose tissue inflammation is largely unexplored.

See accompanying article on page 135

Natural killer T (NKT) cells are innatelike T lymphocytes that recognize glycolipid antigens and are capable of rapidly producing a mixture of T-helper type 1 (T_H1) and T_H2 cytokines, such as interferon (IFN) γ and interleukin (IL) 4 in

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shaping subsequent adaptive immune responses.[6] Thus, NKT cells can function as a bridge between the innate and adaptive immune systems. Caspar-Bauguil et al[7] have reported the presence of significant levels of NKT cells in the stroma-vascular fraction of white adipose tissue by cytofluorometric analysis. However, they have not determined whether NKT cells are involved in adipose tissue inflammation and the development of metabolic disorders, including glucose intolerance in HFD-induced obesity.

Some of the processes involved in adipose tissue inflammation resemble inflammatory processes in atherogenesis.[8] Inflammation during the development of an atherosclerotic lesion is also characterized by monocyte/macrophage and lymphocyte infiltration.[8] These lymphocytes are mainly CD4-positive lymphocytes that express proinflammatory T_H1 cytokines, such as IFN-γ, and orchestrate the inflammatory response in the vascular wall by activating other cells. Previous studies,[9,10] including our own studies, demonstrated that NKT cells were present in atherosclerotic lesions and are critically important in atherogenesis. These findings suggest that NKT cells can also be involved in inflammation within adipose tissue. However, to date, it remains unclear whether NKT cells play a similar role in adipose tissue inflammation.

In the present study, we determined whether NKT cells are involved in HFD-induced glucose intolerance and adipose tissue inflammation by using β_2 microglobulin knockout (KO) mice lacking NKT cells. Moreover, we further examined the effects of NKT cell activation by α-galactosylceramide (αGC), a specific activator for NKT cells,[11] on glucose intolerance and adipose tissue inflammation in HFD-induced obese mice.

Methods

Expanded materials and methods are available in the supplemental files (available online at http://atvb.ahajournals.org).

Experiment 1: The Effects of NKT Cell Depletion on Metabolic Disorders

Male wild-type (WT) (Charles River Japan, Inc, Yokohama, Japan) and KO mice, which lack NKT and T cells on the C57BL/6 background (The Jackson Laboratory, Bar Harbor, Maine), aged 8 weeks, were fed with a standard diet (SD) (WT-SD, n=10; and KO-SD, n=5) or an HFD containing 21% fat and 0.15% cholesterol (WT-HFD, n=10; and KO-HFD, n=14) for 13 weeks. Animals were metabolically phenotyped, including an intraperitoneal glucose tolerance test (ipGTT). Other WT mice, aged 8 weeks, were fed with an SD (n=15) or an HFD (n=15) for 2, 4, or 6 weeks. Afterward, animals underwent euthanasia and organs, including visceral adipose tissue, were dissected.

Experiment 2: The Effects of NKT Cell Activation on Metabolic Disorders

After feeding male WT and KO mice, aged 8 weeks, with an HFD for 13 weeks, phosphate-buffered saline (PBS) (WT-PBS, n=5; and KO-PBS, n=5) or αGC, 0.1 μg/g body weight (Kirin Brewery Company, Ltd, Tokyo, Japan) (WT-αGC, n=5; and KO-αGC, n=5), was injected intraperitoneally. After 8 to 9 days, ipGTT was performed and visceral adipose tissues were dissected. Other WT mice, aged 8 weeks, were injected using PBS (n=9) or αGC, 0.1 μg/g body weight (n=11) intraperitoneally and organs, including visceral adipose tissues, were dissected 1, 4, and 7 days after the injection.

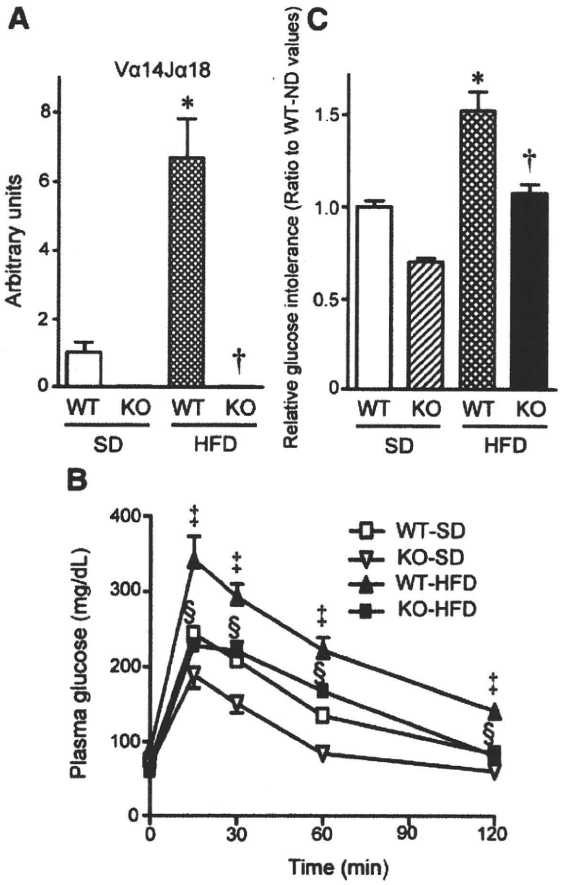

Figure 1. A, Vα14/Jα18 gene expression, an index of natural killer T (NKT) cells, of visceral adipose tissues in experiment 1. B, Plasma glucose concentrations. C, Area under the curve (AUC) values during the intraperitoneal glucose tolerance test (ipGTT). *P<0.01 vs wild type (WT)–standard diet (SD), †P<0.01 vs WT–high-fat diet (HFD), ‡P<0.01 vs WT-SD at each time, and §P<0.01 vs WT-HFD at each time.

The animal care and procedures for the experiments were approved by the Committee of Hokkaido University Graduate School of Medicine on Animal Experimentation.

Results

NKT Cell Depletion Ameliorates Metabolic Disorders in HFD-Fed Mice

To characterize the role of NKT cells in the pathogenesis of HFD-induced glucose intolerance and visceral adipose tissue inflammation, WT and KO mice were fed with either SD or HFD for 13 weeks.

The quantification of NKT cells by Vα14/Jα18 gene expression confirmed that NKT cell infiltration was significantly enhanced in adipose tissue from HFD mice and, more important, was completely abolished in KO groups (Figure 1A).

An HFD did not affect fasting plasma levels of glucose, insulin, and homeostasis model assessment of insulin resistance (HOMA-IR) in WT-HFD and KO-HFD compared with WT-SD (Table 1). However, plasma glucose levels during ipGTT were significantly increased in WT-HFD than in WT-SD, and these values were significantly lower in KO-HFD (Figure 1B). The area under the curve values of plasma glucose

Table. Animal Characteristics in Experiment 1

Characteristic	Animal Group			
	WT-SD (n=10)	KO-SD (n=5)	WT-HFD (n=10)	KO-HFD (n=14)
Body weight, g	29.4 (0.5)	29.4 (1.4)	33.2 (0.6)*	31.4 (0.7)
Blood chemistry results				
Fasting plasma glucose level, mg/dL	78 (8)	50 (8)†	82 (8)	55 (4)‡
Insulin level, ng/mL	0.49 (0.09)	0.27 (0.06)	0.77 (0.22)	0.90 (0.13)
HOMA-IR level	2.10 (0.22)	0.79 (0.21)*	3.29 (0.66)	3.23 (0.68)
Total cholesterol level, mg/dL	102 (2)	103 (3)	181 (10)*	193 (6)*
Leptin level, ng/mL	2.2 (0.5)	2.4 (0.9)	12.9 (2.5)*	16.0 (4.0)*
TNF-α level, pg/mL	37 (5)	13 (4)*	85 (40)	29 (6)§
Adiponectin level, μg/mL	22 (2)	30 (5)	19 (1)	48 (3)‡
Glucagon level, pg/mL	478 (25)	423 (25)	477 (33)	406 (32)
Visceral adipose tissue				
Visceral adipose tissue weight, mg	559 (57)	564 (77)	1388 (131)*	1331 (94)*
Visceral adipose tissue weight/body weight, mg/g	19.7 (1.7)	19.1 (2.4)	42.5 (3.8)*	42.0 (2.3)*
Adipocyte size, μm^2	1697 (156)	1492 (162)	2787 (324)*	2921 (308)*

Abbreviations: HFD, high-fat diet; KO, β_2-microglobulin knockout mice; SD, standard diet; TNF, tumor necrosis factor; WT, wild type (C57BL/6J) mice.

*$P<0.01$ vs WT-SD.

†$P<0.05$ vs WT-SD.

‡$P<0.01$ vs WT-HFD.

§$P<0.05$ vs WT-HFD.

levels during the ipGTT were significantly increased in WT-HFD, and this increase was attenuated in KO-HFD to the WT-SD levels (Figure 1C). These results demonstrated that glucose intolerance seen in HFD-fed mice was significantly ameliorated by the depletion of NKT cells. Plasma total cholesterol and leptin levels were also significantly increased by HFD but were not altered in KO-HFD. The plasma adiponectin level did not change in WT-HFD compared with WT-SD, but significantly increased in KO-HFD. The plasma glucagon level tended to be lower in KO-SD compared with WT-SD, which did not reach statistical significance (Table). An HFD significantly increased the weight of visceral adipose tissue compared with groups fed with SD. An HFD significantly increased the weight of visceral adipose tissue compared with the weight of groups fed with SD. In parallel to visceral adipose tissue weight, adipocyte size measured by morphometric analysis was significantly increased in WT-HFD than in WT-SD. However, these increases were not altered in KO-HFD.

In parallel to the glucose intolerance, the infiltration of F4/80-positive macrophages by immunohistochemical staining was significantly increased in visceral adipose tissues from WT-HFD than WT-SD, and this increase was significantly ameliorated in KO-HFD (Figure 2A and B). Major histocompatibility complex (MHC) class II, CD11c, and arginase gene expression, measured by using real-time reverse transcriptase– polymerase chain reaction (a quantitative index of macrophage activation, M1 macrophage, and M2 macrophage, respectively), demonstrated that infiltrating macrophages possess a predominantly M1 phenotype in WT-HFD mice and an M2 phenotype in KO-HFD mice (Figure 2C–E). Taken together, these data indicated that M1 macrophage infiltration was enhanced in adipose tissue from WT-HFD and that this increase was significantly ameliorated in KO-HFD accompanied by a phenotypic change into M2 macrophage.

To examine the temporal relationship between infiltrating NKT cells and macrophages within obese adipose tissues, WT mice were fed with SD or HFD for 2, 4, or 6 weeks. Quantification of NKT cells by Vα14/Jα18 gene expression demonstrated that NKT cell infiltration was significantly increased after 6 weeks of HFD feeding, whereas macrophages quantified by F4/80 gene expression were not increased during the same period in visceral adipose tissues (supplemental Figure 1A and B). Similarly, in subcutaneous fat tissues, NKT cell infiltration was significantly increased after 4 weeks of HFD feeding, whereas macrophages were not increased during the same period (supplemental Figure 1C and D). More important, macrophages were significantly increased at 13 weeks of HFD feeding. Combining the data from weeks 2 to 6 (supplemental Figure 1) with those from week 13 (Figure 2B), the infiltration of NKT cells preceded that of macrophages in obese adipose tissues. Therefore, the occurrence of glucose intolerance and macrophage infiltration into adipose tissue from HFD-induced obese mice is mediated by NKT cells.

To examine the role of NKT cells in gluconeogenesis, phosphoenolpyruvate carboxykinase and glucose-6-phosphatase gene expression were measured in the hepatic tissues. Hepatic gluconeogenesis tended to be suppressed in KO-SD compared with WT-SD, which did not reach statistical significance (supplemental Figure 2).

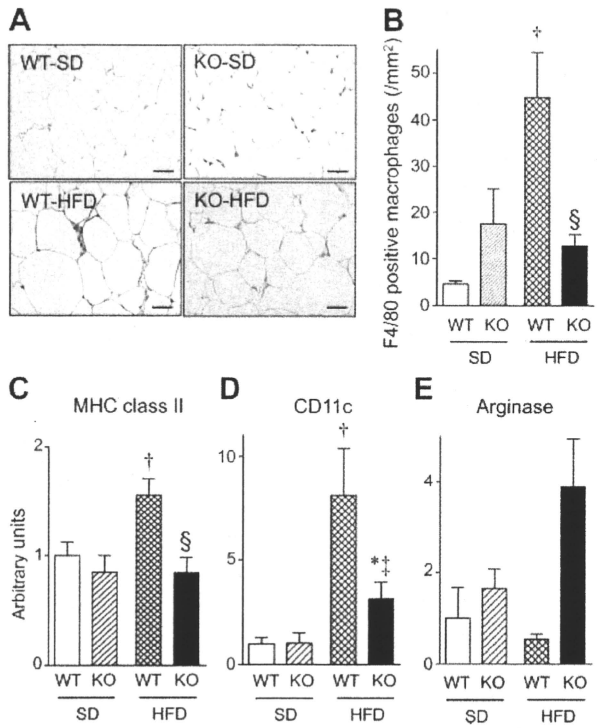

Figure 2. Macrophage infiltration in adipose tissue in experiment 1. A, F4/80 immunohistochemistry. Scale bar, 20 μm. B, The number of F4/80-positive macrophages. C–E, Gene expression of major histocompatibility complex (MHC) class II, CD11c, and arginase, respectively. *$P<0.05$ and †$P<0.01$ vs wild type (WT)–standard diet (SD), and ‡$P<0.05$ and §$P<0.01$ vs WT–high-fat diet (HFD).

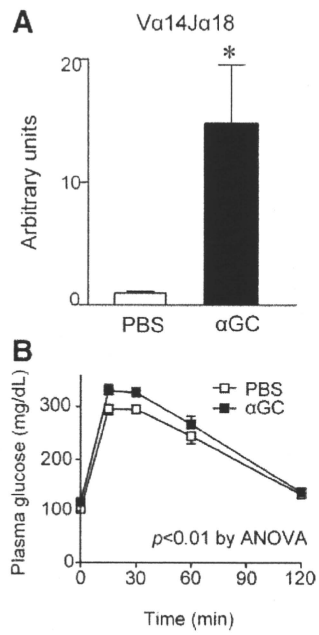

Figure 3. A, Vα14/Jα18 gene expression, an index of natural killer T (NKT) cells, of visceral adipose tissues 8 days after the injection of phosphate-buffered saline (PBS) or α-galactosylceramide (αGC), a specific activator for NKT cells, in experiment 2. B, Plasma glucose concentrations during the intraperitoneal glucose tolerance test (ipGTT) 8 days after PBS or αGC injection. *$P<0.05$ vs PBS.

NKT Cell Activation Exacerbated Metabolic Disorders in HFD-Fed Mice

To further characterize the role of NKT cells in the pathogenesis of HFD-induced glucose intolerance and visceral adipose tissue inflammation, αGC was injected intraperitoneally in WT mice fed HFD for 13 weeks.

αGC did not affect body weight, visceral adipose tissue weight, and adipocyte size in HFD mice 9 days after injection (supplemental Table).

The quantification of NKT cells by Vα14/Jα18 gene expression confirmed αGC significantly enhanced NKT cell infiltration into adipose tissue (Figure 3A). Plasma glucose levels during ipGTT were significantly increased by αGC (15 minutes: 330 [11] vs 296 [11] mg/dL [$P<.05$]; and 30 minutes: 326 [9] vs 295 [8] mg/dL [$P<.05$]) (Figure 3B).

F4/80-positive macrophage infiltration was significantly increased in the adipose tissues for WT mice by αGC (Figure 4A and B). These changes of adipose tissue macrophages by the immunohistochemical analysis were also confirmed by MHC class II and CD11c gene expression (Figure 4C and D). In parallel to macrophage infiltration into the visceral adipose tissue, the injection of αGC significantly increased the expression of MCP-1, TNF-α, IFN-γ, and RANTES genes in HFD mice (Figure 5A–D).

To examine the temporal relationship between infiltrating NKT cells and macrophages, WT mice, aged 8 weeks, were injected using PBS or αGC intraperitoneally and adipose tissues were dissected 1, 4, and 7 days after the injection. The NKT cells and macrophages tended to increase at 4 and 7 days after αGC administration in WT mice (supplemental Figure 3).

To examine the effects of αGC treatment on the metabolic phenotypes of genetically induced obese mice, αGC was injected intraperitoneally in ob/ob mice. Natural killer T cell and macrophage infiltration were significantly increased in αGC-treated ob/ob mice compared with PBS-treated ob/ob mice (supplemental Figure 4A and B). Major histocompatibility complex class II, CD11c, and arginase gene expression were also significantly increased in αGC-treated ob/ob mice (supplemental Figure 4C–E). Similar to diet-induced obese mice, the injection of αGC significantly enhanced the expression of TNF-α, IFN-γ, and RANTES genes, also in ob/ob mice (supplemental Figure 4G–I). Plasma glucose levels during ipGTT in αGC-treated ob/ob mice were comparable to those in PBS-treated ob/ob mice (supplemental Figure 5).

To confirm the specificity of αGC treatment for activating NKT cells, αGC was injected in KO mice fed HFD for 13 weeks. It did not affect NKT cell and macrophage infiltration in the adipose tissues and plasma glucose levels during ipGTT in KO mice (supplemental Figure 6).

To assess the direct relationship between NKT cell activation and adipose tissue inflammation, splenic $CD11b^{+}Gr1^{-}CD4^{-}CD8^{-}B220^{-}$ cells (macrophage-enriched cells) and liver $MHC\text{-}classII^{-}CD8^{-}B220^{-}$ lymphocytes (NKT-enriched cells) were cocultured with or without αGC for 48 hours. Macrophages conditioned with activated NKT cells by αGC secreted a significantly larger amount of MCP-1 into the coculture

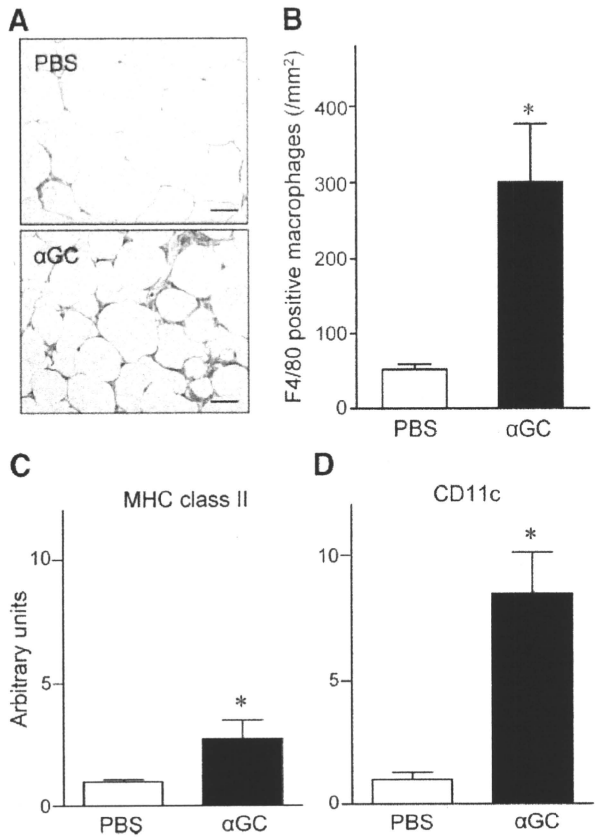

Figure 4. Macrophage infiltration in adipose tissue in experiment 2. A, Demonstrable figures of F4/80 immunohistochemistry. Scale bar, 20 μm. B, The number of F4/80-positive nuclei from mice given phosphate-buffered saline (PBS) and α-galactosylceramide (αGC). C and D, Expression of major histocompatiblity complex (MHC) class II and CD11c genes, respectively, in visceral adipose tissues. *$P<0.05$ vs PBS.

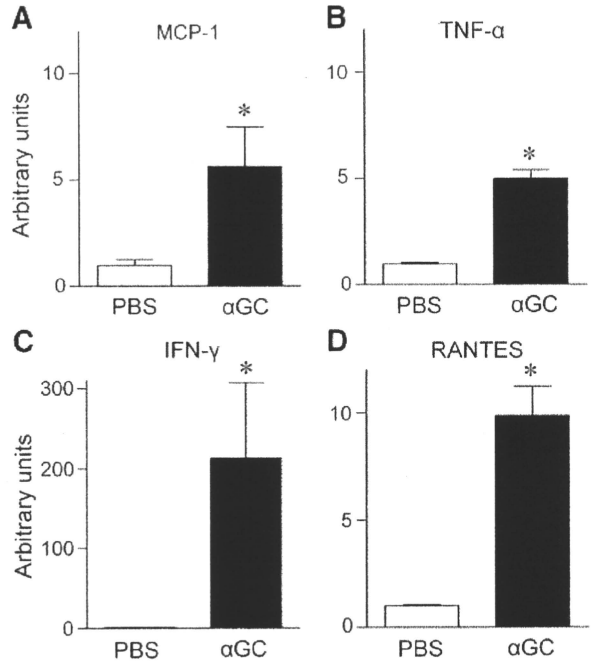

Figure 5. A–D, Expression of monocyte chemoattractant protein (MCP) 1, tumor necrosis factor (TNF) α, interferon (IFN) γ, and regulated upon activation normal T cell expressed secretion (RANTES) genes, respectively, in visceral adipose tissues from mice given phosphate-buffered saline (PBS) and α-galactosylceramide (αGC) in experiment 2. Quantitative reverse transcription (RT)–polymerase chain reaction (PCR) was performed 9 days after PBS or αGC injection. *$P<0.05$ vs PBS.

media compared with unconditioned macrophages (supplemental Figure 7).

Discussion

The present study demonstrated that NKT cells were infiltrated into the visceral adipose tissue in association with macrophages during the development of glucose intolerance in a mouse model of HFD-induced obesity. The depletion of NKT cells in β_2 microglobulin KO mice ameliorated glucose intolerance and visceral adipose tissue inflammation induced by HFD feeding without affecting obesity itself. On the contrary, the activation of NKT cells by αGC exacerbated glucose intolerance and adipose tissue inflammation, including macrophage infiltration and inflammatory cytokine/chemokine gene expression. Therefore, NKT cells may play a pivotal role in the development of glucose intolerance and adipose tissue inflammation associated with HFD-induced obesity.

Visceral obesity has been demonstrated to be associated with macrophage infiltration and inflammation in adipose tissue.[1,2,12] As such, MCP-1 is produced by adipocytes in parallel with increasing adiposity, and mice lacking CCR2, a receptor for MCP-1, exhibit less macrophage infiltration in adipose tissues and a reduction in inflammatory gene expression.[2] However, the development of HFD-induced glucose intolerance was not completely abolished in these mice, suggesting that the other chemokine systems might also contribute to obesity-related adipose tissue inflammation and glucose intolerance.

Early work by cytofluorometric analysis revealed the presence of significant levels of NKT cells in the stromavascular fraction of white adipose tissues.[7] However, the changes of these cells by HFD feeding and even their roles in HFD-induced metabolic disorders have not been examined. In the present study, depleting NKT cells significantly ameliorated glucose intolerance after HFD feeding (Figure 1). Therefore, our study has extended the previous information on the significance of NKT cells by demonstrating that the cell infiltration of these cells into the adipose tissue is involved in the recruitment of macrophages and inflammatory cytokine gene expression during the development of HFD-induced glucose intolerance. However, the present results were not consistent with those of the previous study by Elinav et al,[13] which noted that NKT cells ameliorated glucose intolerance in leptin-deficient ob/ob mice. In their study, the oral administration of liver extracts in ob/ob mice increased hepatic NKT cells and serum levels of IL-10, indicating that the extracts activated NKT cells toward the T_H2 bias, whereas αGC injection stimulated NKT cells toward the T_H1 slant in the present study. Therefore, the discrepancy between these studies might be the result of the differences in the methods of modulating NKT cells and the resultant

changes of cytokines subsequent to NKT cell activation. The differences in the animal models (HFD-induced obese mice vs leptin-deficient ob/ob mice) might also be involved in this discrepancy because the injection of αGC significantly enhanced the expression of arginase in ob/ob mice but not in HFD-induced obese mice.

Previous studies demonstrated that proinflammatory T lymphocytes are also present in visceral adipose tissue and contribute to adipose tissue inflammation and the development of glucose intolerance before the recruitment of macrophages.[4] A recent elegant study by Nishimura et al[14] elucidated the role of T lymphocytes in adipose tissue inflammation in obesity. In their study, many $CD8^+$ effector T cells infiltrated into obese epididymal adipose tissue, preceding macrophage infiltration, in HFD-induced obese mice and initiated the inflammatory cascade that leads to insulin resistance in adipocytes. We could not completely exclude the possibility that T lymphocytes are involved in our model because β_2 microglobulin KO mice used in the present study lack not only NKT cells but also $CD8^+$ T lymphocytes.[15] However, the development of both glucose intolerance and adipose tissue inflammation induced by HFD was significantly exacerbated by the specific activation of NKT cells by using αGC, an activator of NKT cells but not T cells (Figures 3–5). Based on these results, we consider that NKT cells are critically involved in glucose intolerance and adipose tissue inflammation in obese mice.

The NKT cells are a specialized lineage of T cells that recognize glycolipid antigens presented by the MHC class I–like molecule CD1d.[16] The NKT cells mediate various functions rapidly by producing a mixture of T_H1 and T_H2 cytokines, such as IFN-γ and IL-4, in shaping subsequent adaptive immune responses.[6] The present study demonstrated that accumulated macrophages in adipose tissues in αGC-treated mice were classically activated (M1) macrophages, one of the distinct subsets of macrophages categorized as M1 by CD11c (Figure 4).[17,18] In agreement with these findings, the activation of NKT cells was associated with increased gene expression of T_H1-cytokine IFN-γ and MCP-1 in HFD-fed mice (Figure 5). Interferon-γ can also promote the recruitment of monocytes by inducing MCP-1 secretion from periadipocytes, and it could activate other cells, such as macrophages. Therefore, cytokines and chemokines, including IFN-γ and MCP-1, were mechanistically involved in the infiltration of macrophages as a result of NKT cell activation. The NKT cells may orchestrate the inflammatory process in adipose tissue in association with the development of glucose intolerance. The beneficial effects of depleting NKT cells are mostly mediated by the reduction of macrophages. It may be informative to examine whether immunosuppressive agents, such as cyclosporine and tacrolimus, which have been shown to suppress αGC-induced cytokine production in murine NKT cells,[19,20] can ameliorate adipose tissue inflammation and glucose intolerance in our model. However, they also induce glucose intolerance via its toxic effects on the pancreatic islet cells.[21,22] Therefore, these reagents may not be suitable for investigating the role of NKT cells in glucose intolerance in HFD-induced obesity in vivo.

The underlying mechanisms responsible for the activation of NKT cells by the HFD feeding remain established. Based on our results using αGC, a glycosphingolipid derived from marine sponges, sphingolipid ceramide may be a crucial intermediate linking between excess nutrients by HFD and inflammatory cytokines to induce glucose intolerance. In fact, ceramide has been shown to be synthesized by long-chain fatty acids and to induce both inflammation and insulin resistance.[23] In agreement with our results, Rocha et al[5] reported that the HFD feeding increases a number of T cells and IFN-γ gene expression in adipose tissue, suggesting T-cell priming toward the T_H1 slant. However, the HFD feeding has been shown to suppress T_H1 responses in B6 mice by inhibiting toll-like receptor–mediated maturation and proinflammatory cytokine production in dendritic cells.[24] The discrepancy between these studies might be the result of the differences in the tissues studied (visceral adipose tissue lymphocytes vs splenic lymphocytes). More important, the contribution of NKT cells is not mediated by the modulation of adipose tissue weight or adipocyte size because these variables did not differ between HFD-fed groups (Table 1 and Supplemental Table); however, adipocyte cell size has been shown to be an independent predictor of glucose intolerance.[25]

Activated macrophages secrete TNF-α, which can inhibit insulin signal transduction.[26] Obesity itself can trigger adipose tissue inflammation, which leads to the desensitization of insulin action.[27] We have demonstrated that NKT cells may be important in the evolution of atherosclerotic lesions by communicating macrophages through cell-cell interactions and/or secreting inflammatory cytokines.[10] Some of the inflammatory processes involved in atherogenesis (as shown in our previous study) resemble adipose tissue inflammation in the present study. Therefore, NKT cells are considered to mediate chronic inflammation in vascular and adipose tissues and can represent a direct and common soil for the development of atherosclerotic cardiovascular disease and diabetes. An in vivo transfer experiment with isolated NKT cells may provide more direct evidence of the cause-and-effect relationship between NKT cells and glucose intolerance associated with HFD-induced obesity. Nevertheless, αGC has been established to be a specific activator for NKT cells and, in fact, it has been used in a variety of disease models to elucidate the pathogenetic role of NKT cells.[28] Therefore, we used αGC administration to activate NKT cells in the present study. There are several limitations to be acknowledged in the present study. First, we only examined the adipose tissue in the present study and did not assess the contribution of liver or skeletal muscle, which can also determine insulin sensitivity.[1] Fasting plasma glucose level and HOMA-IR were significantly lower in KO-SD than in WT-SD (Table). Knockout-SD mice tended to have lower plasma glucose levels and area under the curve values during ipGTT compared with WT-SD (Figure 1B and C); this finding did not reach statistical significance. These data suggested that the absence of NKT cells could improve glucose metabolism in healthy mice, independently of adipose tissue inflammation. It may be possible that NKT cells affect glucose metabolism via the alterations of gluconeogenesis in the liver and skeletal muscle. However, based on the results that the improvement of glucose metabolism is relatively small in KO-SD mice (Figure 1), we consider that NKT cells may impair glucose

tolerance predominately via promoting adipose tissue inflammation exclusively in HFD-fed mice. Second, there was massive macrophage infiltration in the adipose tissue in our HFD-fed mice even though the weight gain was relatively small. The NKT cell infiltration preceded macrophage infiltration in obese visceral adipose tissues and may play an important role in the early phase of adipose tissue inflammation. Therefore, even though we have not examined how much NKT cells and macrophages infiltrate within adipose tissues during the development of more severe obesity, we consider that the deletion of NKT cells can effectively attenuate the infiltration of macrophages in this setting. In contrast, the activation of NKT cells has been reported to be protective against type 1 diabetes, systemic lupus erythematosus, and infections.[29] Therefore, the inhibition of NKT cells as a therapeutic strategy to prevent and treat metabolic syndrome and cardiovascular disease for obese individuals needs to be used cautiously in the setting of these disease conditions.

In conclusion, the depletion of NKT cells ameliorated chronic inflammation in visceral adipose tissues and suppressed the development of glucose intolerance in HFD-induced obese mice. On the other hand, the activation of NKT cells exacerbated macrophage infiltration in adipose tissue and glucose intolerance with obesity. Therefore, NKT cells enhance chronic inflammation in visceral adipose tissue and contribute to the development of metabolic disorders in obesity. The NKT cells may be the novel therapeutic targets in atherosclerosis, metabolic syndrome, and type 2 diabetes.

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Disclosures

None.

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Hyperuricemia predicts adverse outcomes in patients with heart failure

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ABSTRACT

Background: Hyperuricemia is associated with worse outcomes of patients with chronic heart failure (HF). However, it is unknown in an unselected HF patients encountered in routine clinical practice. We thus assessed the impact of hyperuricemia on long-term outcomes including mortality and rehospitalization among patients hospitalized with worsening HF.

Methods: The Japanese Cardiac Registry of Heart Failure in Cardiology (JCARE-CARD) studied prospectively the characteristics and treatments in a broad sample of hospitalized HF patients and the outcomes were followed for 2.1 years after discharge. Study cohorts ($n=1869$) were divided into 2 groups according to serum uric acid (UA) at discharge; ≥7.4 mg/dL ($n=908$) and <7.4 mg/dL ($n=961$).

Results: Of the total cohort of HF patients, 56% had hyperuricemia defined as UA≥7.0 mg/dl. Patients with UA≥7.4 mg/dL had higher rates of all-cause death, cardiac death, rehospitalization, and all-cause death or rehospitalization due to worsening HF. After multivariable adjustment, higher UA levels were a significant and independent predictor for all-cause death (adjusted hazard ratio [HR] 1.413, 95% confidence interval [CI] 1.094–1.824, $P=0.008$) and cardiac death (adjusted HR 1.399, 95% CI 1.020–1.920, $P=0.037$).

Conclusions: Hyperuricemia was common in patients with HF encountered in clinical practice and higher UA was independently associated with long-term adverse outcomes in these patients.

1. Introduction

High serum uric acid (UA) or hyperuricemia has been well demonstrated to be associated with morbidity and mortality in general population [1–3] as well as in patients with coronary artery disease [4,5]. It is also associated with poor outcomes in patients with mild to severe heart failure (HF) [6–9]. Hyperuricemia in HF may be due to the upregulation of the xanthine oxidase (XO), a key enzyme in the generation of oxygen free radicals. Therefore, it may induce proinflammatory activation [10], impaired oxidative metabolism [11], vascular endothelial dysfunction [12], and exercise intolerance [13,14] in HF. These conditions may well explain the association between hyperuricemia and poor outcome in chronic [6,8] as well as acute HF [9]. However, previous studies enrolled small numbers of HF patients ($n=100$–500) and were performed in a single center [6,8,9]. The impact of hyperuricemia on outcomes has not been assessed in a broad cohort of HF patients. Therefore, the purpose of this study was to examine the prevalence of hyperuricemia in HF patients encountered in routine clinical practice and to determine whether it is independently associated with the long-term outcomes. We analyzed the data from the Japanese Cardiac Registry of Heart Failure in Cardiology (JCARE-CARD), a prospective database of the clinical characteristics, treatments, and outcomes in a broad sample of patients hospitalized with worsening HF in Japan [15–19].

2. Materials and methods

2.1. Study patients

The details of the JCARE-CARD have been described previously [15]. Briefly, eligible patients were those hospitalized due to worsening HF as the primary cause of admission. The patients with acute HF were excluded. For each patient, baseline data obtained at discharge included (1) demography; (2) causes of HF; (3) precipitating causes; (4) comorbidities; (5) complications; (6) clinical status; (7) electrocardiographic and echocardiographic findings; (8) plasma brain-type natriuretic peptide (BNP); and (9) treatments including discharge medications. Histories of hypertension, diabetes mellitus, hyperlipidemia, prior stroke, chronic obstructive pulmonary disease (COPD), smoking, prior myocardial infarction, and sustained ventricular tachycardia/fibrillation (VT/VF) were recorded if they were documented at the discharge of index hospitalization. The definition of each comorbidity was described in our previous report [15]. The diagnosis of atrial fibrillation (AF) was based on a 12-lead standard electrocardiogram performed during the hospitalization.

The JCARE-CARD enrolled a total of 2675 patients hospitalized for HF at 164 participating hospitals. Individual participating hospitals entered the data using a web-based electronic data capture (EDC) system licensed by the JACRE-CARD (www.jcare-

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card.jp). 806 patients were excluded with missing data of serum uric acid, resulting in 1869 patients included in this analysis. They were divided into 2 groups according to serum UA levels at discharge; ≥7.4 mg/dL ($n=908$) and <7.4 mg/dL ($n=961$).

2.2. Outcomes

The status of all patients was surveyed after discharge and the following information was obtained: (1) survival, (2) causes of death, and (3) the rehospitalization due to an exacerbation of HF that required more than continuation of their usual therapy on prior admission. Only patients who survived the initial hospitalization were included in the follow-up analysis. Out of 1869 patients, 104 patients (5.6%) who died during the hospitalization and 145 patients (7.7%) who were missed during the follow-up were excluded from the follow-up analysis. Follow-up data were obtained in 1620 out of 1869 patients (86.7%). Mean post-discharge follow-up was 777 ± 312 days (2.1 ± 0.9 years).

2.3. Statistical analysis

Patient characteristics and treatments were compared using Pearson chi-square test for categorical variables and Mann–Whitney *U* test for continuous variables. Multiple linear regression analysis was used to select those variables that were significantly associated with serum UA levels. The model was obtained by using a stepwise regression selection. Cumulative event-free rates during the follow-up were derived using the method of Kaplan and Meier. The relationship between the serum UA level at baseline and outcomes was evaluated among patients with multivariable adjustment. Baseline clinical variables, treatment factors, and the severity of HF at discharge were used in developing the post-discharge Cox proportional hazard models. A P value of <0.05 was used for criteria for variables to stay in the model. SPSS version 16.0 J for Windows was used for all statistical analyses.

3. Results

3.1. Patient characteristics

Fig. 1 shows the distribution of serum UA among 1869 patients. Mean serum UA level in the study subjects was 7.3 ± 2.4 mg/dL, ranging from 0.3 to 22.5 mg/dL. 1041 (55.7%) patients had hyperuricemia defined as serum UA ≥ 7.0 mg/dL.

The mean age of the total cohort was 71.1 ± 12.9 years and 60.0% was men (Table 1). The causes of HF were ischemic in 32.5%, valvular in 28.5%, hypertensive in 25.9%, and dilated cardiomyopathy in 17.7%. The mean echocardiographic left ventricular ejection fraction (LVEF) was 44.6 ± 16.4%.

Patients with serum UA ≥ 7.4 mg/dL were more often men and significantly higher body mass index (BMI) (Table 1). Causes of HF did not differ between groups. They were more likely to be smoker and have chronic atrial fibrillation and coronary artery bypass grafting (CABG). Serum creatinine and plasma BNP levels were significantly higher and estimated glomerular filtration rate (eGFR) was lower in patients with serum UA ≥ 7.4 mg/dL. They had greater LV end-diastolic and end-systolic diameters and lower LVEF. The implantations of ICD, CRT, and CRT-D were not significantly different between 2 groups.

Patients with serum UA ≥ 7.4 mg/dL were prescribed more by diuretics, especially loop diuretics, and digitalis at discharge (Table 2). However, the use of other medications such as angiotensin converting enzyme (ACE) inhibitor, angiotensin receptor blocker (ARB), and β-blocker did not differ between groups.

3.2. Variables associated with serum UA levels

In a multiple linear regression analysis, younger age [standardized partial regression coefficients (β) 0.183, $P<0.001$], male gender (β 0.092, $P=0.013$), lower eGFR (β 0.405, $P<0.001$), higher hemoglobin concentration (β 0.120, $P=0.004$), and diuretics use (β 0.103, $P=0.003$) were significantly associated with serum UA levels. Low eGFR was the most important factor in this model. However, the multiple correlation coefficient (R^2) of the model entered these five variables was 0.190, indicating that the contribution of these variables to serum UA levels would be minor.

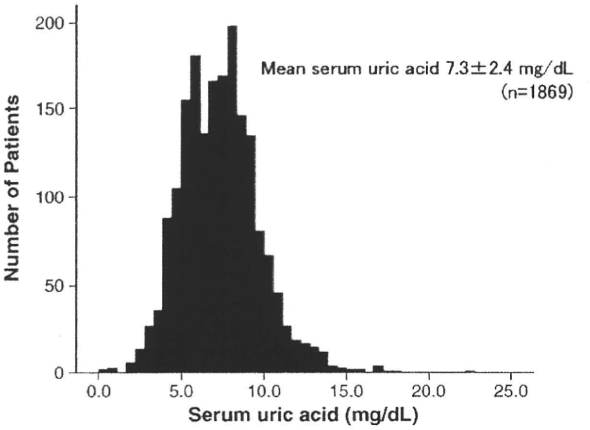

Fig. 1. The distribution of serum UA (mg/dL) at baseline among 1869 patients.

Table 1
Baseline patient characteristics.

Characteristics	Total ($n=1869$)	UA≥7.4 mg/dL ($n=908$)	UA<7.4 mg/dL ($n=961$)	*P* value
Demographics				
Age, yrs (mean ± SD)	71.1 ± 12.9	70.4 ± 14.0	71.8 ± 11.8	0.243
Male, %	60.0	67.4	53.0	<0.001
BMI, kg/m^2	22.3 ± 4.1	22.5 ± 4.1	22.1 ± 4.1	0.013
Causes of heart failure, %				
Ischemic	32.5	33.0	32.0	0.648
Valvular heart disease	28.5	28.7	28.3	0.833
Hypertensive	25.9	24.9	27.0	0.310
Dilated cardiomyopathy	17.7	18.5	17.0	0.383
Hypertrophic cardiomyopathy	1.9	2.1	1.7	0.496
Medical history, %				
Hypertension	53.4	54.4	52.4	0.395
Diabetes mellitus	31.5	32.0	31.1	0.702
Hyperlipidemia	25.0	25.8	24.2	0.436
Prior stroke	16.1	16.7	15.5	0.500
COPD	6.7	7.2	6.2	0.408
Smoking	38.2	43.7	32.9	<0.001
Prior myocardial infarction	27.5	28.2	26.8	0.502
Atrial fibrillation	35.5	38.1	33.0	0.022
Sustained VT/VF	6.5	6.5	6.5	0.968
Previous procedures, %				
PCI	18.5	18.0	19.0	0.590
CABG	9.1	10.6	7.7	0.030
Valvular surgery	7.0	7.1	6.9	0.906
ICD	2.2	2.2	2.2	0.972
CRT	1.6	1.7	1.5	0.830
CRT-D	0.2	0.1	0.2	0.611
Vital signs at discharge				
NYHA functional class	1.8 ± 0.7	1.8 ± 0.7	1.7 ± 0.7	0.006
NYHA classes 3 and 4, %	10.2	11.1	9.3	0.192
Heart rate, bpm	70.6 ± 12.3	70.2 ± 12.4	71.0 ± 12.1	0.156
SBP, mmHg	117.7 ± 19.2	117.1 ± 18.9	118.2 ± 19.4	0.260
DBP, mmHg	66.2 ± 11.9	66.1 ± 12.4	66.3 ± 11.4	0.938
Laboratory data at discharge				
Serum creatinine, mg/dl	1.4 ± 1.2	1.6 ± 1.3	1.2 ± 1.1	<0.001
eGFR, ml/min/1.73 m^2	51.1 ± 25.2	42.4 ± 20.9	58.5 ± 26.1	<0.001
Hemoglobin, g/dL	12.1 ± 2.6	12.0 ± 2.7	12.1 ± 2.6	0.289
Plasma BNP, pg/ml	403 ± 539	485 ± 643	327 ± 405	<0.001
Echocardiographic data at discharge				
LV EDD, mm	55.7 ± 10.3	57.1 ± 10.9	54.4 ± 9.5	<0.001
LV ESD, mm	43.0 ± 12.3	44.8 ± 12.8	41.4 ± 11.6	<0.001
LVEF, %	44.6 ± 16.4	42.8 ± 16.4	46.1 ± 16.3	0.002

BMI, body mass index; COPD, chronic obstructive pulmonary disease; VT/VF, ventricular tachycardia/fibrillation; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; ICD, implantable cardioverter defibrillator; CRT, cardiac resynchronization therapy; CRT-D, cardiac resynchronization therapy device with defibrillator; NYHA, New York Heart Association; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; BNP, brain-type natriuretic peptide; LV, left ventricular; EDD, end-diastolic diameter; ESD, end-systolic diameter; EF, ejection fraction. Values are percent or means ± SD.

3.3. Outcomes

During the follow-up of 2.1 years after hospital discharge, the rates of adverse outcomes were as follows; all-cause death 21.0%, cardiac death 13.5%, rehospitalization due to the worsening HF 36.5%, and all-cause death or rehospitalization 43.9% (Fig. 2). These event rates were significantly higher in patients with serum UA ≥ 7.4 mg/dL.

On multivariate analysis with patients with serum UA < 7.4 mg/dL as the reference, patients with serum UA ≥ 7.4 mg/dL had adverse risk for all-cause death (adjusted hazard ratio [HR] 1.413, 95% confidence interval [CI] 1.094–1.824, $P=0.008$) and cardiac death (adjusted HR 1.399, 95% CI 1.020–1.920, $P=0.037$) (Table 3). Therefore, serum UA levels were significantly associated with long-term adverse outcomes including all-cause death and cardiac death even after adjustment for all other covariates including eGFR and the use of diuretics. They were also associated with

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Table 2
Medication use at hospital discharge.

	Total ($n=1869$)	UA≥7.4 mg/dL ($n=908$)	UA<7.4 mg/dL ($n=961$)	P value
ACE inhibitor, %	36.9	36.1	37.6	0.527
ARB, %	45.8	45.2	46.4	0.617
β blocker, %	48.3	47.9	48.6	0.785
Diuretics, %	88.6	91.4	85.9	<0.001
Loop diuretics, %	80.1	84.4	76.0	<0.001
Thiazide diuretics, %	3.6	4.1	3.2	0.275
Potassium sparing diuretics, %	41.6	41.2	42.0	0.719
Digitalis, %	31.5	34.0	29.2	0.030
Ca channel blocker, %	25.8	27.3	24.4	0.168
Nitrates, %	24.4	24.0	24.8	0.671
Antiarrhythmics, %	16.4	16.9	15.9	0.579
Aspirin, %	47.1	47.9	46.3	0.491
Warfarin, %	40.8	41.8	40.0	0.438
Statin, %	19.8	19.0	20.6	0.406

ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker.

rehospitalization due to worsening HF (unadjusted HR 1.248, $P=0.040$) and all-cause death or rehospitalization (unadjusted HR 1.322, $P=0.013$), which, however, did not reach statistical significance after multivariable adjustment (adjusted HR 1.025, $P=0.801$ and adjusted HR 1.089, $P=0.304$) (Table 3). CABG was not significantly associated with any endpoints including all-cause death, cardiac death, rehospitalization, and all-cause death or rehospitalization. ICD implantation was significantly associated with rehospitalization (adjusted HR 2.094, 95% CI 1.340–3.273, $P=0.001$) and all-cause death or rehospitalization (adjusted HR 1.844, 95% CI 1.186–2.868, $P=0.007$). CRT implantation was associated with cardiac death (adjusted HR 2.668, 95% CI 1.164–6.114, $P=0.020$), rehospitalization (adjusted HR 2.248, 95% CI 1.327–3.809, $P=0.003$), and all-cause death or rehospitalization (adjusted HR 2.009, 95% CI 1.192–3.386, $P=0.009$). In contrast, valvular surgery was associated with lower rates of all-cause death (adjusted HR 0.466, 95% CI 0.238–0.910, $P=0.025$) and cardiac death (adjusted HR 0.419, 95% CI 0.184–0.951, $P=0.038$). However, the inclusion of these procedures as covariates in the Cox regression model did not change our original results shown in Table 3.

The independent predictors associated with all-cause death among those entered into the Cox proportional hazard analysis were serum UA, BMI, eGFR, plasma BNP, age, and NYHA functional class (Table 4). There was 6.8% increase in all-cause death for each 1 mg/dL increase in serum UA level ($P=0.017$).

4. Discussion

The present study demonstrated that hyperuricemia was seen in 56% of the patients hospitalized with HF. They had higher serum creatinine, higher plasma BNP, and lower LVEF and were prescribed more by loop diuretics and digitalis. Importantly, the risk of adjusted long-term adverse outcomes including all-cause death and cardiac death were significantly higher in patients with UA≥7.4 mg/dl.

Even though the association between UA and cardiovascular diseases, including HF, has remained controversial [20,21], previous studies have demonstrated that UA is an independent risk factor for cardiovascular diseases [2,22,23]. Furthermore, experimental studies have identified mechanisms by which UA induces cardiovascular diseases [24,25]. The present results were consistent with these previous reports [6–9,26,27] and extended their prognostic value to a

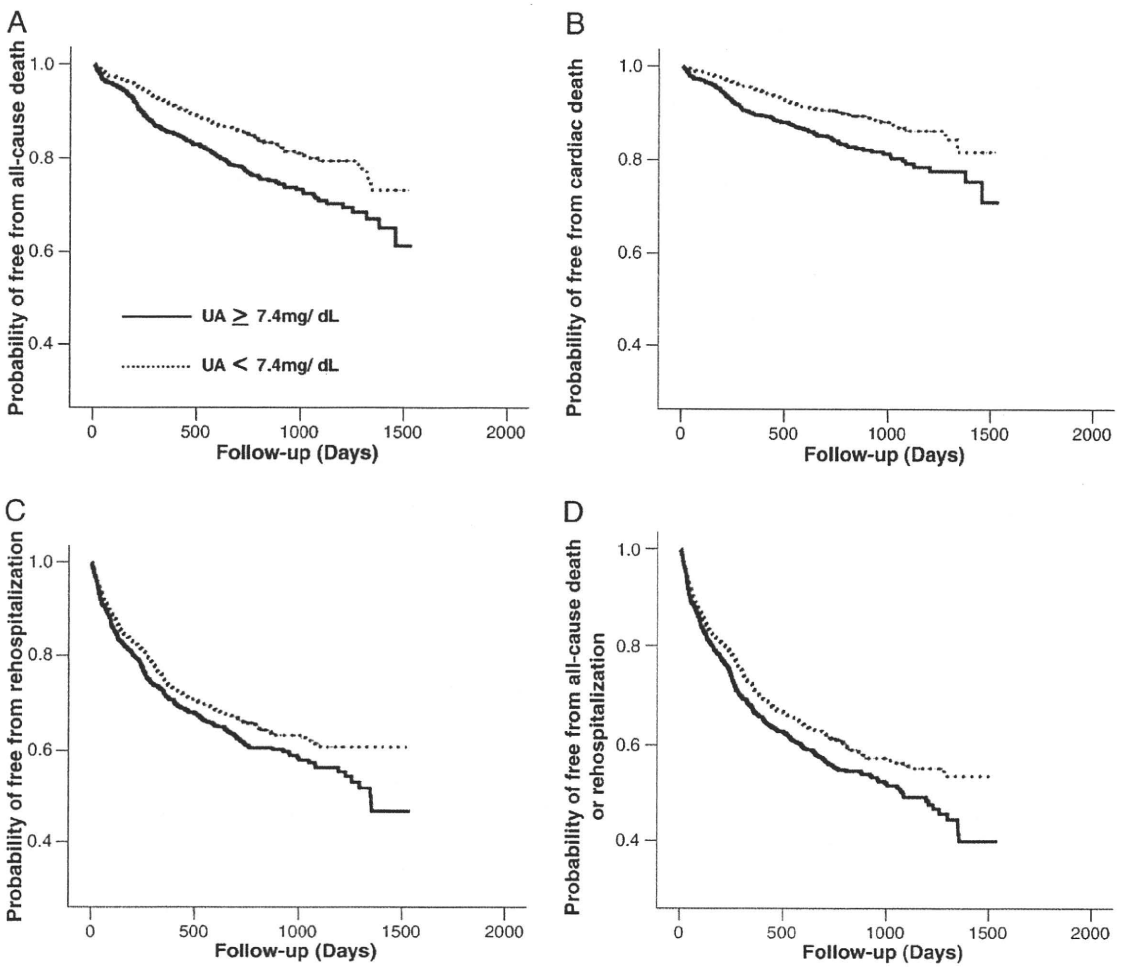

Fig. 2. Kaplan–Meier event-free curves free from all-cause death (A), cardiac death (B), rehospitalization due to worsening HF (C), and all-cause death or rehospitalization (D) comparing patients with serum UA≥7.4 mg/dL (solid lines) and those with serum UA<7.4 mg/dL (dashed lines).

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Table 3
Cox analysis for hazard ratios of outcomes associated with the UA level.

Outcomes	Number (%)					
	UA ≥ 7.4 mg/dl (n = 776)	UA <7.4 mg/dl (n = 844)	Unadjusted HR (95% CI)	P value	Adjusted HR (95% CI)	P value
All-cause death	199 (25.6)	141 (16.7)	1.772 (1.388–2.261)	<0.001	1.413 (1.094–1.824)	0.008
Cardiac death	132 (17.0)	87 (10.3)	1.738 (1.285–2.350)	<0.001	1.399 (1.020–1.920)	0.037
Rehospitalization	303 (39.0)	289 (34.2)	1.248 (1.043–1.494)	0.040	1.025 (0.848–1.239)	0.801
All-cause death or rehospitalization	367 (47.3)	344 (40.8)	1.322 (1.120–1.560)	0.013	1.089 (0.914–1.298)	0.340

The Cox regression model used in the analysis was adjusted for the following covariates: demographics (age, sex, and BMI), medical history (smoking and chronic atrial fibrillation), CABG, NYHA functional class, eGFR, BNP, LVEF, and medication use (diuretics and digitalis). BNP and LVEF at discharge were entered into the model as the categorical variables; i.e. BNP at discharge ≥ 240 pg/ml or <240 pg/ml or unknown and LVEF at discharge < 40% or ≥ 40% or unknown. HR, hazard ratio; CI, confidence interval.

large, non-selected HF population encountered in routine clinical practice and, more importantly, during the long-term follow-up up to 2.1 years by analyzing the large registry data of hospitalized HF patients.

It should be noted that our results were adjusted with all covariates known to have prognostic value in HF and hyperuricemia was demonstrated to be associated with adverse clinical outcomes independent of renal function and diuretic use (Table 3). In the present study, patients with higher UA had more severe renal dysfunction (Table 1). Renal dysfunction causes hyperuricemia via decreased excretion of UA. Moreover, an elevation of UA level itself can lead to renal dysfunction [25,28–32]. In the present study, the multiple linear regression analysis demonstrated that renal function was the most important factor determining UA level. However, the contribution rate of renal function to serum UA levels was low and serum UA levels were independently associated with the adverse outcomes in HF (Tables 3 and 4). These findings have been also reported by other previous studies [10,11,33]. Therefore, even though serum UA levels can be affected by various factors such as age, gender, renal function, and diuretic use, the present study and other previous studies confirmed that hyperuricemia was independently associated with the adverse clinical outcomes in HF.

The normal UA values are usually higher in men than women. The patients with higher UA levels were more often men in the present study (Table 1). Therefore, the association between UA levels and adverse outcomes might be affected by their gender differences. However, the significant impact of serum UA levels on outcomes was consistently observed even after adjustment with gender (Tables 3 and 4). In addition, to exclude the contribution of gender differences of UA levels, we further analyzed by using the different definition of hyperuricemia based on the genders; >7 mg/dL for men and >6 mg/dL for women. Based on this definition, 1112 (59.5%) patients had hyperuricemia. The prevalence of male was the same between hyperuricemia and no hyperuricemia groups (60.3 vs 59.6%, P = 0.770). However, even with the use of different definition of hyperuricemia according to the genders, the relationship between UA and outcomes was consistent with that in our original submission with the UA cut-off values of 7.4 mg/dL.

Table 4
Multivariate predictors of all-cause death by Cox proportional hazard models.

Variables	HR	95% CI	P value
BMI (per 1 kg/m^2 increase)	0.958	0.924–0.993	0.019
eGFR (per 1 ml/min/1.73 m^2 decrease)	1.016	1.010–1.023	<0.001
Serum uric acid (per 1 mg/dL increase)	1.068	1.012–1.127	0.017
Age (per 10 years increase)	1.368	1.214–1.542	<0.001
BNP at discharge ≥240 pg/ml	1.579	1.090–2.287	0.016
NYHA classes 3 and 4 at discharge	1.699	1.165–2.476	0.006

The Cox regression model used in the analysis was adjusted for the following covariates: demographics (age, sex, and BMI), medical history (smoking and chronic atrial fibrillation), CABG, NYHA functional class, eGFR, BNP, LVEF, and medication use (diuretics and digitalis). BNP, LVEF, and NYHA functional class at discharge were entered into the model as the categorical variables; i.e. BNP at discharge ≥ 240 pg/ml or <240 pg/ml or unknown, LVEF at discharge <40% or ≥40% or unknown, and NYHA classes 1 and 2 or 3 and 4. HR, hazard ratio; CI, confidence interval.

There are several mechanisms of hyperuricemia responsible for the increased mortality risk in HF. Serum UA levels may reflect the degree of XO activation in HF [34,35]. XO is one of the major sources of oxygen free radical production and its excess has been shown to be involved in the pathogenesis of HF [36–39]. XO is also shown to impair the regulation of vascular tone [12,33] and reduced vasodilator capacity could lead to exercise intolerance [13,40]. In addition, XO can induce the upregulation of inflammatory cytokines [10]. Hyperuricemia can also reflect an impairment of oxidative metabolism [11]. An inverse relationship between the anaerobic threshold and serum UA concentration has been shown to be present in HF [14]. Finally, hyperuricemia can be a result of renal dysfunction, which may decrease the clearance of UA. However, in the present study as well as other previous studies [11,33], the significant effect of hyperuricemia on outcomes was observed even after the adjustment for risk factors including renal dysfunction.

Several limitations inherent in the design of the registry should be considered. First, the documentation of serum UA levels at hospital discharge might not accurately reflect those after discharge or their changes over time. Second, the information regarding the use of hypouricemiant drugs was not collected in the present study. Similar to the previous studies which also did not collect such information [4–9,27], the critical analysis based on the subgroups with and without the use of hypouricemiant drugs could not be performed. Third, the present study is not a prospective randomized trial and, despite covariate adjustment, other measured and unmeasured factors might have influenced outcomes. For example, serum UA levels have been shown to be higher in patients with postmenopausal state, insulin resistance, elevated leptin level, obstructive sleep apnea, peripheral vascular disease, and movement from rural to urban communities [20]. These factors might be associated with adverse cardiovascular outcomes. Moreover, hyperuricemia is related to inflammation, free radicals and oxidative stress, including XO. However, this study did not collect these data. In addition, the data regarding an indication for surgical treatment were also not collected. However, in the subgroup of prior CABG or valvular surgery higher UA levels were not a significant risk of adverse outcomes either before or after multivariable adjustment. Forth, Cox proportional hazard model has proven to be a useful approach for identifying the relationships of risk factors. However, such approaches must be interpreted with extreme caution when used to determine the covariates. The other hand, the Cox proportional hazard model for survival analysis has gained widespread use from medical researchers. This is mainly due to the fact that this model is quite well suited for the analysis of epidemiological cohort studies and clinical

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trials [41]. In fact, this has been used in the previous studies which assessed the relationship between variables including hyperuricemia and survival [8,27]. Fifth, although the present study demonstrated that low BMI values were significant predictors of all-cause death (Table 4), their values themselves were as low as 22 kg/m^2 compared to those in patients from Europe and United States. However, according to the International Study of Macro-Micro nutrients and Blood Pressure (INTERMAP) study [42], the mean BMI values of Japanese middle-aged men and women were 23.7 and 23.2 kg/m^2, respectively, which were much lower than those of 29.1 and 28.7 kg/m^2 in US population, indicating that the low BMI values in our study patients are a population issue of Japanese. Finally, data were dependent on the accuracy of documentation and abstraction by individual medical centers that participated in this study. However, it was not the objective of this study to restrict enrollment to the narrowly defined population of HF usually included in clinical trials but rather to include a broad range of patients reflecting the current reality of clinical practice. Even though we made an extensive effort to better address and focus the limitation of this study, some major limitation may be still present.

In conclusion, the present study demonstrated that hyperuricemia was common in patients hospitalized with worsening HF and independently associated with long-term adverse outcomes in these patients. Further studies are definitely needed to establish the role of serum UA levels as a potential biomarker for the future risk stratification and a therapeutic target for HF.

Acknowledgments

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Images in Cardiovascular CT

64-Slice MDCT imaging of endocardial cushion defect associated with other cardiac and extracardiac abnormalities

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KEYWORDS:
Congenital heart disease;
Endocardial cushion defect;
Heterotaxy syndrome;
Multidetector computed tomography;
Volume-rendering view

Abstract. Electrocardiographic-gated 64-slice multidetector computed tomography (MDCT) was performed on a 30-year-old man who presented with a complete endocardial cushion defect (ECD) and severe pulmonary hypertension diagnosed when he was 3 years old. Multiplanar reconstruction image showed the common atrium without an atrial septum, a large ventricular septum defect, and a small right ventricle due to a complete atrioventricular canal defect. Three-dimensional CT volume-rending imaging showed a patent ductus arteriosus, dilation of the ascending aorta, and an anomalous-origin right coronary artery. This patient also had heterotaxy syndrome with polysplenia and azygos continuation. MDCT proved to be a good noninvasive imaging method for the evaluation of ECD associated with cardiac as well as extracardiac abnormalities.

A 30-year-old man presented with heart failure and bradycardia (40–45 beats/min). He had been diagnosed with a complete endocardial cushion defect when he was 3 years old. Before permanent pacemaker insertion, a contrast-enhanced electrocardiographic-gated 64-slice multidetector computed tomography (MDCT) was performed to define the cardiac anatomy. Multiplanar reformats showed the common atrium without atrial septum, large ventricular septal defect, and small right ventricle because of complete atrioventricular canal defect (Fig. 1A and B). A volume-rendered image also showed the patent ductus arteriosus, dilatation of the ascending aorta (Fig. 2A), and the anomalous interarterial course of the right coronary artery between the ascending aorta and pulmonary artery (Fig. 2B). This patient also had heterotaxy syndrome with polysplenia and azygos continuation. (Fig. 3A and B).

Conflict of interest: The authors report no conflicts of interest.

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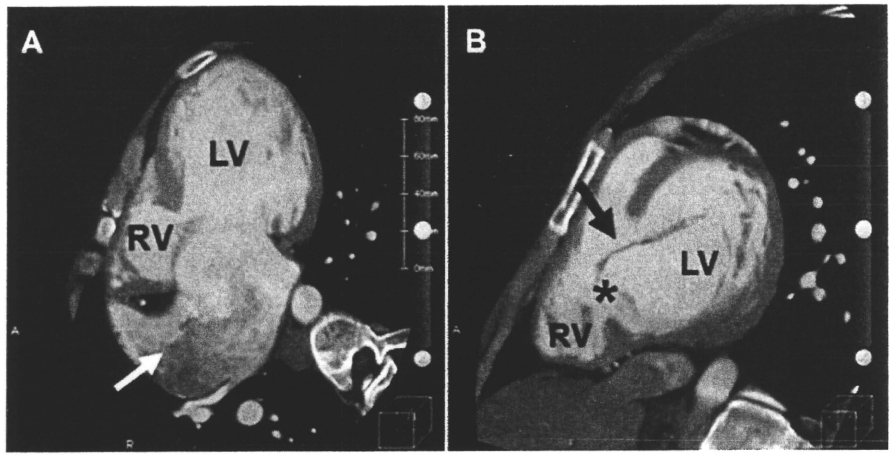

Figure 1 (**A**) Multiplanar reformat showing the common atrium (*white arrow*) and small right ventricle. A movie clip is available in the supplementary material. (**B**) Short-axis multiplanar reformat showing atrioventricular valve leaflets (*black arrow*) straddling the ventricular septum and a large ventricular septal defect (*asterisk*). RV, right ventricle; LV, left ventricle.

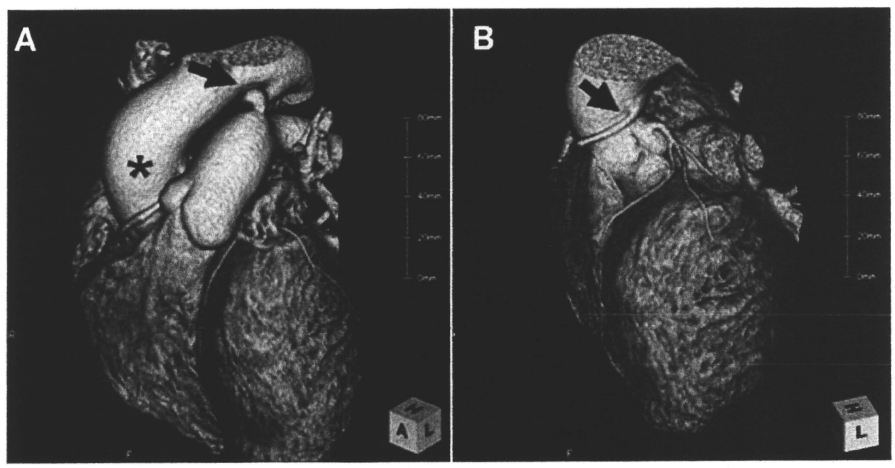

Figure 2 (**A**) Volume-rendered image (left anterior view) showing the patent ductus arteriosus (*black arrow*) and dilated ascending aorta (*asterisk*); (**B**) volume-rendered image without pulmonary artery showing the anomalous origin of the right coronary artery (*black arrow*).

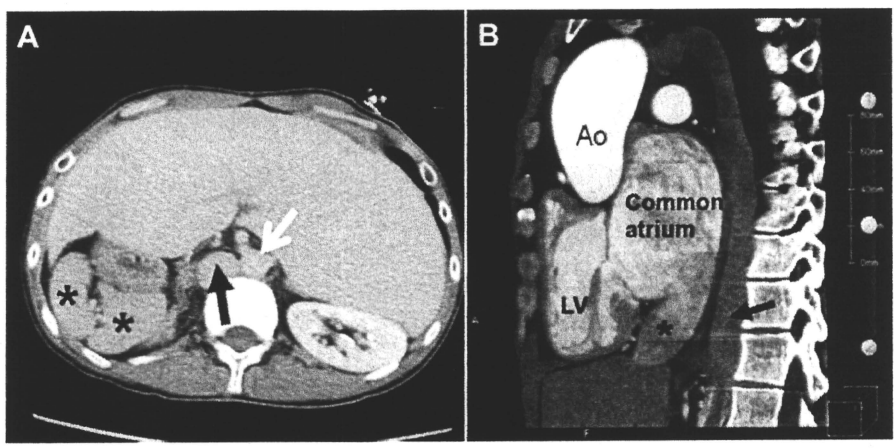

Figure 3 (**A**) Axial contrast-enhanced image showing heterotaxy syndrome with polysplenia (*asterisks*). A dilated azygos vein (*black arrow*) runs beside the descending aorta (*white arrow*). (**B**) Sagittal multiplanar reformat showing the azygos continuation (*black arrow*) and inferior vena cava (*asterisk*). Ao, ascending aorta; LV, left ventricle.

Endocardial cushion defects arise from the abnormal or inadequate fusion of the superior and inferior endocardial cushion, which normally occurs during the fifth week of gestation. They are characterized by a spectrum of cardiac defects involving the atrial septum, ventricular septum, and atrioventricular valves[1] for which MDCT provides a method of comprehensive assessment.[2]

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