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The fasting ^{13}C-glucose breath test is a more sensitive evaluation method for diagnosing hepatic insulin resistance as a cardiovascular risk factor than HOMA-IR

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ABSTRACT

Background: Although we previously reported the fasting ^{13}C-glucose breath test (FGBT) was useful for the diagnosis of hepatic insulin resistance (IR), there has been no report in an actual clinical setting. We therefore performed the FGBT in patients with heart disease to assess the difference in the diagnostic ability of HIR between the FGBT and HOMA-IR; we also assessed the relationship between the FGBT and known cardiovascular risk factors.

Methods: Two hundred patients (100 with ischemic heart disease [IHD], 50 with non-ischemic heart disease [NIHD], and 50 with non-cardiac lifestyle-related disease [NCD]) participated in this study. The data of 40 healthy volunteers [HV] was obtained in our previous study. We evaluated the ^{13}C excretion rate at 120 min (C_{120}) as the indicator of hepatic IR in the FGBT.

Results: The value of C_{120} in each disease group was significantly lower than in HV, but the HOMA-IR in the IHD and NCD groups was not significantly different from that in HV. The value of C_{120} significantly correlated with known cardiovascular risk factors.

Conclusions: These results indicated the FGBT is more sensitive than HOMA-IR for evaluating hepatic IR as a cardiovascular risk factor and is likely useful for managing patients to prevent cardiovascular disease.

1. Introduction

Despite accumulating evidence showing that statins reduce the risk of coronary heart disease in both primary and secondary prevention, a residual risk of roughly 70% still remains [1]. This residual risk presumably includes low high-density-lipoprotein (HDL) cholesterolemia and glucose intolerance based on insulin resistance (IR) [2]. Cardiac diseases have been reported to progress under a glucose intolerant state with low hemoglobin A1C (HbA1C) levels [2], therefore evaluating hepatic IR is important to manage various cardiovascular risk factors.

Glucose clamp tests are recognized as the gold-standard tests for diagnosing IR but are invasive and complicated to use for IR screening. Although the 75-g oral glucose tolerance test (OGTT) is widely used for diagnosing glucose intolerance, this test takes a long time to perform and is stressful for patients, requiring frequent blood sampling. We previously reported that the fasting ^{13}C-glucose breath test (FGBT) is useful for diagnosing hepatic IR and diabetes mellitus (DM) among healthy volunteers and mild glucose intolerance patients [3]. The result of FGBT was calculated from the concentration of the $^{13}CO_2$ in a patient's expired gas. In a fasting state after taking 100 mg of ^{13}C-glucose, the rate of $^{13}CO_2/^{12}CO_2$ in the expired gas of a patient with hepatic IR decreases compared to that of a healthy volunteer. This is because the glycolytic system pathway is suppressed and the gluconeogenesis pathway is activated in a fasting state when a patient develops a hepatic resistant state with impaired glucose tolerance [4].

Homeostatic model assessment insulin resistance (HOMA-IR) is

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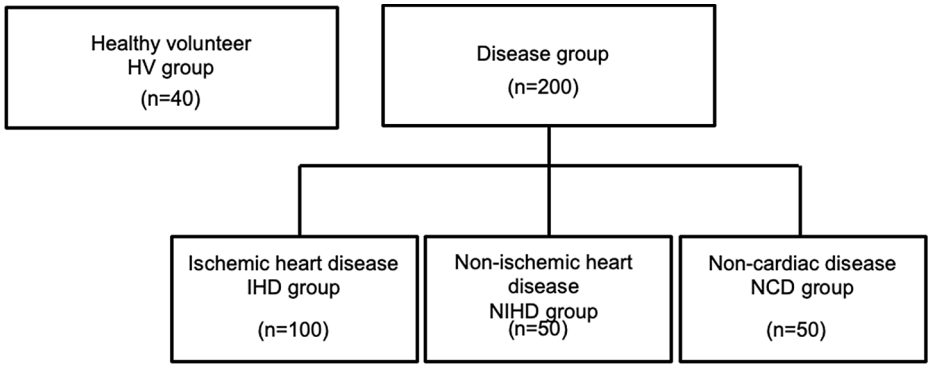

Fig. 1. Study design. HV = healthy volunteer; IHD = ischemic heart disease; NIHD = non-ischemic heart disease; NCD = non-cardiac disease.

widely used as an indicator of IR. However, the reliability of HOMA-IR is reduced in patients with a high fasting blood glucose (FBG) level (> 140 mg/dl) or impaired insulin secretion; such conditions are not an issue with the FGBT. The values of HOMA-IR also reportedly differ among races [5], so whether or not the reference range of HOMA-IR for Caucasoids is applicable to Japanese populations for diagnosing IR remains unclear. Again, this issue does not affect the utility of the FGBT.

The FGBT is a non-invasive and simple test. Furthermore, if the results of the FGBT are found to correlate with residual risk factors, this test may be useful for managing risk factors in the early pathologic stage. To address this issue, we investigated the relationship between the results of the FGBT and the disease profile and biochemical parameters by performing the

Table 1
Patient characteristics.

	IHD (n = 100)		NIHD (n = 50)		NCD (n = 50)		P value
Age (years old)	68.3 ± 8.9		66.0 ± 9.7		66.0 ± 12.3		0.265
Male gender (n, (%))	82	(82.0%)	39	(78.0%)	34	(68.0%)	0.153
Hypertension (n, (%))	47	(47.0%)	20	(40.0%)	34	(68.0%)	0.012
Dyslipidemia (n, (%))	66	(66.0%)	30	(60.%)	33	(66.0%)	0.745
Diabetes (n, (%))	41	(41.0%)	9	(18.0%)	8	(16.0%)	0.001
ischemic heart diseas (n, (%))	100	(100%)	0	(0%)	0	(0%)	–
non ischemic heart disease (n, (%))	11	(11.0%)	50	(100%)	0	(0%)	–
C_{120} (mmol/h)	0.245 ± 0.064		0.244 ± 0.055		0.255 ± 0.060		0.531
BMI (kg/m^2)	24.1 ± 3.1		24.4 ± 2.4		24.3 ± 3.3		0.793
WBC (/mm^3)	5920 ± 1570		5575 ± 1634		5535 ± 1467		0.256
hemoglobin (g/dl)	14.1 ± 1.6		14.0 ± 1.6		14.4 ± 1.4		0.429
platelet (10^4/mm^3)	22.6 ± 5.1		21.7 ± 5.9		24.0 ± 5.8		0.107
TP (g/dl)	7.1 ± 0.4		7.1 ± 0.4		7.2 ± 0.4		0.317
Alb (g/dl)	4.2 ± 0.3		4.2 ± 0.2		4.3 ± 0.2		0.191
T-Bil (mg/dl)	0.7 ± 0.3		0.8 ± 0.3		0.8 ± 0.3		0.348
AST (U/L)	25.5 ± 10.0		23.8 ± 5.9		26.6 ± 8.6		0.274
ALT (U/L)	25.3 ± 12.9		21.3 ± 10.4		26.3 ± 12.8		0.084
ALP (U/L)	235 ± 81.8		204 ± 51.7		212 ± 59.3		0.022
$_\gamma$-GTP (U/L)	37.3 ± 25.5		45.6 ± 31.1		41.5 ± 32.8		0.244
LDH (U/L)	194 ± 36.9		200 ± 37.4		196 ± 29.8		0.627
CPK (U/L)	127 ± 94.7		138 ± 99.7		149 ± 76.2		0.368
HDL-C (mg/dl)	50.7 ± 12.4		55.7 ± 13.5		59.6 ± 18.8		0.002
TG (mg/dl)	120 ± 58.7		114 ± 53.9		134 ± 71.5		0.247
LDL-C (mg/dl)	83.4 ± 24.7		107.7 ± 26.3		109.3 ± 27.6		< 0.001
UA (mg/dl)	5.7 ± 1.0		6.2 ± 1.5		5.8 ± 1.3		0.121
BUN (mg/dl)	16.9 ± 4.4		16.5 ± 4.9		16.1 ± 4.0		0.564
Cr (mg/dl)	0.86 ± 0.21		0.90 ± 0.27		0.85 ± 0.22		0.581
Na (mEq/L)	142 ± 2.0		142 ± 1.8		141 ± 1.9		0.014
K (mEq/L)	4.3 ± 0.4		4.3 ± 0.4		4.2 ± 0.3		0.922
CRP (ng/ml)	0.23 ± 0.5		0.15 ± 0.2		0.12 ± 0.2		0.175
BNP (pg/ml)	30.6 ± 53.4		58.9 ± 61.1		16.0 ± 15.9		< 0.001
FBG (mg/dl)	109 ± 25.4		101 ± 12.3		99 ± 15.8		0.007
HbA1C (%)	6.1 ± 0.7		5.7 ± 0.5		5.7 ± 0.5		< 0.001
IRI (μU/ml)	7.9 ± 7.8		9.9 ± 13.8		6.7 ± 4.0		0.199
HOMA-IR	2.1 ± 2.2		2.7 ± 4.5		1.7 ± 1.2		0.242
eGFR (ml/min/1.73 m^2)	68 ± 15.6		67 ± 17.6		68 ± 15.6		0.873

Abbreviations: Alb, albumin; ALP, alkaline phosphatase; ALT alanine amino transferase; AST, aspartate amino tranferase; BMI, body mass index; BNP, brain natriuretic peptide; BUN, blood urea nitrogen; CPK, creatine phosphokinase; Cr, creatinine; CRP, C-reactive protein; eGFR, estimate glomerular filtration rate; FBG, fasting blood glucose; HbA1C, hemogrobin A1C; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment insulin resistance; IHD, ischemic heart disease; IRI, immunoreactive insulin; K, potassium; LDH, lactate dehydrogenase; LDL-C, low-density lipoprotein cholesterol; Na, sodium; NCD, non-cardiac heart disease; NIHD, non-ischemic heart disease; T-Bil, total bilirubin; TG, triglyceride; TP, total protein; UA, uric acid; WBC, white blood cell; $_\gamma$-GTP, gamma-glutamyl transpeptidase

Values are presented as mean ± SD except for categorical variables.

P value was calculated using the chi-squared test for categorical values and using the one-way ANOVA for continuous values.

FGBT in 200 patients who regularly attended Tokorozawa Heart Center, a cardiovascular center in Saitama, Japan. We also assessed the difference in the results of the FGBT between patients with disease and healthy volunteers, compared with HOMA-IR, a widely used indicator for IR, as the primary outcome, and we evaluated the relationship between the results of the FGBT and known residual risk factors for cardiovascular disease as the secondary outcome.

2. Materials and methods

2.1. Study population

Two hundred patients who regularly attended Tokorozawa Heart Center, a cardiovascular center in Saitama, Japan, were included. Tokorozawa Heart Center is a regional secondary emergency medical facility with 30 beds that specializes in treating cardiovascular disease and primary prevention of cardiovascular disease.

The 200 patients included 100 ischemic heart disease (IHD) patients, 50 non-ischemic heart disease (NIHD) patients, and 50 non-cardiac lifestyle-related disease (NCD) patients (see Fig. 1). The NIHD patients mainly had arrhythmia or non-ischemic heart failure; they were confirmed to have no coronary diseases using coronary angiography or computed tomography before their inclusion in this study. The NCD patients were those with lifestyle-related diseases, such as hypertension, dyslipidemia, and DM, who regularly attended our hospital to manage their risk factors; they were confirmed to have no organic heart disease using echocardiography before their inclusion in this study.

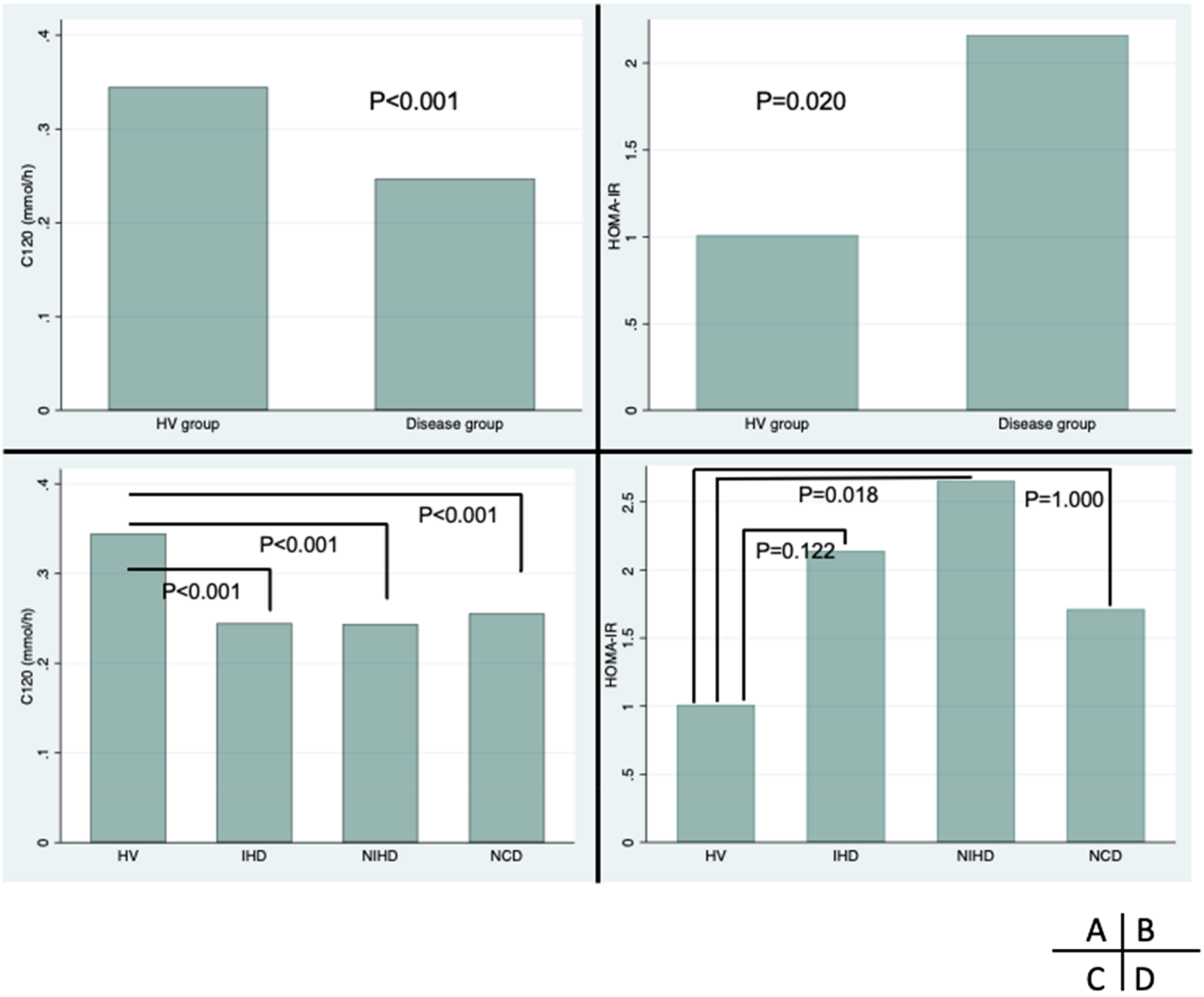

Fig. 2. The difference in the value of C_{120} and HOMA-IR between the HV group and disease group. A: The difference in the mean value of C_{120} (mmol/h) between the HV group and disease group. There was a significant difference between the 2 groups ($p < 0.001$). The P value was calculated using Student's *t*-test. B: The difference in the mean value of HOMA-IR between the HV group and disease group. There was a significant difference between the 2 groups ($p = 0.020$). The P value was calculated using Student's *t*-test. Logarithmic transformation was conducted before analyzing HOMA-IR using Student's *t*-test. C: The difference in the mean value of C_{120} (mmol/h) between the HV group and each disease profile. The value of C_{120} was significantly higher in the HV group than in any disease profile (IHD group, NIHD group, and NCD group: $p < 0.001$, $p < 0.001$, $p < 0.001$ respectively). The P value was calculated using a one-way analysis of variance. The P value between 2 groups was calculated using Scheffe's method to analyze C_{120}. D: The difference in the mean value of HOMA-IR between the HV group and each disease profile. There were no significant differences between the HV group and IHD group or between the HV group and NCD group ($p = 0.122$, $p = 1.000$ respectively). The value of HOMA-IR was significantly lower in the HV group than in the NIHD group ($p = 0.018$). The P value was calculated using a one-way analysis of variance. The P value between 2 groups was calculated using Bonferroni's method for analyzing HOMA-IR. HV = healthy volunteer; IHD = ischemic heart disease; NIHD = non-ischemic heart disease; NCD = non-cardiac disease; C_{120} = ^{13}C excretion rate at 120 min; HOMA-IR = homeostatic model assessment insulin resistance.

The exclusion criteria were (1) < 20 years old or ≥ 85 years old, (2) acute coronary syndrome, (3) end-stage renal disease (including patients receiving hemodialysis), (4) type 1 DM, (5) pregnant or may become pregnant, (6) shock vitals, (7) scheduled to undergo surgery or endoscopic therapy within three months and required to stop antiplatelet therapy, and 8) doctor in charge objected to the patient's participation.

We used the data of 40 healthy volunteers (HV group) for a comparison with the disease group (combined IHD group, NIHD group, and NCD group). These data had been obtained in our previous study [3].

2.2. Outcome evaluation and ethical considerations

The FGBT and fasting blood collection were performed in every patient. The primary outcome was the difference in the value of C_{120} (see details below) using the FGBT and HOMA-IR between the disease groups and HV group. The secondary outcomes were the relationship between the known coronary risk factors and the value of C_{120}.

This study was registered with the University Hospital Medical Information Network-Clinical Trials registry (UMIN-CTR number: UMIN000025662). The Ethics Committee of Tokorozawa Heart Center (Registration Number: 1504) and The Jikei University School of Medicine (Registration Number: 18–188 [4850], 28–105 [8348]) approved this study protocol, which was in accordance with the Declaration of Helsinki, and all patients gave their written informed consent to participate.

2.3. FGBT

The FGBT was performed at 6:00 a.m. in an overnight fasting state (last meal: 21:00). First, patients took 100 mg of glucose labeled with ^{13}C orally after having a control breath sample collected. Two hours later, at rest, patients had their breath sample taken again. ^{13}C-glucose was created by replacing all carbon atoms with ^{13}C. The ^{13}C-glucose used in this study was D-Dlucose-U-$^{13}C6$ (^{13}C: 99 atom%; Chlorella Industry Co., Ltd., Tokyo, Japan). Breath samples were mailed to the Department of Laboratory Medicine, The Jikei University School of Medicine. The $^{13}CO_2$-to-$^{12}CO_2$ ratio was measured using a carbon dioxide carbon isotope ratio analyzer/spectral analyzer POC one (Otsuka Electronics Co., Ltd., Osaka, Japan.). We then calculated the ^{13}C excretion rate (mmol/h) using the $^{13}CO_2$-to$^{12}CO_2$ ratio and patient's body surface area.

Our previous study demonstrated that the area under the curve until 360 min (AUC_{360}) of the ^{13}C excretion kinetic curve after the ingestion of labeled glucose reflected the efficiency of glucose metabolism in the liver [3]. The ^{13}C excretion rate reached a maximum at 120 min after the start of FGBT and the ^{13}C excretion rate at 120 min (C_{120}) showed a strong correlation with the AUC_{360} value [3]. Furthermore, in addition to the AUC_{360} value [3], the C_{120} value showed high diagnostic accuracy in the detection of hepatic IR. Because an AUC_{360} study is time consuming and difficult to perform for large numbers of patients, we used the C_{120} value to evaluate the hepatic IR of patients in this study.

2.4. Biochemical parameters

Venous blood was collected in a fasting state. A complete blood count, parameters reflecting the liver and renal function, serum lipid profile, FBG, fasting immunoreactive insulin levels, hemoglobin A1C (HbA1C), C-reactive protein, and brain natriuretic peptide (BNP) were analyzed. HOMA-IR was calculated by the following equation: HOMA-IR = (FBG × immunoreactive insulin levels)/405.

2.5. Statistical analyses

Categorical variables are presented as the frequency (%). A chi-squared test was used to compare the distribution of categorical variables among groups. Differences in C_{120} values among groups were compared using Student's *t*-test, while differences in the HOMA-IR value were compared using Student's *t*-test, after logarithmic transformation. Quantitative variables were presented as the mean and standard deviation. A parametric analysis was performed when nonparametric parameters showed a parametric distribution after logarithmic transformation. Nonparametric analyses were performed for nonparametric parameters after logarithmic transformation. Differences in the distribution of quantitative variables among three groups were assessed using a one-way analysis of variance. When a significant difference was identified among three groups, Bartlett's test was used to test the homogeneity of variance. Differences between two groups were compared using the Scheffe test if the variables had equal variance or Bonferroni's correction if the variables did not have equal variance. The correlation between C_{120} and quantitative variables was assessed by Pearson's correlation coefficient if a variable was parametrically distributed and by Spearman's correlation coefficient if a variable was not parametrically distributed.

A multiple regression analysis was performed to analyze variables that had a significant correlation with C_{120}. We calculated the variance inflation factor (VIF) to measure the degree of multi-collinearity in the multiple regression analysis. VIFs were calculated by taking a predictor and regressing it against all other predictors in the model. A high correlation with other predictors was represented by a VIF value of > 5,

Table 2
Differences in glucose metabolism parameters between HV group and disease group.

	HV group (n = 62)	Disease group (n = 200) P value vs. HV group			P value between groups
		IHD (n = 100) P value vs. HV group	NIHD (n = 50) P value vs. HV group	NCD (n = 50) P value vs. HV group	
C_{120} (mmol/h)	0.345 ± 0.05	0.247 ± 0.06			
		*P < 0.001			
		0.245 ± 0.06	0.244 ± 0.06	0.255 ± 0.06	P < 0.001
		P < 0.001	P < 0.001	P < 0.001	
HOMA-IR	1.0 ± 0.4	2.2 ± 2.8			
		*P < 0.001			
		2.1 ± 2.2	2.7 ± 4.5	1.7 ± 1.2	P = 0.020
		P = 0.122	P = 0.018	P = 1.000	

Abbrevations: HbA1C, hemogrobin A1C; HOMA-IR, homeostatic model assessment insulin resistance; HV, healthy volunteer; IHD, ischemic heart disease; NCD, non-cardiac heart disease; NIHD, non-ischemic heart disease.
Values are presented as mean ± SD.
P value was calculated using oneway ANOVA.
P value between 2 groups was calculated using the Scheffe's method for analyzing C120.
P value between 2 groups was calculated using the Bonferroni's method for analyzing HOMA-IR.
* P value was calculated using Student's *t* test. Logarithmic transformation was conducted before analyzing HOMA-IR using Student's *t* test.

while no correlation with other predictors was represented as a VIF value of 1. The correlations between HOMA-IR and quantitative variables were analyzed in the same way as C_{120} after logarithmic transformation of HOMA-IR. Two-sided *P* values of < 0.05 were considered to indicate statistical significance. The descriptive assessments and statistical analyses were performed using STATA/IC 15.1 (StataCorp LLC, College Station, TX, USA).

3. Results

We were able to obtain FGBT data and biochemical parameters from all participants. The patient characteristics are shown in Table 1. The value of C_{120} in the disease group was significantly lower than in the HV group (0.245 ± 0.06 vs. 0.345 ± 0.05p < 0.001, Fig. 2A). Although there were no significant differences in the value of C_{120} among the IHD, NIHD, and NCD groups (Table 1), the value of C_{120} in each disease group (IHD group, NIHD group, and NCD group) was significantly lower than in the HV group (0.245 ± 0.06 vs. 0.345 ± 0.05p < 0.0001, 0.244 ± 0.05 vs. 0.345 ± 0.05p < 0.0001, 0.255 ± 0.06 vs. 0.345 ± 0.05p = 0.0008, respectively; Table 2, Fig. 2C). Although the value of HOMA-IR in the overall disease group was significantly higher than in the HV group (2.2 ± 2.8 vs. 1.0 ± 0.4p = 0.020, Fig. 2B), there were no significant differences between the values in the IHD and NCD groups and the HV group (1.0 ± 0.4 vs. 2.1 ± 2.2, p = 0.122, 1.0 ± 0.4 vs. 1.7 ± 1.2, p = 1.000, respectively; Table 2, Fig. 2D).

The value of C_{120} was significantly lower in men (p = 0.024, Table 3) and DM patients (p < 0.001, Table 3) than female and non-DM patients, respectively. The value of C_{120} significantly correlated with the body mass index (BMI) (r = −0.205 p < 0.001), white blood cell (r = −0.209 p = 0.004), hemoglobin (r = −0.139 p = 0.049), gamma-glutamyl transpeptidase (r = −0.201 p < 0.001), HDL-C (r = 0.144 p = 0.042), C-reactive protein (r = −0.195 p = 0.006), FBG (r = −0.360 p < 0.001), HbA1C (r = −0.323 p < 0.001), and HOMA-IR (r = −0.145 p = 0.040) (Table 3). We performed a multiple regression analysis of these parameters, and only HbA1C was an independently significant predictor of C_{120}, as shown in Table 4. We also examined the relationship between HOMA-IR and these parameters. The HOMA-IR value was significantly higher in dyslipidemia patients than patients without dyslipidemia (p = 0.020), but there was no significant difference between DM and non-DM patients (p = 0.304; Table 5). The HOMA-IR significantly correlated with the age (r = −0.184 p = 0.009), BMI (r = 0.447 p < 0.001), white blood cell (r = 0.213 p = 0.003), hemoglobin (r = 0.231 p = 0.001), total bilirubin (r = −0.140 p = 0.049), alanine amino transferase (r = 0.324 p < 0.001), gamma-glutamyl transpeptidase (r = 0.197 p = 0.005), lactate dehydrogenase (r = −0.177 p = 0.012), HDL-C (r = −0.432 p < 0.001), triglyceride (r = 0.410 p < 0.001), BNP (r = −0.190 p = 0.007), and HbA1C (r = 0.185 p = 0.009) (Table 5). The results of the multiple regression analysis showed that the BMI (p < 0.001), HDL-C (p = 0.004), and triglyceride (p = 0.007) were independently significant predictors of the HOMA-IR (Table 6).

Table 3
Differences in C120 about categorical variables and correlation between C120 and quantitative variables.

			P value
categorical variables			
	(+)	(−)	
Male gender	0.242 ± 0.057	0.265 ± 0.071	0.024
Hypertension	0.246 ± 0.051	0.249 ± 0.070	0.732
Dyslipidemia	0.242 ± 0.062	0.256 ± 0.058	0.113
Diabetes	0.224 ± 0.057	0.256 ± 0.060	< 0.001
ischemic heart disease	0.245 ± 0.064	0.249 ± 0.058	0.574
non-ischemic heart disease	0.243 ± 0.055	0.249 ± 0.063	0.535
quantitative variables			
	correlation coefficient		
Age	−0.008		0.911
BMI	−0.205		0.004
WBC	−0.209		0.003
hemoglobin	−0.139		0.005
platelet	0.106		0.135
TP	−0.058		0.412
Alb	0.038		0.594
T-Bil	0.049		0.488
AST	−0.002		0.982
ALT	−0.070		0.327
ALP	−0.075		0.293
$_{\gamma}$-GTP	−0.201		0.004
LDH	0.080		0.263
CPK	0.024		0.737
HDL-C	0.144		0.042
TG	−0.036		0.614
LDL-C	−0.060		0.403
UA	−0.009		0.896
BUN	−0.079		0.269
Cr	−0.014		0.850
Na	−0.030		0.676
K	0.132		0.063
CRP	−0.195		0.006
BNP	−0.037		0.601
FBG	−0.360		< 0.001
HbA1C	−0.323		< 0.001
IRI	−0.101		0.155
HOMA-IR	−0.145		0.040
eGFR	−0.053		0.454

Abbrevations: Alb, albumin; ALP, alkaline phosphatase; ALT alanine amino transferase; AST, aspartate amino tranferase; BMI, body mass index; BNP, brain natriuretic peptide; BUN, blood urea nitrogen; CPK, creatine phosphokinase; Cr, creatinine; CRP, C-reactive protein; eGFR, estimate glomerular filtration rate; FBG, fasting blood glucose; HbA1C, hemogrobin A1C; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment insulin resistance; IHD, ischemic heart disease; IRI, immunoreactive insulin; K, potassium; LDH, lactate dehydrogenase; LDL-C, low-density lipoprotein cholesterol; Na, sodium; NCD, non-cardiac heart disease; NIHD, non-ischemic heart disease; T-Bil, total bilirubin; TG, triglyceride; TP, total protein; UA, uric acid; WBC, white blood cell; $_{\gamma}$-GTP, gamma-glutamyl transpeptidase.
Vaalues are presented as mean ± SD of C120 in the colums of categorical variables.
P value was calculated using the Student's *t* test in categorical variables.
Correlation coefficient and p value were calculated using Pearson's product moment correlation coefficient if parameters were parametrically distributed and using Spearman's rank correlation coefficient if parameters were not parametrically distributed.
Logarithmic transformation was conducted if needed.

4. Discussion

4.1. Discussion

In this study, we performed the FGBT in patients who had cardiovascular disease or lifestyle-related disease requiring medication in an actual clinical setting. The FGBT results (value of C_{120}) in these patients was significantly lower than in HVs. There were no significant differences in the value of C_{120} among the three disease groups, suggesting that the value of C_{120} was already low in the patients with lifestyle-related diseases who had not yet developed cardiovascular disease. Regarding HOMA-IR, there was no significant difference in the value between the IHD group and HV group or between the NCD group and HV group. Although the value of C_{120} in the patients receiving medical intervention with lifestyle-related disease (i.e. the NCD group) was similarly low in the NIHD and IHD groups, the HOMA-IR in the NCD and IHD group did not differ significantly from that in the HV group. These findings suggested that C_{120} is a more sensitive indicator for risk management than HOMA-IR in the early clinical stage.

The value of C_{120} was significantly related to the gender, prevalence of DM, BMI, WBC, hemoglobin, gamma-glutamyl transpeptidase, HDL-C, C-reactive protein, FBG, HbA1C, and HOMA-IR. This suggested that

Table 4
Results of the multiple regression analysis of C120.

	Coefficient	Standard error	P value	95% confidential interval	VIF
Male gender	−0.020	0.012	0.093	−0.044 to 0.003	1.53
BMI	−0.002	0.002	0.186	−0.005 to 0.001	1.35
WBC	−3.3exp(−6)	2.9exp(−6)	0.256	−9.0exp(−6) to 2.4exp(−6)	1.26
$_{\gamma}$-GTP	−0.005	0.008	0.481	−0.020 to 0.010	1.26
HbA1C	−0.027	0.007	< 0.001	−0.041 to −0.013	1.18
hemogrobin	−0.001	0.003	0.862	−0.007 to 0.006	1.58
HOMA-IR	−0.0002	0.006	0.981	−0.013 to 0.012	1.48
HDL-C	−0.005	0.020	0.815	−0.034 to 0.043	1.53

Abbreviations: BMI, body mass index; exp, exponential function; HbA1C, hemogrobin A1C; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment insulin resistance; WBC, white blood cell; VIF, variance inflation factor; $_{\gamma}$-GTP, gamma-glutamyl transpeptidase
Logarithmic transformation was conducted before analyzing if needed.

the results of the FGBT were related to the residual risk factors based on the IR. A multivariate analysis showed that HbA1C was the independent predictor of C_{120}. That meant that DM was the factor most influential on the value of C_{120}. Therefore, to identify the predictors of C_{120} in the non-DM state, we performed a multivariate analysis in the patients whose HbA1C were less than 6.2% (Table S7). The multiple regression analysis showed that the gender and BMI were independent predictors for C_{120}. In contrast, HOMA-IR, which is widely used as an indicator of IR, showed no significant relationship with HbA1C according to a multiple regression analysis, but it was shown to be significantly related to the BMI, HDL-C, and triglyceride. This result was unchanged in the setting of non-DM patients (Table S8). These results suggested that both the FGBT and HOMA-IR were correlated with the residual risk factors of ischemic heart disease, but the FGBT was presumably related to glucose metabolism disorders based on IR, whereas HOMA-IR was related to dyslipidemia based on IR.

Although HOMA-IR is widely used for diagnosing IR and DM [6], the value of HOMA-IR in the Japanese population is reportedly lower than that in Caucasian populations, both in a healthy state and in an insulin-resistant state [5]. Therefore, false negative cases are more frequent in Japanese patients using global standard reference values of HOMA-IR. In addition, the reliability of HOMA-IR was reported to be reduced when the FBG level was > 140 mg/dl [7]. Using HOMA-IR to diagnose glucose metabolism disorders for Japanese patients requires close attention and care because of these problems. HOMA-IR was reported to have an inverse correlation with BNP [8]. Although the same inverse correlation was seen in this study ($n = 200$ $r = -0.190$ $p = 0.007$), HOMA-IR was significantly higher than in the HV group only in the NIHD group (Fig. 2D). Many patients with IR were presumably included, even among heart failure patients, although only the relationship between BNP and HOMA-IR was an inverse correlation. BNP itself may reduce the value of HOMA-IR through several proposed mechanisms [8]. According to this theory, the IR may be underestimated in the NIHD group when evaluated by HOMA-IR because the BNP was significantly higher in the NIHD group than in the other groups. On the other hand, the value of C_{120} did not correlate with the BNP, so an underestimation of hepatic IR might not occur in the NIHD group when they are evaluated by the FGBT.

The cut-off values of C_{120} for diagnosing IR and DM differed between genders in our previous study. The cut-off value of C_{120} for diagnosing IR in men was 0.285 mmol/h (sensitivity 84.6%, specificity 84.2%) whereas that in women was 0.323 mmol/h (sensitivity 88.9%, specificity 85.7%). The cut-off value of C_{120} for diagnosing DM in men was 0.261 mmol/h (sensitivity 100%, specificity 94.7%) whereas that in women was 0.308 mmol/h (sensitivity 100%, specificity 95.2%). In this study, the average value of C_{120} in women was low (0.265 ± 0.071), as was that in non-DM women (0.277 ± 0.075), compared to our previous study. This result seems to suggest that the value of C_{120} was low in patients with cardiac disease or lifestyle-related disease. The multivariate analysis showed that gender was not a significant predictor of the value of C_{120} in DM patients who required medical treatment in this study. Given this finding, the FGBT might not be suitable for diagnosing patients receiving medical intervention, although it may be suitable for evaluating the effects of lifestyle improvement or exercise. To clarify this issue, chronological data are needed. A cohort study rather than a non-cross-sectional study should be performed.

Mizrahi, et al. reported that the breath test using ^{13}C-glucose reliably assessed the changes in the liver glucose metabolism, and the degree of IR evaluated using the HOMA-IR and the OGTT [9]. Hussain, et al. reported that the ^{13}CO2 appearance in exhaled breath following a standard OGTT with ^{13}C-glucose provided a valid surrogate index of the whole-body glucose disposal rate as measured by the golden standard hyperinsulinemic euglycemic clamp, with good accuracy and precision [10]. Maldonado-Hernandez, et al. also reported that the breath test using ^{13}C-glucose for adolescents was a suitable method for IR screening with a reasonable sensitivity and specificity [11].

In those studies, ^{13}C-glucose was used to perform the 75-g OGTT, and frequent breath sampling was needed in order to measure the area under the curve of the ^{13}C excretion rate. In contrast, our method (i.e. FGBT) requires only a small amount of glucose (100 mg) and 2 breath samples (baseline and 2 h after taking glucose), making it easy and simple for patients to perform. We previously reported that the diagnostic ability of the FGBT using C_{120} was equivalent to that of the FGBT using the AUC_{360} required 10 breath samples [3]. In actual clinical settings, the FGBT using C_{120} is far easier on patients than that using the AUC_{360}. The reports mentioned above using the OGTT involved evaluations in a small number of HVs, and there have been no reports involving the breath test using glucose in patients with cardiovascular disease or lifestyle-related disease in actual clinical settings. This study showed that patients with lifestyle-related diseases already had a low value of C_{120} before developing cardiac disease, suggesting that the FGBT is feasible for the management of risk factors.

Several methods for evaluating IR exist, but most require a blood sample and are relatively invasive. The FGBT is a noninvasive and simple method that is correlated with residual risk factors of cardiovascular disease, including glucose metabolism disorders, BMI, dyslipidemia (low-HDL cholesterolemia), and inflammation. The FGBT is presumably useful for managing the risk factors in patients with cardiovascular disease and lifestyle-related disease.

4.2. Limitations

Several limitations associated with the present study warrant mention. Our present study was a single-center study, which might have caused selection bias. In addition, the study periods differed between the disease group (present study) and HV group (previous study). This difference in study period may have affected the results. However, the FGBT is still a simple test, and we used the same method and machine to measure the value of C_{120} in the same place using ^{13}C-glucose produced by the same company. We therefore believe that there was no issue with comparing the data obtained in the present study to those

Table 5
Differences in HOMA-IR about categorical variables and correlation between HOMA-IR and quantitative variables.

			P value
categorical variables			
	(+)	(−)	
Male gender	2.1 ± 3.1	2.0 ± 1.5	0.404
Hypertension	2.3 ± 3.2	2.0 ± 2.3	0.254
Dyslipidemia	2.5 ± 3.7	1.6 ± 1.1	0.042
Diabetes	2.1 ± 1.5	2.2 ± 3.2	0.304
ischemic heart disease	2.1 ± 2.2	2.2 ± 3.3	0.214
non-ischemic heart disease	2.5 ± 4.1	2.0 ± 2.0	0.957
quantitative variables			
	correlation coefficient		
Age	−0.184		0.009
BMI	0.447		< 0.001
WBC	0.213		0.003
hemoglobin	0.231		0.001
platelet	−0.076		0.282
TP	−0.012		0.867
Alb	−0.032		0.652
T-Bil	−0.140		0.049
AST	0.022		0.760
ALT	0.324		< 0.001
ALP	0.061		0.392
γ-GTP	0,1968		0.005
LDH	−0.177		0.012
CPK	−0.104		0.144
HDL-C	−0.432		< 0.001
TG	0.410		< 0.001
LDL-C	0.096		0.175
UA	0.105		0.138
BUN	−0.014		0.848
Cr	0.115		0.106
Na	−0.013		0.857
K	−0.061		0.394
CRP	0.136		0.055
BNP	−0.190		0.007
FBG	0.381		< 0.01
HbA1C	0.186		0.009
IRI	0.968		< 0.001
eGFR	−0.049		0.493

Abbreviations: Alb, albumin; ALP, alkaline phosphatase; ALT alanine amino transferase; AST, aspartate amino tranferase; BMI, body mass index; BNP, brain natriuretic peptide; BUN, blood urea nitrogen; CPK, creatine phosphokinase; Cr, creatinine; CRP, C-reactive protein; eGFR, estimate glomerular filtration rate; FBG, fasting blood glucose; HbA1C, hemogrobin A1C; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment insulin resistance; IHD, ischemic heart disease; IRI, immunoreactive insulin; K, potassium; LDH, lactate dehydrogenase; LDL-C, low-density lipoprotein cholesterol; Na, sodium; NCD, non-cardiac heart disease; NIHD, non-ischemic heart disease; T-Bil, total bilirubin; TG, triglyceride; TP, total protein; UA, uric acid; WBC, white blood cell; γ-GTP, gamma-glutamyl transpeptidase.
Vaalues are presented as mean ± SD of HOMA-IR in the colums of categorical variables.
P value was calculated using the Student's *t* test in categorical variables.
Correlation coefficient and p value were calculated using Pearson's product moment correlation coefficient if parameters were parametrically distributed and using Spearman's rank correlation coefficient if parameters were not parametrically distributed.
Logarithmic transformation was conducted if needed.

from our previous study.

This study was a cross-sectional study, so longitudinal studies may be needed in order to clarify whether or not the FGBT can predict the cardiovascular disease onset risk.

5. Conclusions

The value of C_{120} was significantly lower in the IHD group, NIHD group, and NCD group than in the HV group, in contrast to findings concerning HOMA-IR. The value of C_{120} significantly correlated with the glucose metabolism, BMI, dyslipidemia, and inflammation. Our observations suggest that the FGBT is a useful test for managing cardiovascular risk factors.

Table 6
Results of the multiple regression analysis of HOMA-IR.

	Coefficient	Standard error	P value	95% confidential interval	VIF
Age	−0.007	0.005	0.194	−0.017 to 0.003	1.37
BMI	0.065	0.017	< 0.001	0.031 to 0.098	1.31
WBC	8.3exp(−6)	0.00003	0.795	−0.00005 to 0.00007	1.26
hemogrobin	0.005	0.036	0.884	−0.066 to 0.076	1.57
ALT	0.179	0.120	0.137	−0.058 to 0.416	1.65
LDH	−0.002	0.001	0.166	−0.005 to 0.001	1.19
HDL-C	−0.612	0.210	0.004	−1.027 to −0.198	1.47
TG	0.293	0.107	0.007	0.081 to 0.505	1.48
γ-GTP	0.032	0.088	0.713	−0.141 to 0.206	1.43
BNP	−0.061	0.048	0.202	−0.156 to 0.033	1.45
HbA1C	0.073	0.077	0.347	−0.080 to 0.226	1.20

Abbreviations: ALT alanine amino transferase; BMI, body mass index; BNP, brain natriuretic peptide; exp, exponential function; HbA1C, hemogrobin A1C; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment insulin resistance; LDH, lactate dehydrogenase; TG, triglyceride; WBC, white blood cell; VIF, variance inflation factor; γ-GTP, gamma-glutamyl transpeptidase.
Logarithmic transformation was used before analyzing if needed.

Declaration of Competing Interest

The authors have read the journal's policy on conflicts of interest and have none to declare in association with this manuscript. All authors have read the journal's authorship agreement and have reviewed and approved this manuscript.

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Appendix A. Supplementary data

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