Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

The diabetic heart utilizes ketone bodies as an energy source

Yuji Mizuno[a,*], Eisaku Harada[a], Hitoshi Nakagawa[b], Yoshinobu Morikawa[c], Makoto Shono[a], Fumihito Kugimiya[a], Michihiro Yoshimura[d], Hirofumi Yasue[a]

[a] Division of Cardiovascular Medicine, Kumamoto Aging Research Institute, Kumamoto, Japan
[b] First Department of Internal Medicine, Nara Medical University, Nara, Japan
[c] Division of Cardiovascular Medicine, Minami-nara General Medical Center, Japan
[d] Division of Cardiology, Department of Internal Medicine, Jikei University School of Medicine, Tokyo, Japan

ARTICLE INFO

Article history:
Received 27 May 2017
Accepted 15 August 2017

Keywords:
Cardiac metabolism
Diabetes mellitus
Fatty acids
Glucose
Ketone bodies

ABSTRACT

Background. Diabetic heart is characterized by failure of insulin to increase glucose uptake and increasingly relies on free fatty acids (FFAs) as a source of fuel in animal models. However, it is not well known how cardiac energy metabolism is altered in diabetic hearts in humans. We examined cardiac fuel metabolism in the diabetics as compared to non-diabetics who underwent cardiac catheterization for heart diseases.

Material and Methods. The study subjects comprised 81 patients (male 55, female 26, average age 63.0 ± 10.0 years) who underwent the cardiac catheterization for heart diseases. Thirty-six patients were diagnosed as diabetics (diabetic group) and 45 as non-diabetics (non-diabetic group). Blood samplings were done in both the aortic root (Ao) and coronary sinus (CS) simultaneously and the plasma levels of FFAs, glucose, lactate, pyruvate, total ketone bodies and β-hydroxybutyrate were measured and compared between the two groups.

Results. The myocardial uptake of glucose, lactate and pyruvate were decreased, whereas those of total ketone bodies, β-hydroxybutyrate and acetoacetate were increased in the diabetics as compared to the non-diabetics. However, the myocardial uptakes of FFAs were not significantly increased in the diabetics as compared to the non-diabetics.

Conclusions. Cardiac uptakes of carbohydrate (glucose, lactate and pyruvate) were decreased, whereas those of total ketone bodies and β-hydroxybutyrate were increased in the diabetics as compared to the non-diabetics in humans. Ketone bodies therefore are utilized as an energy source partially replacing glucose in the human diabetic heart.

1. Introduction

The heart has a very high energy demand and has essentially no energy reserves and must therefore continuously produce large amounts of energy or adenosine triphosphate (ATP) at a high rate to sustain contractile function and ionic homeostasis [1–3]. Most of ATP is produced in mitochondria through oxidative phosphorylation and fatty acids and carbohydrates are the primary energy substrates, with fatty acids accounting for 50–75% of ATP production [1–3]. Diabetic hearts however

* *Corresponding author at:* Division of Cardiovascular Medicine, Kumamoto Aging Research Institute, 6-8-1, Yamamuro, Kita-ku, Kumamoto 860-8518, Japan.
E-mail address: mizuno@juryo.or.jp (Y. Mizuno).

https://doi.org/10.1016/j.metabol.2017.08.005

cannot use glucose fully due to shortage of insulin effects and may therefore be forced to switch to almost exclusive use of fatty acids for energy sources [4–6]. Fatty acids however consume more oxygen per mole than glucose and increase thereby the oxidative stress in the cardiac tissue and decrease cardiac efficiency [7–9]. It is not well known however how cardiac fuel metabolism is altered in diabetic hearts in humans. In present study, we examined cardiac fuel metabolism in the diabetic patients as compared to the non-diabetic patients who underwent cardiac catheterization for heart diseases, by assessing cardiac uptake of myocardial fuel substrates including fatty acids, glucose, and ketone bodies.

2. Material and Methods

2.1. Study Subjects

The study subject consisted of 36 patients with type 2 diabetes mellitus (T2DM) (28 men and 8 women, average age 64.2 ± 9.3 years) and 45 patients without DM (27 men and18 women, average age 62.0 ± 10.5 years) who underwent cardiac catheterization from June 2010 to December 2016 at our institution. They included 27 patients with old myocardial infarction, 17 patients with stable angina and 40 patients with chest pain syndrome (Table 1). Patients with acute myocardial infarction, acute infection, overt heart failure (NYHA class II–IV), insulin dependent and/or uncontrolled diabetes mellitus, chronic kidney disease, and other severe illnesses were excluded. Diabetic medications and vasodilators were withheld for >24 h before cardiac catheterization. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). This study was approved by the ethical committee of our institution and written informed consent was obtained from each subject.

2.2. Cardiac Catheterization

Cardiac catheterization was performed in the morning with patients in a fasting state. The method for blood sampling from the coronary sinus (CS) and the aortic root (AO) was previously reported [10,11]. Briefly, a 6F Goodale-Lubin catheter was placed in the CS through intra-jugular vein or femoral vein after the right heart catheterization was completed. A Judkins catheter was placed at the root of the aorta by way of the brachial artery. Blood sampling for FFA, glucose, lactate, pyruvate, ketone bodies and BNP as well as blood gas were then done from the CS and AO simultaneously. Following this, pressure studies, coronary angiography and left ventriculography were done and left ventricular ejection

Table 1 – Comparison of the clinical characteristics between the diabetes mellitus (DM) group and non-DM group.

Variables	DM (n = 36)	Non-DM (n = 45)	P-value
Age (years)	64.2 ± 9.3	62.0 ± 10.5	0.334
Sex (male/female)	28/8	27/18	0.089
Body mass index (kg/m^2)	24.9 ± 3.1	24.3 ± 4.0	0.475
Systolic BP (mm Hg)	156.0 ± 35.3	147.9 ± 27.4	0.261
Diastolic BP (mm Hg)	82.6 ± 16.8	79.2 ± 12.7	0.314
Heart rate (beats/min)	68.8 ± 16.1	66.2 ± 12.9	0.448
Albumin (g/L)	41 (38, 43)	41 (38, 42)	0.690
Total cholesterol (mmol/L)	5.10 ± 1.14	5.16 ± 1.24	0.846
HDL cholesterol (mmol/L)	1.41 ± 0.79	1.37 ± 0.50	0.783
LDL cholesterol (mmol/L)	2.66 ± 0.78	3.01 ± 0.98	0.100
Triglyceride (mmol/L)	2.25 ± 1.72	1.76 ± 1.12	0.132
Fasting glucose (mmol/L)	8.55 (6.05, 11.1)	5.83 (5.33, 6.83)	<0.001
HbA1c (%)	7.2 (6.5, 8.5)	5.7 (5.5, 5.9)	<0.001
Insulin (pmol/L)	37.3 (18.7, 70.3)	34.4 (19.4, 46.6)	0.520
HOMA-IR	1.91 (0.77, 3.29)	1.22 (0.64, 1.69)	0.052
CRP (μg/L)	700 (400, 3300)	1200 (500, 2400)	0.761
Leucocytes (/μL)	6550 (5800, 10,600)	6600 (4900, 8300)	0.274
Hemoglobin (g/L)	139 (124, 149)	137 (130, 149)	0.354
Platelets (10^4/μL)	18.2 (15.5, 25.3)	21.3 (17.3, 26.5)	0.020
eGFR (mL/min/1.73 m^2)	63.6 (54.3, 78.0)	72.6 (54.1, 86.5)	0.761
BNP (AO) (pg/mL)	28.7 (15.8, 128.4)	32.0 (14.9, 104.2)	0.814
Base excess (AO) (mmol/L)	−0.4 (−2.1, 0.7)	0.0 (−1.4, 0.9)	0.497
Old myocardial infarction (n, %)	15 (41.7)	12 (26.7)	0.155
Angina pectoris (n, %)	17 (47.2)	23 (51.1)	0.728
Chest pain syndrome (n, %)	4 (11.1)	10 (22.2)	0.189
Hypertension (n, %)	25 (69.4)	27 (60.0)	0.378
Hyperlipidemia (n, %)	16 (44.4)	20 (44.4)	1.000
Smoking (Current) (n, %)	11 (30.6)	17 (37.8)	0.497

AO, aortic root; BP, blood pressure; BNP, Type-B natriuretic peptide; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein.

fraction (LVEF) was determined. The plasma levels of each substrate at AO minus those at CS, or plasma substrate (AO-CS), were regarded as an index of cardiac substrate uptake.

2.3. Echocardiographic Methods

Echocardiography including two-dimensional, pulsed and continuous wave Doppler, color flow Doppler, and tissue Doppler imaging was performed using the iE33 Ultrasound System (Philips Ultrasound Co. Bothell, U.S.A.). LV and atrial linear dimensions were measured from two-dimensional echocardiographic images and LVEF, early diastolic mitral flow velocity (E)/tissue annular motion velocity (e′) E/e′, LV diastolic dimension (LVDd), LV systolic dimension (LVDs), LV mass index (LVMI), left atrial dimension (LAD), interventricular-septal thickness (IVST), posterior-wall thickness (PWT), relative wall thickness (RWT), stroke volume (SV), and EF were measured and calculated according to the recommendations of American Society of Echocardiography and European Association of Echocardiography [12]. E/e′ (septal) was used as a surrogate marker of LV end-diastolic pressure and e′ as that of LV diastolic function. LV mass was estimated using linear measurements from two dimensional images and indexed to body surface area as LVMI. Echocardiography was performed by the experienced sonographers blinded to clinical information. The inter-observer correlation coefficient of the posterior wall thickness measurement was 0.869 (95% confidence interval 0.757 to 0.980) measured in 50 HF patients.

2.4. Hormonal and Biochemical Analysis

The plasma FFA levels were measured using the enzymatic colorimetric method assay (Wako NEFA-HR2, Wako Diagnostics Inc. Richmond, U.S.A.) on an automatic analyzer (BML Inc., Tokyo, Japan) [13]. The minimum detectable level was 0.0014 mEq/l and the mean intra-assay and inter-assay coefficients of variation (CV) were 0.64% and 1.27%, respectively. The plasma levels of lactate and pyruvate were measured by respectively by means of the oxidase system on an auto-analyzer (Hitachi Inc. Tokyo, Japan). Intra and inter CV of plasma levels of lactate and pyruvate were 1.2%, 2.1% and 0.8%, 0.8% respectively [14]. The levels of total ketone bodies and β-hydroxybutyrate (βOHB) were measured by enzyme circling methods produced by Kinos Inc. Tokyo Japan. The CV of plasma levels of these substances were under 3.0% [15]. Acetoacetate was calculated by total ketone bodies minus βOHB. The other biochemical analyses were done using standard laboratory procedures.

2.5. Statistical Analysis

Data were expressed as mean ± SD. However, when the variable was significantly skewed, the median (25th, 75th percentile) was reported. For continuous variables, differences between the groups were evaluated by unpaired Student *t*-test or Man-Whitney rank-sum test. For discrete variables, differences were expressed as counts and percentages and were analyzed with chi-square test between the groups. Two-tailed $P < 0.05$ was defined as statistical significance. Statistical analysis was performed by using commercially available software (STATA 11.0, STATA Corp., College Station, TX, U.S.A.).

3. Results

Table 1 compares the clinical and biochemical data between the diabetes mellitus (DM) group and non-DM group. There were no significant differences in the clinical and laboratory data between the two groups except higher fasting plasma glucose and HbA1c levels and higher HOMA-IR ($p < 0.001$, $p < 0.001$, and $P = 0.052$, respectively) and lower platelet count ($P = 0.020$) in the DM group than Non-DM group. Of the 36 patients in the DM group, none had been on either insulin injection or oral SGLT2 inhibitors before cardiac catheterization. Sixteen patients were not on any of the anti-diabetic drugs, being diagnosed as T2DM after hospitalization for cardiac catheterization. Ten patients were on metformin (4 with pioglitazone and 2 with vildagliptin), 7 on voglibose, 2 on glimepiride, and 1 was on mitiglinide. However, all these drugs had been withdrawn for >24 h before the cardiac catheterization study, the elimination half-life of all these drugs being <6 h. Table 2 compares the echocardiographic and cardiac catheterization data between the two groups. LV septal and posterior wall thickness were significantly higher ($P = 0.014$ and $P = 0.024$, respectively) and LV e′ significantly lower ($P = 0.026$), with LV E/e′ and LV end-diastolic pressure tending to be higher ($P = 0.126$ and $P = 0.114$, respectively) in the DM than non-DM group. However, there were no significant differences in LVEF, LVDd and LVMI between the two groups. These findings implied that diabetic hearts have LV concentric remodeling and diastolic dysfunction as compared to the nondiabetic hearts.

Table 2 – Comparison of echocardiographic and cardiac catheterization data between the DM group and non-DM group.

Variables	DM (n = 36)	Non-DM (n = 45)	P-value
IVST (mm)	12.8 (11.9, 13.2)	11.4 (9.9, 12.7)	0.014
PWT (mm)	11.3 (11.0, 12.7)	10.8 (9.9, 11.8)	0.024
LVDd (mm)	45.5 (42.7, 49.2)	45.1 (42.2, 47.9)	0.632
LVDs (mm)	28.7 (24.3, 31.0)	28.8 (26.4, 31.3)	0.913
LVMI (g/m^2)	115.5 ± 24.3	110.5 ± 24.9	0.437
E/e′	13.2 (10.6, 16.8)	10.5 (9.7, 13.3)	0.126
e′ (cm/s)	4.0 ± 1.5	7.0 ± 1.8	0.026
LV end-diastolic pressure (mm Hg)	18.0 (14.0, 22.0)	15.0 (13.0, 17.9)	0.114
LVEF (%)	65.8 (51.0,75.0)	68.2 (57.0,77.5)	0.404
Pulmonary wedge pressure (mm Hg)	12.1 ± 5.3	11.4 ± 5.5	0.587
Cardiac index (L/min/m^2)	2.64 ± 0.50	2.63 ± 0.58	0.916

E/e′, ratio of early transmittal velocity to tissue Doppler mitral annular early diastolic velocity; e′, tissue Doppler mitral annular early diastolic velocity; LVDd, left ventricular diastolic dimension; LVDs, left ventricular systolic dimension; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; IVST, interventricular septal thickness; PWT, posterior wall thickness.

Table 3 compares the biochemical and blood gas data between the AO and CS in the DM group and non-D group separately. The plasma levels of FFA, glucose, lactate, pyruvate, total ketone bodies, β-OHB and acetoacetate were lower in the CS than in the AO ($P < 0.0001$, $P < 0.0001$, $P < 0.0001$, $P = 0.001$, $P < 0.0001$, $P < 0.0001$, and $P < 0.0001$, respectively) in the non-DM group and ($P < 0.0001$, $P = 0.001$, $P = 0.001$, $P = 0.6573$, $P < 0.0001$, $P < 0.0001$, and $P < 0.0001$, respectively) in the DM group, indicating that those substances were taken up by the heart in both groups except pyruvate not taken up in the DM group. On the other hands, BNP was significantly higher ($P < 0.0001$) in the CS than in the AO in both groups, indicating that BNP is produced by the heart as we and others have reported [12,13]. The levels of pH and PO2 were lower and those of PCO2, HCO3 and base excess were higher in the CS than in the AO ($P < 0.0001$, respectively) in both groups, indicating that oxygen is consumed and CO_2 produced by the heart.

Table 4 compares the plasma levels of the cardiac substrates in the AO and their cardiac uptakes (AO-CS) between the two groups. The plasma levels of FFA and glucose, total ketone bodies, β-OHB and acetoacetate in the AO were significantly higher ($P = 0.042$, $P < 0.001$, $P = 0.007$, $P = 0.002$ and $P = 0.006$, respectively) in the DM group than the non-DM group. The cardiac uptakes of glucose, lactate, and pyruvate were significantly decreased ($P = 0.013$, $P = 0.001$, and $P = 0.006$, respectively), whereas those of total ketone bodies, β-OHB and acetoacetate were significantly increased ($P = 0.013$, $P = 0.012$ and $P = 0.038$, respectively) in the DM group than the non-DM group (Table 4 and Fig. 1). However, there was no significant difference ($P = 0.704$) in the cardiac uptake of FFA between the groups (Table 4 and Fig. 1), implying that FFA uptake is not significantly increased in the diabetic heart in humans. Table 4 also compares the cardiac extraction fraction [(AO-CS)/AO] of substrates between the 2 groups. There were no differences in the extraction fraction of total ketone bodies, β-OHB and acetoacetate as well as of free fatty acids between the 2 groups, indicating that cardiac ketone body uptake increase in proportion to the circulating levels of ketone bodies, whereas those of lactate and pyruvic acid were significantly decreased

Table 3 – Comparison of the plasma levels of cardiac fuel substrates and blood gas parameters between the AO and CS.

a) DM group

Variables	AO (n = 36)	CS (n = 36)	*P*-value
Free fatty acid (mmol/L)	2.99 (1.4, 3.56)	2.65 (1.1, 3.4)	<0.0001
Glucose (mmol/L)	6.99 (5.83, 7.77)	6.94 (5.72, 5.78)	0.001
Insulin (pmol/L)	37.3 (18.7, 70.3)	36.6 (17.2, 63.1)	0.139
Lactate (mmol/L)	0.65 (0.54, 0.81)	0.49 (0.37, 0.75)	0.001
Pyruvate (μmol/L)	45.4 (34.1, 56.8)	45.4 (22.7, 56.8)	0.657
Total ketone (μmol/L)	369 (188, 444)	216 (105, 263)	<0.0001
β-Hydroxybutyrate (μmol/L)	251 (147, 376)	164 (62, 248)	<0.0001
Acetoacetate (μmol/L)	86 (4, 136)	41 (5,59)	<0.0001
BNP (pg/ml)	41.6 (15.8, 143)	124 (41, 381)	<0.0001
PH	7.40 ± 0.04	7.37 ± 0.03	<0.0001
PO2 (mm Hg)	99.0 ± 27.7	21.6 ± 3.1	<0.0001
PCO2 (mm Hg)	39.5 ± 3.1	50.1 ± 3.8	<0.0001
HCO3- (mmol/L)	24.1 ± 2.5	28.2 ± 2.4	<0.0001
Base excess (mmol/L)	−0.1 (−2.1, 0.9)	1.9 (0.4, 3.2)	<0.0001

b) Non-DM group

Variables	AO (n = 45)	CS (n = 45)	*P*-value
Free fatty acid (mmol/L)	1.9 (0.9, 2.8)	1.7 (0.8, 2.6)	<0.0001
Glucose (mmol/L)	5.33 (5.11.1 5.88)	5.16 (4.83, 5.72)	<0.0001
Insulin (pmol/L)	34.4 (19.4, 46.6)	34.1 (21.5, 51.7)	0.697
Lactate (mmol/L)	0.69 (0.47, 0.82)	0.40 (0.29, 0.47)	<0.0001
Pyruvate (μmol/L)	45.4 (45.5, 90.9)	22.7 (22.7, 45.4)	0.001
Total ketone (μmol/L)	160 (67,302)	82 (45,183)	<0.0001
β-Hydroxybutyrate (μmol/L)	116 (55, 224)	75 (35, 133)	<0.0001
Acetoacetate (μmol/L)	35 (9, 54)	18 (5,31)	<0.0001
BNP (pg/ml)	30 (15, 92)	85.4 (35, 232)	<0.0001
PH	7.41 ± 0.04	7.37 ± 0.03	<0.0001
PO2 (mmHg)	93.8 ± 21.4	21.1 ± 3.5	<0.0001
PCO2 (mmHg)	38.9 ± 4.5	48.8 ± 5.7	<0.0001
HCO3- (mmol/L)	24.2 ± 2.1	27.8 ± 2.8	<0.0001
Base excess (mmol/L)	0.0 (−1.1.1)	1.7 (0.7, 3)	<0.0001

AO, aortic root; CS, coronary sinus; BNP, B type natriuretic peptide.

Table 4 – Comparison of cardiac uptake substances between diabetes mellitus (DM) Group and non-DM group.

Variables	DM (n = 36)	Non-DM (n = 45)	P-value
Cardiac uptake of substrates (AO-CS)			
Free fatty acids (μmol/L)	180 (100, 295)	216 (100, 320)	0.704
Glucose (μmol/L)	55.5 (0, 166.5)	111 (55.1, 222)	0.013
Lactate (μmol/L)	133 (22, 189)	244 (178, 377)	0.001
Pyruvate (μmol/L)	0.0 (0.0, 11.4)	22.7 (11.4, 45.4)	0.006
Total ketone (μmol/L)	123 (70, 217)	69 (29, 115)	0.013
β-Hydroxybutyrate (μmol/L)	91 (56, 142)	52 (20, 86)	0.012
Acetoacetate (μmol/L)	40 (3, 70)	16 (4, 31)	0.038
PO2 (mm Hg)	77.4 ± 26.7	72.6 ± 21.0	0.374
Extraction fraction rate = (AO-CS) × 100/AO			
Free fatty acids (%)	9.7 (3.7, 16.4)	10.5 (5.4, 17.6)	0.335
Glucose (%)	0.7 (−1.0, 2.4)	2.6 (1.1, 4.4)	0.114
Lactate (%)	21.9 (3.7, 34.9)	40.9 (35.0, 47.6)	0.003
Pyruvic acid (%)	0 (0, 25)	50 (12.5, 50)	0.032
Total ketone bodies (%)	40.4 (36.7, 46.6)	42.9 (37.2, 49.5)	0.274
β- Hydroxybutyrate (%)	40.1 (34.0, 44.2)	41.7 (35.9, 45.8)	0.340
Acetoacetate (%)	51.4 (46.2, 64.5)	55.5 (46.5, 61.5)	0.701
PO2 (%)	77.2 ± 5.0	76.4 ± 6.8	0.551

AO, aortic root; CS, coronary sinus.

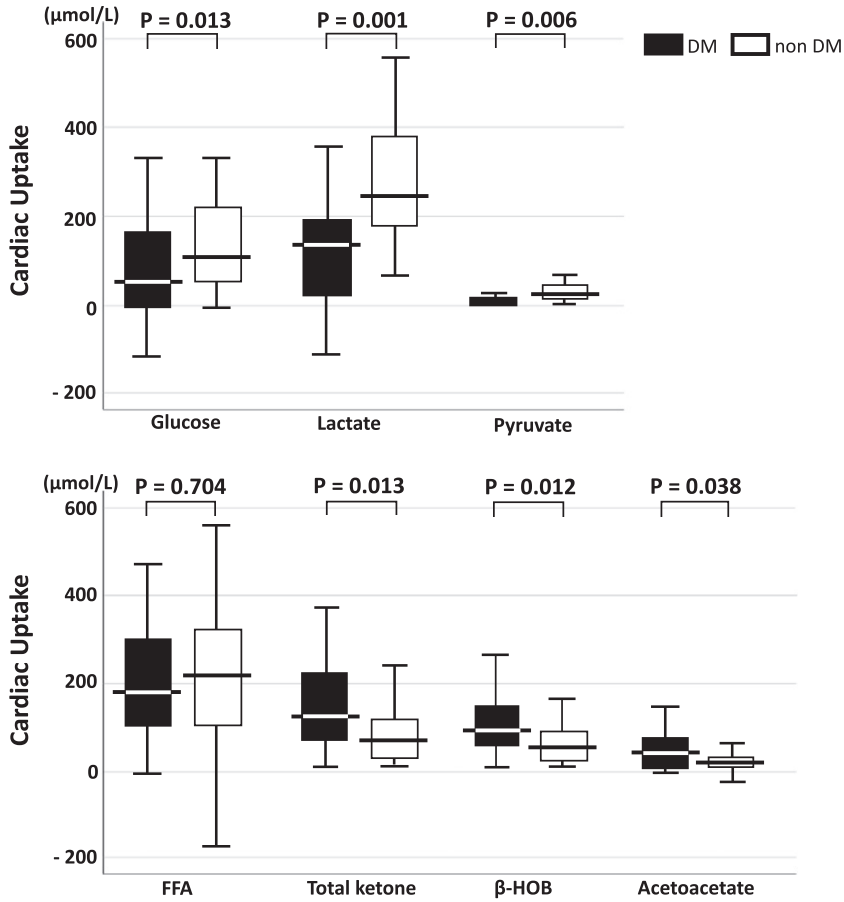

Fig. 1 – Comparison of cardiac uptake of glucose, lactate, pyruvate, FFA, total ketone bodies, and β-hydroxybutyrate (ß-OHB) and acetoacetate between DM patients and non-DM patients.

($P = 0.003$ and $P = 0.032$, respectively) and that of glucose tended to decrease ($P = 0.114$) in the DM group. Cardiac O2 uptake (arterio-venous O2 difference) tended to be elevated in the DM group than the non-DM group ($P = 0.374$) and there was a significant positive correlation between cardiac O2 uptake and plasma glucose levels ($P < 0.001$), implying diabetic heart has cardiac energy inefficiency (Fig. 2).

BNP is produced from the left ventricle (LV) as shown in this study and others and its plasma levels increase in proportion to the severity of the LV dysfunction [10]. There was a significant positive correlation between cardiac uptake of total ketone body and plasma levels of BNP (Ln BNP) in the AO ($P = 0.018$) as shown in Fig. 3, indicating myocardial ketone uptake increases as LV dysfunction deteriorates.

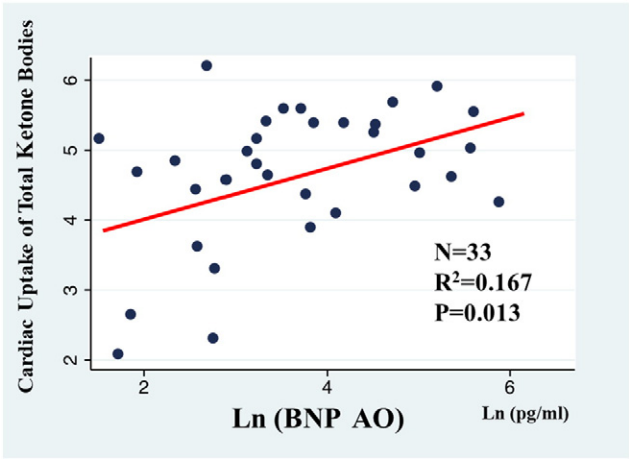

Fig. 3 – Correlation of Ln plasma BNP levels with cardiac total ketone body uptake in the study subjects. There was a significant positive correlation between the Ln plasma BNP levels and cardiac total ketone body uptake.

4. Discussion

In the normal heart, fatty acids and glucose are the major fuels, while lactic acids, ketone bodies and amino acids play minor roles [1–3]. The normal heart has a substantial amount of metabolic flexibility that allows it to switch back and forth between fatty acid and carbohydrate oxidation, depending on the workload of the heart, the energy substrate supply, and hormonal and nutritional states [1–3].

The diabetic heart cannot use the glucose fully due to insulin resistance and may thereby be forced to switch almost exclusively to the fatty acids as a source of energy as demonstrated [4–6]. Diabetic heart has an increased lipid content or cardiac steatosis, which leads to oxidative stress and cardiac dysfunction, as compared to non-diabetic heart [16,17]. However, it is not well known how myocardial substrate oxidation and metabolic flexibility are altered in the diabetic patients.

The present study shows that the plasma levels of FFAs, glucose, total ketone bodies, β-OHB, and acetoacetate were higher at the aortic root and that the myocardial uptakes of glucose, lactate and pyruvate were lower and those of total ketone bodies including β-OHB and acetoacetate were higher in the DM patients than the non-DM patients in the fasting state. There was however no difference in the myocardial uptake of FFAs between the DM and non-DM patients. These findings indicate that diabetic hearts switch partially from carbohydrates (glucose, lactate and pyruvate) to ketone bodies (ß-OHB and acetoacetate) but not to FFAs as energy sources in humans. It is likely that increased cardiac lipids

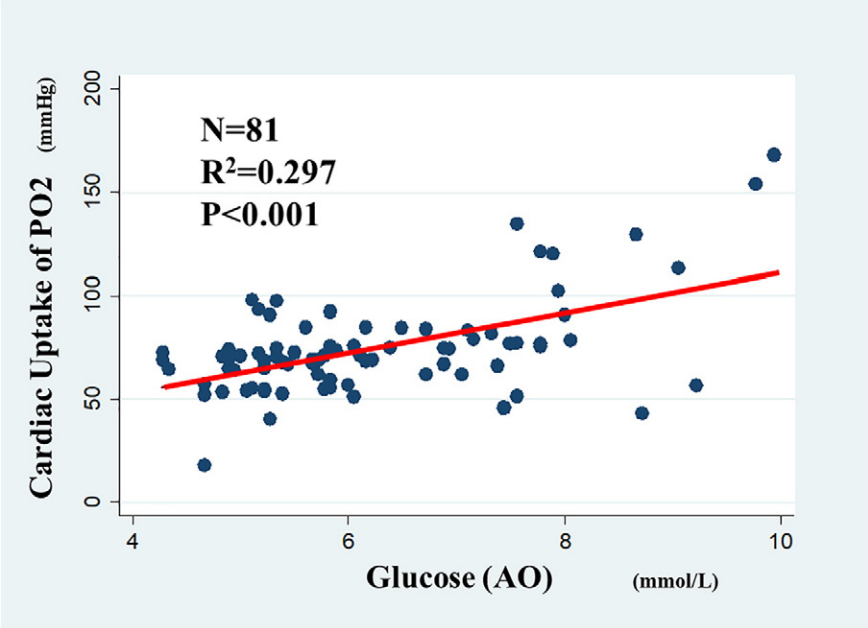

Fig. 2 – Correlation of plasma glucose levels with cardiac oxygen uptake in the study subjects. There was a significant positive correlation between the plasma glucose levels and cardiac oxygen uptake.

with toxic intermediates such as diacylglycerol and ceramide compromise mitochondrial ATP production and increase oxidative stress, which result in suppression of further increase of cardiac FFA uptake even in the presence of impaired glucose oxidation in the established DM in humans [6]. It is also possible that myocardial ischemia in the majority of the study patients may have altered FFA oxidation [1,2]. Furthermore, ketone bodies are avidly taken up by the heart and compete with glucose or FFAs as cardiac energy substrates and suppress FFA uptake [9,18]. Ketone bodies produce ATP more efficiently per molecule of oxygen consumed than glucose or FFAs and thereby increase cardiac efficiency and may thus be a "super fuel" for the heart [9,18–20]. Recent evidence has shown that cardiac ketone oxidation is increased in the failing heart and it is likely that increased ketone oxidation can maintain cardiac energy supply in situation of limited energy production such as DM or heart failure [21–23].

There were no significant differences in the clinical characteristics between the DM and non-DM groups except the increased LV wall thickness and early diastolic dysfunction as indicated by the reduced LV e′ in the group DM (Table 2). These findings imply that the diabetic heart exhibits LV concentric remodeling with diastolic dysfunction and has an increased propensity for heart failure in agreement with previous studies [4–6,17,19]. The present study also shows that cardiac O2 uptake or consumption increases with plasma glucose levels. The present study also revealed that there was a significant positive correlation between the plasma BNP levels and the cardiac uptake of total ketone bodies, indicating that cardiac utilization of ketone bodies increase with deterioration of LV dysfunction [5,8,9,22,23].

4.1. *Clinical Implications*

Ketone bodies are important and efficient fuels of cardiac tissue [7–9,19–25] and increased cardiac uptake of ketone bodies may therefore be an adaptive and compensatory response to the impaired glucose metabolism in the diabetic heart. The control of the energy or hypoxia by selecting cardiac fuel substrates may thus be a novel therapy for DM and heart failure. Indeed, the recent EMPA-REG OUTCOME trial has demonstrated that the administration of a sodium-glucose cotransporter 2 (SGLT2) inhibitor caused an impressive reduction in cardiovascular and all-cause mortality in DM patients at high cardiovascular risk, probably through increased ketone body production and cardiac ketone oxidation as well as hemodynamic effects [9,19,26,27]. Selection of cardiac fuel substances as well as alleviation of insulin resistance, hypoxia, shortage of energy may thus be a novel target for DM and heart failure [28].

4.2. *Study Limitations*

This study was cross-sectional and could not establish the causal relation between cardiac ketone body uptake and DM. The number of the study subjects was small and limited to those who underwent the cardiac catheterization for heart disease and in whom blood sampling could be obtained at both the aortic root and coronary sinus. However, the simultaneous sampling blood at the aorta and coronary sinus enabled to assess cardiac uptakes of energy substrates that are not possible by sampling peripheral venous blood or urine.

5. Conclusions

The cardiac uptakes of glucose, lactate and pyruvate were decreased, whereas those of ketone bodies (ß-OHB and acetoacetate) increased in the diabetic patients as compared to the non-diabetic patients. However, the cardiac uptake of FFAs did not differ between the DM and non-DM patients. Ketone bodies, which are more energy-efficient than glucose or FFAs, are therefore utilized as energy sources partially replacing glucose in human DM heart. Diabetic heart exhibited LV concentric remodeling and diastolic dysfunction and thereby had an increased propensity for heart failure. Ketone bodies are therefore an important and efficient cardiac fuel and may thereby be a novel target for the treatment and prevention of DM and heart failure.

Author Contributions

Conceived and designed the experiments: YM, EH, HY.
Performed the experiments: YM, EH, HN, YM, MS.
Analyzed the data: YM, EH, FK, HY.
Contributed reagents/materials/analyzing tools: YM, EH, MY.
Wrote the paper: YM, HY.

Funding Sources

This study was supported in part by grants from the Japan Vascular Disease Research Foundation, Kyoto, Japan and the Japan Heart Foundation, Tokyo, Japan.

Disclosures

None.

Acknowledgements

We wish to thank many medical staffs at our laboratory and cardiac echo-laboratory and Mrs. Akiko Oda for collecting data as a secretary.

REFERENCES

[1] Stanley WC, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. Physiol Rev 2005; 85(3):1093–129.
[2] Lopaschuk GD, Ussher JR, Folmes CD, Jaswal JS, Stanley WC. Myocardial fatty acid metabolism in health and disease. Physiol Rev 2010;90(1):207–58.
[3] Taegtmeyer H, Young ME, Lopaschuk GD, Abel ED, Brunengraber H, Darley-Usmar V, et al. American heart association council on basic cardiovascular sciences. Assessing

cardiac metabolism: a scientific statement from the american heart association. Circ Res 2016;118(10):1659–701.
[4] Boudina S, Abel ED. Diabetic cardiomyopathy revisited. Circulation 2007;115(25):3213–23.
[5] Fillmore N, Mori J, Lopaschuk GD. Mitochondrial fatty acid oxidation alterations in heart failure, ischaemic heart disease and diabetic cardiomyopathy. Br J Pharmacol 2014;171(8):2080–90.
[6] Chong CR, Clarke K, Levelt E. Metabolic remodeling in diabetic cardiomyopathy. Cardiovasc Res 2017;113:422–30.
[7] Cahill Jr GF. Fuel metabolism in starvation. Annu Rev Nutr 2006;26:1–22.
[8] Janardhan A, Chen J, Crawford PA. Altered systemic ketone body metabolism in advanced heart failure. Tex Heart Inst J 2011;38(5):533–8.
[9] Ferrannini E, Mark M, Mayoux E. CV protection in the EMPA-REG OUTCOME trial: a "Thrifty Substrate" hypothesis. Diabetes Care 2016;39(7):1108–14.
[10] Yasue H, Yoshimura M, Sumida H, Kikuta K, Kugiyama K, Jougasaki M, et al. Localization and mechanism of secretion of B-type natriuretic peptide in comparison with those of A-type natriuretic peptide in normal subjects and patients with heart failure. Circulation 1994;90:195–203.
[11] Mizuno Y, Yoshimura M, Yasue H, Sakamoto T, Ogawa H, Kugiyama K, et al. Aldosterone production is activated in failing ventricle in humans. Circulation 2001;103:2–7.
[12] Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005;18:1440–63.
[13] Shimizu S, Tani Y, Yamada H, Tabata M, Murachi T. Enzymatic determination of serum-free fatty acids: a colorimetric method. Anal Biochem 1980;107:193–8.
[14] Asanuma K, Ariga T, Aida N, Miyasaka A, Nagai Y, Miyagawa T, et al. A new method for simultaneous autoanalysis of plasma levels of lactic acid and pyruvic acid by means of the oxidase system [Jpn]. J Anal Bio-Sci 1985;8:16–24.
[15] Hidaka H. Biochemical tests, organic acids, ketone body and demarcation. Nihon Rinsho (Jpn) 2004;62:676–8.
[16] McGavock JM, Lingvay I, Zib I, Tillery T, Salas N, Unger R, et al. Cardiac steatosis in diabetes mellitus: a 1H-magnetic resonance spectroscopy study. Circulation 2007;116(10):1170–5.
[17] Levelt E, Mahmod M, Piechnik SK, Ariga R, Francis JM, Rodgers CT, et al. Relationship between left ventricular structural and metabolic remodeling in type 2 diabetes. Diabetes 2016;65(1):44–52.
[18] Stanley WC, Meadows SR, Kivilo KM, Roth BA, Lopaschuk GD. β-Hydroxybutyrate inhibits myocardial fatty acid oxidation in vivo independent of changes in malonyl-CoA content. Am J Physiol Heart Circ Physiol 2003;285:H1626–31.
[19] Mudaliar S, Alloju S, Henry RR. Can a shift in fuel energetics explain the beneficial cardiorenal outcomes in the EMPA-REG OUTCOME study? A unifying hypothesis. Diabetes Care 2016;39(7):1115–22.
[20] Cahill Jr GF, Veech RL. Ketoacids? Good medicine? Trans Am Clin Climatol Assoc 2003;114:149–61.
[21] Neubauer S. The failing heart—an engine out of fuel. N Engl J Med 2007;356(11):1140–51.
[22] Aubert G, Martin OJ, Horton JL, Lai L, Vega RB, Leone TC, et al. The failing heart relies on ketone bodies as a fuel. Circulation 2016;133(8):698–705.
[23] Bedi Jr KC, Snyder NW, Brandimarto J, Aziz M, Mesaros C, Worth AJ, et al. Evidence for intramyocardial disruption of lipid metabolism and increased myocardial ketone utilization in advanced human heart failure. Circulation 2016;133(8):706–16.
[24] Shimazu T, Hirschey MD, Newman J, He W, Shirakawa K, Le Moan N, et al. Suppression of oxidative stress by β-hydroxybutyrate, an endogenous histone deacetylase inhibitor. Science 2013;339(6116):211–4.
[25] Cotter DG, Schugar RC, Crawford PA. Ketone body metabolism and cardiovascular disease. Am J Physiol Heart Circ Physiol 2013;304:H1060-6.
[26] Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. EMPA-REG OUTCOME investigators. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med 2015;373(22):2117–28.
[27] Heerspink HJ, Perkins BA, Fitchett DH, Husain M, Cherney DZ. Sodium glucose cotransporter 2 inhibitors in the treatment of diabetes mellitus: cardiovascular and kidney effects, potential mechanisms, and clinical applications. Circulation 2016;134(10):752–7.
[28] Kolwicz Jr SC, Airhart S, Tian R. Ketones step to the plate: a game changer for metabolic remodeling in heart failure? Circulation 2016;133(8):689–91.