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Association of Circulating Ketone Bodies With Functional Outcomes After ST-Segment Elevation Myocardial Infarction

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ABSTRACT

BACKGROUND Circulating ketone bodies (KBs) are increased in patients with heart failure (HF), corresponding with increased cardiac KB metabolism and HF severity. However, the role of circulating KBs in ischemia/reperfusion remains unknown.

OBJECTIVES This study sought to investigate longitudinal changes of KBs and their associations with functional outcomes in patients presenting with ST-segment elevation myocardial infarction (STEMI).

METHODS KBs were measured in 369 participants from a randomized trial on early metformin therapy after STEMI. Nonfasting plasma concentrations of KBs (β-hydroxybutyrate, acetoacetate, and acetone) were measured by nuclear magnetic resonance spectroscopy at presentation, at 24 hours, and after 4 months. Myocardial infarct size and left ventricular ejection fraction (LVEF) were determined by cardiac magnetic resonance imaging at 4 months. Associations of circulating KBs with infarct size and LVEF were determined using multivariable linear regression analyses.

RESULTS Circulating KBs were high at presentation with STEMI (median total KBs: 520 μmol/L; interquartile range [IQR]: 315-997 μmol/L). At 24 hours after reperfusion, KBs were still high compared with levels at 4-month follow-up (206 μmol/L [IQR: 174-246] vs 166 μmol/L [IQR: 143-201], respectively; $P < 0.001$). Increased KB concentrations at 24 hours were independently associated with larger myocardial infarct size (total KBs, per 100 μmol/L: $\beta = 1.56$; 95% confidence interval: 0.29-2.83; $P = 0.016$) and lower LVEF ($\beta = -1.78$; 95% CI: (-3.17 to -0.39; $P = 0.012$).

CONCLUSIONS Circulating KBs are increased in patients presenting with STEMI. Higher KBs at 24 hours are associated with functional outcomes after STEMI, which suggests a potential role for ketone metabolism in response to myocardial ischemia. (Metabolic Modulation With Metformin to Reduce Heart Failure After Acute Myocardial Infarction: Glycometabolic Intervention as Adjunct to Primary Coronary Intervention in ST Elevation Myocardial Infarction (GIPS-III): a Randomized Controlled Trial; NCT01217307) (J Am Coll Cardiol 2021;78:1421-1432) © 2021 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Despite decreasing mortality and morbidity rates over the last decades, myocardial infarction (MI) continues to be a major risk factor for the development of heart failure (HF) (1). Myocardial metabolism in HF has been found to rely on ketone bodies (KBs) as a major cellular energy source, with corresponding upregulation of enzymes involved in KB oxidation and increased

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ABBREVIATIONS AND ACRONYMS

AcAc = acetoacetate

CMR = cardiac magnetic resonance imaging

HbA_{1c} = glycosylated hemoglobin

HF = heart failure

I/R = ischemia/reperfusion

KB = ketone body

LVEF = left ventricular ejection fraction

MI = myocardial infarction

NT-proBNP = N-terminal pro-B-type natriuretic peptide

OHB = hydroxybutyrate

PCI = percutaneous coronary intervention

STEMI = ST-segment elevation myocardial infarction

circulating KB levels (2-4). The upregulation of plasma KBs in HF is considered a consequence of increased hepatic ketogenesis caused by the upregulation of neurohormonal factors such as catecholamines and natriuretic peptides. Both catecholamines and natriuretic peptides stimulate adipocyte lipolysis and promote the release of nonesterified fatty acids, which are important metabolic precursors for ketogenesis (5).

The shift toward KB metabolism is considered adaptive and has been linked to reduced oxidative stress and hemodynamic preservation (6,7). Mice incapable of oxidizing KBs in cardiomyocytes display markedly accelerated HF development in response to ischemia/reperfusion (I/R) and pressure overload (3). KBs are a more efficient source of adenosine triphosphate production compared with glucose and fatty acids because these require more oxygen per molecule adenosine triphosphate produced (8). This could also be important for the replenishment of myocardial adenosine triphosphate in the setting of I/R (8,9). However, little is known about possible changes in KB metabolism and circulating KB levels in humans presenting with acute MI and undergoing percutaneous coronary intervention (PCI). Therefore, our study addressed several objectives. First, we investigated longitudinal changes in circulating KB concentrations after ST-segment elevation myocardial infarction (STEMI). Second, we studied whether plasma KB concentrations were associated with myocardial infarct size and left ventricular ejection fraction (LVEF) at 4 months.

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METHODS

STUDY POPULATION. We measured KBs in archived plasma samples of the GIPS-III (Metabolic modulation with metformin to reduce heart failure after acute myocardial infarction: Glycometabolic Intervention in Adjunct to Primary Percutaneous Coronary Intervention in ST-Segment Elevation Myocardial Infarction; NCT01217307) randomized controlled trial. GIPS-III was designed to assess the effect of 4-month metformin treatment compared with placebo on left ventricular function in patients without diabetes who presented with STEMI. Design and outcomes of this trial were published previously (10,11). Briefly, all patients admitted to the University Medical Center Groningen between January 2011 and May 2013, via the STEMI protocol, were considered eligible for the trial. Inclusion criteria were age older than 18 years, presence of STEMI, and primary PCI with implantation of at least 1 stent with a diameter of at least 3 mm that resulted in Thrombolysis In Myocardial Infarction flow grade 2 or 3 post-PCI. Major exclusion criteria were previous MI, known diabetes, the need for coronary artery bypass graft surgery, severe renal dysfunction, and standard contraindications for cardiac magnetic resonance imaging (CMR). The study protocol of the GIPS-III trial was in accordance with the Declaration of Helsinki and was approved by the local ethics committee (Groningen, the Netherlands) and national regulatory authorities. All patients provided written informed consent.

CHARACTERISTICS DURING HOSPITALIZATION. On admission, standard laboratory assessment and physical examination parameters were measured according to protocol. During hospitalization, blood was sampled at admission (before PCI) and at 3, 6, 9, 12, and 24 hours after PCI to monitor values of cardiac enzymes.

DETECTION OF KBs. Nonfasting blood samples for metabolic profiling were obtained on admission (n = 369), at 24 hours (n = 338), and at 4 months post-PCI (n = 317). EDTA-anticoagulated plasma samples were stored at −80 °C until analyzed. The 3 main KBs, β-hydroxybutyrate (β-OHB), acetoacetate (AcAc), and acetone, were quantified using a Vantera Clinical Analyzer (Labcorp), a fully automated, high-throughput, 400-MHz proton (^{1}H) nuclear magnetic resonance spectroscopy platform. Plasma samples were prepared on board the instrument, and automatically delivered to the flow probe in the nuclear magnetic resonance spectrometer's magnetic field. Data acquisition on the Vantera and spectra data processing were described in greater detail previously (12). Total KBs were defined as the sum of β-OHB, AcAc, and acetone.

CMR. Infarct size and LVEF were measured with CMR at 4-month follow-up. Details on imaging acquisition and analysis were reported elsewhere (10,11). An independent core laboratory (Image Analysis Center, VU University Medical Center, Amsterdam, the Netherlands) evaluated the CMR scans and assessed the primary efficacy measure, blinded for treatment allocation and clinical patient data.

STATISTICAL ANALYSIS. Normally distributed data were presented as mean ± SD. Skewed data were presented as median (interquartile range [IQR]).

TABLE 1 Characteristics at Admission for STEMI (N = 369)

Age at admission, y	58.8 ± 11.6
Women	93 (25.2)
Ethnicity	
Caucasian	355 (96.2)
Asian	10 (2.7)
Black	4 (1.1)
Cardiovascular-related history	
Hypertension	108 (29.3)
Dyslipidemia	231 (62.6)
Current smoking	204 (55.3)
Cerebrovascular accident	3 (0.8)
Peripheral artery disease	0
Previous PCI	4 (1.1)
Medication use at baseline	
β-blocker	38 (10.3)
ACE inhibitor/angiotensin receptor blocker	38 (10.3)
Diuretics	35 (9.5)
Statins	29 (7.9)
Clinical parameters	
Body mass index, kg/m^2	26.9 ± 3.8
Systolic blood pressure, mm Hg	134 ± 23
Diastolic blood pressure, mm Hg	84 ± 15
Heart rate, beats/min	75 ± 16
PCI parameters	
Total ischemic time (min)	174 (118-255)
Single vessel disease	254 (68.8)
Anterior myocardial infarction[a]	144 (39.0)
TIMI flow grade pre-PCI	
0	203 (55.0)
1	27 (7.3)
2	64 (17.3)
3	75 (20.3)
Stent diameter	
<3.5 mm	143 (38.9)
≥3.5 mm	224 (61.1)
TIMI flow grade post-PCI	
2	34 (9.2)
3	335 (90.8)
Myocardial blush grade	
0	10 (2.7)
1	29 (7.9)
2	72 (19.7)
3	255 (69.7)
Laboratory parameters	
CK, U/L	132 (85-213)
CK-MB, U/L	16 (13-25)
Creatinine, μmol/L	72 (62-82)
NT-proBNP, ng/L	81 (40-200)
Glucose, mmol/L	8.3 (7.0-9.6)
HbA_{1c}, %	5.8 (5.6-6.0)
Ketone bodies, μmol/L	
Total ketone bodies	520 (315-997)
β-hydroxybutyrate	369 (227-712)
Acetoacetate	109 (54-216)
Acetone	38 (20-71)

Values are mean ± SD, n (%), or median (interquartile range). [a]Defined as culprit in left anterior descending artery.

ACE = angiotensin-converting enzyme; CK = creatine kinase; CK-MB = creatine kinase myocardial band; HbA_{1c} = glycosylated hemoglobin; NT-proBNP = N-terminal pro-B-type natriuretic peptide; PCI = percutaneous coronary intervention; STEMI = ST-segment elevation myocardial infarction; TIMI = Thrombolysis In Myocardial Infarction.

Continued in the next column

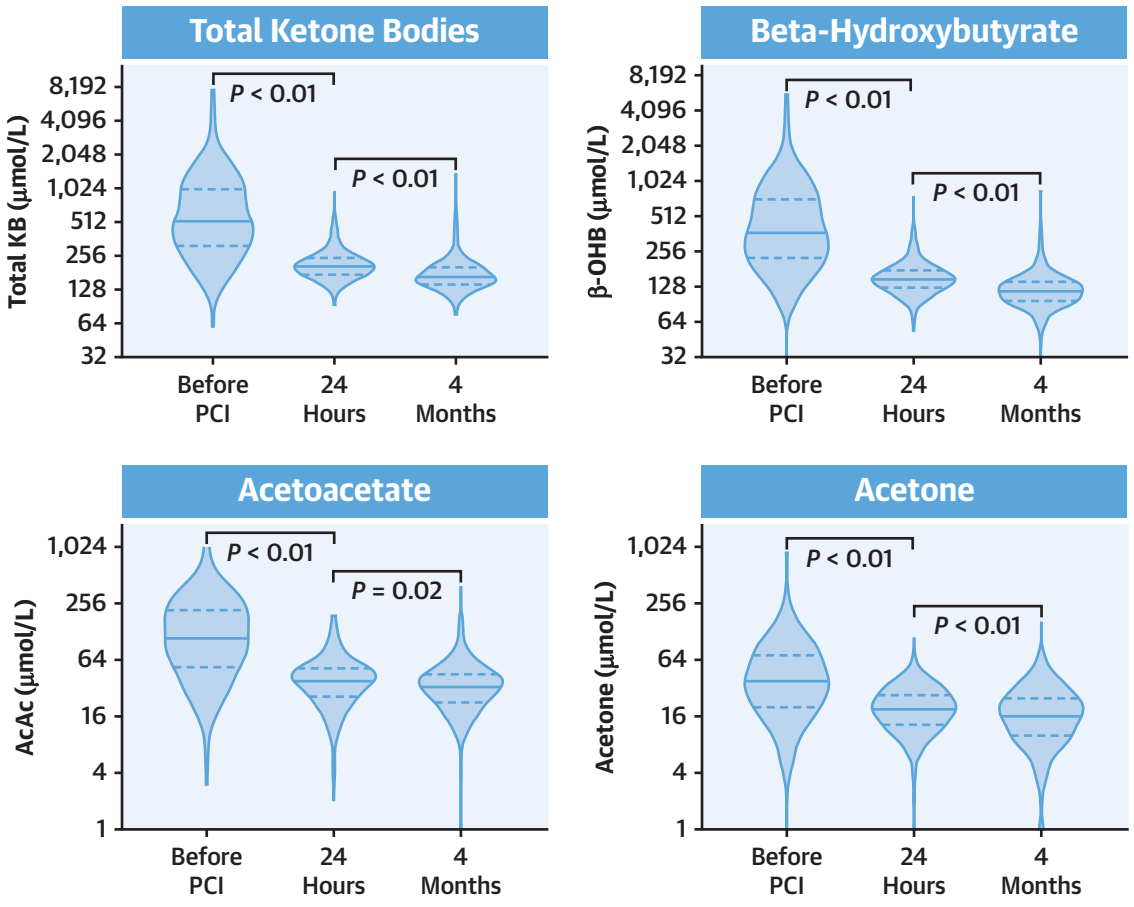
Discrete variables were presented as frequencies and percentages. To compare groups, Student's *t*-tests were used for normally distributed continuous variables, Mann-Whitney *U* tests for skewed continuous variables, and chi-square and Fisher exact tests were used for categorical variables. The Jonckheere-Terpstra test was used to test for a trend in functional outcomes over the KB tertiles. Predictors of KB levels at baseline and 24 hours post-PCI were assessed using age- and sex-adjusted and multivariable regression analyses. Variables with a *P* value <0.10 in age- and sex-adjusted analyses were considered for multivariable analysis. Multivariable models were composed using backward and forward likelihood regression analysis (unless otherwise stated, an identical selection of covariates was chosen by the forward and backward model). Age, sex, and treatment allocation were forced into the multivariable models, and models were checked for absence of collinearity. Subsequently, associations of KBs with infarct size and LVEF were investigated with regression, while correcting for relevant baseline parameters (13). In addition, regression analyses of KBs on log-transformed N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels at baseline, peak NT-proBNP during hospitalization, and NT-proBNP at 4-month follow-up were performed. A 2-tailed *P* value of <0.05 was considered statistically significant. Statistical analyses were performed with STATA software version 15.0 (Stata Corp.). Graphs were drawn in STATA and GraphPad Prism 7.2 and 8.4.2 (GraphPad).

RESULTS

BASELINE CHARACTERISTICS. KBs were measured in 369 patients who presented with STEMI and who participated in the GIPS-III trial. Baseline characteristics of the study population are presented in **Table 1**. Mean age was 59 ± 12 years, and 25% of patients were women.

Total circulating KB concentrations at admission for STEMI amounted to a median of 520 μmol/L (IQR: 315-997 μmol/L) and were positively associated with glycosylated hemoglobin (HbA_{1c}), high-density lipoprotein cholesterol, and lower Thrombolysis In Myocardial Infarction flow pre-PCI (standardized [std] β = 0.25; 95% CI: 0.15-0.36, *P* < 0.001; std

FIGURE 1 **KB Concentrations After ST-Segment Elevation Myocardial Infarction**



Violin plots of circulating ketone body (KB) concentrations at presentation with ST-segment elevation myocardial infarction (STEMI) and at 24 hours and 4 months after reperfusion, showing median **(solid line)** and interquartile ranges **(dashed line)** on a log2 scale. Total KBs and all individual KBs were significantly higher at presentation than at 24-hour follow-up and were higher at 24 hours compared with 4-month follow-up, which suggest that KBs were elevated for a longer period after STEMI, possibly to fuel the heart in need. AcAc = acetoacetate; β-OHB = β-hydroxybutyrate; PCI = percutaneous coronary intervention.

β = 0.22; 95% CI: 0.13-0.32, $P < 0.001$; Thrombolysis In Myocardial Infarction grade 0/1 vs grade 2/3; std β = 0.15; 95% CI: 0.06-0.25; $P = 0.001$, respectively) (Supplemental Table 1). KBs were not associated with ischemic time, culprit location, and the clock time of presentation (which could have been related to overnight fasting that might have occurred in patients who presented in the early morning).

KBs 24 HOURS POST-PCI. All individual KBs and total KB concentrations measured at 24 hours after reperfusion were higher than those at 4-month follow-up (total KBs: 206 μmol/L [IQR: 174-246] vs 166 μmol/L [IQR: 143-201]; $P < 0.001$) (**Figure 1**). Predictors of higher total KBs after 24 hours were larger enzymatic infarct size (std β = 0.16; 95% CI: 0.05-0.27; $P = 0.004$), higher HbA_{1c} (std β = 0.21; 95% CI: 0.07-0.35; $P = 0.004$), and β-blocker use at admission (std β = 0.14; 95% CI: 0.03-0.25; $P = 0.011$) (**Table 2**). Similar associations were observed for the predominant circulating KB, β-OHB (Supplemental Table 2).

KBs AND OUTCOMES. At 4-month follow-up, mean CMR determined infarct size and LVEF were 9.1 ± 7.9% and 53.9 ± 8.5%, respectively. KBs at baseline were not associated with infarct size or LVEF (**Table 3 and Table 4**). At 24 hours after reperfusion, higher concentrations of total KBs and β-OHB were associated with larger myocardial infarct size at 4 months (β = 1.56; 95% CI: 0.29-2.83; $P = 0.016$ per 100 μmol/L increase of total KBs, and β = 2.45; 95% CI: 0.65-4.25; $P = 0.008$ for β-OHB) (**Table 3, Figure 2**). Moreover,

TABLE 2 Age- and Sex-Adjusted and Multivariable Linear Regression Analyses on Plasma Ketone Bodies at 24 Hours

	Age- and Sex-Adjusted			Multivariable		
	Standardized β	95% CI	*P* Value	Standardized β	95% CI	*P* Value
Age, y	0.03	−0.08 to 0.13	0.62	−0.01	−0.12 to 0.10	0.87
Women	0.05	−0.06 to 0.15	0.40	0.05	−0.06 to 0.15	0.40
Metformin treatment	0.03	−0.08 to 0.13	0.62	0.01	−0.10 to 0.11	0.87
PCI parameter						
TIMI flow grade post-PCI 2 vs 3	0.12	0.02 to 0.22	**0.025**			
Myocardial blush grade, 0/1 vs 2/3	0.11	−0.001 to 0.21	0.052			
Proximal culprit[a]	0.10	0.00 to 0.21	0.06			
Medication use						
β-blocker use at baseline	0.16	0.05 to 0.27	**0.003**	0.14	0.03 to 0.25	**0.011**
Statin use at baseline	0.14	0.03 to 0.24	**0.013**			
Metabolic parameter						
HbA_{1C}, %	0.18	0.07 to 0.29	**0.001**	0.21	0.07 to 0.35	**0.004**
Enzymatic infarct size						
Peak CK-MB, U/L	0.10	−0.01 to 0.21	0.07			
Peak CK, U/L	0.16	0.06 to 0.27	**0.003**	0.16	0.05 to 0.27	**0.004**
Peak troponin T, ng/L	0.13	0.03 to 0.24	**0.014**			
Other						
Leucocytes, 10^9/L	0.12	0.01 to 0.23	**0.038**			

[a]Defined as culprit in coronary segments 1, 6, or 11. β: standardized regression coefficients. *P* values <0.05 in **bold**. Next to age and sex, variables with *P* values <0.10 in age- and sex-adjusted analyses were considered for multivariable regression analyses.

Abbreviations as in Table 1.

circulating KBs at 24 hours were also associated with a lower LVEF (per 100 μmol/L increase of total KBs: β = −1.78; 95% CI: −3.17 to −0.39; *P* = 0.012, and for 100 μmol/L β-OHB: β = −2.55; 95% CI: −4.52 to −0.58; *P* = 0.012) (**Table 4, Figure 2**). In addition, functional outcomes were also depicted by tertiles of KBs at 24 hours (**Figure 3**). For total KBs and β-OHB, an increasing trend in infarct size was observed over the tertiles (*P* = 0.016 and *P* = 0.006, respectively). Concerning LVEF, a decreasing trend was only observed for β-OHB (*P* = 0.047). Four months after STEMI, no associations with circulating KBs and infarct size or LVEF were observed (Supplemental Table 3).

NT-proBNP. Associations between KB and NT-proBNP were also assessed. No independent associations were observed between KBs at admission (at presentation and 24 hours after reperfusion) and log NT-proBNP at baseline, log peak NT-proBNP during admission, or log NT-proBNP at 4 months (*P* > 0.05 for all). KBs and β-OHB at 4 months were associated with log NT-proBNP at 4 months (β = 0.13; 95% CI: 0.03-0.23; *P* = 0.013; β = 0.18; 95% CI: 0.02-0.35; *P* = 0.025, respectively) (Supplemental Table 4).

METFORMIN TREATMENT. At baseline, KB concentrations were comparable for both treatment strata (metformin and placebo). Patients treated with metformin 500 mg twice daily, which was initiated within 3 hours after PCI, had higher circulating total KBs and β-OHB concentrations at 24 hours compared with placebo (total KBs: 212 [IQR: 184-248] μmol/L vs 198 [IQR: 170-238] μmol/L, *P* = 0.017) (Supplemental Figure 1, Supplemental Table 5). However, this difference was small, and in regression analysis, metformin treatment was not associated with total KB and β-OHB concentrations (**Table 2**, Supplemental Table 2). In addition, at 4 months, no differences in KB concentrations between the metformin and placebo groups were observed.

DISCUSSION

In HF, ketone bioavailability and cardiac KB metabolism is upregulated. The role of KBs in an I/R setting remains largely unknown. We investigated longitudinal changes of KBs in patients who presented with a first STEMI and underwent primary PCI. We observed that KBs were increased at time of presentation with STEMI. Moreover, higher KB concentrations 24 hours after reperfusion were independently associated with larger infarct size and lower LVEF at 4-month follow-up (**Central Illustration**). Our results indicate that I/R is associated with an increase in KB bioavailability, and that this increase in KBs at 24 hours is associated with impaired functional outcomes. These results uncover a novel role for ketone metabolism in the systemic response to myocardial ischemia, which is likely to be adaptive.

KBs are produced in the liver and serve as metabolic substrates for multiple organs, including the

TABLE 3 Age- and Sex-Adjusted and Multivariable Associations of Plasma Ketone Bodies With Infarct Size Measured at 4-Month Follow-Up

	Age- and Sex-Adjusted			Multivariable[a]		
	β	95% CI	*P* Value	β	95% CI	*P* Value
Admission						
Total ketone bodies, 100 μmol/L	0.11	−0.006 to 0.23	0.06	0.05	−0.06 to 0.17	0.37
β-Hydroxybutyrate, 100 μmol/L	0.13	−0.03 to 0.30	0.10	0.06	−0.11 to 0.21	0.49
Acetoacetate, 10 μmol/L	0.07	0.015 to 0.13	**0.013**	0.04	−0.01 to 0.09	0.13
Acetone, 10 μmol/L	0.16	0.001 to 0.32	**0.048**	0.07	−0.09 to 0.24	0.38
24-h post-PCI						
Total ketone bodies, 100 μmol/L	1.64	0.34 to 2.95	**0.014**	1.56	0.29 to 2.83	**0.016**
β-Hydroxybutyrate, 100 μmol/L	2.31	0.47 to 4.16	**0.014**	2.45	0.65 to 4.25	**0.008**
Acetoacetate, 10 μmol/L	0.37	−0.02 to 0.76	0.07	0.19	−0.18 to 0.57	0.31
Acetone, 10 μmol/L			>0.10			

[a]Each ketone body was modeled separately and adjusted for age, sex, metformin treatment, BMI, TIMI flow pre-PCI, TIMI flow post-PCI, myocardial blush grade, anterior myocardial infarction (defined as culprit in left anterior descending coronary artery), log NT-proBNP and log HbA_{1C} at baseline and statin use at baseline. β: unstandardized regression coefficient. *P* values <0.05 in **bold**.

Abbreviations as in Table 1.

heart and the brain. Hepatic KB synthesis is stimulated during fasting or in other conditions of limited carbohydrate availability, mediated by reductions in the insulin/glucagon ratio (14). In addition, hepatic ketogenesis is also stimulated by increases in sympathetic nervous system activity (14). As a consequence of increased sympathetic tone, catecholamines are released, which, in turn, stimulate adipocyte lipolysis and the release of non-esterified fatty acids, which are important metabolic precursors for ketogenesis (14). Circulating KB concentrations are determined by the balance between the hepatic KB synthesis and their consumption by extrahepatic organs (14). In healthy subjects, circulating KB concentrations can be as low as 19 μmol/L and increase to approximately 120 μmol/L after an overnight fast (2,15). As our patients were studied upon admission in the nonfasting state, and KBs are higher in the fasting state compared with the nonfasting state, it is obvious that KBs were substantially increased at time of presentation with acute MI. KB oxidation in the heart is proportional to the arterial concentrations, which would suggest that the increases in KB concentrations observed in our study were paralleled by enhanced myocardial KB oxidation (4). Increases in circulating KB concentrations have been shown to be paralleled by enhanced myocardial ketone oxidation in patients with HF and arrhythmogenic cardiomyopathy (2,16). However, evidence supporting a similar ketolytic shift in

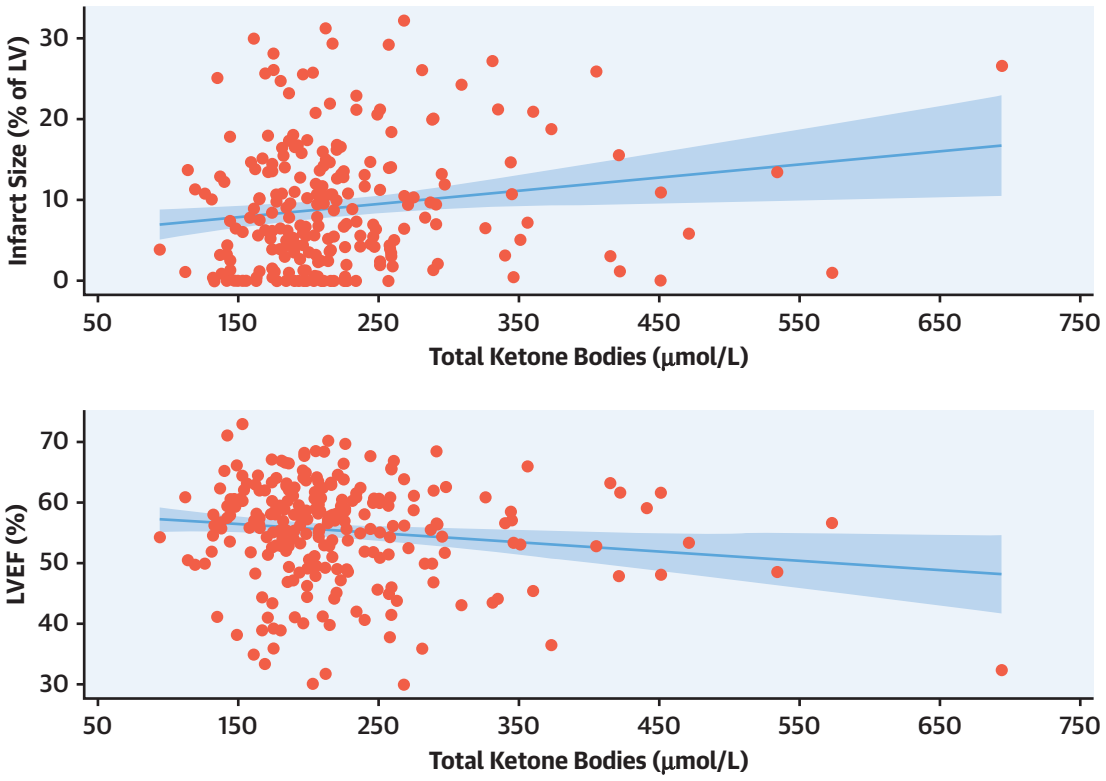
TABLE 4 Age- and Sex-Adjusted and Multivariable Associations of Plasma Ketone Bodies With Left Ventricle Ejection Fraction Measured at 4-Month Follow-Up

	Age- and Sex-Adjusted			Multivariable[a]		
	β	95% CI	*P* Value	β	95% CI	*P* Value
Admission						
Total ketone bodies, 100 μmol/L			>0.10			
β-Hydroxybutyrate, 100 μmol/L			>0.10			
Acetoacetate, 10 μmol/L			>0.10			
Acetone, 10 μmol/L			>0.10			
24 h post-PCI						
Total ketone bodies, 100 μmol/L	−1.57	−2.97 to −0.16	**0.029**	−1.78	−3.17 to −0.39	**0.012**
β-Hydroxybutyrate, 100 μmol/L	−2.04	−4.02 to −0.06	**0.043**	−2.55	−4.52 to −0.58	**0.012**
Acetoacetate, 10 μmol/L	−0.40	−0.82 to 0.02	0.06	−0.30	−0.71 to 0.11	0.15
Acetone, 10 μmol/L			>0.10			

[a]Each ketone body was modeled separately and adjusted for age, sex, metformin treatment, TIMI flow pre- and post-PCI, myocardial blush grade, anterior myocardial infarction (defined as culprit in left anterior descending coronary artery), ischemic time, log NT-proBNP and log HbA_{1C} at baseline and statin use at baseline. β: unstandardized regression coefficient. *P* values <0.05 in **bold**.

Abbreviations as in Table 1.

FIGURE 2 **Functional Outcomes for Individual Points of KBs**

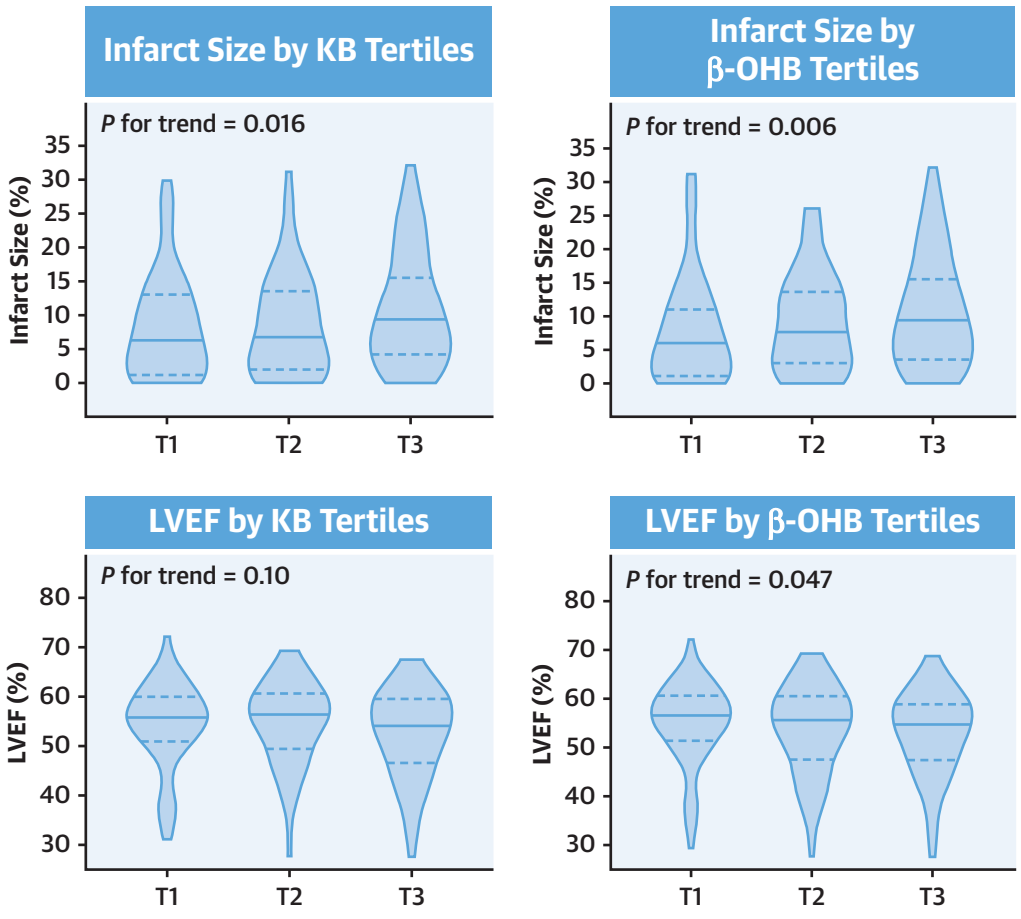


Scatterplot showing **(top)** infarct sizes (n = 236) and **(bottom)** left ventricular ejection fractions (LVEF) (n = 250) for each individual KB observation at 24 hours after reperfusion. The **blue line** depicts the unadjusted regression line with 95% confidence interval **(shaded area)**. The increasing and decreasing regression lines for infarct size and LVEF, respectively, suggest associations between KB and functional outcomes. LV = left ventricle; LVEF = left ventricular ejection fraction; other abbreviation as in Figure 1.

the myocardium of patients with an acute MI is lacking.

Our study was the first to demonstrate that KB concentrations were >3-fold higher in patients who presented with STEMI. We hypothesize that the large increase in circulating KBs at admission was a direct consequence of the stress response to MI, which resulted in a systemic catecholamine surge and free fatty acid release, which predominantly occurred during the first hours after the onset of symptoms (14,17). After presentation with STEMI, catecholamines only gradually decline (18,19), explaining the persistent elevation of KB concentrations at 24 hours after admission, although these concentrations were lower compared with admission. Similar increases in KB concentrations at presentation were detected in experimental models of cerebral ischemia (261-924 μmol/L) (20,21). In addition, Koch et al (20) demonstrated that administration of propranolol, a β-receptor antagonist, reduced hepatic ketogenesis after stroke, which suggested that elevations in circulating KBs after ischemia were the consequence of an increased sympathetic drive. Evidence supporting this catecholamine hypothesis in our study could be sought in the association between KB concentrations and Thrombolysis In Myocardial Infarction grade 0/1 flow. One might speculate that the ongoing ischemia in these patients is likely to be accompanied by an increased sympathetic tone, and consequently, higher ketone bioavailability. Furthermore, there was a negative trend between β-blocker use and KBs (Supplemental Table 1). Increases in KB concentrations were also reported in patients who underwent elective PCI, which suggested that factors associated with myocardial injury might also contribute to KB release (22). In this study, arterial KB concentrations rose instantly, whereas venous KB concentrations increased to a lesser extent. This

FIGURE 3 Functional Outcomes Per Tertiles of KBs



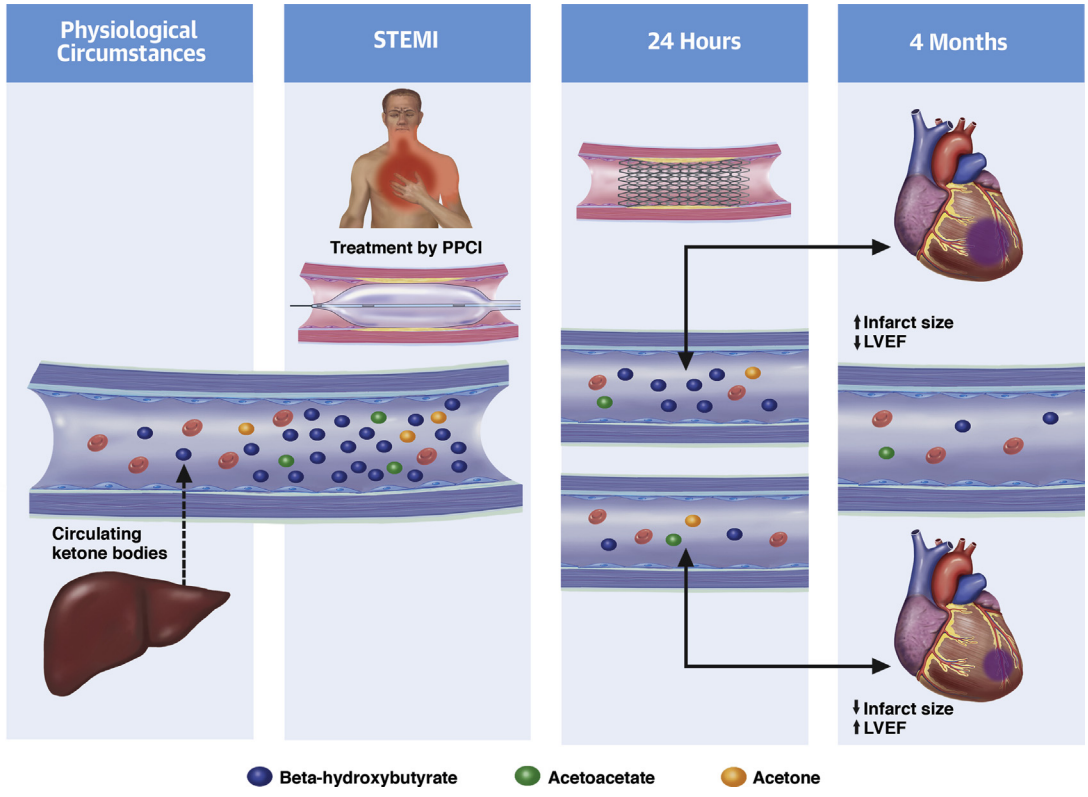
Functional outcomes were presented by tertiles (T) of **(left)** total KB (T1: 94-184 μmol/L, T2: 185-227 μmol/L, T3: 228-967 μmol/L) and **(right)** β-OHB (T1: 54-132 μmol/L, T2: 133-164 μmol/L, T3: 165-759 μmol/L) at 24 hours. **(Solid line)** Median and **(dashed line)** interquartile ranges. For total KB and β-OHB, an increasing trend in infarct size was observed over the tertiles ($P = 0.016$ and $P = 0.006$, respectively). Concerning LVEF, a decreasing trend was only observed for β-OHB ($P = 0.047$). Abbreviations as in Figures 1 and 2.

might also argue against the hypothesis that high KB concentrations were the result from reduced KB use.

We did not observe any associations between KBs at admission and outcomes. A possible explanation is that KB levels at baseline were substantially driven by the initial adrenergic stress response in all patients after MI, and this might have masked associations between KBs at admission and functional outcomes after STEMI. Therefore, we focused our analyses on KBs at 24 hours after reperfusion. We observed that higher circulating KB levels at 24 hours were associated with increased MI size. However, the association with LVEFs post-MI was relatively weak. This might have been due to heterogeneity of LVEFs before MI. Alternatively, KBs might not have directly affected LVEFs, but indirectly affected them via infarct size. Finally, the number of patients with reduced LVEFs was limited, which might have affected the power of our analyses. Further studies should include more patients with larger MI and more severely affected LVEFs to generate better insights on its relation to KBs. To the best of our knowledge, we were the first to investigate and report data on the association between circulating KBs and functional outcomes after MI.

Circulating KB levels at 24 hours were positively associated with HbA_{1C}, β-blocker use on admission, and enzymatic infarct size. The association with HbA_{1C} might be expected, because associations between KBs and type 2 diabetes and insulin resistance

CENTRAL ILLUSTRATION **Circulating Ketone Bodies After ST-Segment Elevation Myocardial Infarction**



de Koning, M.L.Y. et al. J Am Coll Cardiol. 2021;78(14):1421–1432.

Under physiological circumstances circulating ketone bodies (KBs) are close to zero. During ST-segment elevation myocardial infarction (STEMI), KBs are increased and still remain high at 24 hours after reperfusion compared with 4-month follow-up. Moreover, higher total KBs and higher β-hydroxybutyrate (OHB) concentrations at 24 hours after reperfusion were independently associated with larger infarct size and lower left ventricular ejection fraction (LVEF), which suggest that ketone metabolism may play an important role in the response to myocardial ischemia. PPCI = primary percutaneous coronary intervention.

have been previously established (12). The positive association between KBs at 24 hours and β-blocker use at baseline was somewhat surprising but might reflect patients with a worse cardiometabolic risk profile or the adverse effects of β-blockers on lipoprotein metabolism (23,24). However, this remains speculative, and an association between β-blocker use at baseline and HbA_{1c}, for example, was not observed. The higher levels of circulating KBs observed in patients with larger infarct sizes might be the result of enhanced ketogenesis, stimulated by ongoing high catecholamine levels in patients with a large MI (19,25), or could potentially reflect hemodynamic effects (26). No associations between KBs at 24 hours and 4 months and metformin were observed, in contrast to studies (both humans and rats) that reported a significant increase in KBs after metformin therapy (27-29). However, another 2 human studies observed no significant effect of metformin on KBs (30,31). BNPs were linked to increased lipolysis and higher KBs (32,33). In our study, NT-proBNP levels were associated with KBs at 4 months but not at baseline. A possible explanation for the absence of an association in the acute phase of MI might be the influence of catecholamines and acute hemodynamic changes.

This is the first study to demonstrate the correlation between circulating KBs and MI size. Previous studies showed similar associations with disease severity and impaired prognosis in HF with reduced

LVEFs and arrhythmogenic cardiomyopathy (16,34,35). This might suggest that the upregulation of ketone metabolism is a universal cardiac response to stress. Although elevated levels of KBs parallel disease severity, this observation does not necessarily indicate that increased circulating KBs reflect a maladaptive response. Experimental studies demonstrated that mice that lacked KB oxidizing capacity showed a worsened tolerance to I/R combined with pressure overload (3). Furthermore, beneficial effects of exogenous ketone enhancement in experimental MI models were established. Adherence to a ketogenic diet, KB supplementation, and the combination of both before I/R reduced infarct sizes and attenuated left ventricular dysfunction and remodeling post-MI (9,36-38). In addition, treatment with SGLT-2 inhibitors, which enhance ketone bioavailability, was associated with smaller infarct sizes and improved cardiac function in preclinical models as (reviewed by Andreadou et al [39]). To date, studies on KB supplementation in a clinical setting of I/R are lacking, but promising data in patients with HF with reduced EFs and age-matched volunteers suggested increased cardiac output following KB supplementation, even in still physiologic ranges of plasma KB concentrations (40). We hypothesize that KB supplementation may also have a beneficial effect in the STEMI setting due to its oxygen sparing nature and other pleiotropic effects (9,26,37), including inhibition of the NLRP3 inflammasome (41,42), improved myocardial blood flow (43), reduced oxidative stress (7,44), and mitochondrial preservation (37). Moreover, the most prevalent circulating KB, β-OHB, exerts important signaling functions, regulating the activation of multiple stress-response pathways, most prominently by histone deacetylase inhibition, which might also be relevant during I/R (42). However, the possible mechanisms of infarct size reduction and the subsequent prevention of HF continue to be the subject of investigation, and the relative contribution of the possible underlying mechanisms of actions remains to be determined. Future studies, especially those evaluating treatment effects, are warranted.

STUDY STRENGTHS AND LIMITATIONS. The strengths of our study included the serial measurements of circulating KBs, combined with meticulous follow-up. Furthermore, our population consisted of patients without known diabetes, excluding the effects of diabetes mellitus on circulating KBs and KB use (12,45), although a few patients with undiagnosed type 2 diabetes were included. However, adjustment for HbA_{1c} did not change the associations between KB and MI outcomes. In addition to the inherent limitations of a retrospective analysis of prospectively collected data, the specific limitation of our study was the fact that we did not measure hepatic ketone synthesis, plasma non-esterified fatty acids, or cardiac ketone metabolism, and conclusions related to these were speculative. Other limitations were the relatively small myocardial infarct sizes and the low percentage of patients who developed left ventricular dysfunction during follow-up. The results might have been different in a STEMI population at higher risk. Furthermore, nonfasted blood samples were taken, and this might have influenced KB concentrations, although the effect of short-term (overnight) fasting on KBs was limited (46).

CONCLUSIONS

Circulating KB are elevated in patients presenting with STEMI. Higher KBs after 24 hours are associated with functional outcomes after STEMI, suggesting that increased ketone metabolism may play a role in the response to myocardial ischemia.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Blood levels of KBs are elevated in patients with STEMI and associated with infarct size and LVEF after 4 months, suggesting a role for increased ketone metabolism in the response to myocardial ischemia and reperfusion.

TRANSLATIONAL OUTLOOK: Better understanding of the mechanisms linking ketone metabolism with myocardial ischemia and reperfusion may provide new opportunities for therapeutic intervention.

REFERENCES

1. Benjamin EJ, Muntner P, Alonso A, et al. Heart disease and stroke statistics-2019 update: a report from the American Heart Association. *Circulation*. 2019;139:e56–e528.

2. Bedi KCJ, Snyder NW, Brandimarto J, et al. Evidence for intramyocardial disruption of lipid metabolism and increased myocardial ketone utilization in advanced human heart failure. *Circulation*. 2016;133:706–716.

3. Horton JL, Davidson MT, Kurishima C, et al. The failing heart utilizes 3-hydroxybutyrate as a metabolic stress defense. *JCI Insight*. 2019;4:e124079.

4. Murashige D, Jang C, Neinast M, et al. Comprehensive quantification of fuel use by the failing and nonfailing human heart. *Science*. 2020;370:364–368.

5. Lafontan M, Sengenes C, Galitzky J, et al. Recent developments on lipolysis regulation in humans and discovery of a new lipolytic pathway. *Int J Obes Relat Metab Disord*. 2000;24(Suppl 4):S47–S52.

6. Schugar RC, Moll AR, André d'Avignon D, et al. Cardiomyocyte-specific deficiency of ketone body metabolism promotes accelerated pathological remodeling. *Mol Metab*. 2014;3:754–769.

7. Uchihashi M, Hoshino A, Okawa Y, et al. Cardiac-specific bdh1 overexpression ameliorates oxidative stress and cardiac remodeling in pressure overload-induced heart failure. *Circ Heart Fail*. 2017;10:e004417.

8. Veech RL. The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins Leukot Essent Fatty Acids*. 2004;70:309–319.

9. Zou Z, Sasaguri S, Rajesh KG, Suzuki R. dl-3-Hydroxybutyrate administration prevents myocardial damage after coronary occlusion in rat hearts. *Am J Physiol Heart Circ Physiol*. 2002;283:H1968–H1974.

10. Lexis CPH, van der Horst ICC, Lipsic E, et al. Effect of metformin on left ventricular function after acute myocardial infarction in patients without diabetes: the GIPS-III randomized clinical trial. *JAMA*. 2014;311:1526–1535.

11. Lexis CPH, van der Horst ICC, Lipsic E, et al. Metformin in non-diabetic patients presenting with ST elevation myocardial infarction: rationale and design of the glycometabolic intervention as adjunct to primary percutaneous intervention in ST elevation myocardial infarction (GIPS)-III trial. *Cardiovasc Drugs Ther*. 2012;26:417–426.

12. Garcia E, Shalaurova I, Matyus SP, et al. Ketone bodies are mildly elevated in subjects with type 2 diabetes mellitus and are inversely associated with insulin resistance as measured by the lipoprotein insulin resistance index. *J Clin Med*. 2020;9:321.

13. Baul PB, Deepak AD, Kakkar M, Modi S. Effect of atorvastatin on blood ketone levels and glycemic control in patients with type 2 diabetes mellitus: a single arm pilot study. *Diabetes Metab Syndr*. 2020;14:1333–1337.

14. Kadir AA, Clarke K, Evans RD. Cardiac ketone body metabolism. *Biochim Biophys Acta Mol Basis Dis*. 2020;105:165739.

15. Flores-Guerrero JL, Westenbrink BD, Connelly MA, et al. Association of beta-hydroxybutyrate with development of heart failure: sex differences in a Dutch population cohort. *Eur J Clin Invest*. 2021;51:e13468.

16. Song JP, Chen L, Chen X, et al. Elevated plasma β-hydroxybutyrate predicts adverse outcomes and disease progression in patients with arrhythmogenic cardiomyopathy. *Sci Transl Med*. 2020;12:eaay8329.

17. Vetter NJ, Strange RC, Adams W, Oliver MF. Initial metabolic and hormonal response to acute myocardial infarction. *Lancet*. 1974;1:284–288.

18. Gupta DK, Jewitt DE, Young R, Hartog M, Opie LH. Increased plasma-free-fatty-acid concentrations and their significance in patients with acute myocardial infarction. *Lancet*. 1969;2:1209–1213.

19. Valori C, Thomas M, Shillingford J. Free noradrenaline and adrenaline excretion in relation to clinical syndromes following myocardial infarction. *Am J Cardiol*. 1967;20:605–617.

20. Koch K, Berressem D, Konietzka J, et al. Hepatic ketogenesis induced by middle cerebral artery occlusion in mice. *J Am Heart Assoc*. 2017;6:e005556.

21. Baranovicova E, Grendar M, Kalenska D, et al. NMR metabolomic study of blood plasma in ischemic and ischemically preconditioned rats: an increased level of ketone bodies and decreased content of glycolytic products 24 h after global cerebral ischemia. *J Physiol Biochem*. 2018;74:417–429.

22. Di Marino S, Viceconte N, Lembo A, et al. Early metabolic response to acute myocardial ischaemia in patients undergoing elective coronary angioplasty. *Open Heart*. 2018;5:1–8.

23. Kasiske BL, Ma JZ, Kalil RS, Louis TA. Effects of antihypertensive therapy on serum lipids. *Ann Intern Med*. 1995;122:133–141.

24. Ripley TL, Saseen JJ. β-blockers: a review of their pharmacological and physiological diversity in hypertension. *Ann Pharmacother*. 2014;48:723–733.

25. Opie LH. Metabolism of free fatty acids, glucose and catecholamines in acute myocardial infarction. Relation to myocardial ischemia and infarct size. *Am J Cardiol*. 1975;36:938–953.

26. Yurista SR, Chong C-R, Badimon JJ, Kelly DP, de Boer RA, Westenbrink BD. Therapeutic potential of ketone bodies for patients with cardiovascular disease: JACC state-of-the-art review. *J Am Coll Cardiol*. 2021;77:1660–1669.

27. Tessari P, Tiengo A. Metformin treatment of rats with diet-induced overweight and hypertriglyceridemia decreases plasma triglyceride concentrations, while decreasing triglyceride and increasing ketone body output by the isolated perfused liver. *Acta Diabetol*. 2008;45:143–145.

28. Nattrass M, Hinks L, Smythe P, Todd PG, Alberti KG. Metabolic effects of combined sulphonylurea and metformin therapy in maturity-onset diabetice. *Horm Metab Res*. 1979;11:332–337.

29. Cuyàs E, Fernández-Arroyo S, Buxó M, et al. Metformin induces a fasting- and antifolate-mimicking modification of systemic host metabolism in breast cancer patients. *Aging (Albany NY)*. 2019;11:2874–2888.

30. Gregorio F, Ambrosi F, Marchetti P, et al. Low dose metformin in the treatment of type II non-insulin-dependent diabetes: clinical and metabolic evaluations. *Acta Diabetol Lat*. 1990;27:139–155.

31. Kong SH, Koo BK, Moon MK. Effects of dapagliflozin on endothelial function, renal injury markers, and glycemic control in drug-naïve patients with type 2 diabetes mellitus. *Diabetes Metab J*. 2019;43:711–717.

32. Lafontan M, Moro C, Berlan M, et al. Control of lipolysis by natriuretic peptides and cyclic GMP. *Trends Endocrinol Metab*. 2008;19:130–137.

33. Kashiwagi Y, Nagoshi T, Inoue Y, et al. Close linkage between blood total ketone body levels and B-type natriuretic peptide levels in patients with cardiovascular disorders. *Sci Rep*. 2021;11:6498.

34. Yokokawa T, Sugano Y, Shimouchi A, et al. Exhaled acetone concentration is related to hemodynamic severity in patients with non-ischemic chronic heart failure. *Circ J*. 2016;80:1178–1186.

35. Marcondes-Braga FG, Batista GL, Gutz IGR, et al. Impact of exhaled breath acetone in the prognosis of patients with heart failure with reduced ejection fraction (HFrEF). One year of clinical follow-up. *PLoS One*. 2016;11:e0168790.

36. Šnorek M, Hodyc D, Šedivý V, et al. Short-term fasting reduces the extent of myocardial infarction and incidence of reperfusion arrhythmias in rats. *Physiol Res*. 2012;61:567–574.

37. Yu Y, Yu Y, Zhang Y, et al. Treatment with D-β-hydroxybutyrate protects heart from ischemia/reperfusion injury in mice. *Eur J Pharmacol*. 2018;829:121–128.

38. Yurista SR, Matsuura TR, Silljé HHW, et al. Ketone ester treatment improves cardiac function and reduces pathologic remodeling in preclinical models of heart failure. *Circ Heart Fail*. 2021;14:e007684.

39. Andreadou I, Bell RM, Bøtker HE, Zuurbier CJ. SGLT2 inhibitors reduce infarct size in reperfused ischemic heart and improve cardiac function during ischemic episodes in preclinical models. *Biochim Biophys Acta Mol Basis Dis*. 2020;1866:165770.

40. Nielsen R, Møller N, Gormsen LC, et al. Cardiovascular effects of treatment with the ketone body 3-hydroxybutyrate in chronic heart failure patients. *Circulation*. 2019;139:2129–2141.

41. Youm Y-H, Nguyen KY, Grant RW, et al. The ketone metabolite β-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. *Nat Med*. 2015;21:263–269.

42. Newman JC, Verdin E. β-Hydroxybutyrate: a signaling metabolite. *Annu Rev Nutr*. 2017;37:51–76.

43. Gormsen LC, Svart M, Thomsen HH, et al. Ketone body infusion with 3-hydroxybutyrate reduces myocardial glucose uptake and increases blood flow in humans: a positron emission tomography study. *J Am Heart Assoc*. 2017;6:1–11.

44. Shimazu T, Hirschey MD, Newman J, et al. Suppression of oxidative stress by β-hydroxybutyrate, an endogenous histone deacetylase inhibitor. *Science*. 2013;339:211–214.

45. Mizuno Y, Harada E, Nakagawa H, et al. The diabetic heart utilizes ketone bodies as an energy source. *Metabolism*. 2017;77:65–72.

46. Evans M, Cogan KE, Egan B. Metabolism of ketone bodies during exercise and training: physiological basis for exogenous supplementation. *J Physiol*. 2017;595:2857–2871.

KEY WORDS myocardial infarction, metabolism, ketone bodies, ischemia/reperfusion, metformin

APPENDIX For the supplemental tables and a figure, please see the online version of this paper.