Thiol-based cardioprotection

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CHAPTER 2

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 KBs 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 (NCT 01217307). Non-fasting plasma concentrations of KBs (beta-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 to levels at 4 months 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: β=1.56, 95% confidence interval (CI): 0.29 to 2.83, $P=0.016$) and lower LVEF (β=-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, suggesting a potential role for ketone metabolism in response to myocardial ischemia.

INTRODUCTION

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 circulating KB levels.[2–4] The upregulation of plasma KBs in HF is considered a consequence of increased hepatic ketogenesis due to the upregulation of neurohormonal factors such as catecholamines and natriuretic peptides. Both catecholamines and natriuretic peptides stimulate adipocyte lipolysis and promote the release of non-esterified fatty acids, important metabolic precursors for ketogenesis.[5]

The shift towards KB metabolism is considered adaptive and has been linked to reduced oxidative stress and hemodynamic preservation.[6,7] Mice incapable of oxidizing KB 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 as 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 myocardial infarction undergoing percutaneous coronary intervention (PCI). Therefore, our study addressed several objectives. First, we investigated longitudinal changes in circulating KB concentrations after ST-segment elevation MI (STEMI). Secondly, we studied whether plasma KB concentrations are associated with myocardial infarct size and left ventricular ejection fraction (LVEF) at 4 months.

METHODS

Study population

We measured KBs in archived plasma samples of the Glycometabolic Intervention in Adjunct to Primary Percutaneous Coronary Intervention in ST-Segment Elevation Myocardial Infarction trial (GIPS-III, NCT 01217307). The GIPS-III was designed to assess the effect of 4 months metformin treatment compared to placebo on left ventricular function in non-diabetic patients presenting 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, the presence of STEMI and primary PCI with implantation of at least 1 stent with a diameter of at least 3 mm resulting in Thrombolysis In Myocardial Infarction (TIMI) 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
Non-fasting 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 three 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 (NMR) spectroscopy platform. Plasma samples were prepared on board the instrument, and automatically delivered to the flow probe in the NMR spectrometer's magnetic field. Data acquisition on the Vantera® and spectra data processing have been 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 months follow-up. Details on imaging acquisition and analysis have been reported elsewhere.[10,11] An independent core laboratory (Image Analysis Center, VU 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 ± standard deviation (SD). Skewed data were presented as median (interquartile range [IQR]). 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's exact tests 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 uni- and multivariable regression analyses. Variables with a *P*-value <0.1 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 and NT-proBNP at 4 months follow-up were performed. A two-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% was female.

Table 1 | Characteristics at admission for STEMI

Characteristic	Total (n=369)
Age at admission, years, mean ± SD	58.8 ± 11.6
Female sex, n (%)	93 (25.2%)
Ethnicity, n (%)	
Caucasian	355 (96.2%)
Asian	10 (2.7%)
Black	4 (1.1%)
Cardiovascular related history, n (%)	
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, n (%)	
β-blocker	38 (10.3%)
ACE Inhibitor/Angiotensin Receptor Blocker	38 (10.3%)
Diuretics	35 (9.5%)
Statins	29 (7.9%)
Clinical parameters, mean ± SD	
Body Mass Index, kg/m^2	26.9 ± 3.8
Systolic blood pressure, mmHg	134 ± 23
Diastolic blood pressure, mmHg	84 ± 15
Heart rate, beats/min	75 ± 16

PCI parameters	
Total ischemic time (min), median [IQR]	174 [118-255]
Single vessel disease, n (%)	254 (68.8%)
Anterior myocardial infarction, n (%)[a]	144 (39.0%)
TIMI flow pre-PCI, n (%)	
0	203 (55.0%)
1	27 (7.3%)
2	64 (17.3%)
3	75 (20.3%)
Stent diameter, n (%)	
<3.5 mm	143 (38.9%)
≥3.5 mm	224 (61.1%)
TIMI flow post-PCI, n (%)	
2	34 (9.2%)
3	335 (90.8%)
Myocardial blush grade, n (%)	
0	10 (2.7%)
1	29 (7.9%)
2	72 (19.7%)
3	255 (69.7%)
Laboratory parameters, median [IQR]	
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]
Beta-hydroxybutyrate	369 [227-712]
Acetoacetate	109 [54-216]
Acetone	38 [20-71]

[a] *defined as culprit in LAD*

Abbreviations: ACE, angiotensin converting enzyme; CK, creatine kinase; CK-MB, creatine kinase-myocardial band; HbA_{1c}, glycosylated hemoglobin; IQR, interquartile range; NT-proBNP, N-terminal pro-b-type natriuretic peptide; PCI, percutaneous coronary intervention; SD, standard deviation; STEMI, ST-segment elevation myocardial infarction; TIMI, Thrombolysis in Myocardial Infarction.

Total circulating KB concentrations at admission for STEMI amounted to 520 [315-997] (median [IQR], µmol/L), and were positively associated with glycosylated hemoglobin (HbA_{1C}), High-density lipoprotein cholesterol and a lower TIMI flow pre-PCI (standardized (std) β=0.25, 95% confidence interval (CI): 0.15 to 0.36, *P*<0.001; std β=0.22, 95% CI: 0.13 to 0.32, *P*<0.001; TIMI 0/1 vs. 2/3 std β=0.15, 95% CI: 0.06 to 0.25, *P*=0.001, respectively, **Supplementary 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 which presented in the early morning).

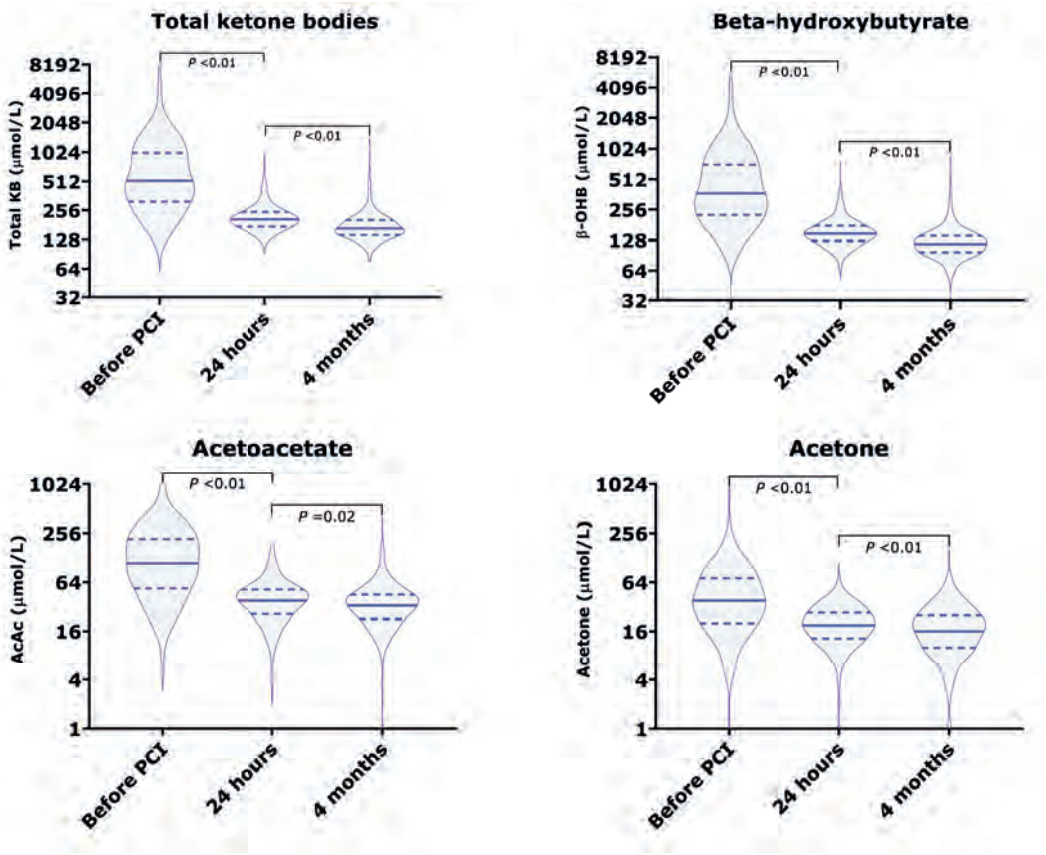


Figure 1 | KB concentrations after STEMI

Violin plots of circulating KB concentrations at presentation with 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 hours follow-up and higher at 24 hours compared with 4 months follow-up, suggesting that KBs are elevated for a longer period after STEMI, possibly to fuel the heart in need. Abbreviations: AcAc, acetoacetate; β-OHB, Beta-Hydroxybutyrate; KBs, ketone bodies.

KBs 24 hours post-PCI

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

Table 2 | Age- and sex-adjusted and multivariable linear regression analyses on plasma KBs at 24 hours

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

[a] *defined as culprit in coronary segment 1, 6, or 11*

β: standardized regression coefficients. P-values <0.05 in ***bold*** *print*

Next to age and sex, variables with P-values<0.1 in age- and sex-adjusted analyses were considered for multivariable regression analyses.

Abbreviations: CI, confidence interval; CK, creatine kinase; CK-MB, creatine kinase-myocardial band; HbA_{1c}, glycosylated hemoglobin; PCI, percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction.

KBs and outcomes

At 4 months 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**, **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 to 2.83, *P*=0.016 per 100 µmol/L increase of total KBs and β=2.45, 95% CI: 0.65 to 4.25, *P*=0.008 for β-OHB; **Table 3**, **Figure 2**). Moreover, circulating KBs at 24 hours were also associated with lower LVEF (per 100 µmol/L 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**).

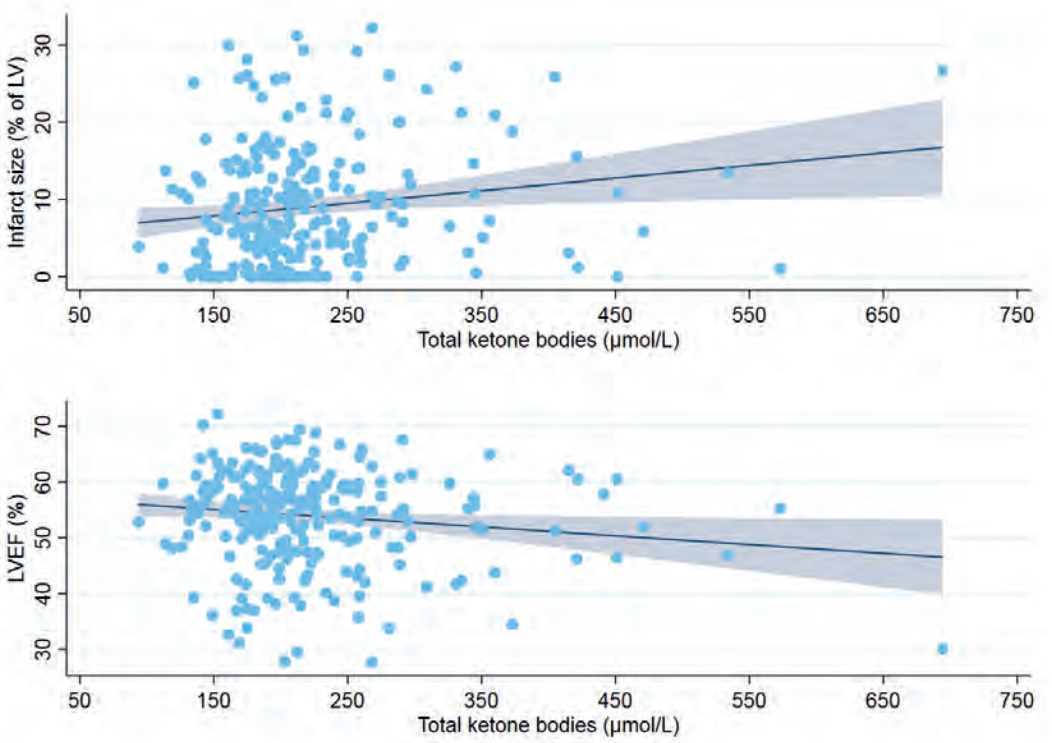


Figure 2 | Functional outcomes for individual points of KBs

Scatterplot showing infarct sizes (top, n=236) and LVEF (bottom, n=250) for each individual KB observation at 24 hours after reperfusion. The blue line depicts the unadjusted regression line with 95% confidence interval (bluish-gray). The increasing and decreasing regression lines for infarct size and LVEF, respectively, suggest associations between KB and functional outcomes. Abbreviations: KBs, ketone bodies; LV, left ventricle; LVEF, left ventricular ejection fraction.

Table 3 | Age- and sex-adjusted and multivariable associations of plasma KBs with infarct size measured at 4 months follow-up

	Age- and sex-adjusted				Multivariable [a]			
Variable	**β**	**95% CI lower limit**	**95% CI upper limit**	***P*-value**	**β**	**95% CI lower limit**	**95% CI upper limit**	***P*-value**
Admission								
Total ketone bodies, 100 µmol/L	0.11	-0.006	0.23	0.06	0.05	-0.06	0.17	0.37
Beta-hydroxybutyrate, 100 µmol/L	0.13	-0.03	0.30	0.10	0.06	-0.110	0.21	0.49
Acetoacetate, 10 µmol/L	0.07	0.015	0.13	**0.013**	0.04	-0.01	0.09	0.13
Acetone, 10 µmol/L	0.16	0.001	0.32	**0.048**	0.07	-0.09	0.24	0.38
24 hours post-PCI								
Total ketone bodies, 100 µmol/L	1.64	0.34	2.95	**0.014**	1.56	0.29	2.83	**0.016**
Beta-hydroxybutyrate, 100 µmol/L	2.31	0.47	4.16	**0.014**	2.45	0.65	4.25	**0.008**
Acetoacetate, 10 µmol/L	0.37	-0.02	0.76	0.065	0.19	-0.18	0.57	0.31
Acetone, 10 µmol/L				>0.10				

β: unstandardized regression coefficient. P-values <0.05 in ***bold*** *print.*

[a] Each ketone body was modelled separately and adjusted for age, sex, metformin treatment, BMI, TIMI flow pre- and 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.

Abbreviations: BMI, body mass index; CI, confidence interval; HbA_{1C}, glycosylated hemoglobin; NT-proBNP, N-terminal pro-b-type natriuretic peptide; PCI, percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction.

Table 4 | Age- and sex-adjusted and multivariable associations of plasma KBs with left ventricle ejection fraction measured at 4 months follow-up

	Age- and sex-adjusted				Multivariable [a]			
Variable	**β**	**95% CI lower limit**	**95% CI upper limit**	***P*-value**	**β**	**95% CI lower limit**	**95% CI upper limit**	***P*-value**
Admission								
Total ketone bodies, 100 µmol/L				>0.10				
Beta-hydroxybutyrate, 100 µmol/L				>0.10				
Acetoacetate, 10 µmol/L				>0.10				
Acetone, 10 µmol/L				>0.10				
24 hours post-PCI								
Total ketone bodies, 100 µmol/L	-1.57	-2.97	-0.16	**0.029**	-1.78	-3.17	-0.39	**0.012**
Beta-hydroxybutyrate, 100 µmol/L	-2.04	-4.02	-0.06	**0.043**	-2.55	-4.52	-0.58	**0.012**
Acetoacetate, 10 µmol/L	-0.40	-0.82	0.02	0.06	-0.30	-0.71	0.11	0.15
Acetone, 10 µmol/L				>0.10				

*β: unstandardized regression coefficient. P-values <0.05 in **bold** print.*

[a] Each ketone body was modelled 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.

Abbreviations: CI, confidence interval; HbA_{1C}, glycosylated hemoglobin; NT-proBNP, N-terminal pro-b-type natriuretic peptide;PCI, percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction.

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 (**Supplementary Table 3**).

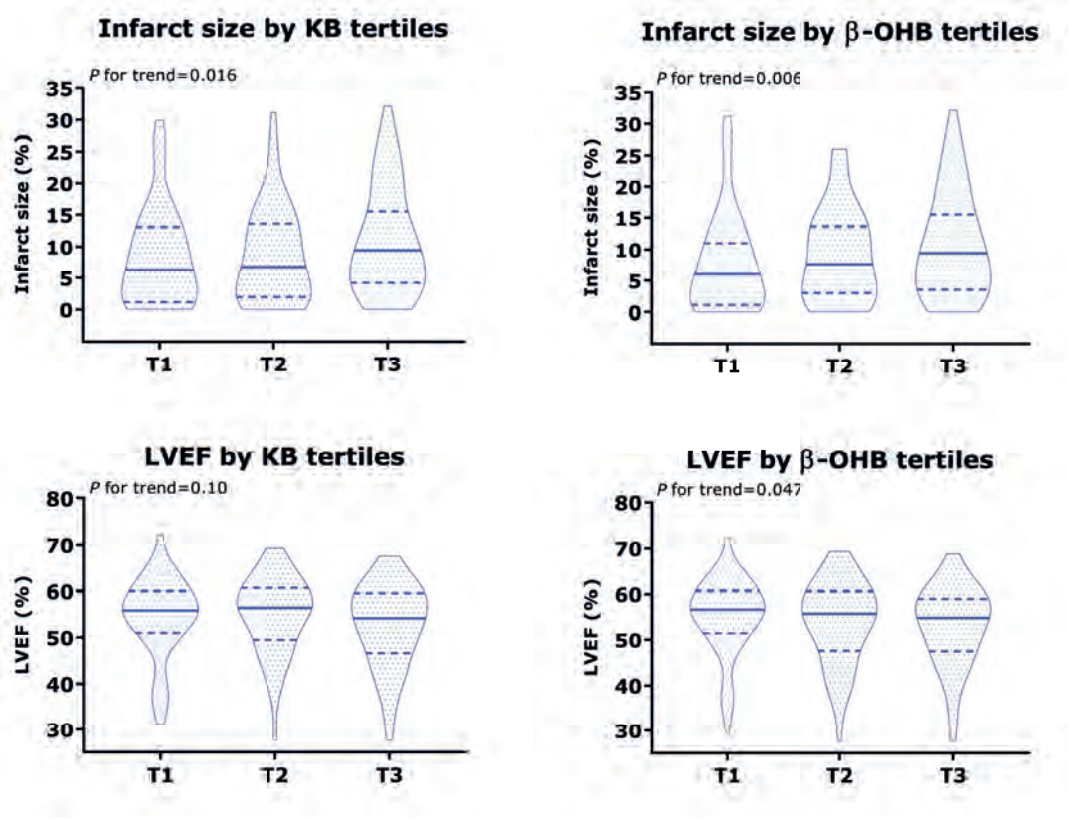


Figure 3 | Functional outcomes per tertiles of KBs

Functional outcomes were presented by tertiles of total KBs (left; T1: 94-184 µmol/L, T2: 185-227, T3: 228-967) and β-OHB (right; T1: 54-132 µmol/L, T2: 133-164, T3: 165-759) at 24 hours. Median and interquartile ranges are depicted by a solid and dashed line, respectively. 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). Abbreviations: β-OHB, Beta-Hydroxybutyrate; KBs, ketone bodies; LVEF, left ventricular ejection fraction.

NT-proBNP

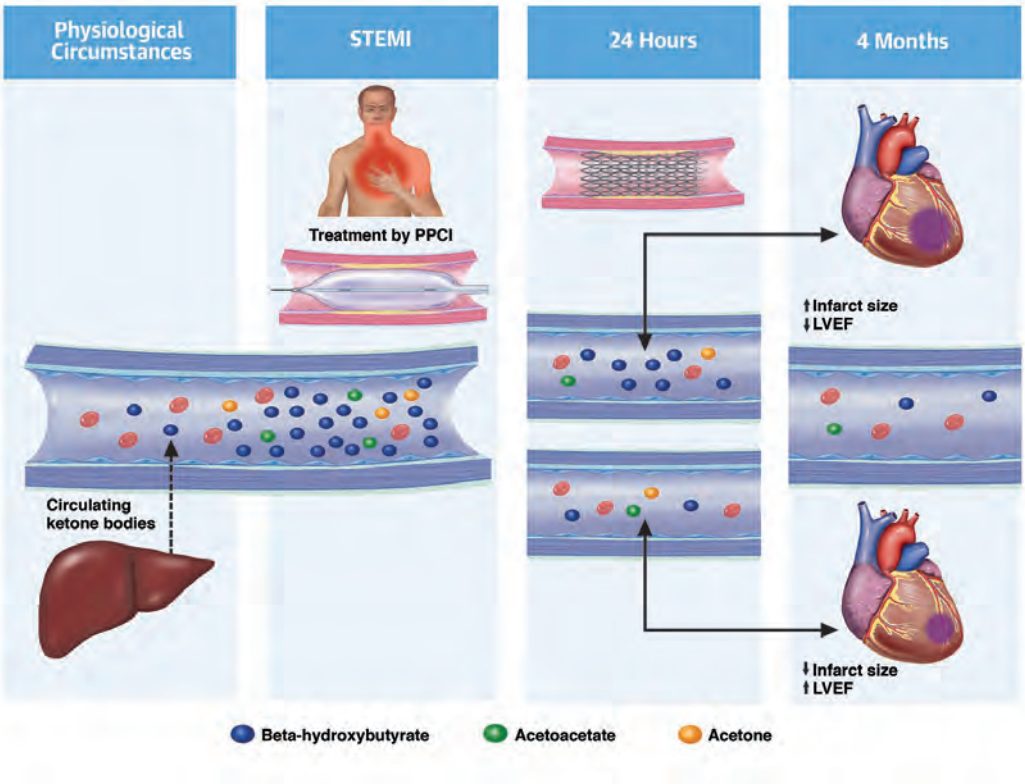
Associations between KBs and NT-proBNP were assessed. No independent associations were observed between KBs at admission (at presentation and 24 hours after reperfusion) and log NT-proBNP at baseline, log peak NT-proBNP or log NT-proBNP at 4 months (P>0.05 for all). KBs and β-OHB at 4 months were associated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

Metformin treatment

At baseline KB concentrations were comparable for both treatment strata (metformin and placebo). Patients treated with metformin 500 mg b.i.d., which was initiated within 3 hours after PCI, did have higher circulating total KBs and β-OHB concentrations at 24 hours, compared with placebo (total KBs: 212 μmol/L [184-248] vs 198 μmol/L [170-238], P=0.017; **Figure S1**, **Supplementary 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**, **Supplementary Table 2**). Also at 4 months no differences in KB concentrations between the metformin and placebo group 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 presenting with a first STEMI undergoing primary PCI. We observed that KB were increased at time of presentation with STEMI. Moreover, higher KBs concentrations at 24 hours after reperfusion were independently associated with larger infarct size and lower LVEF at 4 months 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.



Central illustration | Circulating KBs after STEMI

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

KBs are produced in the liver and serve as metabolic substrates for multiple organs, including the 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, 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 around 120 μmol/L after an overnight fast.[2,15] As our patients were studied upon admission in the non-fasting state, and KBs are higher in the fasting compared with the non-fasting 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 studies are paralleled by enhanced myocardial KB oxidation.[4] Indeed, 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 the myocardium of patients with an acute MI is lacking.

Our study is the first to demonstrate that KB concentrations are more than 3-fold higher in patients presenting with STEMI. We hypothesize that the large increase in circulating KBs at admission is a direct consequence of the stress response to MI, resulting in a systemic catecholamine surge and free fatty acid release, which predominantly occurs 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 lower compared to admission. Similar increases in KB concentrations at presentation have been detected in experimental models of cerebral ischemia (261-924 μmol/L).[20,21] In addition, Koch *et al*. demonstrated that administration of propranolol, a β-receptor antagonist, reduced hepatic ketogenesis after stroke, suggesting that elevations in circulating KB after ischemia are the consequence of an increased sympathetic drive.[20] Evidence supporting this catecholamine hypothesis in our study may be sought in the association between KB concentrations and TIMI 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 (**Supplementary Table 1**). Increases in KB concentrations have also been reported in patients undergoing elective PCI, suggesting that factors associated with myocardial injury may also contribute to KB release.[22] In this study, arterial KB concentrations rose instantly, while venous KB concentrations increased to a lesser extent. This might also argue against the hypothesis that high KB concentrations are the result from reduced KB utilization.

We did not observe any associations between KBs at admission and outcomes. A possible explanation is that KB levels at baseline are 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. We therefore focused our analyses on KBs at 24 hours after reperfusion. We observed that higher circulating KB levels at 24 hours were associated with increased myocardial infarct size. However, the association with LVEF post-MI was relatively weak. This might be due to heterogeneity of LVEF prior to MI. Alternatively, KBs might not directly affect LVEF, but indirectly via infarct size. Finally, the number of patients with reduced LVEF is limited, which might affect the power of our analyses. Further studies should include more patients with large MI and more severely affected LVEF to generate better insights on its relation to KBs. To our best knowledge, we are 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} could be expected, since associations between KBs and type 2 diabetes and insulin resistance have been established before.[12] The positive association between KBs at 24 hours and β-blocker use at baseline was somewhat surprising, but might be a reflection of patients with a worse cardiometabolic risk profile or 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 with studies (both humans and rats) that reported a significant increase in KBs after metformin therapy.[27–29] However, another two human studies observed no significant effect of metformin on KBs.[30,31] BNPs have been 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 may 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 have shown similar associations with disease severity and impaired prognosis in HF with reduced LVEF and arrythmogenic 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. In fact, experimental studies demonstrate that mice that lacked KB oxidizing capacity, showed worsened tolerance to I/R combined with pressure overload.[3] Furthermore, beneficial effects of exogenous ketone enhancement in experimental MI models have been 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] Also 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[39]). To date, studies on KB supplementation in a clinical setting of I/R are lacking, but promising data in HFrEF patients and age-matched volunteers suggested increased cardiac output following KB supplementation, even in still physiological ranges of plasma KB concentrations.[40] We hypothesize that KB supplementation may also have a beneficial effect in 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 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

Strengths of our study comprise 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 KBs and MI outcomes. In addition to the inherent limitations of a retrospective analysis of prospectively collected data, the specific limitation of our study is the fact that we did not measure hepatic ketone synthesis, plasma non-esterified fatty acids or cardiac ketone metabolism, and conclusions related to these are speculative. Other limitations are the relatively small myocardial infarct sizes and the low percentage of patients developing left ventricular dysfunction during follow-up. The results may have been different in a STEMI population at higher risk. Furthermore, non-fasted 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 KBs 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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SUPPLEMENTARY MATERIAL

Supplementary Table 1 | Age- and sex-adjusted and multivariable linear regression analyses of baseline parameters with total KBs at admission

Variable	Age- and sex-adjusted Standardized β	95% CI lower limit	95% CI upper limit	*P*-value	Multivariable Standardized β	95% CI lower limit	95% CI upper limit	*P*-value
Age, years	0.06	-0.04	0.16	0.24	-0.01	-0.10	0.09	0.86
Female sex	0.02	-0.08	0.12	0.72	-0.08	-0.18	0.01	0.08
TIMI flow pre-PCI, 0/1 vs. 2/3	0.17	0.07	0.27	**0.001**	0.15	0.06	0.25	**0.001**
Anterior myocardial infarction [a]	0.09	-0.01	0.20	0.07				
Leucocytes, 10^9/L	0.12	0.01	0.22	**0.027**				
Glucose, mmol/L	0.28	0.18	0.38	**<0.001**				
HbA_{1c}, %	0.36	0.26	0.46	**<0.001**	0.25	0.15	0.36	**<0.001**
HDL-cholesterol, mmol/L	0.20	0.10	0.30	**<0.001**	0.22	0.13	0.32	**<0.001**
Triglycerides, mmol/L	-0.09	-0.19	0.00	0.06				
Dyslipidemia in medical history	-0.10	-0.20	0.00	0.06				
β-blocker use at baseline	-0.10	-0.20	0.01	0.06				
Statin use at baseline	-0.10	-0.20	0.00	0.06				

*P-values <0.05 are **bold** printed*

[a] defined as culprit in left anterior descending coronary artery

Next to age and sex, variables with P-values <0.1 in age- and sex-adjusted analyses were considered for multivariable regression analysis

Abbreviations: CI, confidence interval; HbA_{1c}, glycosylated hemoglobin; HDL, high density lipoprotein; KBs, ketone bodies; PCI, percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction.

Supplementary Table 2 | Age-and sex-adjusted and multivariable linear regression analyses on Beta-Hydroxybutyrate at 24 hours

	Age- and sex-adjusted				Multivariable			
Variable	**Standardized β**	**95% CI lower limit**	**95% CI upper limit**	***P*-value**	**Standardized β**	**95% CI lower limit**	**95% CI upper limit**	***P*-value**
Age, years	0.07	-0.04	0.17	0.23	0.03	-0.08	0.14	0.61
Female sex	0.08	-0.03	0.18	0.17	0.07	-0.04	0.18	0.19
Metformin treatment	0.05	-0.06	0.15	0.38	0.03	-0.07	0.14	0.55
TIMI flow post-PCI, 2 vs. 3	0.12	0.02	0.23	**0.022**				
Proximal culprit [a]	0.09	-0.01	0.20	0.09				
Leucocytes, 10^9/L	0.13	0.02	0.24	**0.017**				
HbA_{1c}, %	0.17	0.06	0.28	**0.002**	0.18	0.04	0.32	**0.010**
β-blocker use at baseline	0.18	0.07	0.28	**0.001**	0.16	0.05	0.26	**0.004**
Diuretic use at baseline	0.13	0.02	0.24	**0.026**				
Statin use at baseline	0.14	0.04	0.25	**0.009**				
Hypertension at baseline	0.11	0.00	0.22	**0.044**				
Peak CK-MB, U/L	0.10	0.00	0.21	0.06				
Peak CK, U/L	0.16	0.05	0.26	**0.004**	0.15	0.04	0.26	**0.006**
Peak Troponin T, ng/L	0.12	0.01	0.22	**0.031**				

*P-values <0.05 are **bold** printed*

[a] defined as culprit in coronary segment 1, 6, or 11.

Next to age and sex and treatment allocation, variables with P-values <0.1 in age- and sex-adjusted analyses were considered for multivariable regression analysis.

Abbreviations: CI, confidence interval; CK, creatine kinase; CK-MB, creatine kinase-myocardial band; HbA_{1c}, glycosylated hemoglobin; PCI, percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction.

Supplementary Table 3 | Age- and sex-adjusted and multivariable associations of plasma KBs at 4 months with infarct size and left ventricle ejection fraction measured at 4 months follow

	Age- and sex-adjusted				Multivariable [a]			
Variable	β	95% CI lower limit	95% CI upper limit	*P*-value	β	95% CI lower limit	95% CI upper limit	*P*-value
Infarct Size								
Total KBs, 100 µmol/L	-0.43	-1.33	0.47	0.34				
Beta-hydroxybutyrate, 100 µmol/L	-0.86	-2.30	0.58	0.24				
Acetoacetate, 10 µmol/L	-0.04	-0.32	0.24	0.78				
Acetone, 10 µmol/L	-0.26	-0.88	0.37	0.42				
Left ventricular ejection fraction								
Total KBs, 100 µmol/L	0.90	-0.02	1.83	0.055	0.77	-0.11	1.64	0.085
Beta-hydroxybutyrate, 100 µmol/L	1.51	0.03	2.98	**0.045**	1.07	-0.35	2.48	0.14
Acetoacetate, 10 µmol/L	0.21	-0.08	0.50	0.16				
Acetone, 10 µmol/L	0.46	-0.18	1.10	0.16				

β: unstandardized regression coefficient. P-values <0.05 in ***bold*** *print.*

[a] Each ketone body was modelled 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 at baseline and HbA_{1c} at 4 months follow-up.

Abbreviations: CI, confidence interval; HbA_{1c}, glycosylated hemoglobin; KBs, ketone bodies; NT-proBNP, N-terminal pro-b-type natriuretic peptide; PCI, percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction.

Supplementary Table 4 | Age- and sex-adjusted and multivariable associations of plasma KBs with log NT-proBNP at 4 months follow-up

	Age- and sex-adjusted				Multivariable [a]			
	β	95% CI lower limit	95% CI upper limit	*P*-value	β	95% CI lower limit	95% CI upper limit	*P*-value
Total KBs baseline, 100 µmol/L	0.02	0.01	0.04	**0.003**	0.01	-0.005	0.02	0.23
β-OHB baseline, 100 µmol/L	0.03	0.01	0.05	**0.005**	0.01	-0.01	0.03	0.24
Total KBs 24h, 100 µmol/L	0.12	-0.02	0.26	0.094	0.07	-0.05	0.20	0.26
β-OHB 24h, 100 µmol/L	0.13	-0.06	0.32	0.183	0.10	-0.07	0.28	0.24
Total KBs 4 months, 10 µmol/L	0.12	0.004	0.23	**0.042**	0.13	0.03	0.23	**0.013**
β-OHB 4 months, 10 µmol/L	0.15	-0.03	0.33	0.102	0.18	0.02	0.35	**0.025**

*β: unstandardized regression coefficient. P-values <0.05 in **bold** print.*

[a] Each KB was modelled separately and adjusted for age, sex, metformin treatment, BMI, TIMI-flow pre PCI, TIMI-flow post-PCI, myocardial blush grade and anterior myocardial infarction (defined as culprit in left anterior descending coronary artery), ischemic time, statin use at baseline (for KB at baseline and 24 hours) and log NT-proBNP at baseline.

Abbreviations: BMI, body mass index; β-OHB, Beta-Hydroxybutyrate; CI, confidence interval; h, hours; KB, ketone bodies; NT-proBNP, N-terminal pro-b-type natriuretic peptide; PCI, percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction.

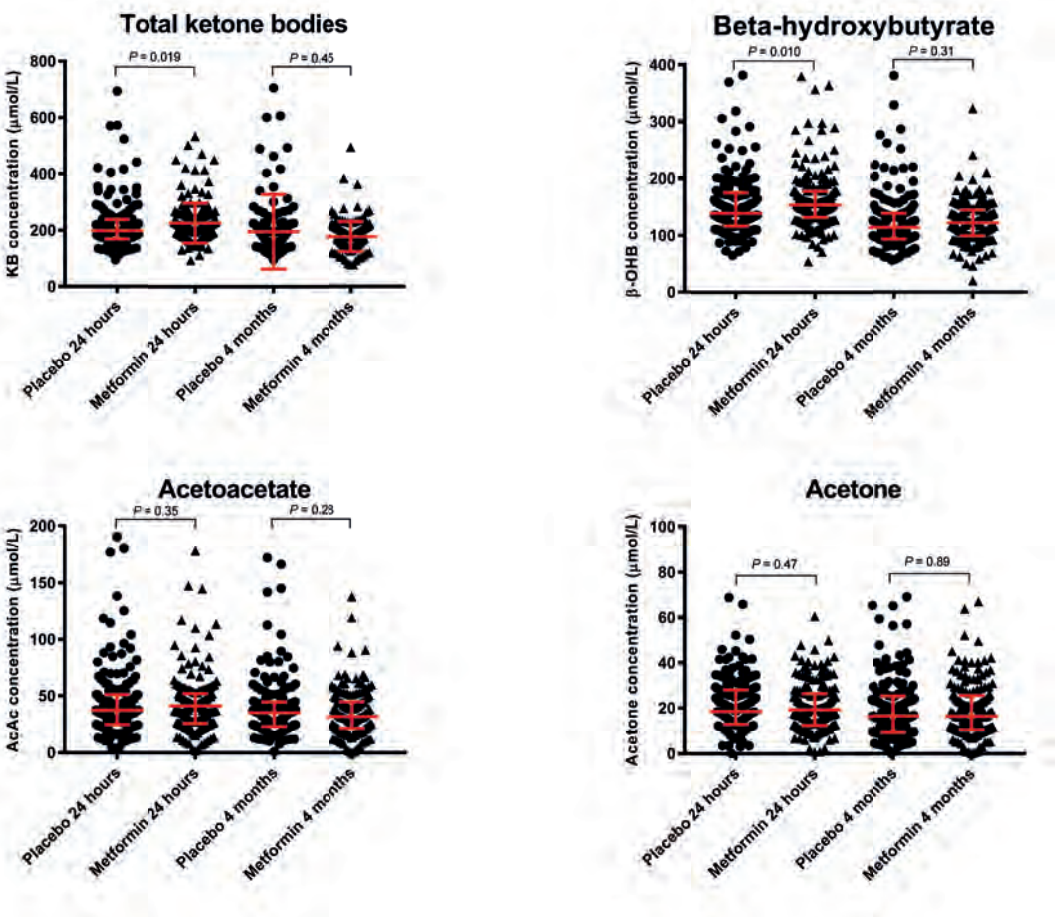
Supplementary Table 5 | KB levels (µmol/L) per treatment strata, depicted as median and IQR

	Placebo n=188	**Metformin n=191**	***P*-value**
Admission			
Total KBs	528 [317-933]	519 [315-1084]	0.81
Beta-hydroxybutyrate	362 [219-683]	376 [232-758]	0.65
Acetoacetate	111 [59-203]	106 [49-229]	0.96
Acetone	35 [20-67]	39 [20-75]	0.73
24 hours post-PCI			
Total KBs	198 [170-238]	212 [184-248]	**0.019**
Beta-hydroxybutyrate	139 [117-175]	154 [132-178]	**0.010**
Acetoacetate	37 [25-51]	41 [26-52]	0.35
Acetone	19 [13-28]	19 [13-27]	0.47
4 months post-PCI			
Total KBs	163 [141-196]	168 [145-205]	0.45
Beta-hydroxybutyrate	114 [94-139]	122 [99-145]	0.31
Acetoacetate	36 [23-45]	32 [21-45]	0.28
Acetone	16 [9-25]	16 [10-25]	0.89

Wilcoxin rank-sum test

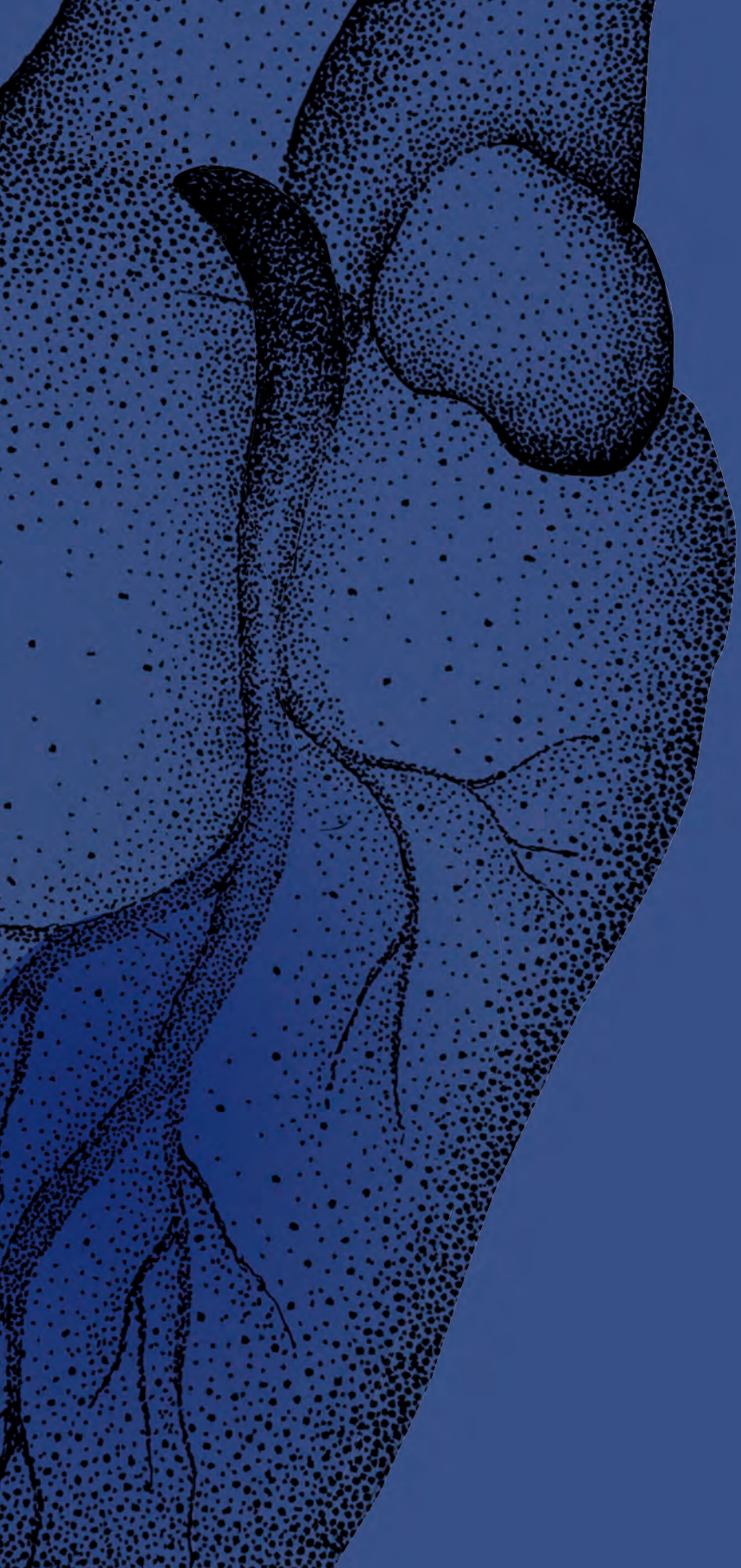
*P-values <0.05 are **bold** printed*

Abbreviations: IQR, interquartile range; KBs, ketone bodies, PCI, percutaneous coronary intervention.



Supplementary Figure 1 | KBs stratified by treatment allocation

Concentrations of circulating KBs at 24 hours and 4 months follow-up stratified by treatment allocation, depicted as median and interquartile range in red. Abbreviations: AcAc, acetoacetate; β-OHB, Beta-Hydroxybutyrate; KBs, ketone bodies



CHAPTER 2

Ketone Bodies: Universal Cardiac Response to Stress?

Complimentary editorial

Salva R. Yurista, Anthony Rosenzweig, Christopher T. Nguyen

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Over the past decade, there has been significant interest in the role of ketone bodies (KBs) both within and outside the medical arena because of their reported beneficial effects in the heart.[1] In a fed state, circulating KB (mainly acetone, acetoacetate, β-hydroxybutyrate) concentrations are low and elevation of KBs occurs during prolonged fasting or when consuming a ketogenic diet or ketone supplements (eg, 1,3-butanediol, medium-chain triglyceride, ketone salts, ketone ester). Ketogenesis, a metabolic pathway that produces KBs, can also be augmented by lipolytic hormones (eg, glucagon, cortisol, and catecholamines) through their actions on adipocytes, which yields more release of free fatty acids to be used in the ketogenic pathway.[1,2]

The heart is generally recognized as a "metabolic omnivore" with the ability to oxidize different energy substrates to generate adenosine triphosphate and shift from one fuel source to another, depending on metabolic demand, neurohormonal status, and substrate availability. Accumulating evidence suggests that metabolic perturbations contribute to the progression of heart failure (HF), and the failing heart reprograms energy metabolism towards increased KB use.[3–6] Likewise, increasing delivery of readily processed fuels in the form of KBs (through supplementation or other means) results in augmented myocardial ketone oxidation and/or enhanced ventricular function.[3,4,7–9] These findings can be explained by the expected role of KBs as "energy-efficient" substrates and potentially by pleiotropic effects of KBs in the heart.[1,2] Increased ketogenesis with sodium-glucose co-transporter inhibitors is considered a possible mechanism that contributes to their cardiovascular benefits.[10]

KBs require lower amounts of oxygen per molecule of adenosine triphosphate generated than that of fatty acid oxidation, and therefore, may provide supplemental fuel for the failing heart to overcome bioenergetic insufficiency, although the efficiency is not higher than glucose. The debate over whether KBs represent a significant contributor to overall cardiac bioenergetics is still ongoing. A recent study demonstrated that KBs contribute to approximately 6% of total cardiac adenosine triphosphate production in healthy subjects and that this percentage can increase 3-fold in the failing heart. The uptake of ketones in HF with a preserved (HFpEF) and reduced (HFrEF) ejection fraction at least, in part, depends on circulating concentrations of KBs.[11]

Myocardial infarction (MI) remains the most common cause of HFrEF, and cardiac metabolic changes occurring during ischemia likely contribute to HF development.[12] Although previous studies of KBs have focused mainly on HF, their role in MI is unclear. In this issue of the *Journal*, de Koning *et al*[13] explore changes in KBs in the setting of ischemia/reperfusion. Their study was conducted in 369 patients without diabetes who presented with ST-segment elevation myocardial infarction (STEMI) and who were enrolled in a prospective, single-center, double-blind, randomized placebo-controlled trial of early metformin treatment after STEMI called the GIPS-III (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) trial. First, the investigators sought to determine the longitudinal changes in circulating

ketone concentrations. Using nuclear magnetic resonance spectroscopy, circulating KBs were measured at presentation, 24 hours after primary percutaneous coronary intervention, and 4 months after STEMI presentation. The investigators discovered that the patients had elevated total KBs at presentation, which remained elevated at 24 hours after percutaneous coronary intervention. Moreover, plasma KBs concentration at admission were not correlated with culprit vessel location or the ischemic time window. Although there were no non-STEMI, healthy, or disease control subjects included, KBs levels were compared with the concentrations at 4-month follow-up. Next, the investigators evaluated functional outcomes, namely, MI size and left ventricular ejection fraction (LVEF) using cardiac magnetic resonance at 4 months for associations. Elevated total KBs and β-hydroxybutyrate concentrations at 24 hours after percutaneous coronary intervention were independently associated with both larger infarct size and lower LVEFs.

These findings form an essential basis for our understanding of the role of KBs in ischemia/reperfusion. Increased circulating ketone concentrations and/or myocardial ketone oxidation that were associated with poor functional outcome were also reported in other clinical contexts, including HFrEF, HFpEF, diabetes, and arrhythmogenic cardiomyopathy.[3,5,6,14–17] Previous studies discovered that acute myocardial ischemia and infarction immediately induced catecholamine release, and catecholamine excess was recognized as an important mechanism in the progression of HF and cardiomyopathy.[18] Because catecholamines exert lipolytic activity on adipocytes, catecholamine surge may contribute to increased KB concentrations. In HF, increases in inflammatory cytokines and natriuretic peptides may also stimulate ketogenesis.[2] Taken together, these observations suggest a ketogenic shift is a common cardiac response to stress (**Figure 1**). In addition, increased KB concentrations may serve as biomarkers that are associated with disease severity at least in some contexts. Although the current study was designed to reveal associations and did not test the functional effects of KBs after STEMI, there are multiple reasons to believe modulating cardiac ketone oxidation by increasing KBs availability could have therapeutic benefits in STEMI. These include the well-documented and substantial changes that occur in cardiac energy metabolism during ischemia, the supplemental metabolic substrate and potential pleiotropic cardiac effects of KBs, and the positive impact of circulating KB levels on cardiac ketone oxidation rates.

The investigators are to be praised for performing meticulous collection, analyses, and interpretation of the data, as well measurement of KB concentrations in fed subjects without diabetes to avoid the confounding effects of diabetes and overnight fasting. However, as acknowledged by the investigators, this study was limited by a low prevalence left ventricular dysfunction and relatively small infarcts; therefore, it might not generalizable to a broader population. Future research with larger sample sizes and an appropriate control group could fruitfully validate the kinds of conclusions that can be drawn from this study. Furthermore, incorporation of direct measurement of cardiac arterio-venous KB gradients into future studies would enable assessment of human KB use and production in STEMI, providing additional insights into the mechanisms underlying dynamic regulation of this important metabolic pathway in STEMI.

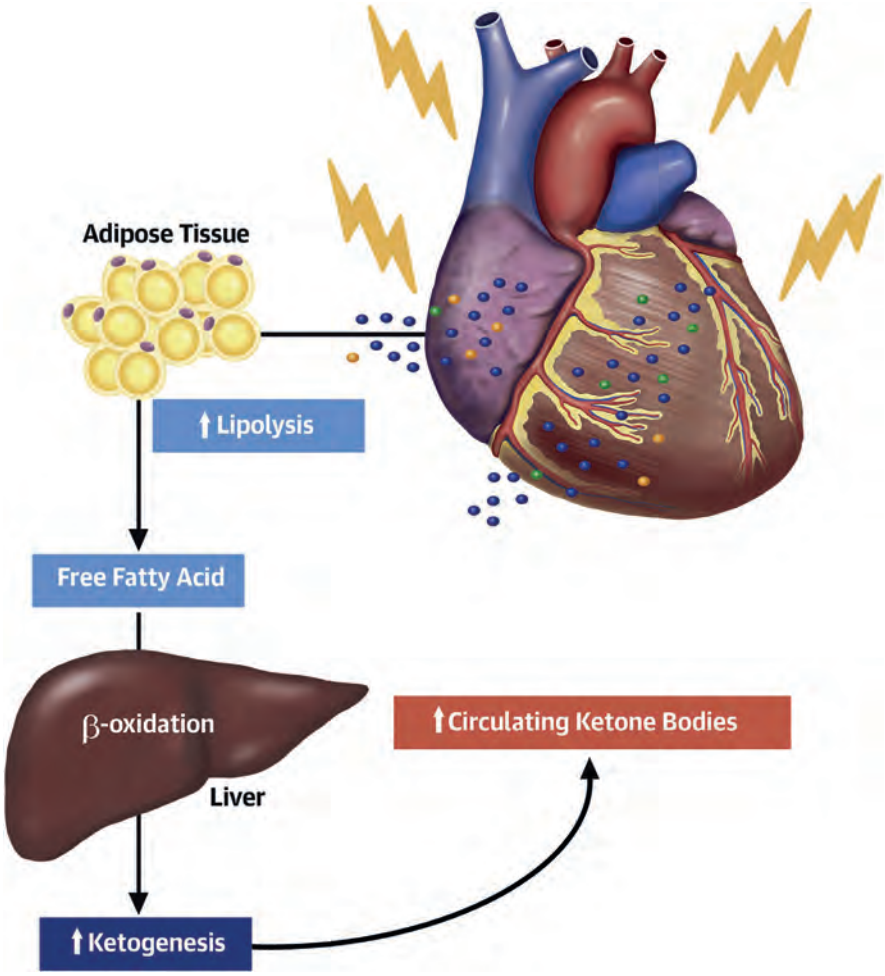

Figure 1 | Ketogenic Shift Is a Universal Cardiac Response to Stress

When different cardiac stressors exist (e.g., in myocardial ischemia and/or infarction, heart failure), the heart will release neurohormones (e.g., catecholamines), cytokines, and natriuretic peptides, which can act synergistically, inducing lipolysis on adipocytes and result in increased ketogenesis.

Recent work such as the current study by de Koning *et al*[13] has heightened scientific interest in KBs and their potential cardiac benefits. Although the appeal of enhancing KBs as a therapeutic approach is understandable, additional rigorous preclinical and clinical studies will be required to test this therapeutic hypothesis and determine the mechanisms contributing to any benefits observed. Ongoing clinical trials have the potential to provide new data addressing these important questions and could shed considerable light on the role of KBs in cardiovascular disease and their potential as therapeutic targets.

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