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RESEARCH ARTICLE

Ketone monoester ingestion improves cardiac function in adults with type 2 diabetes: a double-blind, placebo-controlled, randomized, crossover trial

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Abstract

Type 2 diabetes (T2D) is a metabolic disease associated with cardiovascular dysfunction. The myocardium preferentially uses ketones over free fatty acids as a more energy-efficient substrate. The primary aim was to assess the effects of ketone monoester (K_{me}) ingestion on cardiac output index ($\dot{Q}$i). The secondary aims were to assess the effects of K_{me} ingestion on markers of cardiac hemodynamics, muscle oxygenation, and vascular function at rest, during and following step-incremental cycling. We undertook a double-blind, randomized, crossover design study in 13 adults [age, 66 ± 10 yr; body mass index (BMI), 31.3 ± 7.0 kg·m^{-2}] with T2D. Participants completed two conditions, where they ingested a K_{me} (0.115 g·kg^{-1}) or a placebo taste-matched drink. Cardiac function was measured using thoracic impedance cardiography, and muscle oxygenation of the calf was determined via near-infrared spectroscopy. Macrovascular endothelial function was measured by flow-mediated dilation (FMD), and microvascular endothelial function was measured via transdermal delivery of acetylcholine (ACh) and insulin. Circulating β-hydroxybutyrate [β-Hb] was measured throughout. K_{me} ingestion raised circulating β-Hb throughout the protocol (peak 1.9 mM; $P = 0.001$ vs. placebo). K_{me} ingestion increased $\dot{Q}$i by 0.75 ± 0.5 L·min^{-1}·m^{-2} ($P = 0.003$), stroke volume index by 7.2 ± 4.5 mL·m^{-2} ($P = 0.001$), and peripheral muscle oxygenation by 9.9 ± 7.1% ($P = 0.001$) and reduced systemic vascular resistance index by −420 ± −225 dyn·s^{-1}·cm^{-5}·m^{-2} ($P = 0.031$) compared with the placebo condition. There were no differences between K_{me} and placebo in heart rate ($P = 0.995$), FMD ($P = 0.542$), ACh max ($P = 0.800$), and insulin max ($P = 0.242$). Ingestion of K_{me} improved $\dot{Q}$i, stroke volume index, and peripheral muscle oxygenation but did not alter macro- or microvascular endothelial function in people with T2D.

NEW & NOTEWORTHY For the first time, we show that acute ketone monoester ingestion (K_{me}) can increase cardiac output and stroke volume and reduce systemic vascular resistance at rest and during exercise in sodium glucose transporter inhibitors naïve (i.e. no drug-induced ketosis) people with type 2 diabetes. Acute K_{me} ingestion improves peripheral skeletal muscle oxygenation during moderate intensity and maximal exercise. K_{me} has no effect on macro- or microvascular endothelial function in people with type 2 diabetes.

β-hydroxybutyrate; cardiovascular hemodynamics; diabetes; exercise; muscle oxygenation

INTRODUCTION

Type 2 diabetes (T2D) is a chronic, progressive metabolic disease, with the epidemic expected to reach ∼630 million people globally by 2045 (1). Chronic hyperglycemia leads to endothelial dysfunction within both the macro- and microvasculature (2). T2D contributes to 11.3% of deaths globally, of which cardiovascular disease (CVD) remains the leading cause of mortality (3). Indeed, people with T2D have a significantly increased risk of myocardial infarction, ischemic heart disease, and heart failure (4). The initial treatment for T2D includes diet and exercise advice, and if these strategies fail to reverse the decline in glucose control, pharmaceutical therapy is indicated (5). Physical activity,

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particularly that which is moderate-to-vigorous in intensity, can reduce CVD risk; however, adherence remains low (6). Therefore, alternative or additive strategies to improve the cardiovascular health of people with T2D are needed.

Physiologically, people with T2D reportedly find physical activity and exercise difficult (7). From a physiological perspective, people with T2D are characterized by attenuated increases in cardiac output ($\dot{Q}$) during exercise (8) and endothelial dysfunction (9), both of which are factors associated with an increased oxygen cost of exercise (10) and impaired muscle oxygen delivery (11). Insulin resistance, which characterizes T2D, decreases the individual's capacity to use glucose. Glucose is the most efficient energy substrate, providing 2.58 units of adenosine triphosphate (ATP) per molecule of oxygen and, instead, insulin resistance increases the reliance on the less metabolically efficient free fatty acids (FFAs; 2.33 units of ATP per molecule of oxygen) (10). Interestingly, a "thrifty substrate" hypothesis has been proposed for people with T2D, where myocardial substrate metabolism shifts toward the more energy-efficient ketone bodies (2.50 units of ATP per unit of O_2) (12). This is supported by evidence in human hearts (13) and people with T2D and heart failure with preserved ejection fraction (HFpEF) (14), where ketone became the major fuel source for the heart after infusion of ketone bodies (3-hydroxybutyrate; β-Hb).

Interestingly, a recent study demonstrated that acute β-Hb infusion in people with heart failure increased $\dot{Q}$ and stroke volume (SV) (15). Although infusion of β-Hb has provided a promising physiological insight, as a viable treatment option, it is an impractical solution with minimal ecological validity. Exogenous ketones, however, can also be ingested, typically in the form of the ketone monoester (K_{me}) (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate, and have been shown to rapidly increase blood [β-Hb] within 30 min in healthy people (16) and people with heart failure (15). Indeed, a recent study in a cohort of people with T2D and HFpEF demonstrated that K_{me} ingestion improves cardiac function (14). However, the majority of the cohort (62%) were on sodium-glucose transporter (SGLT2) inhibitor treatment, a glucose-lowering drug that induces endogenous ketosis (17) and improves cardiovascular function (18). Although these results are promising for the use of K_{me} ingestion as an add-on cardiovascular treatment, ∼5%–10% of people with T2D are intolerant to SGLT2 inhibitors (19) and to date, there are no studies assessing the cardiovascular effect of K_{me} ingestion in people with T2D, who are SGLT2 inhibitor naïve.

The primary aim of the current study was to investigate the acute effects of K_{me} ingestion on $\dot{Q}$, at rest, during step-incremental cycling exercise, and during recovery in adults with T2D, not on SGLT2 inhibitors treatment. Secondary outcomes aimed to investigate the acute effects of K_{me} ingestion on vascular function, peripheral muscle (de)oxygenation, and ventilation at rest, during step-incremental cycling exercise and recovery in this cohort. We hypothesized that K_{me} ingestion would improve central hemodynamics, vascular function, peripheral muscle (de)oxygenation, and ventilation in adults with T2D.

METHODS

Ethical Approval

The present study used a double-blind, placebo-controlled, randomized, crossover study design to assess the effects of acute ingestion of K_{me} on cardiovascular and metabolic parameters at rest, during step-incremental cycling exercise, and during the postexercise recovery period, in adults with T2D. The experimental design comprised an initial screening visit, followed by two experimental conditions, completed in a randomized order. Participants were recruited from primary care and local databases (see Fig. 1). Favorable ethics opinion was granted by the Berkshire A NHS Research Ethics Committee (20/SC/0055) and Health Research Authority, and the study was preregistered on the ClinicalTrials.gov website (NCT04854330). All experimental testing took place in laboratories within the School of Sport, Health and Exercise Science at the University of Portsmouth, UK.

Participant Characteristics

In total, 13 adults (6 males and 7 females) with T2D [according to the World Health Organization (WHO) criteria], with glycated hemoglobin ($HbA1_c$) > 48 ($mmol \cdot mol^{-1}$), were recruited. All female participants were postmenopausal. Participants were excluded if they had significant renal impairment (estimated glomerular filtration rate <30 $mL \cdot min^{-1} \cdot 1.73\ m^2$), uncontrolled hypertension (systolic blood pressure; SBP >180 mmHg), body mass index (BMI) >40 $kg \cdot m^{-2}$, history of myocardial infarction or cerebrovascular events, were contraindicated to exercise, currently taking SGLT2i (i.e., empagliflozin and dapagliflozin) (20) and/or GLP-1 receptor agonists, and were on a low- carbohydrate ketogenic diet or unwilling or unable to provide fully written informed consent.

Our primary outcome was the difference in cardiac output index ($\dot{Q}i$) between conditions. An a priori sample size calculation was performed to estimate the required *n*. For 80% power with an a-level set at $P < 0.05$ (two-tailed) to detect a 1 SD difference [similar to previous studies that used a similar design, outcome measure, and the same device (21)], 13 people were required for this study. To account for dropouts, we aimed to recruit 16 adults with T2D.

Pretest Requirements

Participants attended a baseline screening visit, during which safety assessments, including resting electrocardiogram, and screening blood ($HbA1_c$, full blood count, liver, and estimated glomerular filtration rate), were undertaken, followed by familiarization with the testing environment and assessments required for study involvement. Participants were subsequently asked to continue their regular medication treatment and to record their diet and physical activity using self-reported diaries for the 24-h period before each experimental visit. For visit two, they were asked to repeat their diet and physical activity behaviors from their first experimental visit. Before each experimental visit, participants were also required to refrain from alcohol, smoking, and strenuous exercise for 24 h and caffeine for 12 h before each visit. Participants were fasted overnight and consumed a standardized breakfast [2 granola bars (crunchy oats and honey, Nature Valley, UK; 42 g

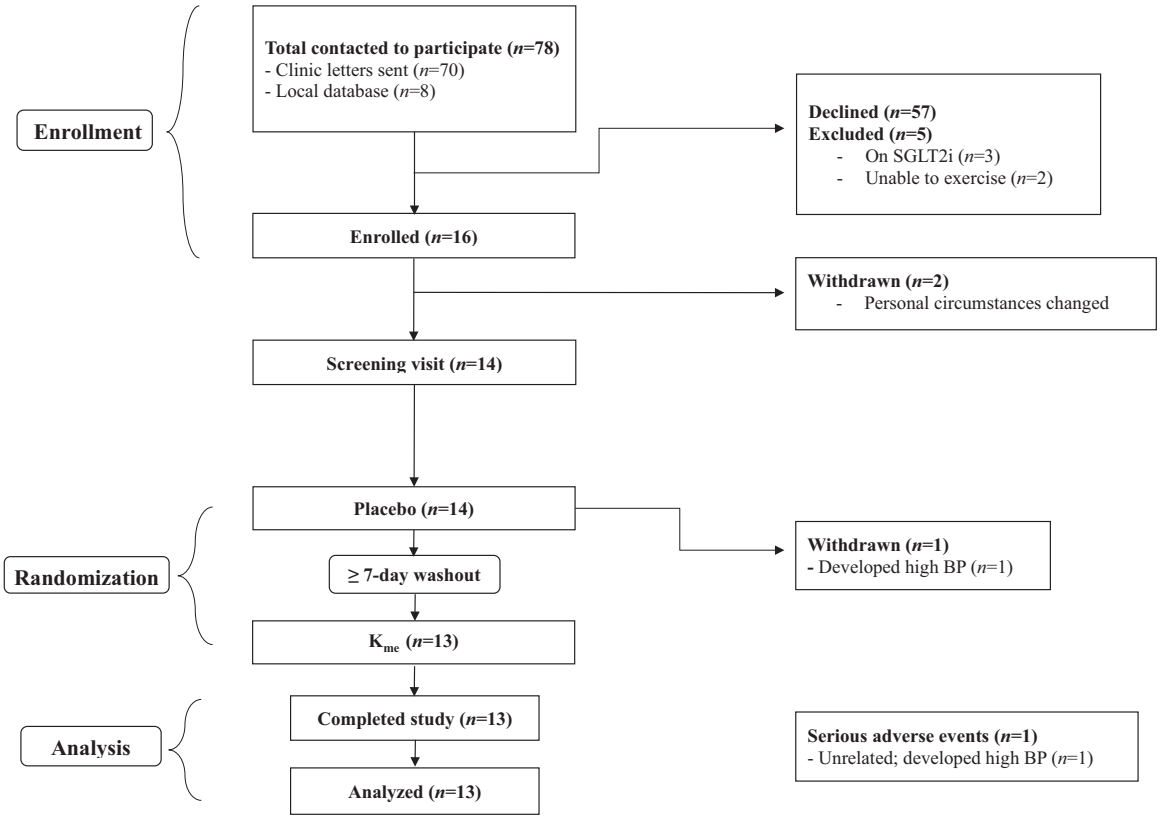

Figure 1. Participant consort flowchart. BP, blood pressure; K_{me}, ketone monoesters, SGLT2i, sodium glucose transporter inhibitors.

total, fat 7.2 g, sugars 11.9 g)] 3 h before each visit. All experimental visits occurred in the morning (8:30 AM ± 1 h start).

Experimental Visits

On each experimental visit (see Fig. 2), participants consumed either a K_{me} or a placebo supplement (drink) in a randomized order. Based on current literature, transient rises in circulating [β-Hb] are back to baseline within 24 h (22). Each visit was separated by a minimum of a 7-day washout to ensure that there were no remaining circulating [β-Hb] and that participants were fully recovered from the exercise. At the start of the protocol, an indwelling intravenous catheter (BD Nexiva, Becton Dickinson Infusion Therapy Systems Inc., Sandy, UT) was inserted into the antecubital vein for repeated blood sampling throughout the protocol. Thirty-minute postingestion (23) and supine rest, participants underwent measures of cardiac function, microvascular and macrovascular endothelial function, peripheral muscle (de)oxygenation, and pulmonary gas exchange and ventilation. Central hemodynamics were noninvasively measured and blood samples were collected at rest, during cycling exercise and recovery (5 min), whereas near-infrared spectroscopy (NIRS), pulmonary gas exchange, and ventilation measures were recorded at rest and during exercise. Assessment of micro- and macrovascular endothelial function was undertaken at rest (pre-exercise) and 15 min following cycling exercise to volitional exhaustion. Experimental procedures are described in detail below.

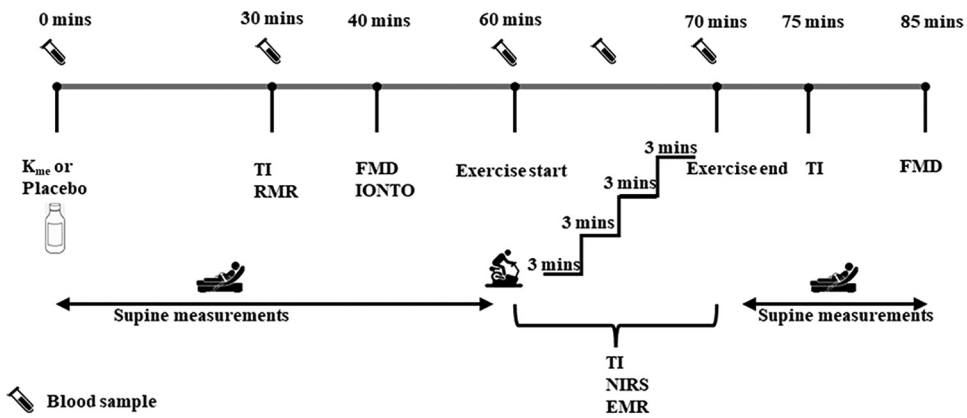

Figure 2. Experimental protocol timeline. EMR, exercise metabolic rate; FMD, flow-mediated dilation; IONTO, iontophoresis; RMR, resting metabolic rate; TI, thoracic impedance cardiography.

Supplementation

Diabetic ketoacidosis is a serious consideration when designing ketone studies in people with impaired insulin function (24). We therefore chose to use the lowest possible dose that we felt could induce ketosis (13). A commercially available K_{me} supplement, in the form of I-3-hydroxybutI(*R*)-3-hydroxybutyrate [HVMN Ltd, Florida; 0.115 $g \cdot kg^{-1}$ (0.3 $mL \cdot kg^{-1}$) body mass] and a color, volume, and taste-matched placebo drink [containing ketone-placebo-flavor-mix (HVMN), bitter flavor (denatonium benzoate, Bittrex) and water] were poured into identical amber nontransparent glass bottles. Concealed allocation, undertaken by an independent laboratory technician, was used to blind participants and assessors to their randomized order of the K_{me} and placebo conditions. Specifically, a computer program (https://www.randomizer.org) was used to randomly allocate participants in order of conditions 1:1.

Exercise Protocol

Participants performed a step incremental cycling test on an electronically braked cycle ergometer (Lode Corival, Groningen, The Netherlands) until volitional exhaustion. It is likely that not all participants made it to a maximal effort; however, this was not the focus of the study. Initially, participants performed 3 min of unloaded (0 W) baseline cycling at 50 revolutions per minute (rpm), after which the work rate increased in a stepwise manner to complete 3-min steady-state constant work rate transitions. Step increments of 10 W, 20 W, or 30 W were used, based on the familiarization session of each participant, established based on expected fitness and gas exchange responses from familiarization. The rate of perceived exertion was measured every minute via a 6–20 Borg Scale (25). Volitional exhaustion was defined as the point at which participants were unable or willing to maintain the required cadence for >5 consecutive seconds despite strong verbal encouragement or a respiratory exchange ratio (RER) ≥1.1. Moderate-intensity exercise was reported as the highest common stage (average resistance 45 ± 11 W) at which participants reached a steady-state $\dot{V}O_2$ response [i.e., below their gas exchange threshold (GET)].

Thoracic Impedance Cardiography

Central hemodynamics were noninvasively assessed using thoracic impedance cardiography at rest (supine and seated positions), during exercise, and during the postexercise recovery period (5-min supine rest), (Q-Link PhysioFlow, Manatec Biomedical, Poissy, France). Transthoracic bioimpedance quantifies the mechanical activity of the heart by measuring the high frequency alternating electrical current (66 kHz) of low magnitude (4.5 mA peak to peak) toward the thorax between the electrodes positioned on the neck and the electrodes positioned on the xiphoïd process. Impedance cardiography measures changes in transthoracic impedance during cardiac ejection to calculate SV, which is then multiplied by HR to provide an estimate of $\dot{Q}$ and has been validated against the "gold standard" of the direct Fick principle (26).

After careful skin preparation, six electrodes were positioned according to the manufacturers' guidelines; two over the carotid artery above the supraclavicular fossa, two anteriorly in the xiphoid region; and two in locations corresponding to the V1 and V6 positions used for conventional ECG monitoring. By detecting and measuring the difference of thoracic impedance over time, this system estimates measures of $\dot{Q}$, $\dot{Q}$i ($\dot{Q}$ corrected for body surface area (BSA= 0.007184 × (Height (cm) 0.725) × (Weight (kg) 0.425))), SV and SV normalized to BSA (SVi), contractility index (CTI) (the SBP to end-systolic volume ratio), heart rate (HR) ventricular ejection time (VET), early diastolic filling ratio (EDFR); ejection fraction (EF), end-diastolic volume (EDV), left cardiac work index (LCWi), systemic vascular resistance (SVR), and SVR normalized to BSA (SVRi).

Macrovascular Endothelial Function

Macrovascular endothelial function was assessed by measuring brachial artery flow-mediated dilation (FMD) at rest, 30-min postingestion, and 15-min postexercise. FMD was measured in the supine position on the right arm, with the cuff placed distal to the olecranon process. A 10-MHz multifrequency linear array ultrasound probe was attached to a duplex ultrasound machine (Micromaxx, Bothell, WA) and used to image the brachial artery in the distal third of the upper arm (see Online Supplement; Supplemental Fig. S1). The Doppler angle of insonation was maintained at 60°. Following 60 s of diameter recording, the cuff was rapidly inflated to 220 mmHg (Moor VMS-PRES, Moor Instruments, UK), and inflation was maintained for 5 min. Diameter recording resumed 30 s before rapid cuff deflation and continued for 3 min thereafter.

Microvascular Endothelial Function

Acetylcholine (ACh) and insulin (INS) were delivered transdermally via iontophoresis to the volar aspect of the right forearm, 30-min postingestion of each drink. In brief, following cleaning the skin surface with water for injection, two perspex rings were attached to the skin, with one acting as an anode and the other the cathode. These electrodes were connected to the iontophoresis controller (MIC 2, Moor Instruments, UK). The anode chamber was filled with ∼0.5 mL of ACh (Sigma-Aldrich), with [1%] dissolved in water for injection. The cathode chamber was filled with ∼0.5 mL of INS (Humulin, Eli Lilly) with [0.01%] dissolved in water for injection. The protocol for electrical pulses included: four pulses at 25 μA, followed by a single pulse of 50 μA, 100 μA, 150 μA and, finally, 200 μA. These pulses lasted for 20 s, with 120-s intervals between each pulse, during which no current was applied. Laser Doppler probes (VP1T/7, Moor Instruments) connected to a perfusion monitor (Moor VMS-LDF, Moor Instruments) were used to assess cutaneous vascular conductance (CVC) throughout.

Peripheral Muscle Oxygenation

Oxygenation status of the *m. gastrocnemius medialis* was measured continuously, at 10 Hz, using a commercially available near-infrared spectroscopy system (NIRS; Artinis Portamon, Elst, The Netherlands) during seated rest and step-incremental cycling exercise. The NIRS device samples the microcirculation of subcutaneous tissue at a depth of 2–3 cm. Given that the present cohort was overweight and

individuals with obesity [BMI: 31 ± 7 kg·m^2 (Table 1)] and to avoid capturing data with a low signal-to-noise ratio, the site of *m. gastrocnemius medialis* was chosen as the area of interrogation, as it presents with lower subcutaneous adipose tissue compared with the commonly used *m. vastus lateralis*. This is in line with previous reports in obese and individuals with T2D (27). To ensure consistent placement between individuals and visits, the probe was placed over the belly of *m. gastrocnemius medialis* (10 cm inferior to the tibial tubercle and 10 cm lateral to the anterior tibial crest), the distance from the medial malleolus to the center of the probe, and the distance from the anterior border of the tibia to the center of the probe were measured. After marking the NIRS probe placement area, the placement site was initially cleaned and shaved. The probe was secured with tape (Kinesio Tex) and a dark elastic bandage to minimize extraneous light interference with the near-infrared signal. The light source and detector of the NIRS have the capacity to determine scaled absolute values of [oxygenated hemoglobin + myoglobin] (O_2Hb) and [deoxygenated, hemoglobin + myoglobin] (HHb) in tissue [Lindkvist and Grönlund (28)]. The tissue saturation index (TSI%) and total hemoglobin [tHb] bound to oxygen in the microcirculation of the volume of tissue being studied were also derived.

Pulmonary Gas Exchange and Ventilation

Breath-by-breath changes in pulmonary gas exchange and ventilation were noninvasively measured (Clinical Metabolic Cart, COSMED Ltd, Rome, Italy) for 5 min at rest in the supine position and throughout the cycling exercise. Before each measurement, gas and volume calibrations were performed as per the manufacturer's guidelines. Of the available parameters, the focus herein was on minute ventilation ($\dot{V}_E$), pulmonary oxygen uptake ($\dot{V}_{O_2}$), and carbon dioxide production ($\dot{V}_{CO_2}$).

Table 1. *Participant characteristics*

Variable	Means ± SD or %
Age, yr	65.5 ± 10.0
Men, *n* (%)	6 (46)
Height, cm	168.3 ± 0.2
Body mass, kg^{-1}	89.9 ± 28
BMI, kg·m^2	31.3 ± 7.0
Waist:hip ratio	0.9 ± 0.1
Race	
Caucasian	11 (85)
South Asian	2(15)
Clinical information	
$HbA1_c$, mmol·mol^{-1}	51.3 ± 2.9
Diabetes duration, yr	9.3 ± 8
Systolic BP, mmHg	148.7 ± 21
Diastolic BP, mmHg	83.3 ± 11
Medication	
Metformin, %	(6) 46.2
Insulin, %	(2) 15.4
ACEi and ARB, %	(7) 53.9
Statins, %	(8) 61.5

Data are expressed as means ± SD or as a percentage unless otherwise stated. $n = 13$. ACEi, ACE inhibitors; ARB, angiotensin receptor blockers; BMI, body mass index; BP, blood pressure; HbA1c; glycated hemoglobin.

Biochemical Analysis

Circulating [β-Hb] and troponin-T (TNT) were measured pre- and postingestion of K_{me} or placebo (at 30-min postingestion; at the end of each stage of moderate-intensity cycling exercise and during the 5-min postexercise recovery period). Measures were assessed in duplicate using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Biomatik, Ontario, Canada). Assays were analyzed via a plate reader (SpectraMax i3x, Molecular Devices, UK). Circulating blood [glucose] and [lactate] were measured using an automated glucose and lactate analyzer at 30-min postingestion, during exercise and exercise recovery (Biosen C-Line, EKF, Cardiff, UK).

Data Handling and Analysis

Thoracic impedance cardiography.

Data were recorded continuously (at 1-s intervals) at rest (supine and seated upright), during step-incremental cycling exercise, and postexercise recovery. Before each measurement, signal quality verification was performed, and data points with signal quality <90% were excluded as per the manufacturer's guidelines. The final 30 s of each phase [supine rest; seated rest; moderate-intensity cycling; max stage (highest common maximal stage reached by the participants); recovery (5-min postexercise)] were averaged.

Macrovascular endothelial function.

Analysis of brachial artery diameter and time to peak (TTP) dilation was performed using a custom-designed edge-detection and wall-tracking software that is largely independent of investigator bias (Cardiovascular Suite; Quipu, Pisa, Italy). FMD was calculated using *Eq. 1* and expressed as the percentage change in vessel diameter.

$$(\text{peak diameter} \times \text{baseline diameter})/\text{baseline diameter}. \quad (1)$$

Microvascular endothelial function. Forearm microvascular endothelial function was assessed via iontophoresis, with data recorded using an acquisition system (PowerLab, AD Instruments, Australia) and software (LabChart 7, ADInstruments, Australia). Skin blood flow responses were calculated using *Eq. 2* and expressed as CVC.

$$\text{CVC} = \text{skin flux}/\text{mean arterial pressure; flux} \cdot \text{mmHg}^{-1}. \quad (2)$$

The average skin blood flow for both ACh and INS was calculated over the final 20 s of the intervals between each successful pulse (i.e., 100–120 s following each pulse) (29). Maximal skin blood flow (i.e., the peak CVC) and area under the curve (AUC) were calculated for each participant. BP was measured on the contralateral arm to the site of iontophoresis using an automated BP monitor (Omron M5, Omron, Kyoto, Japan) before and after each iontophoresis protocol to calculate mean arterial pressure (MAP).

Peripheral muscle oxygenation.

Changes between conditions in [TSI], [O_2Hb], and [HHb] were recorded continuously at rest (supine and seated) and during exercise. NIRS-derived data were measured at a frequency of 10 Hz and averaged into 1-s time bins. Subsequently, a 30-s average at the end of each steady-

state phase was derived (supine rest; seated rest; moderate-intensity exercise and maximal exercise stage).

Pulmonary gas exchange and ventilation.
$\dot{V}O_{2peak}$ was determined as the highest 30-s average of $\dot{V}O_2$ during the test. The last 30 s of each phase (supine rest; seated rest; moderate-intensity exercise and maximal exercise stage) were averaged.

Statistical Analysis

Normality of the outcome data was established based on kurtosis and skewness analyses (30), and linearity of the data was established via a Kolmogorov–Smirnov test (31). A between-conditions (K_{me} vs. placebo), single-factor analysis of variance via a linear mixed model (LMM) was used to compare microvascular function at rest and peak oxygen consumption during the incremental cycling test. A two-factor LMM analysis was used to compare time (changes across each testing stage) and between conditions (K_{me} vs. placebo) for thoracic impedance variables (SV; SVi; $\dot{Q}$; $\dot{Q}$i; CTI; VET; EF; EDV, EDFR, LCWi, SVR; SVRi) cardiopulmonary parameters (minute ventilation; oxygen consumption; carbon dioxide production; respiratory exchange ratio; FMD, TTP dilation); TSI, [O_2Hb], [HHb], and blood biomarkers (β-Hb; TNT; blood glucose; lactate). Mean differences of the grand means are reported in figures and text. Statistically significant interactions and main effects were further investigated with multiple comparisons using Fischer's least significant approach (32). Analyses were conducted using SPSS (v. 28; IBM, Armonk, NY). and statistical significance was set at $P \leq 0.05$. Data are presented as means and 95% confidence interval (CI) unless otherwise stated.

RESULTS

Participants

In total, 16 participants consented to take part in this trial (Fig. 1; Table 1), with $n = 13$ completing the primary outcome. The range of the BMI among our cohort was 21.5–39.9 kg·m^{-2} with only four (30.7%) of our participants being considered within the healthy weight range (BMI < 25 kg·m^{-2}). One serious adverse event (unrelated to the trial) was reported; specifically, the hospital admission was due to hypertension. Of the three participants who did not complete the trial, one developed hypertension and the personal circumstances of the other two changed precluding them from taking part.

Effect of K_{me} Ingestion on Circulating [β-Hydroxybutyrate]

A condition × time interaction was observed for [β-Hb] ($P = 0.001$), with higher concentrations observed in the K_{me} versus placebo (mean difference: 1.3 mM; 95% CI: 1.1–1.3, $P = 0.001$; Fig. 3*A*). Post hoc comparisons demonstrated that, before ingestion, [β-Hb] levels were not different between K_{me} and placebo (mean difference: 0.08 mM; 95% CI: 0.02–0.12, $P = 0.987$). Circulating levels of [β-Hb] were higher following K_{me} ingestion compared with placebo at all timepoints of the experimental protocol and peaked at 30 min postingestion (mean difference: 1.9 mM; 95% CI: 1.8–2.0, $P = 0.001$).

Effect of K_{me} Ingestion on Central Hemodynamics

K_{me} ingestion increased $\dot{Q}$i (mean difference: 0.75 L·min^{-1}·m^{-2}; 95% CI: 0.26–1.25, $P = 0.003$; Fig. 3*B*), SVi (mean difference: 7.2 mL·m^{-2}; 95% CI: 3.8–10.5, $P = 0.001$; Fig. 3*C*), and decreased SVRi (mean difference: −420 dyn·s·cm^{-5}·m^{-2}; 95% CI: −134 to 706, $P = 0.004$; Fig. 3*D*) compared with placebo. K_{me} ingestion had no effect on HR ($P = 0.995$; Fig. 3*E*). No supplement × time interaction was observed for any of the hemodynamic parameters, although a main effect for time was evident (see Supplemental Table S1).

Post hoc comparisons revealed that, compared with placebo, K_{me} ingestion increased SVi during supine rest (mean difference: 10.4 mL·m^{-2}; 95% CI: −2.8 to 18.0, $P = 0.008$), during moderate-intensity cycling (mean difference: 4.0 mL·m^{-2}; 95% CI: 0.2–15.4, $P = 0.044$), and during exercise recovery (mean difference: 8.8 mL·m^{-2}; 95% CI: 1.2–16.3, $P = 0.024$). K_{me} ingestion increased CTI (mean difference: 70.3; 95% CI: 16.0–124.6, $P = 0.012$) at maximal exercise stage, whereas SVRi was lower during supine rest (mean difference: 887 dyn·s·cm^{-5}·m^2; 95% CI: −1,526 to −248, $P = 0.007$) and during postexercise recovery (mean difference: 847 dyn·s·cm^{-5}·m^{-2}; 95% CI: −1,486 to −208, $P = 0.010$) compared with placebo.

Effect of K_{me} on Macro- and Microvascular Endothelial Function

K_{me} had no effect on FMD (i.e., macrovascular endothelial function) during seated rest (mean difference: 0.7%; 95% CI: −1.7 to 3.3, $P = 0.542$; Fig. 4*A*) or during postexercise recovery (mean difference: 1.5%; 95% CI: 0.5–4.1, $P = 0.259$; Fig. 4*C*), and no effect on TTP dilation during seated rest (mean difference: 20.9 s; 95% CI: 5.9–47.7, $P = 0.121$; Fig. 4*B*) or postexercise recovery (mean difference: 9.5 s; 95% CI: −10.5 to 36.3, $P = 0.474$; Fig. 4*D*) compared with placebo.

K_{me} had no effect on resting microvascular, Ach-mediated, endothelial function in the forearm (ACh AUC mean difference: 0.35 flux·mmHg^{-1}; 95% CI: −0.75 to 1.45, $P = 0.502$; Fig. 4*E*; ACh Max; mean difference: 0.28; 95% CI: 0.27–0.21, $P = 0.800$; Fig. 4*F*) compared with placebo. K_{me} ingestion impaired insulin-mediated microvascular endothelial function compared with placebo for AUC (mean difference 0.21 flux·mmHg^{-1}; 95% CI: −0.38 to −0.51, $P = 0.014$; Fig. 4*G*) but not the maximum response (mean difference: 0.40 flux·mmHg^{-1}; 95% CI: −0.03 to 0.11, $P = 0.242$; Fig. 4*H*). K_{me} ingestion had no effect on resting systolic (mean difference: 1.8 ± 4.5 mmHg; 95% CI: 0.6–2, $P = 0.536$) and resting diastolic blood pressure (mean difference: 1.2 ± 3.7 mmHg; 95% CI: 0.4–1.6, $P = 0.742$).

Effect of K_{me} on Peripheral Muscle Oxygenation

K_{me} ingestion increased TSI% compared with placebo (mean difference: 9.9%, 95% CI: 4.3–15.4, $P = 0.001$). Post hoc analysis revealed that TSI% was higher during moderate-intensity cycling (mean difference: 9.8%; 95% CI: 2.3–20, $P = 0.045$) and max cycling (mean difference: 14.7%; 95% CI: 3.8–25.6, $P = 0.009$) compared with placebo. K_{me} ingestion had no effect on O_2Hb [mean difference: 0.21 arbitrary unit (A.U.); 95% CI: −1.7 to 2.1, $P = 0.812$] or [HHb] (mean difference: 0.47 A.U.; 95% CI: −2.8 to 1.8, $P = 0.691$). No significant interactions (supplement × time) were observed for muscle oxygenation

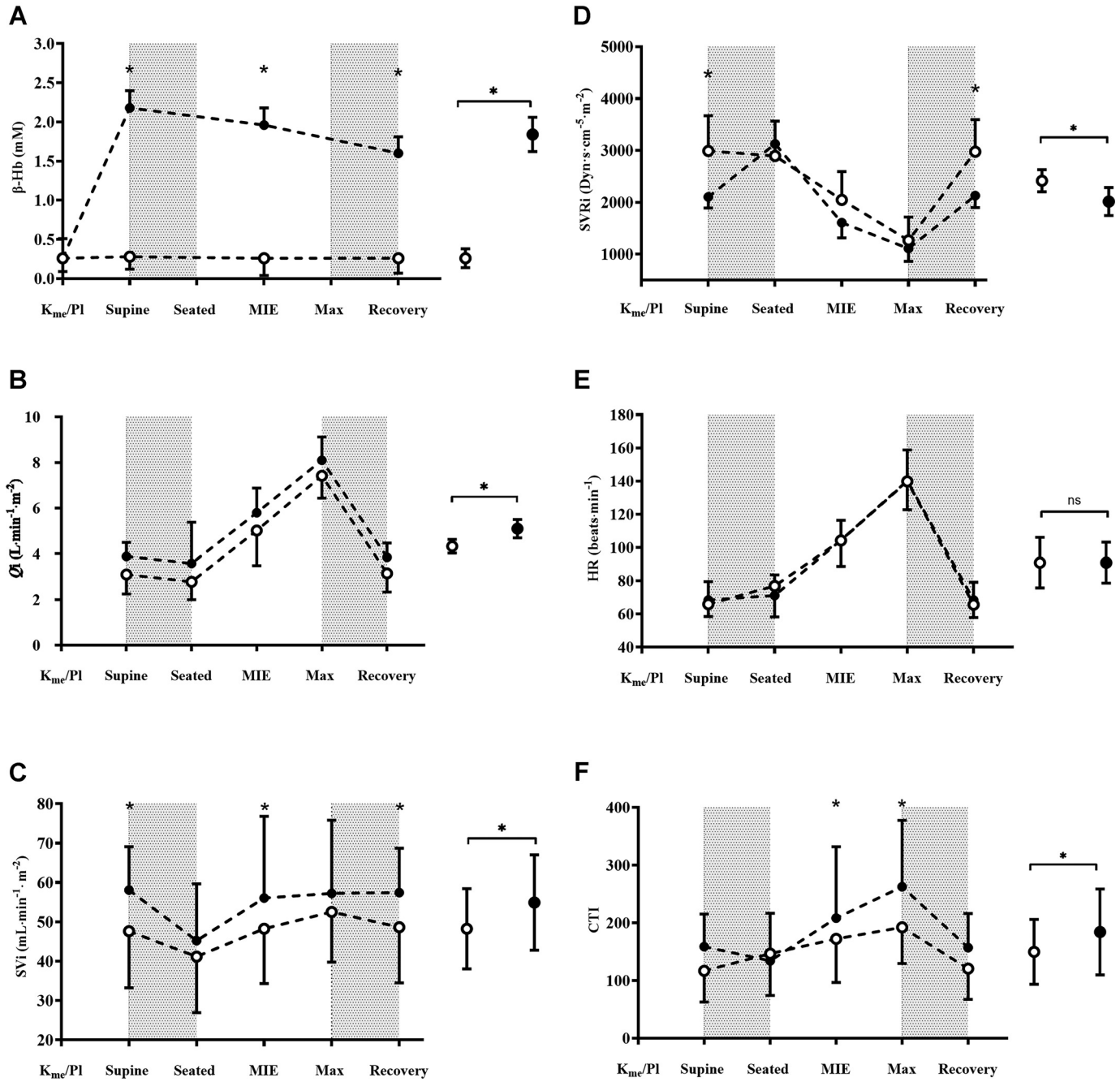

Figure 3. Mean cardiac function response to K_{me} ingestion (black circles) vs. placebo (open circles) at rest (gray shade), during, and after exercise. *A*: mean β-Hb response with grand means. *B*: mean $\dot{Q}$i response with grand means. *C*: mean SVI response with grand means. *D*: mean HR response with grand means. *E*: mean SVRi response with grand means *F*: mean CTI response with grand means. Data are presented as means ± SD. *Significantly different to placebo. Significance is set at $P \leq 0.05$. β-Hb, β-hydroxybutyrate; CTI, contractility index; HR, heart rate; K_{me}/Pl ingestion, ketone monoesters/placebo; Max, maximal intensity exercise; MIE, moderate intensity exercise; $\dot{Q}$i, cardiac output index; seated, seated rest; supine, supine rest; SVi, stroke volume index; SVRi, systemic vascular resistance index.

when exploring TSI% ($P = 0.709$; Fig. 5*C*), O_2Hb ($P = 0.889$; Fig. 5*D*), or [HHb] ($P = 0.754$; Fig. 5*E*).

Effect of K_{me} on Pulmonary Gas Exchange

K_{me} had no effect on $\dot{V}O_{2peak}$ (K_{me} 17.1 ± 2.6 mL·kg·min^{-1} vs. Placebo 17.0 ± 3.1 mL·kg·min^{-1}; mean difference: 0.1 mL·kg·min^{-1}; 95% CI: −1.5 to 1.5, $P = 0.990$), $\dot{V}O_2$ (mean difference: 33.3 mL·min^{-1}; 95% CI: −134 to 127, $P = 0.960$), or $\dot{V}E$ (mean difference: 1.8 mL·min^{-1}; 95% CI: −7.5 to 4.1, $P = 0.558$). No significant interactions (supplement × time) were observed for $\dot{V}O_2$, ($P = 0.789$; Fig. 5*A*) or $\dot{V}E$ ($P = 0.876$; Fig. 5*B*).

Effect of K_{me} Ingestion on [TNT], [Glucose], and [Lactate]

K_{me} had no effect on [TNT] compared with placebo (mean difference: 0.001 pg·mL^{-1}; 95% CI: −0.007 to 0.008, $P =$

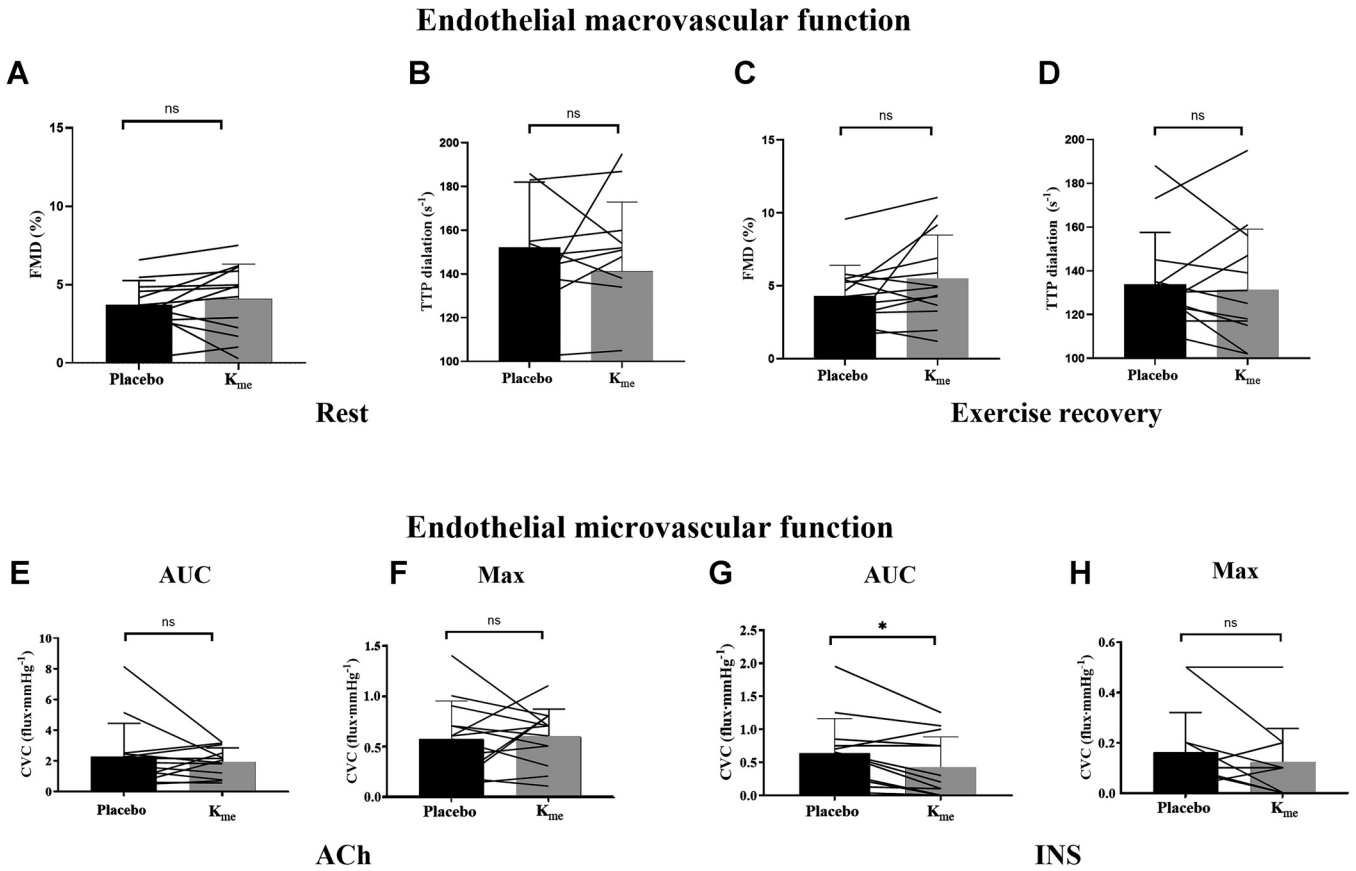

Figure 4. Macro- and microvascular response after K_{me} (gray columns) and placebo (black columns) ingestion. *Difference from placebo. FMD at rest (*A*); TTP at rest (*B*); FMD recovery (*C*); TTP recovery (*D*); AUC of ACh (*E*); Max ACh (*F*); AUC of INS (*G*); Max INS depicts the resting microvascular response to K_{me} and placebo ingestion (*H*). ACh, acetylcholine; AUC, area under the curve; CVC, cutaneous vascular conductance; FMD, flow-mediated dilatation; INS, insulin; Max, maximum; TTP, time to peak.

0.853; see Supplement Fig. S1*A*). No supplement × time interaction was observed for [TNT] ($P = 0.680$), nor a time effect ($P = 0.784$) was observed. K_{me} ingestion had no effect on blood [glucose] (mean difference: 0.7 mmol·L^{-1}; 95% CI: −0.8 to 0.7, $P = 0.858$, see Supplement Fig. S1*B*) or blood [lactate] (mean difference: 0.3 mmol·L^{-1}; 95% CI: −0.9 to 0.3, $P = 0.323$, see Supplement Fig. S1*C*) compared with placebo. No supplement × time interaction was observed for blood [glucose] ($P = 0.747$) or blood [lactate] ($P = 0.695$).

DISCUSSION

This was the first trial to evaluate the acute effects of K_{me} ingestion on central hemodynamics at rest, during and in the recovery of step-incremental cycling exercise, in SGLT2 inhibitor naïve adults with T2D. Our secondary outcomes assessed the acute effects of K_{me} ingestion on vascular function, peripheral muscle (de)oxygenation, and ventilatory responses. The principal novel findings were that, compared with placebo, acute K_{me} ingestion induced profound [β-Hb] increases, given the dose, and improved central hemodynamics and peripheral muscle oxygenation at rest and during exercise However, K_{me} ingestion did not improve micro- and macrovascular endothelial function or alter ventilation.

K_{me} Ingestion and Circulating [β-Hb]

In the present study, we show that oral ingestion of 0.115 g·kg^{-1} of K_{me} can induce profound [β-Hb] increases given the dose in people with T2DM (i.e., >[β-Hb] 0.5 mM ketosis threshold (33), with [β-Hb] levels rising from 0.1 mM (preingestion [β-Hb] levels) to 2.2 ± 0.2 mM. Previous studies that either infused (15) or ingested (13) comparable dosages led to similar [β-Hb] rises of 3.3 ± 0.4 mM and 2.1 ± 0.3 mM, respectively, in people with heart failure. Infusion of β-Hb typically leads to higher circulating concentrations compared with ingestion (34), which may explain the slightly higher [β-Hb] observed by Nielsen et al. (15) versus Monzo et al. (13). Nevertheless, our data indicate that K_{me} ingestion may be a viable option for inducing acute ketosis due to the ease of rapidly and noninvasively increasing plasma [β-Hb] in SGLT2 inhibitor naïve people with T2D.

Effect of K_{me} Ingestion on Central Hemodynamics

Cardiac abnormalities, such as myocardial dysfunction (35) and reduced $\dot{Q}$ (36), have been observed in people with T2D. In the current cohort of people with T2D, resting $\dot{Q}$i during placebo condition was lower (2.8–3.1 ± 0.8 L·min^{-1}·m^{-2}) compared with normative healthy values (≥3.3 L·min^{-1}·m^{-2}) (37); however, $\dot{Q}$i was closer to the normative values following K_{me} ingestion (3.9 ± 0.6 L·min^{-1}·m^{-2}). Importantly, they are

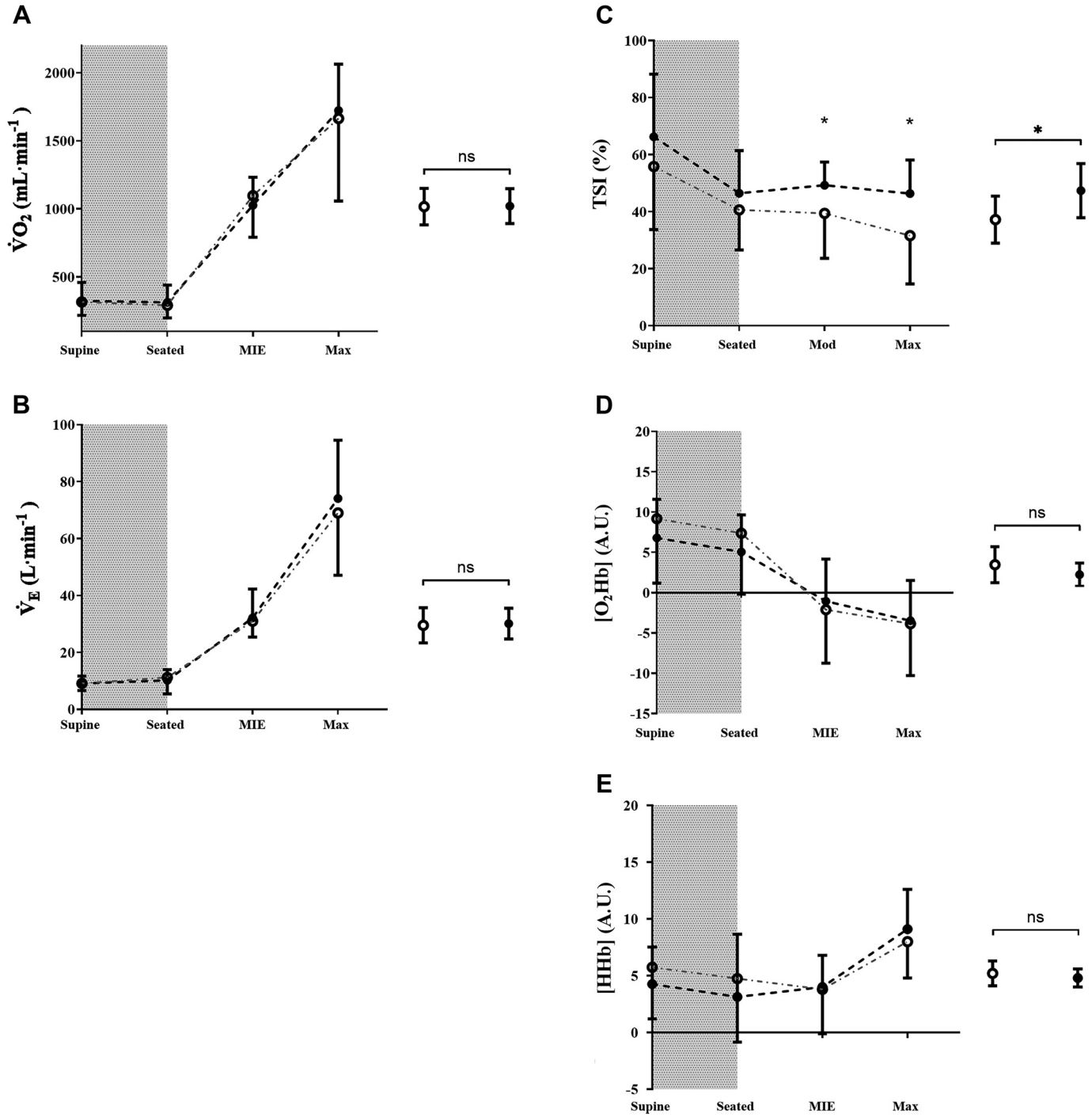

Figure 5. Respiratory and muscle oxygenation parameters at rest and during exercise after K_{me} and placebo ingestion response with grand means. *Difference from placebo. *A*: mean $\dot{V}O_2$ response with grand means. *B*: mean $\dot{V}E$ response with grand means. *C*: mean TSI response with grand means. *D*: mean [O_2Hb] response with grand means. *E*: mean [HHb]. Data are expressed as means ± SD. Significance is set at $P \leq 0.05$. [HHb], deoxygenated hemoglobin combined with myoglobin; MIE, moderate intensity exercise; RQ, respiratory quotient; seated, seated rest; supine, supine rest; [O_2Hb], oxygenated hemoglobin combined with myoglobin; TSI, tissue saturation index; $\dot{V}E$, minute ventilation; $\dot{V}O_2$, oxygen consumption.

determinants known to limit oxygen delivery and physiological function (38) and are related to increased cardiac-related mortality (39). We show that acute K_{me} ingestion can increase $\dot{Q}i$ and SVi at rest and during subsequent exercise compared with placebo. In particular, we demonstrate that K_{me} ingestion elicits similar increases in $\dot{Q}$ (2.0 ± 0.3 vs. 1.6 ± 0.4 L·min^{-1}) and reductions in systemic vascular resistance (30 vs. 31%) to that seen with K_{me} infusion in individuals with heart failure (15). Therefore, this relatively inexpensive drink has the potential to acutely reduce the cardiovascular burden associated with T2D.

The present observations are in line with earlier acute studies in animal models of both swine (40) and rats (41), which both showed that increased circulating [β-Hb] led to

improved central hemodynamics ($\dot{Q}$i; SVi) purported due to improved ATP turnover and reduced inflammation. People with T2D have impaired $\dot{Q}$ (36), which is at least in part due to insulin-resistant cardiomyocytes (42). This is partially attributed to the fact that the diabetic heart relies predominantly on the metabolism of the FFAs (2.33 ATP per molecule of oxygen) instead of glucose (2.58) (43), which can increase cardiac afterload by ~10% compared with glucose oxidation (44). Given that the cardiomyocytes of people with T2D have been shown to preferentially switch substrate utilization from FFAs to ketone bodies when ketone body availability is increased (13), it is plausible to assume that the utilization of a more efficient substrate after K_{me} ingestion may have led to the improved $\dot{Q}$i and SVi observed in the present study. Indeed, a recent chronic study in a cohort of people with T2D and HFpEF demonstrated that oral K_{me} ingestion improved cardiac function (14); however, the majority of the cohort (62%) were on sodium-glucose transporter (SGLT2) inhibitors treatment, a glucose-lowering drug that increases [β-Hb] levels (17) and improves cardiovascular function (18). The current study is the first to show that acute ingestion of K_{me} can increase $\dot{Q}$i and SVi in people with T2D who are SGLT2 inhibitor naïve.

The observed increase in $\dot{Q}$i and SVi may partly be caused by reduced SVRi. In fact, previous studies have been supportive of the vasodilatory effects of K_{me} in mice (45) and healthy individuals (46). However, despite the observed reduction in SVRi, we did not observe any changes in either micro- or macrovascular endothelial function between K_{me} and placebo. Although we did not observe any differences in HR between conditions, we did observe an increase in SVi and consequently $\dot{Q}$i after K_{me} ingestion compared with placebo. We therefore speculate that the Frank–Starling relationship [i.e., the relationship between the initial length of myocardial fibers and the force generated by cardiac contraction (47)] may have shifted upward due to increased contractility, as shown previously in murine myocyte models (48). We therefore speculate that the observed improvements in $\dot{Q}$i and SVi in this study may be attributed to a direct effect of β-Hb on the myocardium rather than β-Hb-induced vasodilation.

Effect of K_{me} on Macro- and Microvascular Endothelial Function at Rest and after Exercise

This is the first study to examine the effect of acute $K_{me\ ingestion}$ on vascular function in humans. Previous studies in animals demonstrated acute improvements in microvascular endothelial function after β-Hb infusion (45). Indeed, in individuals with obesity, there is a positive correlation between [β-Hb] and changes in macrovascular endothelial function, measured by FMD (46). However, we show that, compared with placebo, K_{me} ingestion did not affect micro- or macrovascular endothelial function in individuals with T2D. It is possible that the dose of K_{me} in the current study was not adequate to evoke a vasodilatory response in these individuals. Indeed, people with T2D are characterized by hyperglycemia that impairs vascular function (2), which also suggests we may need a larger vasodilatory stimulus than the current dose of K_{me} to have an effect. Interestingly, increased [β-Hb] promotes protection against oxidative stress through upregulation of antioxidant defense genes (49), which ultimately leads to improved endothelial function. Therefore, we cannot preclude that maintaining increased levels of β-Hb via chronic ingestion of K_{me}, could lead to improvements in the vascular function of people with T2D.

Effect of K_{me} on Skeletal Muscle Oxygenation

Impairments in skeletal muscle oxygenation are well-described in people with T2DM. In brief, these include reduced capillary density (50), reduced perfusion with concomitant reductions in blood flow, and heterogeneous distribution of blood flow accompanied by increased levels of [HHb] (51). We observed an increase in the TSI of the *m. gastrocnemius medialis* during exercise after K_{me} ingestion compared with placebo. This increase in TSI is in line with studies showing increases in skeletal muscle oxygenation in athletes (52) and in people with cardiogenic shock (53) with similar dosing strategy (~1.0 and 0.5 $g \cdot kg^{-1}$ body mass, respectfully) although with higher [β-Hb] bioavailability (~4.0 mM and ~2.8 mM respectfully). These results are unsurprising, given that ketone uptake by skeletal muscle has been observed in plasma [β-Hb] levels ranging from ~0.8 to 1.7 $mmol \cdot L^{-1}$ (54).

We report improved $\dot{Q}$i and SVi in combination with improved TSI. The improvement in O_2 delivery (TSI) is likely driven by the increase in $\dot{Q}$i that may support a better matching between delivery and muscle O_2 demand. Indeed, we show a trend toward significance in ΔTSI and in Δ$\dot{Q}$i between K_{me} and placebo ($r = 2.03$, $P = 0.094$). Future studies should explore this relationship further given that people with T2D have impaired vascular function (55, 56). The impaired vascular function may explain some of the reluctance to exercise in people with T2D given the discomfort they experience. It is possible that K_{me} may have the potential for improving the ability to undertake physical activity and derive the associated benefits on quality of life, but future research is needed to verify this possibility.

Effect of K_{me} on Resting and Exercise Cardiopulmonary Parameters

We show that K_{me} ingestion did not alter ventilatory responses in people with T2D compared with placebo. This is in line with data in healthy individuals (52), which demonstrates no change in pulmonary gas exchange following K_{me} ingestion. Increased availability and oxidation of β-Hb lead to an increased availability of [acetyl-CoA] within the citric acid cycle (in rodent models), which may ultimately lead to a reduced oxygen cost of exercise at the same absolute exercise intensity (57). Higher doses of K_{me} have been significantly associated with lower oxygen consumption (58), which may be due to an improved phosphate: O_2 (P/O) ratio. However, given the risk of ketoacidosis, we are unlikely to be able to supplement people with T2D with larger doses of K_{me}, which might elicit the reduction in $\dot{V}O_2$, seen in other populations. These data suggest that, in individuals with T2D, our current dosing strategy is unlikely to make the skeletal muscle more efficient at using oxygen.

Safety

We reported one serious adverse event, but this was unrelated to the trial. In comparison with Nielsen et al. (15), who

reported a clinically meaningful (59) increase in HR by 7 beats/min during K_{me} infusion (~0.18 g·kg^{-1}), in people with heart failure (15), we observed no differences in HR between conditions after K_{me} ingestion (~0.11 g·kg^{-1}). Given that an increase in HR is associated with a worse prognosis in people with cardiac dysfunction (60), current findings may be useful for optimizing a dose and administration mode (infusion or ingestion) of K_{me} in individuals with increased cardiovascular burden. Pharmacokinetic and dynamic responses to differing doses of Kme ingestion studies in healthy and metabolically compromised groups are warranted.

Limitations

The current study did not obtain a nonsupplemental baseline of cardiac hemodynamics; however, given the trial design (double-blind placebo-controlled), it is likely that the observed changes are a true reflection of the effect of the supplement over time. The current study did not measure pH levels. Increases in pH have previously been shown with infusion of K_{me} (61); however, studies that have used similar doses of K_{me} reported only marginally increased pH levels (15) or lower pH following K_{me} ingestion in healthy individuals (58). Any increases are unlikely to affect the result or to induce any adverse events. In addition, our ultrasound did not have the capability to measure the diameter and blood flow simultaneously, and therefore, we could not report shear rate or reactive hyperemia. This is a limitation for our assessment of macrovascular endothelial function, it is however unlikely to have altered our findings as the relationship between FMD and postdeflation shear rate appears to be age-dependent, given that it only applies when investigating young adults (62). Our study was acute in nature and therefore we cannot preclude that longer-term interventions may have adverse effects or that any improvements acutely are only transient or that participants may develop tachyphylaxis. Finally, although impedance cardiography is a validated noninvasive method against the Fick principle (26), it is also known to overestimate measurements of SV and $\dot{Q}$ (63). However, given the trial design (i.e., a crossover, where each participant is in their own control), both conditions are likely to be slight overestimates of $\dot{Q}$ and therefore should not affect our interpretation of the data. Finally, the current study did not include a healthy matched control group, and hence, we are unable to determine whether the response in the present study is consistent with that seen in a healthy cohort.

Conclusions

In conclusion, we show, for the first time in SGLT2 inhibitor naive people with T2D, that acute K_{me} ingestion improves central hemodynamics at rest and during exercise. Acute K_{me} ingestion also improved peripheral muscle oxygenation yet had no apparent impact on endothelial function or ventilation compared with placebo. The beneficial effects on $\dot{Q}$i and SVi are likely driven by an increase in [β-Hb] metabolism by the myocardium, resulting in a higher ATP yield for a given amount of oxygen. K_{me} ingestion has the potential to be an adjunct treatment to improve $\dot{Q}$i and SVi in people with T2D; however, larger and longer-term efficacy and safety trials are warranted.

DATA AVAILABILITY

The full anonymized dataset has been made open access and freely available as Supplemental Material on the University of Portsmouth repository (https://doi.org/10.17029/89279950-3254-495a-b1aa-86573108b55c).

SUPPLEMENTAL MATERIAL

Supplemental Table and Supplemental Fig. S1: https://doi.org/10.17029/bd9df2d4-25bf-4e1e-89d9-ab1d06252dac.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.P. and A.I.S. conceived and designed research; M.P., K.F., C.E., H.M., and A.I.S. performed experiments; M.P., K.F., H.M., and A.I.S. analyzed data; M.P., Z.L.S., and A.I.S. interpreted results of experiments; M.P. and A.I.S. prepared figures; M.P. and A.I.S. drafted manuscript; M.P., Z.L.S., K.F., C.E., T.J.J., J.C., H.M., J.S., M.C., M.I.B., W.D.S., J.P.L., and A.I.S. edited and revised manuscript; M.P., Z.L.S., K.F., C.E., T.J.J., J.C., H.M., J.S., M.C., M.I.B., W.D.S., J.P.L., and A.I.S. approved final version of manuscript.

REFERENCES

1. **Saeedi P**, **Petersohn I**, **Salpea P**, **Malanda B**, **Karuranga S**, **Unwin N**, **Colagiuri S**, **Guariguata L**, **Motala AA**, **Ogurtsova K**, **Shaw JE**, **Bright D**, **Williams R**; **IDF Diabetes Atlas Committee.** Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract* 157: 107843, 2019. doi:10.1016/j.diabres.2019.107843.
2. **Stratton IM**, **Adler AI**, **Neil HA**, **Matthews DR**, **Manley SE**, **Cull CA**, **Hadden D**, **Turner RC**, **Holman RR.** Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ* 321: 405–412, 2000. doi:10.1136/bmj.321.7258.405.
3. **Zheng Y**, **Ley SH**, **Hu FB.** Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol* 14: 88–98, 2018. doi:10.1038/nrendo.2017.151.
4. **Kannel WB**, **McGee DL.** Diabetes and cardiovascular disease. The Framingham study. *JAMA* 241: 2035–2038, 1979. doi:10.1001/jama.241.19.2035.
5. **Qaseem A**, **Wilt TJ**, **Kansagara D**, **Horwitch C**, **Barry MJ**, **Forciea MA**, **Fitterman N**, **Balzer K**, **Boyd C**, **Humphrey LL**, **Iorio A**, **Lin J**, **Maroto M**, **McLean R**, **Mustafa R**, **Tufte J**; **Clinical Guidelines Committee of the American College of Physicians.** Hemoglobin A1c targets for glycemic control with pharmacologic therapy for nonpregnant adults with type 2 diabetes mellitus: a guidance statement update from the American College of Physicians. *Ann Intern Med* 168: 569–576, 2018. doi:10.7326/M17-0939.
6. **Ponzo V**, **Rosato R**, **Tarsia E**, **Goitre I**, **De Michieli F**, **Fadda M**, **Monge T**, **Pezzana A**, **Broglio F**, **Bo S.** Self-reported adherence to diet and preferences towards type of meal plan in patient with type 2 diabetes mellitus. A cross-sectional study. *Nutr Metab Cardiovasc Dis* 27: 642–650, 2017. doi:10.1016/j.numecd.2017.05.007.

7. **Reusch JEB**, **Bridenstine M**, **Regensteiner JG.** Type 2 diabetes mellitus and exercise impairment. *Rev Endocr Metab Disord* 14: 77–86, 2013. doi:10.1007/s11154-012-9234-4.
8. **Pinto TE**, **Gusso S**, **Hofman PL**, **Derraik JG**, **Hornung TS**, **Cutfield WS**, **Baldi JC.** Systolic and diastolic abnormalities reduce the cardiac response to exercise in adolescents with type 2 diabetes. *Diabetes Care* 37: 1439–1446,2014. doi:10.2337/dc13-2031.
9. **Rask-Madsen C**, **King GL.** Vascular complications of diabetes: mechanisms of injury and protective factors. *Cell Metab* 17: 20–33, 2013. doi:10.1016/j.cmet.2012.11.012.
10. **Regensteiner JG**, **Bauer TA**, **Reusch JE**, **Brandenburg SL**, **Sippel JM**, **Vogelsong AM**, **Smith S**, **Wolfel EE**, **Eckel RH**, **Hiatt WR.** Abnormal oxygen uptake kinetic responses in women with type II diabetes mellitus. *J Appl Physiol (1985)* 85: 310–317, 1998. doi:10.1152/jappl.1998.85.1.310.
11. **Bauer TA**, **Reusch JE**, **Levi M**, **Regensteiner JG.** Skeletal muscle deoxygenation after the onset of moderate exercise suggests slowed microvascular blood flow kinetics in type 2 diabetes. *Diabetes Care* 30: 2880–2885, 2007. doi:10.2337/dc07-0843.
12. **Murray AJ**, **Knight NS**, **Cole MA**, **Cochlin LE**, **Carter E**, **Tchabanenko K**, **Pichulik T**, **Gulston MK**, **Atherton HJ**, **Schroeder MA**, **Deacon RM**, **Kashiwaya Y**, **King MT**, **Pawlosky R**, **Rawlins JN**, **Tyler DJ**, **Griffin JL**, **Robertson J**, **Veech RL**, **Clarke K.** Novel ketone diet enhances physical and cognitive performance. *FASEB J* 30: 4021–4032, 2016. doi:10.1096/fj.201600773R.
13. **Monzo L**, **Sedlacek K**, **Hromanikova K**, **Tomanova L**, **Borlaug BA**, **Jabor A**, **Kautzner J**, **Melenovsky V.** Myocardial ketone body utilization in patients with heart failure: the impact of oral ketone ester. *Metabolism* 115: 154452, 2021. doi:10.1016/j.metabol.2020.154452.
14. **Gopalasingam N**, **Berg-Hansen K**, **Christensen KH**, **Ladefoged BT**, **Poulsen SH**, **Andersen MJ**, **Borlaug BA**, **Nielsen R**, **Møller N**, **Wiggers H.** Randomized crossover trial of 2-week ketone ester treatment in patients with type 2 diabetes and heart failure with preserved ejection fraction. *Circulation* 150: 1570–1583, 2024. doi:10.1161/CIRCULATIONAHA.124.069732.
15. **Nielsen R**, **Møller N**, **Gormsen LC**, **Tolbod LP**, **Hansson NH**, **Sorensen J**, **Harms HJ**, **Frøkiær J**, **Eiskjaer H**, **Jespersen NR**, **Mellemkjaer S**, **Lassen TR**, **Pryds K**, **Bøtker HE**, **Wiggers H.** Cardiovascular effects of treatment with the ketone body 3-hydroxybutyrate in chronic heart failure patients. *Circulation* 139: 2129–2141, 2019. doi:10.1161/CIRCULATIONAHA.118.036459.
16. **Cox PJ**, **Kirk T**, **Ashmore T**, **Willerton K**, **Evans R**, **Smith A**, **Murray AJ**, **Stubbs B**, **West J**, **McLure SW**, **King MT**, **Dodd MS**, **Holloway C**, **Neubauer S**, **Drawer S**, **Veech RL**, **Griffin JL**, **Clarke K.** Nutritional ketosis alters fuel preference and thereby endurance performance in athletes. *Cell Metab* 24: 256–268, 2016. doi:10.1016/j.cmet.2016.07.010.
17. **Qiu H**, **Novikov A**, **Vallon V.** Ketosis and diabetic ketoacidosis in response to SGLT2 inhibitors: basic mechanisms and therapeutic perspectives. *Diabetes Metab Res Rev* 33: e2886, 2017. doi:10.1002/dmrr.2886.
18. **Ferrannini E**, **Mark M**, **Mayoux E.** CV Protection in the EMPA-REG OUTCOME trial: a 'thrifty substrate' hypothesis. *Diabetes Care* 39: 1108–1114, 2016. doi:10.2337/dc16-0330.
19. **Pittampalli S**, **Upadyayula S**, **Mekala HM**, **Lippmann S.** Risks vs benefits for SGLT2 inhibitor medications. *Fed Pract* 35: 45–48, 2018.
20. **Zinman B**, **Wanner C**, **Lachin JM**, **Fitchett D**, **Bluhmki E**, **Hantel S**, **Mattheus M**, **Devins T**, **Johansen OE**, **Woerle HJ**, **Broedl UC**, **Inzucchi SE**; **EMPA-REG OUTCOME Investigators.** Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med* 373: 2117–2128, 2015. doi:10.1056/NEJMoa1504720.
21. **Rampuri ZH**, **Collins SÉ**, **Mickelsen BSA**, **Brotto AR**, **Beaudry RI**, **van Diepen S**, **Stickland MK.** The effect of inhaled nitric oxide on maximal oxygen consumption during exercise in acute hypoxia: a randomized double-blind crossover trial. *J Appl Physiol (1985)* 136: 514–524, 2024. doi:10.1152/japplphysiol.00767.2022.
22. **Falkenhain K**, **Daraei A**, **Little JP.** The effect of novel exogenous ketone supplements on blood beta-hydroxybutyrate and glucose. *J Diet Suppl* 21: 38–52, 2024. doi:10.1080/19390211.2023.2179152.
23. **Myette-Côté É**, **Neudorf H**, **Rafiei H**, **Clarke K**, **Little JP.** Prior ingestion of exogenous ketone monoester attenuates the glycaemic response to an oral glucose tolerance test in healthy young individuals. *J Physiol* 596: 1385–1395, 2018. doi:10.1113/JP275709.
24. **Robinson C**, **Cochran E**, **Gorden P**, **Brown RJ.** Management of diabetic ketoacidosis in severe insulin resistance. *Diabetes Care* 39: e116–e118, 2016. doi:10.2337/dc16-0635.
25. **Crawford DA**, **Drake NB**, **Carper MJ**, **DeBlauw J**, **Heinrich KM.** Validity, reliability, and application of the session-RPE method for quantifying training loads during high intensity functional training. *Sports (Basel)* 6: 84, 2018. doi:10.3390/sports6030084.
26. **Richard R**, **Lonsdorfer-Wolf E**, **Charloux A**, **Doutreleau S**, **Buchheit M**, **Oswald-Mammosser M**, **Lampert E**, **Mettauer B**, **Geny B**, **Lonsdorfer J.** Non-invasive cardiac output evaluation during a maximal progressive exercise test, using a new impedance cardiograph device. *Eur J Appl Physiol* 85: 202–207, 2001. doi:10.1007/s004210100458.
27. **Soares RN**, **Murias JM.** Near-infrared spectroscopy assessment of microvasculature detects difference in lower limb vascular responsiveness in obese compared to lean individuals. *Microvasc Res* 118: 31–35, 2018. doi:10.1016/j.mvr.2018.01.008.
28. **Lindkvist M**, **Grönlund C.** Absolute quantifications of oxy- and deoxyhemoglobin concentrations by combination of differentially resolved spectroscopy and spatially resolved spectroscopy. *Spectrosc Lett* 48: 170–172, 2015. doi: 10.1080/00387010.2013.867887.
29. **Eglin CM**, **Costello JT**, **Bailey SJ**, **Gilchrist M**, **Massey H**, **Shepherd AI.** Effects of dietary nitrate supplementation on the response to extremity cooling and endothelial function in individuals with cold sensitivity. A double blind, placebo controlled, crossover, randomised control trial. *Nitric Oxide* 70: 76–85, 2017. doi:10.1016/j.niox.2017.09.005.
30. **Kim HY.** Statistical notes for clinical researchers: assessing normal distribution (2) using skewness and kurtosis. *Restor Dent Endod* 38: 52–54, 2013. doi:10.5395/rde.2013.38.1.52.
31. **Yadolah Dodge** (Editor)**.** Kolmogorov–Smirnov Test BT. In: *The Concise Encyclopedia of Statistics*. New York: Springer, 2008, p. 283–287. doi:10.1007/978-0-387-32833-1_214.
32. **Rothman KJ.** No adjustments are needed for multiple comparisons. *Epidemiology* 1: 43–46, 1990. doi:10.1097/00001648-199001000-00010.
33. **Newman JC**, **Verdin E.** β-hydroxybutyrate: a signaling metabolite. *Annu Rev Nutr* 37: 51–76, 2017. doi:10.1146/annurev-nutr-071816-064916.
34. **Mangoni AA**, **Jackson SH.** Age-related changes in pharmacokinetics and pharmacodynamics: basic principles and practical applications. *Br J Clin Pharmacol* 57: 6–14, 2004. doi:10.1046/j.1365-2125.2003.02007.x.
35. **Scognamiglio R**, **Avogaro A**, **Casara D**, **Crepaldi C**, **Marin M**, **Palisi M**, **Mingardi R**, **Erle G**, **Fasoli G**, **Dalla Volta S.** Myocardial dysfunction and adrenergic cardiac innervation in patients with insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 31: 404–412, 1998. doi:10.1016/s0735-1097(97)00516-0.
36. **Waddingham MT**, **Edgley AJ**, **Tsuchimochi H**, **Kelly DJ**, **Shirai M**, **Pearson JT.** Contractile apparatus dysfunction early in the pathophysiology of diabetic cardiomyopathy. *World J Diabetes* 6: 943–960, 2015. doi:10.4239/wjd.v6.i7.943.
37. **Carlsson M**, **Andersson R**, **Bloch KM**, **Steding-Ehrenborg K**, **Mosén H**, **Stahlberg F**, **Ekmehag B**, **Arheden H.** Cardiac output and cardiac index measured with cardiovascular magnetic resonance in healthy subjects, elite athletes and patients with congestive heart failure. *J Cardiovasc Magn Reson* 14: 51, 2012. doi:10.1186/1532-429X-14-51.
38. **Regensteiner JG**, **Sippel J**, **McFarling ET**, **Wolfel EE**, **Hiatt WR.** Effects of non-insulin-dependent diabetes on oxygen consumption during treadmill exercise. *Med Sci Sports Exerc* 27: 875–881, 1995.
39. **Mosenzon O**, **Alguwaihes A**, **Leon JLA**, **Bayram F**, **Darmon P**, **Davis TME**, **Dieuzeide G**, **Eriksen KT**, **Hong T**, **Kaltoft MS**, **Lengyel C**, **Rhee NA**, **Russo GT**, **Shirabe S**, **Urbancova K**, **Vencio S**; **CAPTURE Study Investigators.** CAPTURE: a multinational, cross-sectional study of cardiovascular disease prevalence in adults with type 2 diabetes across 13 countries. *Cardiovasc Diabetol* 20: 154, 2021. doi:10.1186/s12933-021-01344-0.
40. **Yurista SR**, **Eder RA**, **Welsh A**, **Jiang W**, **Chen S**, **Foster AN**, **Mauskapf A**, **Tang WHW**, **Hucker WJ**, **Coll-Font J**, **Rosenzweig A**, **Nguyen CT.** Ketone ester supplementation suppresses cardiac inflammation and improves cardiac energetics in a swine model of acute myocardial infarction. *Metabolism* 145: 155608, 2023. doi:10.1016/j.metabol.2023.155608.
41. **Sato K**, **Kashiwaya Y**, **Keon CA**, **Tsuchiya N**, **King MT**, **Radda GK**, **Chance B**, **Clarke K**, **Veech RL.** Insulin, ketone bodies, and

mitochondrial energy transduction. *FASEB J* 9: 651–658, 1995. doi:10.1096/fasebj.9.8.7768357.
42. **Saotome M**, **Ikoma T**, **Hasan P**, **Maekawa Y.** Cardiac insulin resistance in heart failure: the role of mitochondrial dynamics. *Int J Mol Sci* 20: 3552, 2019. doi:10.3390/ijms20143552.
43. **Karwi QG**, **Lopaschuk GD.** CrossTalk proposal: ketone bodies are an important metabolic fuel for the heart. *J Physiol* 600: 1001–1004, 2022. doi:10.1113/JP281004.
44. **Barsotti A**, **Giannoni A**, **Di Napoli P**, **Emdin M.** Energy metabolism in the normal and in the diabetic heart. *Curr Pharm Des* 15: 836–840, 2009. doi:10.2174/138161209787582066.
45. **McCarthy CG**, **Chakraborty S**, **Singh G**, **Yeoh BS**, **Schreckenberger ZJ**, **Singh A**, **Mell B**, **Bearss NR**, **Yang T**, **Cheng X**, **Vijay-Kumar M**, **Wenceslau CF**, **Joe B.** Ketone body β-hydroxybutyrate is an autophagy-dependent vasodilator. *JCI Insight* 6: e149037 2021. doi:10.1172/jci.insight.149037.
46. **Walsh JJ**, **Neudorf H**, **Little JP.** 14-day ketone supplementation lowers glucose and improves vascular function in obesity: a randomized crossover trial. *J Clin Endocrinol Metab* 106: e1738–e1754, 2021. doi:10.1210/clinem/dgaa925.
47. **Delicce AV**, **Makaryus AN.** Physiology, frank starling law. In: *StatPearls*. Treasure Island, FL: StatPearls Publishing. 2023.
48. **Klos M**, **Morgenstern S**, **Hicks K**, **Suresh S**, **Devaney EJ.** The effects of the ketone body β-hydroxybutyrate on isolated rat ventricular myocyte excitation-contraction coupling. *Arch Biochem Biophys* 662: 143–150, 2019. doi:10.1016/j.abb.2018.11.027.
49. **Shimazu T**, **Hirschey MD**, **Newman J**, **He W**, **Shirakawa K**, **Le Moan N**, **Grueter CA**, **Lim H**, **Saunders LR**, **Stevens RD**, **Newgard CB**, **Farese RV**, **de Cabo R**, **Ulrich S**, **Akassoglou K**, **Verdin E.** Suppression of oxidative stress by β-hydroxybutyrate, an endogenous histone deacetylase inhibitor. *Science* 339: 211–214, 2013. doi:10.1126/science.1227166.
50. **Groen BB**, **Hamer HM**, **Snijders T**, **van Kranenburg J**, **Frijns D**, **Vink H**, **van Loon LJ.** Skeletal muscle capillary density and microvascular function are compromised with aging and type 2 diabetes. *J Appl Physiol (1985)* 116: 998–1005, 2014. doi:10.1152/japplphysiol.00919.2013.
51. **Frisbee JC**, **Wu F**, **Goodwill AG**, **Butcher JT**, **Beard DA.** Spatial heterogeneity in skeletal muscle microvascular blood flow distribution is increased in the metabolic syndrome. *Am J Physiol Regul Integr Comp Physiol* 301: R975–R986, 2011. doi:10.1152/ajpregu.00275.2011.
52. **Poffé C**, **Robberechts R**, **Podlogar T**, **Kusters M**, **Debevec T**, **Hespel P.** Exogenous ketosis increases blood and muscle oxygenation but not performance during exercise in hypoxia. *Am J Physiol Regul Integr Comp Physiol* 321: R844–R857, 2021. doi:10.1152/ajpregu.00198.2021.
53. **Berg-Hansen K**, **Christensen KH**, **Gopalasingam N**, **Nielsen R**, **Eiskjær H**, **Møller N**, **Birkelund T**, **Christensen S**, **Wiggers H.** Beneficial effects of ketone ester in patients with cardiogenic shock: a randomized, controlled, double-blind trial. *JACC Heart Fail* 11: 1337–1347, 2023. doi:10.1016/j.jchf.2023.05.029.
54. **Mikkelsen KH**, **Seifert T**, **Secher NH**, **Grøndal T**, **van Hall G.** Systemic, cerebral and skeletal muscle ketone body and energy metabolism during acute hyper-D-β-hydroxybutyratemia in post-absorptive healthy males. *J Clin Endocrinol Metab* 100: 636–643, 2015. doi:10.1210/jc.2014-2608.
55. **Meza CA**, **La Favor JD**, **Kim DH**, **Hickner RC.** Endothelial dysfunction: is there a hyperglycemia-induced imbalance of NOX and NOS? *Int J Mol Sci* 20: 3775, 2019. doi:10.3390/ijms20153775.
56. **Evans M**, **McSwiney FT**, **Brady AJ**, **Egan B.** no benefit of ingestion of a ketone monoester supplement on 10-km running performance. *Med Sci Sports Exerc* 51: 2506–2515, 2019. doi:10.1249/mss.0000000000002065.
57. **Suissa L**, **Kotchetkov P**, **Guigonis JM**, **Doche E**, **Osman O**, **Pourcher T**, **Lindenthal S.** Ingested ketone ester leads to a rapid rise of acetyl-CoA and competes with glucose metabolism in the brain of non-fasted mice. *Int J Mol Sci* 22: 524 2021. doi:10.3390/ijms22020524.
58. **Dearlove DJ**, **Harrison OK**, **Hodson L**, **Jefferson A**, **Clarke K**, **Cox PJ.** The effect of blood ketone concentration and exercise intensity on exogenous ketone oxidation rates in athletes. *Med Sci Sport Exerc* 53: 505–516 2021. doi:10.1249/MSS.0000000000002502.
59. **Vazir A**, **Claggett B**, **Jhund P**, **Castagno D**, **Skali H**, **Yusuf S**, **Swedberg K**, **Granger CB**, **McMurray JJ**, **Pfeffer MA**, **Solomon SD.** Prognostic importance of temporal changes in resting heart rate in heart failure patients: an analysis of the CHARM program. *Eur Heart J* 36: 669–675, 2015. doi:10.1093/eurheartj/ehu401.
60. **Fox K**, **Ford I**, **Steg PG**, **Tendera M**, **Robertson M**, **Ferrari R**; **BEAUTIFUL investigators.** Heart rate as a prognostic risk factor in patients with coronary artery disease and left-ventricular systolic dysfunction (BEAUTIFUL): a subgroup analysis of a randomised controlled trial. *Lancet* 372: 817–821, 2008. doi:10.1016/s0140-6736(08)61171-x.
61. **Balasse EO.** Kinetics of ketone body metabolism in fasting humans. *Metabolism* 28: 41–50, 1979. doi:10.1016/0026-0495(79)90166-5.
62. **Thijssen DH**, **Bullens LM**, **van Bemmel MM**, **Dawson EA**, **Hopkins N**, **Tinken TM**, **Black MA**, **Hopman MT**, **Cable NT**, **Green DJ.** Does arterial shear explain the magnitude of flow-mediated dilation? A comparison between young and older humans. *Am J Physiol Heart Circ Physiol* 296: H57–H64, 2009. doi:10.1152/ajpheart.00980.2008.
63. **Kemps HM**, **Thijssen EJ**, **Schep G**, **Sleutjes BT**, **De Vries WR**, **Hoogeveen AR**, **Wijn PF**, **Doevendans PA.** Evaluation of two methods for continuous cardiac output assessment during exercise in chronic heart failure patients. *J Appl Physiol (1985)* 105: 1822–1829, 2008. doi:10.1152/japplphysiol.90430.2008.