

**Prior ingestion of exogenous ketone monoester attenuates the glycemic response
to an oral glucose tolerance test in healthy young individuals**

Etienne Myette-Côté¹, Helena, Neudorf¹, Hossein Rafiei¹, Kieran Clarke² and
Jonathan Peter Little¹

¹School of Health and Exercise Sciences, University of British Columbia Okanagan.

²Department of Physiology, Anatomy, and Genetics, University of Oxford, Oxford, UK.

Running head: Ketone monoester and glucose control

Address for correspondence:

Dr Jonathan P. Little

University of British Columbia Okanagan

School of Health and Exercise Sciences

3333 University Way

Kelowna, BC V1V 1V7

Ph: 250-807-9876

Email: jonathan.little@ubc.ca

Key words: ketone bodies, D-beta-hydroxybutyrate, glucose control, non-esterified fatty acids, insulin sensitivity.

ABSTRACT

The recent development of exogenous ketone supplements allows direct testing of the metabolic effects of elevated blood ketones without the confounding influence of widespread changes experienced with ketogenic diets or prolonged fasting. The main objectives of this study were two-fold: 1) To determine whether acute oral ingestion of ketone monoester (KME; (R)-3-hydroxybutyl (R)-3-hydroxybutyrate) impacts plasma glucose levels during a standardized oral glucose tolerance test (OGTT). 2) To compare changes in insulin concentrations and estimates of insulin sensitivity after acute KME supplementation. Twenty healthy participants (n=10 males/females) aged between 18-35 years took part in a randomized (blinded?) crossover study. After an overnight fast, participants consumed a KME supplement (ΔG° ; 0.45 ml/kg body weight) or placebo (water) 30 minutes before completing a 75 gram OGTT. Blood samples were collected every 15-30 minutes over a period of 2.5 hours. KME acutely raised blood D-beta-hydroxybutyrate (β -OHB) to 3.2 ± 0.6 mM within 30 minutes with levels remaining elevated throughout the entire OGTT. Compared to placebo, KME significantly decreased glucose area under the curve (AUC; -16%, $P = 0.001$), non-esterified fatty acid (NEFA) AUC (-44%, $P < 0.001$) and C-peptide incremental AUC ($P = 0.005$), while improving oral glucose insulin sensitivity index by ~11% ($P = 0.001$). In conclusion, a KME supplement that acutely increased β -OHB levels up to ~3 mM improved glucose tolerance in healthy humans. The improvement in glucose tolerance did not appear to be driven by an increase in insulin secretion, but was accompanied by improved markers of insulin sensitivity, possibly related to the β -OHB-mediated reduction in circulating NEFA. These results suggest that ketone monoester supplements could have therapeutic potential in the management and prevention of metabolic disease.

INTRODUCTION

The ketone bodies, D-beta-hydroxybutyrate (β -OHB) and acetoacetate, are produced by the liver under conditions of starvation, very-low carbohydrate intake and prolonged glycogen-depleting exercise [1-3]. Several studies, including classical work by Cahill et al. [4,5], have demonstrated that β -OHB, the main ketone body in circulation, can act as an alternative energy substrate for metabolically active tissues, such as the brain, heart, kidneys and skeletal muscles. More recently, β -OHB has been shown to have several cellular signaling functions including acting as an endogenous histone deacetylase inhibitor, a ligand for cell surface receptors, and an inhibitor of the NLRP3 inflammasome [6]. These findings suggest that, in addition to its well-known role as a fat-derived energy source, β -OHB can modify an array of physiological functions.

Metabolic abnormalities, including insulin resistance and glucose intolerance are major public health concerns that require novel and efficient prevention and treatment interventions. Several groups have successfully utilized low-carbohydrate ketogenic diets (LCKD) in the management of hyperglycemia in people with insulin resistance and type 2 diabetes [7-10]. Among them, Tay et al. 2015 showed a 1% decrease in hemoglobin A1c after 52 weeks of a LCKD in type 2 diabetes [10]. A LCKD has been proposed as the first approach in diabetes management [11], and has shown encouraging results as a treatment of obesity, cancer and neurological diseases [12-15].

A unique metabolic consequence of the LCKD is an increase in circulating β -OHB from normal resting values of ~ 0.1 - 0.2 mmol/l to levels of ~ 0.5 to 3.5 mmol/l; a state which has been termed nutritional ketosis [16,17]. It is not possible in LCKD studies to determine whether the rise in β -OHB has direct effects on glucose control and metabolic function due the myriad of confounding factors that accompany severe carbohydrate restriction (e.g., decreased body mass, lowered insulin, and elevated lipolysis).

To date, most studies exploring the direct effects of elevated plasma ketones on markers of metabolic control in human and animal models employed ketone infusion methods [18-24]. A consistent finding in studies in which β -OHB is infused to levels ≥ 1.0 mmol/l is a reduction in glucose and circulating free fatty acids; potentially related to reduced hepatic glucose output and inhibition of adipose tissue lipolysis, respectively. Lowering glucose and free fatty acids could be of potential value for individuals with glucose intolerance and insulin resistance, but infusing β -OHB is not a practical therapeutic strategy. In the past few years, the emergence of exogenous ketone supplements allows researchers to study the isolated effects of elevated ketones without the presence of metabolic keto-adaptations or the use of infusions.

Exogenous ketone supplements can be ingested in the form of ketone salts (KS) (e.g., sodium-potassium β -OHB) or ketone esters (KE) (available in monoester and diester forms). So far, a limited number of studies have tested the effect of KS and KE on circulating metabolites in rats [25-27]. Consistent with the ketone infusion studies mentioned above, exogenous ketone supplements decrease circulating glucose and free fatty acids. The high amount of salt contained in the KS supplement, along with potential gastrointestinal distress, limit its therapeutic utility and can be avoided by consuming a ketone monoester (KME) supplement [28]. KME supplementation using (R)-3-hydroxybutyl (R)-3-hydroxybutyrate has been shown to provide a safe [29] and novel strategy for rapidly increasing blood β -OHB to approximately 3 mM within ~30 minutes in healthy humans [30,31]. Once ingested, a non-racemic KME drink will be metabolized into the D isoform of beta-hydroxybutyrate, the isoform produced by endogenous ketogenesis [32,33]. Therefore, oral consumption of KME may be an interesting alternative for increasing β -OHB and improving metabolic control.

Further investigation is necessary to explore the metabolic effects of ketone supplementation in humans before KME can be considered as a therapeutic option. The objectives of this study were threefold: 1) To determine whether acute ingestion of KME; (R)-3-hydroxybutyl (R)-3-hydroxybutyrate impacts plasma glucose levels during a standardized oral glucose tolerance test (OGTT). 2) To compare changes in insulin concentrations and estimates of insulin sensitivity after acute KME supplementation. 3) To observe the time course of blood ketone levels after ingestion of an acute dose of KME followed by a glucose drink. In order to do so, we conducted a randomized, placebo-controlled crossover experiment in healthy young males and females. Due to the novelty of KME and the unknown responses on glucose tolerance and insulin sensitivity when these supplements are consumed prior to an OGTT we conducted this initial study in healthy participants in order to determine the normal physiological responses. We hypothesized that, when compared to a placebo, a single dose of KME taken 30 minutes prior a 2-hour oral glucose tolerance test (OGTT) would reduce glucose area under the curve (AUC) and improve oral glucose insulin sensitivity (OGIS) index.

METHODS

Research design

The study was approved by the University of British Columbia Clinical Research Ethics Board. The experimental design involved an initial visit for screening and baseline testing and two experimental conditions that each required a 3-hour visit to the laboratory. On experimental visits, participants arrived at the laboratory following a ≥ 10 -hour overnight fast. According to a randomized crossover design, participants consumed either ketone or placebo supplement followed 30 minutes later by a 75 gram glucose drink for a standard 2-hour OGTT. At least 48 hours later, participants returned to the lab following a similar overnight fast and performed the alternate experimental condition.

Participants

Twenty healthy participants (10 males and 10 females) aged between 18-35 years were recruited and provided written informed consent during an initial screening visit. Exclusion criteria included 1) currently taking any medications (except for birth control for females), 2) following a low-carbohydrate diet or consuming nutritional ketone supplements, 3) considered a competitive athlete engaged in competition or intensive training, 4) waist circumference >102 centimeters for men or >88 centimeters for women. Participants' baseline characteristics are presented in Table 1.

Baseline testing

Body weight (kg), height (cm), blood pressure (mmHg) and waist circumference (cm) were measured using standardized methods. Participants were given a 24-hour food log to complete on the day prior their first experimental condition. They were also told to avoid exercising and to not consume alcohol for 24 hours prior to the experimental trials.

Experimental trials

Participants reported to the laboratory for experimental trials after an overnight fast (≥ 10 hours). The research coordinator confirmed with them that the 24-hour food log had been completed, that no exercise had been performed and that no alcohol had been consumed on the day before. An indwelling intravenous catheter (BD Nexiva, Becton Dickinson Infusion Therapy Systems Inc., Utah, USA) was then inserted into the antecubital vein for repeated blood sampling. At each time point, blood was drawn into 1x2ml ethylenediamine tetraacetic acid (EDTA) and 1x4ml serum tubes (BD Vacutainer, Becton Dickinson Infusion Therapy Systems Inc., Utah, USA) for isolation of plasma and serum. Seven intravenous blood draws were performed for each condition. The first collection (-30 min) occurred immediately before the consumption of the ketone or placebo supplement. Participants consumed a KME supplement in the form of (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate

(ΔG° from T ΔS° Ltd, UK; 0.45 ml/kg body mass or 482 mg/kg body mass) ingested with water and vanilla-flavoured stevia (SweetLeaf) in a total volume of 100 ml. Immediately following ingestion of the ketones, participants were given 20 ml of calorie-free Gatorade (G2) in attempts to remove any remaining flavour of the supplement. In the placebo condition, participants consumed 100 ml of water and vanilla-flavoured stevia (SweetLeaf) followed by the same 20 ml calorie-free Gatorade (G2). Thirty minutes later another blood sample was collected (0 min), then followed immediately by the consumption of a 75 g oral glucose tolerance test drink (Thermo Scientific, Fisher Scientific Company, Middletown, USA). Another five blood draws occurred at 15, 30, 60, 90 and 120 minutes after ingestion of the glucose drink. At each time point, β -OHB was measured in whole blood (Precision Neo; Abbott Laboratories, Witney, UK). After the trial was completed, participants were asked to complete a gastrointestinal distress questionnaire and to guess whether they had the placebo or ketone supplement by answering the following; “What condition do you think you were in today?” Before leaving the laboratory, a copy of the 24-hour food log was provided to participants who were asked to repeat the exact diet 24 hours before the following visit. Subjects were also reminded to not perform exercise or to consume alcohol 24 hours prior to the next visit.

Participants returned to the laboratory (≥ 10 hour fast) at least 48 hours later to complete the alternate condition. Adherence to the 24-hour diet, exercise and alcohol guidelines were confirmed upon arrival in the laboratory. If no significant deviations were observed, the protocol for the third visit was the same as the previous one, except that participants received the alternate supplement (ketone or placebo). Female participants completed both experimental conditions in the follicular phase (between 3-9 days after the beginning of their menstrual cycle).

Blood samples

Upon collection, 10 μ L of a dipeptidyl peptidase (DPP-4) inhibitor (Cat. No. DPP4-010, Millipore, MA, USA) was added to the EDTA tube to prevent the degradation of active glucagon-like peptide-1 (GLP-1). The EDTA and serum tubes were centrifuged at 1,500 $\times g$ for 15 min at 4°C. Following centrifugation, plasma and serum samples were stored in a -80°C freezer until assays were performed. Blood metabolites were analyzed using the following commercially available kits; Serum non-esterified fatty acids (NEFA) (Wako Diagnostics HR Series, CA, USA) and serum glucose (Glucose hexokinase, Pointe Scientific INC, MI, USA) were analyzed on a Chemwell 2910 automated analyzer (Awareness Technologies, Palm City, USA). Serum C-peptide (C-peptide, Meso Scale Discovery, MD, USA) and plasma GLP-1 (M/R active GLP-1 (7-36) amide, Meso Scale

Discovery, MD, USA) were analyzed on a MESO Quickplex SQ 120. Serum insulin (Human Insulin ELISA, Crystal Chem, IL, USA) and serum adiponectin (Rapid Human Adiponectin Immunoassay kit, Antibody and Immunoassay Services, Li Ka Shing Faculty of Medicine, The University of Hong Kong) were analyzed on an iMark™ Microplate Absorbance Reader (Bio Rad, CA, USA). All assays were run in duplicate. The coefficient of variation for duplicate samples was 4.2% for serum NEFA, 2.9% for serum glucose, 7.8% for serum C-peptide, 4.1% for plasma GLP-1, 4.9% for serum insulin, and 4.0% for serum adiponectin.

Oral glucose insulin sensitivity index

The oral glucose insulin sensitivity (OGIS) index was computed using the model-based method proposed by Mari et al. [34].

Visual Analogue Scale

At 120 minutes of each experimental condition, participants were asked to fill out visual analog scales (VAS) [35] for the following five symptoms; nausea, urge to vomit, bloating, belching and cramps. Participants were asked to fill out the VAS based on their global experience and symptoms throughout the 2.5-hour experimental condition.

Statistical Analysis

Data were analyzed using SPSS v.21 (SPSS Inc., Chicago, IL, USA). Normality was assessed using Q-Q plots and Shapiro-Wilk tests within each experimental condition. Two-hour area under the curve (AUC) and incremental AUC (iAUC) were calculated using GraphPad Prism v.6.0 (GraphPad Software Inc., San Diego, CA, USA) and included time points 0 to 120. Area under the curves and iAUCs were compared between experimental conditions using paired Student's t-tests. VAS differences between conditions were assessed using a Wilcoxon signed-rank test. A linear mixed-effects model including time points -30 to 120 (Condition and Time as fixed factors and Subject as random factor) was used to determine the treatment effects. Significant interactions were followed up with pre-planned contrasts comparing Placebo to KME within each time point using Bonferroni corrections for multiple comparisons. Cohen's *d* effect size was calculated for all of the significant pre-planned comparisons. Significance was set at $P < 0.05$. Data in Table 1 and figures are presented as mean (SD) whereas non-parametric data in Table 2 are presented as median with range.

RESULTS

Baseline characteristics of the participants are presented in Table 1. The β -OHB, glucose, insulin, and C-peptide responses over time are presented in Figure 1. As expected, a significant condition X time interaction was found for β -OHB ($P < 0.001$), with all time points except -30 ($P = 0.577$) being higher after KME supplementation compared to placebo (all $P < 0.001$, Figure 1A). Also as expected, significant main effects of time were found for glucose, insulin and C-peptide (all $p < 0.001$). There was a main effect of condition observed for glucose ($P < 0.001$, Figure 1B) with glucose being lower in the KME condition. No significant effects of condition or condition X time interactions were found for insulin (respectively $P = 0.971$ and $P = 0.871$; Figure 1C) or C-peptide ($P = 0.078$, $P = 0.489$; Figure 1D).

Areas under the curve for β -OHB, glucose, insulin, and C-peptide are presented in Figures 2 and 3. As expected, the KME supplement significantly increased β -OHB AUC compared to placebo (1104%, $P < 0.001$, $d = 8.9$) (Figure 2A). As compared to placebo, the KME supplement significantly decreased glucose AUC (-16%, $P = 0.001$, $d = 0.9$, Fig 2B) and glucose iAUC (-37%, $P = 0.029$, $d = 0.6$) (Fig 3A). While no differences were observed between KME and placebo for the insulin AUC: (-3%, $P = 0.710$, $d = 0.1$, Fig 2C), insulin iAUC: (-15%, $P = 0.087$, $d = 0.4$, Fig 3B) or C-peptide AUC (-9%, $P = 0.151$, $d = 0.4$, Fig 2D), C-peptide iAUC showed a significant decrease in the KME condition (-21%, $P = 0.005$, $d = 0.9$, Fig 3C). Oral glucose insulin sensitivity index improved by ~11% in the KME condition ($P = 0.001$, $d = 1.0$, Fig 4).

A condition x time interaction was observed for serum NEFA ($P < 0.001$; Figure 5A). Pre-planned contrasts comparing the two conditions within each time point revealed significant differences at time 0 ($P < 0.001$, $d = 1.7$), 15 ($P < 0.001$, $d = 1.9$), 30 ($P < 0.001$, $d = 1.4$), 60 ($P = 0.001$, $d = 0.8$), 90 ($P = 0.004$, $d = 0.8$) and 120 ($P = 0.005$, $d = 1.0$). NEFA AUC was also decreased by ~44% after KME as compared to Placebo ($P < 0.001$, $d = 1.8$, Fig 5B). A significant condition X time interaction was seen for GLP-1 ($P = 0.002$), with GLP-1 being lower at time 30 in the KME condition ($P < 0.001$, $d = 1.4$; Fig 6A). There were no significant effects of condition, time, or condition X time interactions for adiponectin (all $P > 0.239$). Gastrointestinal symptoms were generally low to non-existent with medians of zero for all symptoms except nausea where the median was 1 (out of 100 mm) in the ketone condition (Table 2). Wilcoxon signed rank tests revealed no significant differences between conditions for symptoms of nausea, urge to vomit, bloating, belching and cramps (all $P > 0.05$; Table 2).

DISCUSSION

The objective of this study was to determine if a single dose of KME consumed 30 minutes before a 2-hour OGTT could improve glucose tolerance in young healthy individuals. Our results demonstrate that in individuals with fasting glucose levels within the normal range (4.0-6.0 mmol/l) [36], 2-hour total and incremental glucose AUCs are significantly lowered by a single dose of KME supplement as compared to a placebo. This improvement in glucose tolerance following the ingestion of KME was accompanied by a decrease in circulating NEFA levels and an improvement in the OGIS index, a marker of insulin sensitivity. The decrease in C-peptide iAUC under the KME condition also supports the notion that exogenous ketone supplementation may lower glucose via improved insulin sensitivity.

At the time of writing this manuscript, the first data in humans reporting a glucose lowering effect of KME supplements in the resting state were published [37]. This recent publication demonstrated that a KME supplement ingested following the consumption of a standard meal decreased glucose levels from 5.5 to 4.7 mmol/l over the 4-hour study. This decrease of ~15% is of similar magnitude as the 16% decrease in glucose AUC observed in our study. Our study adds to these findings and extends the glucose lowering effect to KME consumption prior to ingestion of glucose, suggesting that pre-meal supplementation with exogenous ketones could improve glucose tolerance. The hypoglycemic action of ketone bodies has been shown in β -OHB infusion studies in both humans and animals [18-24,38]. Among them, Miles et al. (REF here) and Mikkelsen et al. (REF here) showed that β -OHB, infused to levels of ~2.0 mM, significantly decreased circulating glucose and suppressed endogenous glucose production by ~20% in healthy males [18,38]. The glucose-lowering action of ketones may be due to a direct effect on hepatic glucose production, as it has been shown to occur in the absence of changes in insulin or glucagon [21,24,39-41]. However, ketone body infusion has also been shown in some studies to stimulate pancreatic beta-cell insulin secretion [42-44]. Previous studies using KME supplement have observed a two-fold increase in insulin levels during a post-exercise hyperglycemic clamp [45] and a small increase in the fasting state [37,46] whereas no change was seen following a post-exercise high-dose protein-carbohydrate drink [31]. The potential insulinogenic action of ketone bodies remains a matter of debate, but our results clearly indicate that if indeed KME stimulates insulin secretion, this effect is not additive to the stimulus of a standardized 75-gram glucose drink. Contrary to a potential increase in insulin secretion following the use of exogenous ketones, we observed a decrease in C-peptide iAUC during the OGTT. Miles et al. reported an increase in C-peptide levels

following the infusion of β -OHB in fasted humans [18]. The discrepancy between this report and our findings might come from the fact that our study involved ingestion of exogenous ketones followed by glucose instead of isolated infusion of ketones in the basal state. The decrease in C-peptide was accompanied by a decrease in incretin hormone glucagon-like peptide-one (GLP-1) in the KME condition. This observation is in agreement with Stubbs et al. who reported a decrease in GLP-1 following a KME supplement in the fasting state [46]. GLP-1 is produced by the gut in response to food (or carbohydrate) ingestion and acts to potentiate insulin secretion [47]. Thus, a decrease in GLP-1 might be linked to the observed decrease in C-peptide iAUC following the KME supplement. More studies are needed to determine the underlying mechanisms linking KME ingestion to improved glucose tolerance, including further exploration of effects on insulin sensitivity, insulin secretion and GLP-1 involvement.

Our study was not able to directly test mechanisms such as hepatic glucose production or muscle glucose uptake, but our results suggest that improved insulin sensitivity could be potentially responsible for the improved glucose tolerance after KME ingestion. While starvation and a KD increase the release and utilization of NEFA [48-50], β -OHB can directly inhibit lipolysis via agonism of the nicotinic acid receptor GR109A (also known as HM74A, PUMA-G) on adipocytes [51]. In line with our results, several studies involving ketone infusion have reported a significant decrease in NEFA levels in both fasting and fed conditions [18,24,37,38]. Noteworthy, the KME supplement in our study was able to immediately decrease NEFA in the fasted state (-30 vs. 0 minute time points) and further decrease NEFA levels on top of the anti-lipolytic effect of glucose ingestion (time points 15-120 minutes). Since lower levels of NEFA are associated with better insulin sensitivity, we suggest that the potential improvement in insulin sensitivity in the KME condition could be driven by a decrease in circulating NEFA [52-54]. The absence of change in adiponectin levels following KME is in accordance with a previous study using KS and suggests that this protein was not involved in the insulin sensitivity improvement [26]. Adiponectin is an adipokine that is associated with improved insulin sensitivity [55], the secretion of which can be stimulated through the nicotinic acid receptor [56].

Some limitations of our study should be acknowledged. First, this study was conducted with healthy young individuals, so the results may not apply to populations with metabolic impairments. Given that KME is so new, we aimed to study the response in healthy individuals to reduce the confounding influence of insulin resistance, beta-cell dysfunction, and medications, all of which could confound interpretation of OGTT results. Along this line, more studies are needed to determine the possible impact of KME

supplementation in individuals with impaired glucose tolerance. Secondly, the use of the oral glucose insulin sensitivity index limits mechanistic interpretation of our findings, compared to a more direct assessment of insulin sensitivity such as a hyperinsulinemic-euglycemic clamp or stable isotope glucose tracers. However, the OGIS correlates well with clamp-derived measures in healthy individuals and, if KME is to be used therapeutically to lower glucose, we feel it is important to demonstrate efficacy in improving glucose tolerance as ultimately individuals consume carbohydrates, which contribute to hyperglycemia in real-life. Finally, our study could not rule out potential effects of the KME on digestion and absorption of the glucose drink, so future studies could explore gastric emptying to better understand the mechanisms behind the improved glucose tolerance following the KME supplement.

In conclusion, a KME supplement that acutely increased β -OHB levels up to ~3 mmol/l improved glucose tolerance in healthy humans. The improvement in glucose tolerance was not driven by increased insulin secretion, but was accompanied by improved markers of insulin sensitivity, possibly related to the β -OHB-mediated reduction in circulating NEFA. These acute effects on improved glucose tolerance and insulin sensitivity, along with lower NEFA, suggest that ketone monoester supplements could have therapeutic potential in the management and prevention of metabolic disease.

REFERENCES:

1. Balasse EO, Fery F (1989) Ketone body production and disposal: effects of fasting, diabetes, and exercise. *Diabetes Metab Rev* 5: 247-270.
2. Yancy WS, Jr., Olsen MK, Guyton JR, Bakst RP, Westman EC (2004) A low-carbohydrate, ketogenic diet versus a low-fat diet to treat obesity and hyperlipidemia: a randomized, controlled trial. *Ann Intern Med* 140: 769-777.
3. Robinson AM, Williamson DH (1980) Physiological roles of ketone bodies as substrates and signals in mammalian tissues. *Physiol Rev* 60: 143-187.
4. Cahill GF, Jr. (1976) Starvation in man. *Clin Endocrinol Metab* 5: 397-415.
5. Owen OE, Felig P, Morgan AP, Wahren J, Cahill GF, Jr. (1969) Liver and kidney metabolism during prolonged starvation. *J Clin Invest* 48: 574-583.
6. Youm YH, Nguyen KY, Grant RW, Goldberg EL, Bodogai M, et al. (2015) The ketone metabolite beta-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. *Nat Med* 21: 263-269.
7. Hussain TA, Mathew TC, Dashti AA, Asfar S, Al-Zaid N, et al. (2012) Effect of low-calorie versus low-carbohydrate ketogenic diet in type 2 diabetes. *Nutrition* 28: 1016-1021.
8. Guldbrand H, Dizdar B, Bunjaku B, Lindström T, Bachrach-Lindström M, et al. (2012) In type 2 diabetes, randomisation to advice to follow a low-carbohydrate diet transiently improves glycaemic control compared with advice to follow a low-fat diet producing a similar weight loss. *Diabetologia* 55: 2118-2127.
9. McKenzie AL, Hallberg SJ, Creighton BC, Volk BM, Link TM, et al. (2017) A Novel Intervention Including Individualized Nutritional Recommendations Reduces Hemoglobin A1c Level, Medication Use, and Weight in Type 2 Diabetes. *JMIR Diabetes* 2: e5.
10. Tay J, Luscombe-Marsh ND, Thompson CH, Noakes M, Buckley JD, et al. (2015) Comparison of low-and high-carbohydrate diets for type 2 diabetes management: a randomized trial. *The American journal of clinical nutrition* 102: 780-790.
11. Feinman RD, Pogozelski WK, Astrup A, Bernstein RK, Fine EJ, et al. (2015) Dietary carbohydrate restriction as the first approach in diabetes management: critical review and evidence base. *Nutrition* 31: 1-13.
12. Stafstrom CE, Rho JM (2012) The ketogenic diet as a treatment paradigm for diverse neurological disorders. *Frontiers in pharmacology* 3.
13. Paoli A, Rubini A, Volek J, Grimaldi K (2013) Beyond weight loss: a review of the therapeutic uses of very-low-carbohydrate (ketogenic) diets. *European journal of clinical nutrition* 67: 789-796.
14. Klement RJ, Sweeney RA (2016) Impact of a ketogenic diet intervention during radiotherapy on body composition: I. Initial clinical experience with six prospectively studied patients. *BMC research notes* 9: 143.
15. Martin K, Jackson CF, Levy RG, Cooper PN (2016) Ketogenic diet and other dietary treatments for epilepsy. *The Cochrane Library*.
16. van Delft R, Lambrechts D, Verschuure P, Hulsman J, Majoie M (2010) Blood beta-hydroxybutyrate correlates better with seizure reduction due to ketogenic diet than do ketones in the urine. *Seizure* 19: 36-39.
17. Johnston CS, Tjonn SL, Swan PD, White A, Hutchins H, et al. (2006) Ketogenic low-carbohydrate diets have no metabolic advantage over nonketogenic low-carbohydrate diets. *The American journal of clinical nutrition* 83: 1055-1061.
18. Miles JM, HAYMOND MW, GERICH JE (1981) Suppression of glucose production and stimulation of insulin secretion by physiological concentrations of ketone bodies in man. *The Journal of Clinical Endocrinology & Metabolism* 52: 34-37.

19. Shaw J, Wolfe RR (1984) Influence of beta-hydroxybutyrate infusion on glucose and free fatty acid metabolism in dogs. *American Journal of Physiology-Endocrinology and Metabolism* 247: E756-E764.
20. Henry RR, Brechtel G, Lim K-H (1990) Effects of ketone bodies on carbohydrate metabolism in non-insulin-dependent (type II) diabetes mellitus. *Metabolism* 39: 853-858.
21. Binkiewicz A, Sadeghi-Nejad A, Hochman H, Loridan L, Senior B (1974) An effect of ketones on the concentrations of glucose and of free fatty acids in man independent of the release of insulin. *The Journal of pediatrics* 84: 226-231.
22. Müller MJ, Paschen U, Seitz HJ (1984) Effect of ketone bodies on glucose production and utilization in the miniature pig. *Journal of Clinical Investigation* 74: 249.
23. Beylot M, Khalfallah Y, Riou J, Cohen R, Normand S, et al. (1986) Effects of ketone bodies on basal and insulin-stimulated glucose utilization in man. *The Journal of Clinical Endocrinology & Metabolism* 63: 9-15.
24. Balasse E, Ooms H (1968) Changes in the concentrations of glucose, free fatty acids, insulin and ketone bodies in the blood during sodium\ -hydroxybutyrate infusions in man. *Diabetologia* 4: 133-135.
25. Kesi SL, Poff AM, Ward NP, Fiorelli TN, Ari C, et al. (2016) Effects of exogenous ketone supplementation on blood ketone, glucose, triglyceride, and lipoprotein levels in Sprague–Dawley rats. *Nutrition & metabolism* 13: 9.
26. de Oliveira Caminhoto R, Komino ACM, de Fatima Silva F, Andreotti S, Sertié RAL, et al. (2017) Oral β -hydroxybutyrate increases ketonemia, decreases visceral adipocyte volume and improves serum lipid profile in Wistar rats. *Nutrition & Metabolism* 14: 31.
27. Ari C, Kovács Z, Juhasz G, Murdun C, Goldhagen CR, et al. (2017) Exogenous ketone supplements reduce anxiety-related behavior in Sprague-Dawley and Wistar Albino Glaxo/Rijswijk rats. *Frontiers in molecular neuroscience* 9: 137.
28. Veech RL (2004) The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins, leukotrienes and essential fatty acids* 70: 309-319.
29. Clarke K, Tchabanenko K, Pawlosky R, Carter E, King MT, et al. (2012) Kinetics, safety and tolerability of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate in healthy adult subjects. *Regulatory Toxicology and Pharmacology* 63: 401-408.
30. Cox PJ, Kirk T, Ashmore T, Willerton K, Evans R, et al. (2016) Nutritional ketosis alters fuel preference and thereby endurance performance in athletes. *Cell metabolism* 24: 256-268.
31. Vandoorne T, De Smet S, Ramaekers M, Van Thienen R, De Bock K, et al. (2017) Intake of a Ketone Ester Drink during Recovery from Exercise Promotes mTORC1 Signaling but Not Glycogen Resynthesis in Human Muscle. *Frontiers in physiology* 8.
32. Desrochers S, Dubreuil P, Brunet J, Jette M, David F, et al. (1995) Metabolism of (R, S)-1, 3-butanediol acetoacetate esters, potential parenteral and enteral nutrients in conscious pigs. *American Journal of Physiology-Endocrinology and Metabolism* 268: E660-E667.
33. Tate RL, Mehman MA, Tobin RB (1971) Metabolic fate of 1, 3-butanediol in the rat: conversion to β -hydroxybutyrate. *Journal of Nutrition* 101: 1719-1726.
34. Mari A, Pacini G, Murphy E, Ludvik B, Nolan JJ (2001) A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. *Diabetes care* 24: 539-548.

35. Flint A, Raben A, Blundell J, Astrup A (2000) Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International journal of obesity* 24: 38.
36. Cheng A (2013) Canadian Diabetes Association Clinical Practice Guidelines Expert Committee: Canadian diabetes association 2013 clinical practice guidelines for the prevention and management of diabetes in Canada. *Can J Diabetes* 37: S1-S212.
37. Stubbs BJ, Cox PJ, Evans RD, Santer P, Miller JJ, et al. (2017) On the metabolism of exogenous ketones in humans. *Frontiers in Physiology* 8: 848.
38. Mikkelsen KH, Seifert T, Secher NH, Grøndal T, van Hall G (2015) Systemic, cerebral and skeletal muscle ketone body and energy metabolism during acute hyper-D- β -hydroxybutyratemia in post-absorptive healthy males. *The Journal of Clinical Endocrinology & Metabolism* 100: 636-643.
39. Senior B, Loridan L (1968) Direct regulatory effect of ketones on lipolysis and on glucose concentrations in man. *Nature* 219: 83-84.
40. Sherwin R, Hendler R, Felig P (1975) Effect of ketone infusions on amino acid and nitrogen metabolism in man. *Journal of Clinical Investigation* 55: 1382.
41. Balasse E (1970) Inhibition of free fatty acid oxidation by acetoacetate in normal dogs. *European journal of clinical investigation* 1: 155-160.
42. Madison LL, Mebane D, Unger RH, Lochner A (1964) The hypoglycemic action of ketones. II. Evidence for a stimulatory feedback of ketones on the pancreatic beta cells. *Journal of Clinical Investigation* 43: 408.
43. Balasse E, Ooms H, Lambilliotte J (1970) Evidence for a stimulatory effect of ketone bodies on insulin secretion in man. *Hormone and Metabolic Research* 2: 371-372.
44. Jenkins D, Hunter W, Goff D (1970) Ketone bodies and evidence for increased insulin secretion. *Nature* 227: 384-385.
45. Holdsworth DA, Cox PJ, Kirk T, Stradling H, Impey SG, et al. (2017) A ketone ester drink increases postexercise muscle glycogen synthesis in humans. *Medicine and science in sports and exercise* 49: 1789.
46. Stubbs BJ, Cox PJ, Evans RD, Cyranka M, Clarke K, et al. (2017) A Ketone Ester Drink Lowers Human Ghrelin and Appetite. *Obesity*.
47. Seino Y, Fukushima M, Yabe D (2010) GIP and GLP-1, the two incretin hormones: similarities and differences. *Journal of diabetes investigation* 1: 8-23.
48. Cahill Jr GF (2006) Fuel metabolism in starvation. *Annu Rev Nutr* 26: 1-22.
49. Volek JS, Fernandez ML, Feinman RD, Phinney SD (2008) Dietary carbohydrate restriction induces a unique metabolic state positively affecting atherogenic dyslipidemia, fatty acid partitioning, and metabolic syndrome. *Progress in lipid research* 47: 307-318.
50. Schwarz J-M, Neese RA, Turner S, Dare D, Hellerstein MK (1995) Short-term alterations in carbohydrate energy intake in humans. Striking effects on hepatic glucose production, de novo lipogenesis, lipolysis, and whole-body fuel selection. *Journal of Clinical Investigation* 96: 2735.
51. Taggart AK, Kero J, Gan X, Cai T-Q, Cheng K, et al. (2005) (D)- β -hydroxybutyrate inhibits adipocyte lipolysis via the nicotinic acid receptor PUMA-G. *Journal of Biological Chemistry* 280: 26649-26652.
52. Boden G, Lebed B, Schatz M, Homko C, Lemieux S (2001) Effects of acute changes of plasma free fatty acids on intramyocellular fat content and insulin resistance in healthy subjects. *Diabetes* 50: 1612-1617.
53. Griffin ME, Marcucci MJ, Cline GW, Bell K, Barucci N, et al. (1999) Free fatty acid-induced insulin resistance is associated with activation of protein kinase C θ and alterations in the insulin signaling cascade. *Diabetes* 48: 1270-1274.

54. Bachmann OP, Dahl DB, Brechtel K, Machann J, Haap M, et al. (2001) Effects of intravenous and dietary lipid challenge on intramyocellular lipid content and the relation with insulin sensitivity in humans. *Diabetes* 50: 2579-2584.
55. Yadav A, Kataria MA, Saini V, Yadav A (2013) Role of leptin and adiponectin in insulin resistance. *Clinica Chimica Acta* 417: 80-84.
56. Plaisance EP, Lukasova M, Offermanns S, Zhang Y, Cao G, et al. (2009) Niacin stimulates adiponectin secretion through the GPR109A receptor. *American Journal of Physiology-Endocrinology and Metabolism* 296: E549-E558.

Table 1. Characteristics of participants

Variable	Mean (SD)
Number of participants (n)	20
Male (n)	10
Female (n)	10
Age (years)	25.4 (4.1)
Body mass index (kg/m ²)	22.1 (2.2)
Waist circumference (cm)	70.2 (7.2)
Systolic blood pressure (mmHg)	116 (8)
Diastolic blood pressure (mmHg)	76 (8)

Data are presented as mean (SD).

Table 2. Gastrointestinal symptoms assessed at the end of the ketone monoester (KME) supplement and placebo experimental trials.

	Nausea		Urge to Vomit		Bloating		Belching		Cramps	
	Range	Median	Range	Median	Range	Median	Range	Median	Range	Median
Placebo	0-15	0	0-4	0	0-9	0	0-40	0	0-10	0
Ketone	0-40	1	0-22	0	0-33	0	0-32	0	0-8	0
Wilcoxon signed-rank tests										
P values	0.06		0.09		0.60		0.92		0.89	
Effect sizes										
Cohen's d	0.5		0.6		0.3		0.1		0.1	

Results are presented in millimetres (0-100).

FIGURE CAPTIONS

Figure 1. Beta-Hydroxybutyrate (β -OHB), glucose, insulin and C-Peptide responses following a single dose of ketone monoester supplement or placebo. Supplements were consumed in the fasted state followed 30 minutes later by a 75 gram oral glucose tolerance test (OGTT). (A) β -OHB; (B) Glucose; (C) Insulin; and (D) C-peptide. * $P < 0.001$ vs. placebo within time point, Bonferroni adjusted post-hoc. † $P < 0.001$ significant main effect of condition.

Figure 2. Two-hour area under the curve (AUC) following a single dose of ketone monoester supplement or placebo. Supplements were consumed in the fasted state followed 30 minutes later by a 75 gram oral glucose tolerance test (OGTT). (A) Beta-Hydroxybutyrate (β -OHB) AUC; (B) Glucose AUC; (C) Insulin AUC; and (D) C-peptide AUC. * $P = 0.001$ vs. placebo.

Figure 3. Two-hour incremental area under the curve (iAUC) following a single dose of ketone monoester supplement or placebo. Supplements were consumed in the fasted state followed 30 minutes later by a 75 gram oral glucose tolerance test (OGTT). (A) Beta-hydroxybutyrate iAUC; (B) Glucose iAUC; (C) Insulin AUC; and (D) C-peptide iAUC. ** $P < 0.01$ vs. placebo, * $P < 0.05$ vs. placebo.

Figure 4. Oral glucose insulin sensitivity index following a single dose of ketone monoester supplement or placebo. Supplements were consumed in the fasted state followed 30 minutes later by a 75 gram oral glucose tolerance test (OGTT). * $P = 0.001$ vs. placebo.

Figure 5. Non-esterified fatty acids (NEFA) following a single dose of ketone monoester supplement or placebo. Supplements were consumed in the fasted state followed 30 minutes later by a 75 gram oral glucose tolerance test (OGTT). (A) NEFA response over time; and (B) Two-hour area under the curve.. ** $P < 0.001$ vs. placebo. * $P < 0.01$ vs. placebo within time point, Bonferroni adjusted post-hoc.

Figure 6. Glucagon-like peptide (GLP-1) and adiponectin responses following a single dose of ketone monoester supplement or placebo. Supplements were consumed in the fasted state followed 30 minutes later by a 75 gram oral glucose tolerance test (OGTT). (A) GLP-1; and (B) Adiponectin.

* $P < 0.01$ vs. placebo within time point, Bonferroni adjusted post-hoc.

Figure 1

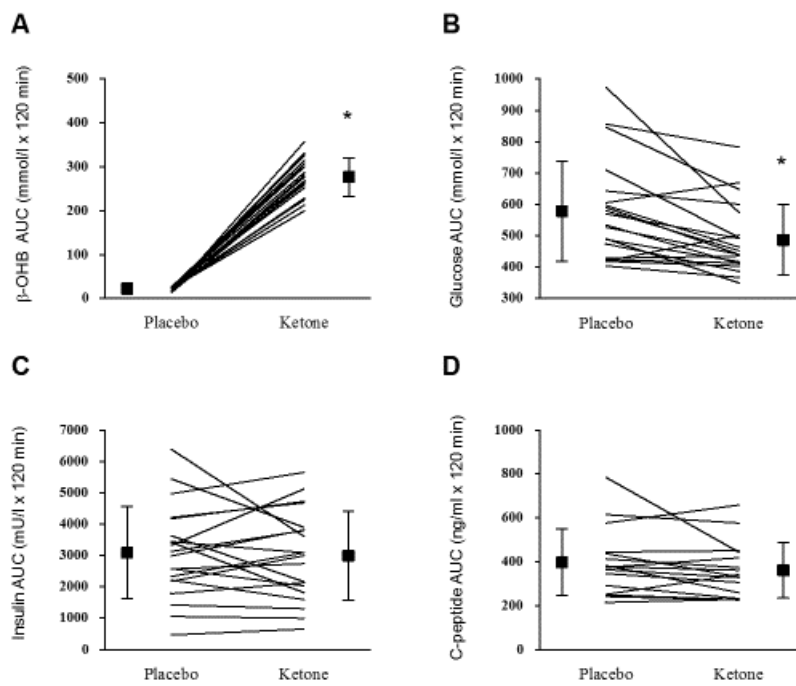
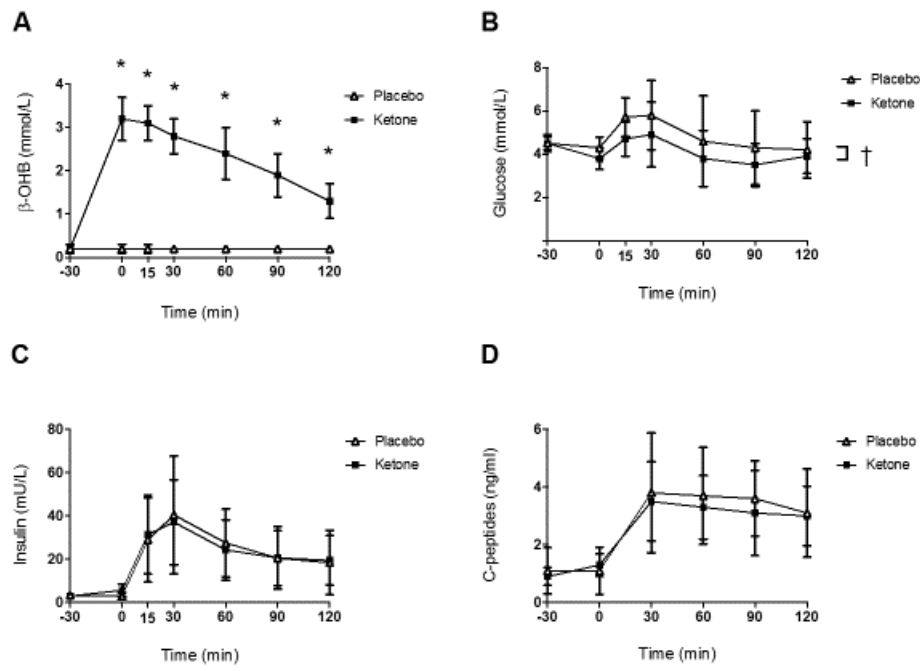


Figure 2

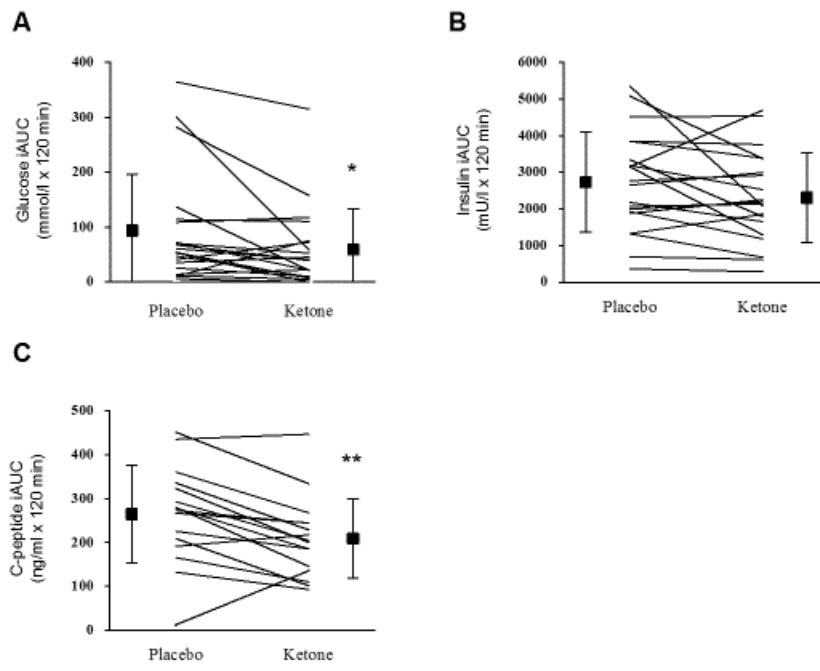


Figure 3

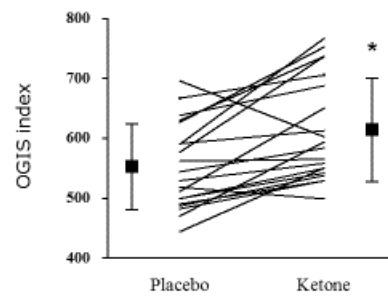


Figure 4

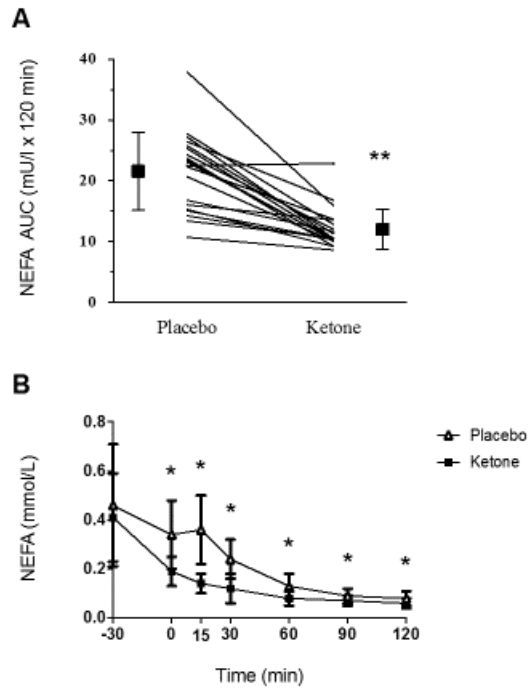


Figure 5

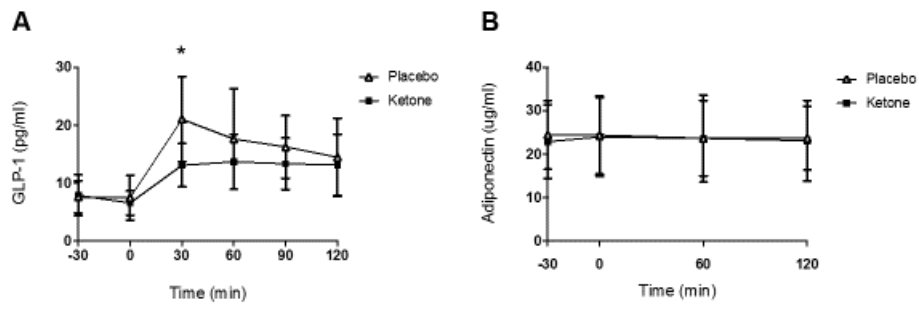


Figure 6