Exogenous D-β-Hydroxybutyrate Lowers Blood Glucose
by Decreasing the Availability of L-Alanine
for Gluconeogenesis

Adrian Soto-Mota, Nicholas G. Norwitz, Rhys D. Evans, Kieran Clarke

Affiliation: Department of Physiology, Anatomy and Genetics
University of Oxford
Oxford OX1 3PT
United Kingdom

Corresponding author: Dr Adrian Soto
Department of Physiology, Anatomy and Genetics
Sherrington Building
University of Oxford
Parks Road
Oxford OX1 3PT
United Kingdom
Phone: ±44 1865 272472
Email: adrian.soto@dpag.ox.ac.uk
ABSTRACT

Background
Interventions that acutely increase blood ketone concentrations simultaneously lower blood glucose levels, although the explanation for this phenomenon is unknown. The hypoglycaemic effect of acute ketosis is greater in people with type 2 diabetes (T2D) in whom gluconeogenesis contributes significantly to hyperglycaemia. L-alanine is a gluconeogenic substrate secreted by skeletal muscle at higher levels in people with T2D. As infusion of ketones lower circulating L-alanine blood levels, we sought to determine whether supplementation with L-alanine would attenuate the hypoglycaemic effect of an exogenous ketone ester (KE) supplement.

Methods
This crossover study involved 10 healthy human volunteers who fasted for 24 hours prior to the ingestion of 25 g of D-β-hydroxybutyrate (βHB) in the form of a KE drink (ΔG®) on two separate visits. During one of the visits participants additionally ingested 2 g of L-alanine to see if L-alanine supplementation would attenuate the hypoglycaemic effect of the KE drink. Blood L-alanine, L-glutamine, glucose, βHB, free fatty acids (FFA), lactate, and C-peptide were measured every fifteen minutes for 120 minutes after ingestion of the KE, with or without L-alanine.

Findings
The KE drinks elevated blood βHB concentrations from negligible levels to 4.5 ± 1.24 mmol/L, lowered glucose from 4.97 to 3.77 ± 0.4 mmol/L, and lowered and L-alanine from 0.56 to 0.41 ± 0.9 mmol/L. L-alanine in the KE drink elevated blood L-Alanine to 0.68 ± mmol/L, but had no significant effect on blood βHB, L-glutamine, FFA, lactate, nor C-peptide concentrations. By contrast, L-alanine supplementation significantly attenuated the ketosis-induced drop in glucose from 28% to 16% (p<0.001).

Conclusions
The hypoglycaemic effect of acutely elevated βHB is partially due to βHB decreasing L-alanine availability as a substrate for gluconeogenesis.
Funding

Adrian Soto-Mota is funded by CONACYT and INCMSNZ. The KE drinks were provided by TdeltaS Ltd, Oxford.

KEY WORDS: Alanine, D-β-hydroxybutyrate, Diabetes, Exogenous Ketosis, Gluconeogenesis, Hyperglycaemia, Hypoglycaemia, Ketone Bodies
INTRODUCTION

In the fed state, the human brain relies almost exclusively on glucose as an energy source. However, in the fasted state or during carbohydrate scarcity, the human body has two adaptive mechanisms: (i) Gluconeogenesis, which promotes the catabolism of muscle tissue. (ii) Ketogenesis, which produces primarily \( \text{D-\(\beta\)-hydroxybutyrate (\(\beta\)HB)} \) from fat stores and provides an alternative fuel substrate for the brain. This latter option fuels the energy expensive brain, while at the same time preserving valuable lean tissue. Consistent with this adaptive muscle-sparing model, \( \beta \)HB inhibits gluconeogenesis¹.

In type 2 diabetes (T2D), excessive gluconeogenesis underlies hyperglycemia². Thus, one could predict that \( \beta \)HB, by inhibiting gluconeogenesis³, could help manage hyperglycemia in T2D. Consistent with this hypothesis, ketogenic diets have been shown to effectively reverse T2D⁴,⁵. While most of the benefit of ketogenic diets in T2D likely comes from therapeutic carbohydrate reduction, it is not yet clear to what degree \( \beta \)HB itself might play a supporting therapeutic role. In fact, in both animals and humans, exogenous infusion of \( \beta \)HB are alone sufficient to decrease blood glucose⁶–⁹.

The development of an enantiomerically pure medical-grade exogenous ketone ester (KE) for human consumption, following on the possibility that \( \beta \)HB could itself benefit persons with T2D, has inspired interest in the exploration of exogenous ketosis for T2D¹⁰. This interest is bolstered by the observation that the hypoglycemic effect of ketosis is larger in people living with T2D than in non-diabetic adults¹¹. And, as of yet, no mechanism has been found to explain this difference.

Gluconeogenesis is main hyperglycaemic mechanism in people living with T2D²,¹². Ketosis, by providing an alternate source of energy to the brain, preserves muscle tissue¹³ and ketones limit the secretion of L-alanine, a gluconeogenic substrate, by muscle cells¹¹,¹⁴.
Thus, it is plausible that ketones decrease blood glucose (and do so to a greater extent in those with T2D) by reducing L-alanine availability for gluconeogenesis. This study sought to test this hypothesis by investigating whether L-alanine supplementation attenuates the hypoglycaemic effect of exogenously induced ketosis.
METHODS

I) Ethics

This study was pre-registered at ISRCTN16169021 and received Ethics approval by the East of England - Cambridge South Research Ethics Committee on February 18th, 2019 (Reference 18/EE/0115). All participants were older than 18 years old and signed an informed consent form approved by the same Research Ethics Committee.

This study was conducted in accordance with the guidelines set forth by the International Conference on Harmonisation Guidelines for Good Clinical Practice, and the Declaration of Helsinki regarding the treatment of human subjects in a study\textsuperscript{15}. Female potential participants in risk of being pregnant were excluded.

II) Statistical analysis

Sample size was calculated to detect a blood glucose difference of at least 0.9 mM between groups with a statistical power of 80% and an alpha probability of 0.05. Therefore, six participants per group were needed. To achieve a sex and age balanced cohort and to allow for dropouts, a total of 10 participants were recruited and all completed the study. Sample size calculations were performed using G*Power version 3.1\textsuperscript{16}.

All statistical tests and analysis were performed using Excel\textsuperscript{®} (Microsoft, United States). Data, presented as means ± standard deviations (SD), were analysed using one-way repeated measurements ANOVA and adjusted with Bonferroni’s correction for multiple comparisons. Differences were considered significant at p < 0.05. Since baseline blood concentrations of L-alanine and L-glutamine have large interpersonal variations due to body composition, comparisons were made using change from baseline.
Materials and procedures

10 healthy participants engaged in a randomized crossover experiment in which they came to the lab on two occasions after a 24 hour fast. On one visit, participants drank a mix 25 g of the ΔG® KE ((R)-3- hydroxy butyl (R)-3-hydroxybutyrate, provided by TdeltaS Ltd., United Kingdom) diluted in 25 mL water. On the other visit, participants consumed the same drink with an additional 2g of L-alanine (provided by Hard Eight Nutrition LLC, United States) to match skeletal muscle release of L-alanine per two hours².

After ingestion, the KE monoester bond is cleaved by esterases in the gut wall, yielding βHB and butanediol in equal amounts. Both are absorbed into the portal circulation and the latter is taken up by the liver, where it is converted to βHB by alcohol dehydrogenase. βHB leaves hepatocytes via monocarboxylate transporters. Pharmacokinetic studies have shown that, in the fasted and resting states, the βHB monoester can induce ketosis for 3–4 h, with a peak at ~1 h².

²

Blood samples were collected through a venous cannula at baseline and every fifteen minutes for 120 minutes. Glucose, βHB, lactate, and free fatty acids were assayed using a commercial semi-automated bench-top analyser (ABX Pentra, Montpellier, France). L-alanine, L-glutamine and human C-peptide were measured using kits (by Abcam, United Kingdom: ab83394, ab145659, and ab178641 respectively).
RESULTS

Participant anthropometric characteristics

Ten healthy participants, five males and five females, were enrolled in this crossover trial. All participants were of normal weight (mean BMI 23.2 kg/m² ± 2.4) and mean age was 40 years ± 16 years. Table 1 summarises anthropometric characteristics of our participants. Ingestion of the KE drink was well-tolerated by all participants and none presented symptomatic hypoglycaemia.

| Table 1. Participant characteristics |
|-------------------------------------|
| Sex       | 5 (F), 5 (M) |
| Age (years) | 40 (16) |
| Height (cm) | 176 (15) |
| Weight (kg) | 73 (18) |
| BMI (kg/m²) | 23.2 (2.4) |

Data are expressed as mean (Standard deviation).

Blood L-alanine, L-glutamine and glucose concentration changes after ketone ester ingestion, with and without L-alanine.

In the absence of L-alanine supplementation, inducing acute ketosis with the KE raised blood βHB concentration to 4.5 ± 1.24 mmol/L and L-glutamine by 9% ± 4% (absolute change from 1.099 to 1.223 ± 0.045 mmol/L). By contrast, exogenous ketosis lowered blood L-alanine by 26% ± 9.3% (absolute change from 0.563 to 0.4409 ± 0.053 mmol/L) and blood glucose by 28% ± 8% (absolute change from 4.97 to 3.77 ± 0.4 mmol/L). Compared to baseline, changes in βHB, glucose, and alanine, but not glutamine, were significantly different (p < 0.05).

In the presence of L-alanine supplementation, inducing ketosis raised blood βHB concentration to 4.6 ± 1.35 mmol/L and L-glutamine by 10% ± 6% (absolute change from 1.12 to 1.26 ± 0.05 mmol/L).
As expected, L-alanine supplementation led to a net increase in L-alanine by 16% (absolute change from 0.543 to 0.682 ± 0.051 mmol/L), thus maintaining L-alanine availability for gluconeogenesis. Correspondingly, blood glucose decreased by a lesser amount, only by 17% ± 5% (absolute change from 5.01 to 4.03 ± 0.3 mmol/l). Again, changes in βHB, glucose and L-alanine, but not L-glutamine, were significant (p<0.05). These results are described in detail in Table 2 and illustrated in Figure 1.

### Table 2. Blood L-alanine, L-glutamine and glucose concentration changes after inducing acute ketosis with and without L-alanine supplementation.

| Timepoint | βHB (mmol/L) | βHB + A (mmol/L) | Glucose (mmol/L) | Glucose + A (mmol/L) | Glutamine (mmol/L) | Glutamine + A (mmol/L) | Alanine (mmol/L) | Alanine + A (mmol/L) |
|-----------|--------------|------------------|-----------------|---------------------|--------------------|------------------------|------------------|---------------------|
| 0         | 0.6          | 0.5              | 4.97            | 5.01                | 1.11               | 1.09                   | 0.56             | 0.54                |
| 15        | 3.4          | 3.2              | 4.52            | 4.64                | 0.90               | 0.88                   | 0.49             | 0.57                |
| 30        | 3.9          | 4.0              | 4.11            | 4.31                | 0.89               | 0.81                   | 0.43             | 0.63                |
| 45        | 3.8          | 4.3              | 3.77            | 4.03                | 0.86               | 0.82                   | 0.40             | 0.68                |
| 60        | 4.3          | 4.7              | 3.79            | 4.07                | 0.93               | 0.91                   | 0.41             | 0.64                |
| 75        | 4.5          | 4.6              | 3.91            | 4.15                | 1.16               | 1.01                   | 0.44             | 0.59                |
| 90        | 4.1          | 4.2              | 3.99            | 4.37                | 1.26               | 1.19                   | 0.49             | 0.55                |
| 120       | 3.7          | 3.6              | 4.1             | 4.51                | 1.31               | 1.23                   | 0.51             | 0.54                |

Data are expressed as means from n = 10.

All measurements were performed by triplicate.
FIGURE 1. Blood L-alanine, L-glutamine and glucose concentration changes after inducing acute ketosis with and without L-alanine supplementation.

Data are presented as the mean ± SD (n = 10 participants, measurements by triplicate).
**L-alanine supplementation attenuated the hypoglycaemic effect of acute ketosis.**

2 g L-alanine significantly reduced the magnitude of the hypoglycaemic effect of exogenous ketosis at 30, 45, 75 and 90 min after KE administration (p<0.001). Even when the difference was not statistically significant, glucose levels were consistently lower at all timepoints when L-alanine was supplemented (Table 2 and Figure 2).

![Glucose AUC (KE) vs Glucose AUC (KE + L-Ala)](image)

**FIGURE 2. L-alanine supplementation attenuated the hypoglycaemic effect of ketosis.**

Data are presented as mean AUC (n = 10 participants, measurements by triplicate). Y axis = percent change from baseline of blood glucose concentration. X axis = Time (minutes) after drinking 25 of βHB ketone monoester. Mean change after baseline at every timepoint was analysed with a one-way repeated measures ANOVA and found to be statistically significant after a Bonferroni correction for multiple comparisons (p ≤ 0.006) as indicated by *.

**L-alanine supplementation did not impact changes in blood lactate, free fatty acids (FFA) and C-peptide during acute ketosis.**

Compared to baseline, FFA concentrations were significantly lower at most timepoints 30 minutes after KE ingestion. C-peptide was moderately elevated in both groups at the 30 minutes timepoint. Blood lactate, FFA and C-peptide concentrations were similar at all timepoints after ingestion of KE regardless of L-alanine supplementation, as in Figure 3.
FIGURE 3. Blood lactate, free fatty acids (FFA) and C-peptide during acute ketosis with and without L-alanine. Mean change after baseline was analysed with a one-way repeated measures ANOVA. Statistical significance after a Bonferroni correction ($p<0.001$).

* Indicates change from baseline, not difference between groups.
DISCUSSION

These data suggest that a decrease in L-alanine availability contributes to the hypoglycaemic effect of acute ketosis. However, it is also clear that the hypoglycaemic effect of acute ketosis is a result of multiple mechanisms.

Glucose-alanine cycle: pyruvate in disguise

During prolonged fasting, L-alanine blood levels decrease more than those of any other amino acid, largely because of hepatic uptake of L-alanine to fuel gluconeogenesis. This phenomenon prompted the discovery of the glucose-alanine cycle, in which pyruvate is transformed into L-alanine (pyruvate in disguise) via transamination in skeletal muscle. L-alanine is released to the bloodstream, taken up by the liver, and transformed back into pyruvate to fuel gluconeogenesis\(^\text{18}\). Thus, any reduction in intramuscular pyruvate would result in lower L-alanine levels in the blood and less gluconeogenesis.

Ketone oxidation decreases intramuscular pyruvate via multiple mechanisms. (i) First, ketone oxidation reduces glycolysis in skeletal muscle\(^\text{14}\), therefore, decreasing pyruvate production. (ii) Second, ketone body oxidation increases acetyl CoA levels\(^\text{19}\). Acetyl CoA is an allosteric activator of pyruvate carboxylase, thus, driving the conversion of pyruvate to oxaloacetate\(^\text{20}\). (iii) In addition to decreasing pyruvate for transamination into L-alanine, ketosis also directly decreases L-alanine production by reducing skeletal muscle protein breakdown\(^\text{13}\).

Furthermore, in the liver, L-alanine supports gluconeogenesis. L-alanine allosterically inhibits the liver isozyme of pyruvate kinase, the enzyme responsible for the last step of glycolysis\(^\text{21}\). Thus, L-alanine restriction would favour glycolysis over gluconeogenesis. L-alanine is also a potent glucagon secretion agonist\(^\text{22}\), and glucagon stimulates gluconeogenesis. Again, L-alanine restriction is predicted to downregulate gluconeogenesis in the liver.
The hypoglycaemic effect of ketone body oxidation is likely pleiotropic. As observed in this study, the ability of exogenously induced to reduce circulating L-alanine as fuel for gluconeogenesis cannot account for the entire hypoglycaemic effect of KE ingestion. Other mechanisms are at play.

Exogenously induced ketosis can stimulate insulin secretion\textsuperscript{23,24}. However, even while we observed a transient C-peptide spike at 30 minutes after KE ingestion, it should be noted that this does not necessarily translate into an equal magnitude, one-to-one, spike in systemic circulating insulin due to insulin liver extraction. What is more, even if we assume this C-peptide change correlates with an equal magnitude insulin change, the latter would not be large or long enough to account for the entire hypoglycaemic effect observed.

There is also evidence that a decrease in gluconeogenesis and glucose section into the blood, rather than an increase in insulin-mediated glucose clearance from the blood, accounts for most of the hypoglycaemic effect of ketosis\textsuperscript{3}. For example, exogenous ketone infusions in patients with type 1 diabetes demonstrate that acute ketosis lowers blood glucose\textsuperscript{11} even in the absence of insulin. Thus, it is unlikely that the minor insulinogenic effect of exogenous ketosis accounts for most of the hypoglycaemic effect KE ingestion.

The hypoglycaemic effect of acute ketosis is also not restricted to the fasting state. Inducing acute ketosis lowers the postprandial glycaemic curve after a dextrose challenge without inducing significant differences in insulin secretion\textsuperscript{25}. One of the proposed mechanisms is that \(\beta\text{HB}\)-mediated inhibition of lipolysis\textsuperscript{26} depletes the blood supply of fatty acids, driving an increase in glucose uptake. However, this has not been observed after the ingestion of niacin, which also blocks lipolysis by acting on the same receptor as \(\beta\text{HB}\) and has no effect on gluconeogenesis\textsuperscript{27}.
As gluconeogenesis contributes to elevations in blood glucose in the post-absorptive state, βHB-induced attenuation of gluconeogenesis could also explain (at least partially) the postprandial attenuation of the glycaemic curve that has been previously observed.

It is further worth nothing that glycerol is a gluconeogenic substrate. However, evidence suggests that, in the fasting humans, L-alanine and lactate, not glycerol, are the major gluconeogenic substrates, not glycerol. Since ketosis also reduces the availability of glycerol by inhibiting lipolysis, ketosis could be potentially beneficial for individuals in whom gluconeogenesis is pathologically elevated regardless of which substrate is the most important contributor to gluconeogenesis. But, again, L-alanine is probably more relevant.

It is unlikely that this is a KE-specific effect. While the hepatic conversion of butanediol derived from ketone ester into βHB would alter hepatocyte NAD+/NADH balance (therefore altering endogenous glucose production), studies comparing the ingestion of equimolar quantities of ketone salts and ketone esters (that lack a butanediol component) demonstrate hypoglycaemic effects of similar magnitudes.

Figure 4 summarises some of the mechanisms whereby exogenously induced acute ketosis could lower blood glucose concentration.
**FIGURE 4. Summary of the mechanisms whereby ketosis lowers blood glucose.**

*In skeletal muscle:* βHB increases the concentration of acetyl CoA\(^{19}\), which inhibits pyruvate dehydrogenase and activates pyruvate carboxylase\(^{20}\). As a result, more pyruvate is transformed into oxaloacetate. Additionally, the rise in acetyl CoA inhibits phosphofructokinase-1 (PFK1), downregulating glycolysis, and therefore, decreasing pyruvate production\(^{28}\). Through these two mechanisms, pyruvate levels are reduced and there is less pyruvate available for transamination into L-alanine. βHB also decreases protein breakdown, further reducing L-alanine production\(^{14}\). *In adipose tissue:* βHB inhibits lipolysis via de PUMA-G receptor\(^{26}\), reducing the release of glycerol, a minor gluconeogenic substrate. *In the pancreas:* βHB promotes insulin release by the beta cells\(^{24,29}\). Furthermore, there is less L-alanine to stimulate glucagon release by the alpha cells\(^{22}\). The decrease in gluconeogenic substrates (L-alanine and glycerol) and inhibitory hormonal signaling (increase in insulin/glucagon ratio; and L-alanine allosterically inhibits pyruvate kinase\(^{21}\)) cause a decrease in gluconeogenesis by the liver (and also possibly other peripheral tissues, like the intestines, that can perform gluconeogenesis). The net result of acute ketosis induced by exogenous βHB is a small increase in peripheral glucose uptake, due to a small increase in insulin, and a larger decrease in glucose release by gluconeogenic tissues.
**Limitations of this study**

First, the relative contribution of the different gluconeogenic organs (liver, kidneys, and intestine) and of the different gluconeogenic substrates (lactate, glycerol, alanine, and glutamine), varies depending on whether a person is in the fed or fasted state.\(^{30,31}\). However, there is evidence suggesting that, in the context of short-term fasting, alanine is the most important contributor via liver gluconeogenesis.\(^{32–36}\).

Since all participants in this study were fasted for control purposes, we cannot extrapolate these insights to other metabolic states where there are different relative contributions from the kidneys and the intestines. Future studies may choose to investigate the same question in postprandial participants or using labelled substrates.

Additionally, while unlikely, an unexpected interaction between L-alanine supplementation and exogenously induced ketosis cannot be entirely ruled out. Thus, a future study should include an independent L-alanine arm to compare the impact of L-alanine on glycaemia in the absence of ketosis. Additional informative measurements for future studies we did not include in this experiment are nitrogen balance and glucagon levels.

Finally, it has been proposed that glycerol is the most relevant gluconeogenic substrate.\(^{37}\). Even if this is true, since acute ketosis also reduces the availability of glycerol, the overall conclusion of ketosis being potentially therapeutic for individuals in whom gluconeogenesis is pathologically elevated holds true.
CONCLUSION

These data demonstrate, in healthy humans, that exogenous ketosis results in a reduction in L-alanine availability to fuel gluconeogenesis and that this contributes to the hypoglycaemic effects of acute ketosis, even independent of carbohydrate restriction. These data provide insight into the mechanisms whereby ketosis itself could provide therapeutic benefit in insulin resistant individuals in whom gluconeogenesis is pathologically elevated.
ACKNOWLEDGMENTS

The authors thank Vicky Ball for her diligent support. Dr. Adrian Soto-Mota thanks the Consejo Nacional de Ciencia y Tecnología (CONACYT, Mexico) and the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (FUNSAED, Mexico) for their financial support.

AUTHOR CONTRIBUTIONS

All authors contributed significantly to the design, execution, analysis and reporting of this study.

DECLARATION OF INTERESTS

The intellectual property covering the uses of ketone bodies and ketone esters are owned by BTG Plc, Oxford University Innovation Ltd and the US National Institutes of Health. Professor Kieran Clarke, as an inventor, will receive a share of the royalties under the terms prescribed by each institution. Professor Clarke is a director of TdeltaS Ltd, a company spun out of the University of Oxford to develop products based on the science of ketone bodies in human nutrition. The other authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this paper.
REFERENCES

1. Cahill, G. F. & George, F. C. Fuel Metabolism in Starvation. *Annual Review of Nutrition* **26**, 1–22 (2006).

2. Consoli, A., Nurjhan, N., Reilly, J. J., Bier, D. M. & Gerich, J. E. Mechanism of increased gluconeogenesis in noninsulin-dependent diabetes mellitus. Role of alterations in systemic, hepatic, and muscle lactate and alanine metabolism. *Journal of Clinical Investigation* **86**, 2038–2045 (1990).

3. BEYLOT, M. et al. Metabolic effects of a D-β-hydroxybutyrate infusion in septic patients. *Critical Care Medicine* **22**, 1091–1098 (1994).

4. Hallberg, S. J. et al. Effectiveness and Safety of a Novel Care Model for the Management of Type 2 Diabetes at 1 Year: An Open-Label, Non-Randomized, Controlled Study. *Diabetes Therapy* **9**, 583–612 (2018).

5. Lim, E. L. et al. Reversal of type 2 diabetes: Normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol. *Diabetologia* **54**, 2506–2514 (2011).

6. Nakatani, T., Yasuda, K., Ozawa, K. & Tobe, T. Changes in Blood Glucose Levels in Relation to Blood Ketone Body Ratio following Hypertonic Glucose Infusion in 70% Hepatectomized Rabbits. *European Surgical Research* **16**, 303–311 (1984).

7. Felts, P. W., Crofford, B. & Park, C. R. Effect of Infused Ketone Bodies on Glucose Utilization in the Dog. *Journal of Clinical Investigation* vol. **43** (1964).

8. Muller, M. J., Paschen, U. & Seitz, H. J. Effect of ketone bodies on glucose production and utilization in the miniature pig. *The Journal of clinical investigation* **74**, 249–261 (1984).

9. Stubbs, B. J. et al. On the metabolism of exogenous ketones in humans. *Frontiers in Physiology* **8**, 848 (2017).

10. Soto-Mota, A., Norwitz, N. G. & Clarke, K. Why a D-β-hydroxybutyrate monoester? *Biochemical Society Transactions* **48**, 51–59 (2020).

11. Sherwin, R. S., Hendler, R. G. & Felig, P. Effect of diabetes mellitus and insulin on the turnover and metabolic response to ketones in man. *Diabetes* **25**, 776–784 (1976).

12. Magnusson, I., Rothman, D. L., Katz, L. D., Shulman, R. G. & Shulman, G. I. Increased rate of gluconeogenesis in type II diabetes mellitus. A 13C nuclear magnetic resonance study. *Journal of Clinical Investigation* **90**, 1323–1327 (1992).

13. Cox, P. J. et al. Nutritional Ketosis Alters Fuel Preference and Thereby Endurance Performance in Athletes. *Cell Metabolism* **24**, 256–268 (2016).

14. Wu, G. & Thompson, J. R. The effect of ketone bodies on alanine and glutamine metabolism in isolated skeletal muscle from the fasted chick. *Biochemical Journal* **255**, 139–144 (1988).

15. WMA. WMA DECLARATION OF HELSINKI – ETHICAL PRINCIPLES FOR Scientific Requirements and Research Protocols. *World Medical Association* **29–32** (2013).
16. Erdfelder, E., Faul, F., Buchner, A. & Lang, A. G. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behavior Research Methods* **41**, 1149–1160 (2009).

17. Clarke, K. *et al.* Kinetics, safety and tolerability of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate in healthy adult subjects. *Regulatory Toxicology and Pharmacology* **63**, 401–408 (2012).

18. Felig, P., Pozefsk, T., Marlis, E. & Cahill, G. F. Alanine: Key Role in Gluconeogenesis. *Science* **167**, 1003–1004 (1970).

19. Sato, K. *et al.* Insulin, ketone bodies, and mitochondrial energy transduction. *The FASEB Journal* **9**, 651–658 (1995).

20. Jitrapakdee, S. *et al.* Structure, Mechanism and Regulation of Pyruvate Carboxylase. *Biochem J* **413**, 369–387 (2008).

21. Van Veelen, C. W. M., Rijksen, G., Vlug, A. M. C. & Staal, G. E. J. *Correlation between Alanine inhibition of pyruvate kinase and composition of K-M hybrids. Clinica Chimica Acta* vol. 110 (1981).

22. Müller, W. A., Faloona, G. R. & Unger, R. H. The effect of alanine on glucagon secretion. *The Journal of clinical investigation* **50**, 2215–2218 (1971).

23. Balasse, E., Ooms, H. & Lambilliotte, J. Evidence for a stimulatory effect of ketone bodies on insulin secretion in man. *Hormone and Metabolic Research* **2**, 371–372 (1970).

24. Biden, T. J. & Taylor, K. W. Effects of ketone bodies on insulin release and islet-cell metabolism in the rat. *Biochemical Journal* **212**, 371–377 (1983).

25. Myette-Côté, É., Caldwell, H. G., Ainslie, P. N., Clarke, K. & Little, J. P. A ketone monoester drink reduces the glycemic response to an oral glucose challenge in individuals with obesity: a randomized trial. *The American Journal of Clinical Nutrition* **110**, 1491–1501 (2019).

26. Taggart, A. K. P. *et al.* (D)-β-hydroxybutyrate inhibits adipocyte lipolysis via the nicotinic acid receptor PUMA-G. *Journal of Biological Chemistry* **280**, 26649–26652 (2005).

27. Wang, W., Basinger, A., Neese, R. A., Christiansen, M. & Hellerstein, M. K. Effects of nicotinic acid on fatty acid kinetics, fuel selection, and pathways of glucose production in women. *American journal of physiology. Endocrinology and metabolism* **279**, E50-9 (2000).

28. Usenik, A. & Legiša, M. Evolution of allosteric citrate binding sites on 6-phosphofructo-1-kinase. *PLoS ONE* **5**, e15447 (2010).

29. Beylot, M. *et al.* Effects of ketone bodies on basal and insulin-stimulated glucose utilization in man. *Journal of Clinical Endocrinology and Metabolism* **63**, 9–15 (1986).

30. Chiasson, J. L., Liljenquist, J. E., Sinclair Smith, B. C. & Lacy, W. W. Gluconeogenesis from alanine in normal postabsorptive man. Intrahepatic stimulatory effect of glucagon. *Diabetes* **24**, 574–584 (1975).

31. Chiasson, J. L. *et al.* Effects of insulin at two dose levels on gluconeogenesis from alanine in fasting man. *Metabolism: Clinical and Experimental* **29**, 810–818 (1980).
32. Sarabhai, T. & Roden, M. Hungry for your alanine: When liver depends on muscle proteolysis. *Journal of Clinical Investigation* vol. 129 4563–4566 (2019).

33. Shah, A. M. & Wondisford, F. E. Tracking the carbons supplying gluconeogenesis. *Journal of Biological Chemistry* vol. 295 14419–14429 (2020).

34. Martin, G. *et al.* Glutamine gluconeogenesis in the small intestine of 72 h-fasted adult rats is undetectable. *Biochemical Journal* 401, 465–473 (2007).

35. Stumvoll, M. *et al.* Human kidney and liver gluconeogenesis: Evidence for organ substrate selectivity. *American Journal of Physiology - Endocrinology and Metabolism* 274, (1998).

36. Petersen, K. F., Dufour, S., Cline, G. W. & Shulman, G. I. Regulation of hepatic mitochondrial oxidation by glucose-alanine cycling during starvation in humans. *Journal of Clinical Investigation* 129, 4671–4675 (2019).

37. Wang, Y., Kwon, H., Su, X. & Wondisford, F. E. Glycerol not lactate is the major net carbon source for gluconeogenesis in mice during both short and prolonged fasting. *Molecular Metabolism* 31, 36–44 (2020).