2020-07-28

The Effects of Exercise on the Fasting Ketone Production Curve: A Randomized Crossover Study

Landon S. Deru
Brigham Young University

The Effects of Exercise on the Fasting Ketone Production Curve:

A Randomized Crossover Study

Landon S. Deru

A thesis defense submitted to the faculty of
Brigham Young University
in partial fulfilment of the requirements for the degree of

Master of Science

Bruce Bailey, Chair
Benjamin Bikman
Lance Davidson
Larry Tucker

Department of Exercise Sciences

Brigham Young University

ABSTRACT

The Effects of Exercise on the Fasting Ketone Production Curve:
A Randomized Crossover Study

Landon S. Deru
Department of Exercise Sciences, BYU
Master of Sciences

Elevated ketone production and utilization results in a host of health benefits. The aim of this study was to assess the rate of ketone production during a prolonged fast and to evaluate how an initial bout of exercise influences this production. Mood and hunger, along with plasma insulin and glucagon, were also assessed.

In this crossover study, 20 adult subjects (11 Male, 9 Female) completed two 36-hour fasts, with one protocol requiring the subject to complete an intense treadmill exercise session at the beginning of the fast. Ketone levels were assessed via blood ketone meter and recorded every two hours. Subjective mood and hunger ratings were also recorded every two hours. Venipuncture was performed every 12 hours to assess plasma insulin and glucagon.

The mean area under the ketone production curve for the nonexercise intervention was 19.19 ± 2.59 mmol/L and 27.49 ± 2.59 mmol/L for the exercise intervention, resulting in a significant 8.30 mmol/L difference between conditions (95% probability interval was 1.94 to 14.82 mmol/L). The mean time to nutritional ketosis was 21.07 ± 2.95 hours with fasting alone, and 17.5 ± 1.69 hours when combined with exercise (posterior probability = 0.89). There was a significant decrease in insulin over time ($F(3,133) = 61.75$, $p < 0.0001$). There was also a significant increase in glucagon over time ($F(3,133) = 21.10$, $p < 0.0001$). Hunger and stomach discomfort did not differ between conditions. Anger ($F(10,394) = 2.74$, $p = 0.0028$), depression ($F(10,394) = 2.91$, $p = 0.0016$), tension ($F(10,394) = 2.29$, $p = 0.0128$), vigor ($F(10,394) = 11.65$, $p < 0.0001$), and fatigue ($F(10,394) = 10.60$, $p = 0.0001$) increased over the course of the fast, but did not differ between conditions.

Completing aerobic exercise at the beginning of a 36-hour fast results in significantly more ketone production. The impact of exercise on ketone production comes at little or no impact on hunger, stomach discomfort and negative moods. A difference in time to achieving nutritional ketosis between conditions may exist, but this was not observed in this study.

Keywords: ketosis, ketogenesis, blood ketone, fasting, exercise, beta-hydroxybutyrate

Table of Contents

List of Tables

List of Figures

Introduction

Alzheimer's disease, stroke, heart disease and diabetes all rank among the leading causes of death in the United States (Centers for Disease Control and Prevention, 2019). Many behavioral and pharmacological strategies have been employed to combat the accelerated incidence of these ailments. Increasing evidence indicates that states of elevated plasma ketones, termed ketosis, may improve prognosis for each of these conditions (Abbasi, 2018; Kashiwaya et al., 1994; Kashiwaya et al., 2000; Mattson et al., 2018). Ketosis is a therapeutic intervention for the treatment of multiple conditions, including epilepsy (Kang et al., 2007), obesity (Abbasi, 2018; Naude et al., 2014), psychosomatic diseases (Yamamoto et al., 1979), hypertension (Goldhamer et al., 2002), heart failure (Wu et al., 2016), and chronic pain (Michalsen et al., 2006) and it has been used as adjunctive therapy to combat cancer (Winters et al., 2017).

In addition to these benefits Kashiwaya et al. (2000), found that beta-hydroxybutyrate (BHB), the body's predominant ketone, protects against neurodegeneration and toxin-induced nerve damage commonly seen in Parkinson's and Alzheimer's diseases. Enhanced synaptic plasticity and neurogenesis, resistance to neuronal degeneration, and enhanced recovery from nerve injury, all function as added neurological benefits of nutritional ketosis (Mattson et al., 2018). Work done by Shimazu et al. (2013) and Haces et al. (2008) found that BHB increases histone acetylation, which induces the expression of certain genes that reduce oxidative stress and protects against cellular damage. Additionally, mechanisms have been discovered that describe the role of ketones in decreasing inflammation (Youm et al., 2015), improving mitochondrial respiration and ATP production (Maalouf et al., 2007; Tieu et al., 2003), and in signaling their own negative feedback loop (Ahmed et al., 2009; Taggart et al., 2005). Consequently, ketones (which until recently were considered "metabolic garbage"; (Harvey et

al., 2019) have been determined to be fundamental metabolic and signaling mediators, even when glucose is abundant (Puchalska & Crawford, 2017).

Several studies suggest that many of the benefits described are still achieved even if ketosis is not maintained for an extended period of time. For example, Anson et al. (2003) found that alternate-day fasting resulted in beneficial effects that met or exceeded those of caloric restriction when observing reductions in serum glucose and insulin levels as well as increased resistance of neurons in the brain to excitotoxic stress. Additionally, recent works by Anton et al. (2018), Harvie et al. (2013) and Mattson et al. (2017) each describe the "metabolic switch" as a transition from utilizing glucose as a major fuel source to utilizing fatty acids and ketones. This switch is uniquely beneficial as the chronically overburdened glucose oxidation pathway receives respite while the fatty acid and ketone pathways take over, a state known as metabolic flexibility. The mechanisms explaining how ketones act in benefiting each of these pathways are still being discovered, but research suggests that some of the benefits of being in nutritional ketosis results (at least in part) from the actions of the ketones themselves (Puchalska & Crawford, 2017).

The state of nutritional ketosis is most readily achieved through either carbohydrate restriction, complete calorie restriction (fasting), or a combination. Fasting has recently gained popularity as a means for weight loss and other health benefits (Kerndt et al., 1982). While many studies have used fasting to induce a state of nutritional ketosis (Balasse et al., 1978; Bergqvist, 2005; Browning et al., 2012; Cahill, 1976; Finnell et al., 2018; Longo & Mattson, 2014), the time course to achieve this state has not been well described (Harvey et al., 2018). An appreciation of this time course in healthy individuals would provide an essential foundation for

future research in discovering the physiological pathways and health benefits associated with this state.

To our knowledge, no other study has objectively measured the time course of ketone production in a fasted state compared to that of a fasted state with an initial bout of intense exercise. The aim of this study was to determine the time course of ketone body production during a prolonged (36-hour) fast and to evaluate how an initial bout of exercise influences this time course. Anton et al. (2018) recommends that randomized controlled trials should use biomarkers of the metabolic switch as a measure of compliance and the magnitude of negative energy balance during the fasting period. BHB, insulin, and glucagon are each biomarkers that can be used to observe these effects (Muoio & Newgard, 2008). A secondary aim of the study was to evaluate how mood and hunger changed over the period of the fast, with and without an initial bout of exercise.

We hypothesized that the area under the ketone production curve would be greater during the exercise condition compared to the nonexercise condition over the course of a 36-hour fast. We also postulated that the timeframe for achieving nutritional ketosis would be significantly less when the fast was combined with an initial bout of exercise compared to fasting alone. Additionally, we expected plasma insulin to decrease and plasma glucagon to increase more dramatically when fasting was combined with exercise. When considering hunger, we expected no difference in perceived hunger between the two fasting protocols (Sumithran et al., 2013). Finally, we hypothesized moods of tension, depression, anger, fatigue, and confusion to increase and vigor to decrease throughout the fasts of the exercise day compared to the nonexercise day.

Materials and Methods

Design

A randomized crossover design with counterbalanced treatment conditions was used to compare the influence of fasting alone to fasting combined with vigorous exercise on the ketone production curve. These two conditions included a 36-hour water-only fast beginning at 8:00 pm and ending at 8:00 am, 36 hours later. Multiple studies have demonstrated water-only fasting up to and exceeding 36 hours to be safe and well tolerated for healthy participants (Balasse et al., 1978; Browning et al., 2012; Cahill, 2006). Approval from the university's Institutional Review Board was obtained prior to initiating any aspect of this study.

Participants completed two treatment conditions, with a minimum six-day washout between each session. Using randomizer.org, condition order was randomly assigned to participant numbers prior to the study (Suresh, 2011). The participant numbers were assigned to participants chronologically from the time they joined the study. Prior to each laboratory session, participants were screened for contraindications to participation as outlined below. The outcome variables we measured were body mass index (BMI), percent body fat, fat mass, hunger, thirst, stomach discomfort, mood, capillary BHB ketone levels, plasma glucagon, and plasma insulin.

Testing Conditions

On the day the fast was initiated, participants were asked to maintain normal eating behaviors and hydration patterns to avoid excess caloric consumption in preparation for the fast. Participants arrived at the lab having not eaten for four hours. Only water was allowed leading up to the visit. After screenings and assessments, a standardized meal was provided and a 36-hour fast initiated. This was a water-only fast, meaning no other food or beverages were allowed during the fasting period. Participants were instructed to stay hydrated throughout the fast.

Noncaloric, electrolyte and/or caffeinated beverages/additives were not allowed. Gum chewing was also prohibited.

Based on random assignment, participants either proceeded with a fasting-only regimen or participated in the exercise regimen 30 minutes following the initiation of the fasting period. During the testing period, participants were required to complete hunger and mood assessments and check and record capillary ketone levels every two hours, except during sleeping hours. Additionally, they returned to the lab for venous blood draws every 12 hours beginning at 8:00 pm following the standardized meal.

Participants

Twenty healthy adults (11 male and 9 female) were recruited through word of mouth, advertisements, and fliers in the local community. Participants were adults 18 years of age or older and must have been weight-stable for the past three months. Participants were required to be capable of participating in vigorous physical activity without restrictions as measured by a Physical Activity Readiness Questionnaire (PAR-Q). The PAR-Q contains questions regarding contraindications to physical activity. Any 'yes' response on the PAR-Q excluded participants. Exclusion also occurred if participants did not provide proper written consent or if they met any of the following exclusion criteria:

1. Diagnosed with a chronic disease (i.e., cancer, heart/liver/kidney disease)
2. Diagnosed with a metabolic disease (i.e., Type I and Type II diabetes)
3. Diagnosed with orthopedic impairments (i.e., joint replacements or arthritis)
4. Diagnosed with an eating disorder (i.e., anorexia, bulimia or binge eating disorder)
5. Taking medications that alter metabolism, appetite or neurological function (i.e., insulin, metformin, amphetamine-based ADHD medications, depression and anxiety medications

such as selective serotonin reuptake inhibitors, serotonin and norepinephrine inhibitors and benzodiazepines; (Verhaegen & Van Gaal, 2017)

6. Food allergies (i.e., nuts, celiac disease or gluten intolerance or lactose intolerance)
7. Habitually consume 60 mg or more of caffeine daily (Johnston et al., 2003)
8. Pregnant or lactating
9. Postmenopausal (Morrow et al., 1981)
10. Underweight or obese (BMI < 18.5 or > 30 or a body weight of less than 110 lb; (*National Institutes of Health*, 2019)
11. Currently participating in ketogenic, carbohydrate or calorie restricted diets
12. Currently fasting more than once per week
13. Irregular sleeping patterns (including graveyard or swing shifts)

Measurements

Anthropometric Measurements

Body weight and height were measured for all participants at the beginning of each session. Weight was measured using a digital scale (Seca, Hamburg, Germany) accurate to ± 0.1 kg with participants dressed in athletic shorts and a t-shirt with shoes removed. Height was measured by a stadiometer accurate to ± 0.1 cm (Seca, Hamburg, Germany). Body mass index (BMI) was calculated as weight (in kilograms) divided by the square of height (in meters). A GE iDXA (GE, Fairfield, CT) was used to assess fat mass, percent body fat and visceral adipose tissue (Bailey et al., 2018; Speakman et al., 2001; Tataranni et al., 1996). Visceral fat was calculated using the CoreScan application of the GE iDXA (Mohammad et al., 2017; Rothney et al., 2013). Calibration of the DXA scan took place at the beginning of each testing day using a manufacturer-provided calibration block. Scans were analyzed using Encore software version 17.

Mood and Hunger Ratings

Participants were asked to rate their mood and energy levels using the Brunel Mood Scale (BRUMS), every two hours while recording capillary ketone levels. The 24-item BRUMS measures six identifiable mood states (Tension, Depression, Anger, Vigor, Fatigue, and Confusion) through a self-report inventory on a 5-point Likert Scale from 0 (not at all) to 4 (extremely) based on current feelings. The BRUMS questionnaire has been validated by Rohlfs (2005) as a sensitive and trustworthy replacement for the Profile of Mood States (POMS) questionnaire in detecting mood states for adolescent and adult populations. Hunger, thirst and discomfort were assessed using a Visual Analog Scale (Stubbs et al., 2000).

Plasma Insulin and Glucagon

Two 4 ml EDTA (with anticoagulant) tubes of blood were taken from each participant at the median cubital vein within 30 seconds of tourniquet application. Venipuncture took place every 12 hours (time 0, 12, 24, and 36 hours) in the human performance lab. For processing, each 4 ml tube was inverted to allow for mixture. Both samples were centrifuged for 15 minutes at 1500 xg at 3 °C within 10 minutes of collection, after which the plasma was aliquoted and placed in separate vials. Both plasma samples were stored in a −80 °F freezer until ready for analysis. Insulin and glucagon levels were quantified using standard 96-well microplate ELISA kits according to the manufacturer's instructions.

Capillary Ketone Assessment

Capillary BHB ketone measurements were assessed on the even hours during the fasting session (with the exception of sleep time) using the Precision Xtra portable ketone meter (Abbott Laboratories, Abington, NC). Prior to each fasting session, these meters were calibrated using manufacturer control solutions. A 5-µl capillary blood sample was applied to an electrochemical

strip inserted into the sensor. The Precision Xtra portable ketone monitor was demonstrated by Byrne et al. to be accurate in measuring real-time whole blood capillary BHB compared to venous whole blood reference samples up to blood levels of 6 mmol/L (Byrne, 2000).

Procedures

Screening

Potential participants for the study were sent an email containing a link to an online survey. The online questionnaire was used to ensure participants met inclusion criteria. Candidates must have answered each question properly in order to qualify for the study. As part of the online survey, participants were asked to report any food allergies, complete a food preference questionnaire, and the PAR-Q. The food preference questionnaire was used to ensure that participants would be able to eat the standardized meals. Qualifying candidates were invited to participate in the study and were instructed to arrive prepared to exercise. They were also instructed to avoid caffeine consumption and other stimulants on the testing day as well as to refrain from vigorous physical activity for the 24-hour period prior to testing. Adherence to the pretest day protocols were assessed at the beginning of each session. If pretest protocols had not been followed, the participant was rescheduled.

Orientation. Informed consent was given by participants prior to participation in any aspect of this study. Participants reported to the Human Performance Research Lab at the university for each assessment. Each participant was informed of the main purpose of the study and familiarized with the testing procedures. Training for proper portable ketone meter use took place in accordance with manufacturer guidelines, and participants were given a copy of these testing instructions. Using Qualtrics online survey software (Qualtrics, Provo, UT), participants logged their own capillary ketone blood levels and filled out the questionnaires relating to mood

and hunger every two hours. Participants were reminded to take and record these measurements via automated text messages. Participants were oriented to the Qualtrics software and given login information during the initial orientation. Participants were asked to go about their normal activities of daily living during the testing period and to avoid exercise or strenuous activity, including strength or cardiovascular training, yard work, hiking or other moderate activity. Participants were also asked to maintain their normal sleeping patterns.

Standardized Meals. Participants were given a standardized meal to initiate each fast. The energy needs for each participant were estimated using equations validated by Hall et al. (2011). This equation uses height (cm), weight (kg), age (years) and gender to predict basal metabolic rates (BMR) and has been validated for accuracy and reliability (Hall et al., 2011). An activity factor of 1.55 was used to estimate total daily energy requirements (World Health Organization, 1985). Meals were standardized based on macronutrient content (60% CHO, 25% fat, 15% protein). Participants were given 25% (BMR x 1.55 x 0.25) of their daily caloric requirements in the standardized meal. The same foods were given on both test days and participants were instructed to consume all the food provided for each meal. Meal adherence was assessed in each session by direct observation by the researchers. Any noncompliance (not eating all food and/or eating other foods) required participants to redo that particular lab session on a different day when they had followed food protocols. Meals were designed to represent a reasonable amount of food to provide participants with adequate food intake (Hoffman & Polich, 1998).

Treatment Sessions. Participants were asked to eat normally leading up to the fast and abstain from food for four hours prior to the standardized meal and initiation of the fast to prevent prefast caloric loading and to normalize measured blood markers (Plumelle et al., 2014).

Consent, orientation, instruction, hunger assessment, anthropometric data, demographic information, and bloodwork were collected during the initiation of each session. Participants returned to the lab for anthropometric measurements and bloodwork at 12, 24, and 36 hours of fasting where they were reminded to stay hydrated according to testing protocols. Testing procedures and protocols were reviewed with each participant prior to initiation of each fasting session.

Based on random assignment, participants completed either an exercise condition or a nonexercise condition first. During the nonexercise condition, after all measurements were taken (including a DXA scan) and after consuming a standardized meal, participants immediately proceeded with normal daily activity. During the exercise condition, all measurements were taken (excluding a DXA scan), followed by an exercise regimen 30 minutes after consuming the standardized meal.

Exercise Protocol. Participants exercised on a treadmill at a grade and speed that brought their estimated heart rate reserve (HRR) to 70%. Exercise at this intensity is classified as intense (Jette et al., 1990) and was used because it has been shown to maximize glucose oxidation during aerobic exercise as compared to lower-intensity training (Purdom et al., 2018). Participants exercised in this manner until an equivalent number of calories was expended as given in the standardized meal. The formula used to calculate the participant's target heart rate was:

$$\text{Target HR} = [\%\ \text{target intensity} \times (HR_{max} - HR_{rest})] + HR_{rest}\ \text{(Solheim et al., 2014))} \quad (1)$$

Maximal HR estimation was calculated using the following formula:

$$HR_{max} = 208 - (0.7 \bullet \text{age})\ \text{(Roy \& McCrory, 2015)} \quad (2)$$

Participants were fitted with a strap-on heart rate monitor (Garmin, Olathe, KS) and instructed to be seated for 5 minutes to establish resting HR (HRrest). Once HR calculations were complete,

subjects began the exercise. The research assistants adjusted the speed and grade to meet the target HR within the first 5 minutes of exercise. Once this speed and grade were set, they were not adjusted for the remainder of the exercise intervention. If a participant was unable to maintain the exercise at this intensity, they were allowed to take a 60-second break, after which the exercise was resumed.

The length of exercise was individualized in order to expend a similar number of calories as consumed from the standardized meal. This calculation was based on the standard ACSM-established metabolic calculation converting oxygen to kcal by multiplying liters of oxygen by 5. The equations that were used are presented below.

ACSM Metabolic Equation:

$$VO_2\ ml.kg^{-1}min^{-1} = (0.2 \bullet S) + (0.9 \bullet S \bullet G) + 3.5 \quad (3)$$

Equations to Estimate Time on Treadmill:

$$min = (E/5 \bullet 1000)/(kg((0.2 \bullet S) + (0.9 \bullet S \bullet G) + 3.5)) \quad (4)$$

E = calculate energy based on standardized meal in kcal

kg = weight of the participant in kilograms

S = speed of the treadmill in meters per minute

G = grade of the treadmill

min = time on treadmill

All calculations were performed in a preset, protected spreadsheet to ensure accuracy. Energy expenditure was verified using indirect calorimetry (COSMED, Rome, Italy).

Statistical Analysis

Participant data are reported as means and standard deviations. Condition and gender were the primary factors in the models. The two conditions being considered were fasting without exercise and fasting with exercise.

Area Under the Beta-Hydroxybutyrate Curve Analysis

Using "R" version 4.0.1 (Vienna, Austria) and JAGS version 4.3.0 statistical software, the area under the treatment curves was analyzed by using the trapezoidal area under each curve with one observation per subject by treatment. The Bayesian paradigm was used as the basis for the analyses. Posterior probability densities for the parameters of interest were generated using Markov chain Monte Carlo methods. These posterior probability densities could then be used to make appropriate inferential statements. Posterior probabilities exceeding 0.95 were taken to be statistically significant. All chains from the posterior densities were evaluated to determine if convergence was achieved.

Timeframe to Achieve Nutritional Ketosis Analysis

Using the standard 0.5 mmol/L (Gibson et al., 2015; Guerci et al., 2003), the timeframe to achieve nutritional ketosis for both males and females under the two treatment conditions was also evaluated using the Bayesian paradigm. To evaluate this timeframe, an estimate of the curve was needed. After considering a number of possible curves, a cubic curve was found to fit best. The coefficients of the curve were estimated using a Bayesian hierarchical model. The best-fit model had hierarchical parameters (meaning the coefficients for each subject were treated as a draw from the population of possible coefficients) for the intercept, linear, and quadratic coefficients. A hierarchical paradigm was not used on the cubic coefficients.

Plasma Insulin and Glucagon Analysis

In order to determine the impact of exercise and fasting on insulin and glucagon levels, linear mixed models were fit in PC-SAS version 9.4 (Cary, NC). Condition (exercise and nonexercise), order of condition (exercise first and exercise second), and visit number were the fixed effects used in these models. Participant was used as a random effect. The interaction between condition, order, and visit number were evaluated for all models. The LS Means procedure was used to evaluate significant main and interactive affects. To evaluate any potential moderating effect of gender, the mixed models were repeated but this time included gender and evaluated the two-way interactions of gender and condition, gender and order, and gender and visit. Alpha was set to 0.05.

Hunger, Thirst, Stomach Discomfort, and Mood Analyses

Hunger, thirst, stomach discomfort, and mood data were analyzed using PC-SAS (v. 9.4) statistical software. The mean and standard deviation of all responses were calculated, and the scores were graphed over the time of the fast. The two conditions (exercise and nonexercise) were compared for each response across the span of the 36-hour fasting period. Responses from the visual analog scales for hunger, thirst and stomach discomfort were evaluated independently. All responses of the 24-item BRUMS mood questionnaire were divided into the six mood factors assessed (anger, depression, tension, vigor, fatigue, and confusion). Once sorted, each mood factor score was summed for a score between zero and sixteen. Linear mixed models were fit to determine the impact of exercise and fasting on mood over the 36-hour fast. Condition (exercise and nonexercise), order of condition (exercise first and exercise second), and visit number were the fixed effects used in these models. Participant was used as a random effect. The interaction between condition, order, and visit number were evaluated for all models. The LS

Means procedure was used to evaluate significant main and interactive effects. Alpha was set to 0.05. To evaluate any potential moderating effect of gender, the mixed models were repeated but and it was found that gender did not predict any outcomes.

Results

We screened 31 individuals for eligibility. Of those screened, 11 were disqualified for reasons outlined in Figure 1. The remaining 20 participants were randomly allocated to condition order. Eleven men and nine women were recruited and all participants completed all aspects of the study. The demographic characteristics of the participants are outlined in Table 1. The standardized meal fed to participants prior to each fast was 614.84 ± 85.18 kcal. Measured energy expenditure during the exercise bout on the fast and exercise day was 587.55 ± 120.13 kcal. The average METs during the prescribed exercise was 9.14 ± 1.37. The average respiratory quotient (R) throughout the prescribed exercise was 0.95, indicating that the major fuel source for the exercise was glucose (Muoio, 2014).

Area Under the Beta-Hydroxybutyrate Curve

Area under the curve for plasma BHB was calculated for each participant. The 95% posterior probability interval for the interaction between condition and gender was −6.62 to 18.94 mmol/L. Because this interval included zero, we concluded there was no interaction. Similarly, the 95% posterior probability interval for the effect of gender was −7.53 to 8.27 mmol/L. Because this interval included zero, we again concluded no gender effect. Because no differences in gender were observed, men and women were analyzed together.

The mean area under the curve for the nonexercise intervention was 19.19 ± 2.59 mmol/L, while the mean area under the curve for the exercise condition was 27.49 ± 2.59 mmol/L. The 95% posterior probability interval for the treatment effect (difference in area under

the curve between conditions) was 1.94 to 14.82 mmol/L. Because the posterior probability distribution interval of the treatment differences did not include zero, we concluded that the exercise treatment had a significantly larger area under the curve than the nonexercise treatment at the 0.95 level.

Time to Achieve Nutritional Ketosis

The hierarchical model was used to estimate the time to nutritional ketosis for each condition. The posterior estimate of the mean time to nutritional ketosis was 21.07 ± 2.95 hours with fasting alone, and 17.5 ± 1.69 hours when fasting was combined with exercise (see Figure 2). This represents a 3.43-hour reduction in time to nutritional ketosis when the fast was started with exercise. The posterior probability that the time to nutritional ketosis was different between conditions was 0.89. Because we had previously determined a posterior probability exceeding 0.95 was need to achieve significance, this difference was not determined to be statistically significant.

Plasma Insulin and Glucagon

For insulin, there was a significant main effect of time ($F(3,133) = 61.75$, $p < 0.0001$) showing a decrease in insulin over time, but no significant effect for condition ($F(1,133) = 0.11$, $p = 0.7430$) nor an interaction between condition and time ($F(3,133) = 2.08$, $p = 0.1055$). For glucagon, there was a significant main effect for time ($F(3,133) = 21.10$, $p < 0.0001$) showing an increase in glucagon over time. There was also a significant effect of condition on glucagon ($F(1,133) = 37.93$, $p < 0.0001$) showing a higher glucagon level on the exercise day compared to the nonexercise day, but no interaction between condition and time ($F(3,133) = 2.56$, $p = 0.0576$). There was a significant main effect for both time ($F(3,133) = 10.29$, $p < 0.0001$) and

condition ($F(1,133) = 14.67$, $p = 0.0002$) for the insulin:glucagon ratio, but no interaction of condition-by-time ($F(3,133) = 1.31$, $p = 0.2731$ (see Figure 3).

Hunger, Thirst and Stomach Discomfort

There was a significant main effect for hunger and time ($F(10,394) = 41.60$, $p < 0.0001$) showing that hunger increased significantly from baseline at hours 12–36 ($p < 0.01$), but no significant interaction between condition-by-time ($F(10,394) = 0.33$, $p = 0.9735$). There was a significant main effect for the interaction of time and thirst ($F(10,394) = 1.96$, $p = 0.0364$), and a significant interaction between condition and time ($F(10,394) = 1.98$, $p = 0.0339$) showing that participants were significantly more thirsty immediately after their exercise bout, but there was no difference between conditions after that. There was a significant main effect for stomach discomfort and time ($F(10,394) = 7.11$, $p < 0.0001$) suggesting that stomach discomfort increased significantly from baseline at hours 12–36, but no significant effect of condition-by-time ($F(10,394) = 0.48$, $p = 0.9047$) (Figure 4).

Mood

There was a significant main effect for time for the following moods: anger ($F(10,394) = 2.74$, $p = 0.0028$), depression ($F(10,394) = 2.91$, $p = 0.0016$), tension ($F(10,394) = 2.29$, $p = 0.0128$), vigor ($F(10,394) = 11.65$, $p < 0.0001$), and fatigue ($F(10,394) = 10.60$, $p = 0.0001$) showing that negative moods increased from baseline, while vigor decreased from baseline over the course of the fast. There was a significant main effect of condition for confusion ($F(10,394) = 4.63$, $p = 0.0320$) showing more confusion on the nonexercise day compared to the day with exercise (see Figure 5). No other significant main effects of condition were observed.

Discussion

The main purpose of the study was to evaluate how an initial bout of exercise impacts BHB ketone production over a 36-hour fast. We found that completing a bout of vigorous aerobic exercise at the beginning of the 36-hour fast increased the production of BHB by 43.3%, compared to fasting alone, an effect that was independent of gender (see Figure 5). The average 3.5-hour difference in time to ketosis observed is this study was not statistically significant. These findings help to address the paucity of research on the moderators of ketone production during a water-only fast.

Understanding the time course to nutritional ketosis and the impact of fasting and exercise on ketone production are particularly meaningful when considering the potential health benefits of BHB that have been highlighted in a number of recent studies. For example, ketones have shown promising cardiovascular results through the reduction of blood pressure in those with hypertension (Goldhamer et al., 2002), and reduction of total cholesterol, triglycerides, LDL cholesterol, and atherosclerotic plaque formation (Malinowski et al., 2019). Ketones have also been linked to a reduction in HbA1C (Gibas & Gibas, 2017), enhanced insulin sensitivity (Sutton et al., 2018), and weight loss in diabetics (Grajower & Horne, 2019). Both Mattson et al. (Mattson et al., 2017) and Harvie et al. (Harvie et al., 2013) suggest that these benefits are achieved even with intermittent exposure to BHB.

There are very few studies that have evaluated the time course of ketone production during a prolonged fast. These studies have a number of limitations that make it difficult to generalize their results. For example, Haymond et al. (1982) measured BHB every four hours over the course of an 86-hour fast in ten healthy men and ten healthy women. While mean BHB levels were not reported at each measurement time, the graph provided suggests that adults

achieved nutritional ketosis between 16–18 hours. Our fasting-only group hit nutritional ketosis after 21.07 ± 2.95 hours, which is several hours slower. Browning et al. (2012) measured BHB every four hours over the course of a 48-hour fast in nine healthy men and nine healthy women. While mean BHB levels were not reported at each measurement time, the graphs provided suggest that adults achieve nutritional ketosis within 24–26 hours, which is several hours longer than observed in the fasting-only group in our study (Browning et al., 2012). Neither of these studies controlled any aspect of diet coming into the fast which may directly alter the time course to ketosis, especially if the participants prepared for the fast by consuming more food. The difference of time course in the Browning study may also be explained by the fact that the population included only overweight and obese adults (Browning et al., 2012).

One factor thought to alter the time course of ketone production is gender. It has been suggested that women tend to enter nutritional ketosis faster than men. This gender difference was observed in a study by Haymond et al. (1982). However, we did not observe this gender difference in our study under either condition. In fact, gender did not alter any of the relationships observed in this study. Our results are supported by Browning et al. (2012) who also found no such difference between genders in fasted conditions.

We hypothesized that exercise would result in more BHB production and a shorter time to ketosis. While increased BHB production was observed in this study in the area under the curve analysis, the study was likely not powered sufficiently to observe a difference in time to ketosis. The basis for this hypothesis was related to reducing both muscle and liver glycogen, which would facilitate the switching of metabolic fuels from glucose to fat. Schranner et al. (2020) concluded that an acute bout of endurance exercise raises ketone levels significantly a few hours after the exercise, but has little effect immediately after the exercise. The exercise

prescribed in the study was calculated to closely match the calories of the standardized meal given to the participants at the beginning of the fast and the intensity was chosen to target glucose utilization specifically. Affectively, the exercise bout in our study allowed earlier consumption of stored glycogen, and subsequent use of fatty acids and ketones for metabolic fuel. Further exploration of this relationship is warranted to determine if time to ketosis was reduced with exercise. The results of this study did not reach significance, but suggest that this is likely the case.

While understanding ketone production trends was the main focus of this study, we also assessed insulin and glucagon levels, as these two hormones both exert strong influences on lipolysis and ketone production (Muoio & Newgard, 2008). As seen in Figure 2, insulin and the insulin:glucagon ratio experience the most dramatic changes within the first 12 hours of fasting, after which they seem to level off. The reduction in plasma insulin seen in this study is supported by the work of Sutton et al. (2018) and supports the notion that fasting does not have to extend beyond 12 hours to appreciate the majority of the reduction in plasma insulin. The marked reduction in the insulin:glucagon ratio indicates that hydrolysis of adipose tissue and triacylglycerol was taking place (Capozzi et al., 2020). Interestingly, glucagon inclined steadily in both conditions over the duration of both fasts with the exercise condition remaining markedly higher. While these trends are interesting and follow the established counter-regulatory roles of insulin and glucagon on each other to protect against hypoglycemia, insulin seems to play a larger role in the ketogenic process than glucagon and strongly inhibits ketosis (McGarry & Foster, 1977). In fact, Capozzi et al. (2020) demonstrated that glucagon is not necessary to facilitate an increase in ketone production in response to a fast.

A secondary question that was addressed in this study was how fasting altered feelings of hunger, thirst, stomach discomfort and affect and how the perceptions differed with or without exercise. This purpose was added to the study in order to examine the utility of adding exercise at the beginning of a fast. Regardless of the potential benefits of combining fasting with exercise, compliance would be difficult to maintain if it presented unpleasant side effects. Wegman et al. (2015) and Heilbronn et al. (2005) both found a significant increase in hunger and stomach discomfort over the duration of intermittent fasting. Our results confirm these findings, and also show that while both hunger and stomach discomfort increased throughout each fast, ratings did not change with the addition of exercise. Thus, combining exercise with fasting increases ketone production without increasing subjective ratings of hunger or fasting discomfort.

In addition to hunger and stomach discomfort, we also evaluated subjective feelings of mood. We found that anger, depression, tension and fatigue increased over time throughout the fast, while vigor decreased. These results are consistent with Appleton and Baker (2015) who found that fasting was associated with negative affect. However, in our study, the changes in mood were not different between conditions. To our knowledge, this is the first study that has observed the influence on mood of combining exercise with fasting. Because feelings of anger, confusion, depression, fatigue, tension and vigor are not swayed by exercise, adding exercise to a fast would increase the potential benefits of fasting without perpetuating a negative mood.

The enhanced production of BHB observed when combining exercise with fasting can benefit individuals participating in a variety of dietary practices designed to optimize ketone body production. While various fasting protocols such as time-restricted eating, alternate-day fasting and prolonged fasting are currently popular, some fasting styles may be more beneficial for the building of BHB than others. For example, someone practicing a 16-hour fast, followed

by an 8-hour eating window would likely not reach nutritional ketosis, even when beginning the fast with exercise. Even an 18- or 20-hour fast would likely be inadequate to reach nutritional ketosis without exercise. Most people would likely be in nutritional ketosis if they fasted 24 hours or more. However, we also recognize that there may be benefits from achieving BHB levels below those of 0.5 mmol/L, and that a shorter fasting period may be more tolerable and improve compliance. However, literature is lacking in evidence to support specific claims relating to benefits of lower BHB levels.

Strengths, Limitations, and Future Research

Strengths

Several strengths should be taken into account when considering the results of this study, including that this was a randomized controlled trial with human subjects. While there are randomized controlled trials evaluating BHB and fasting, much of this literature is from the early to mid-1900s with methods and outcomes that are not well described. Additional strength comes from providing the standardized meals to initiate each fast. Doing so allowed us to ensure that differences were a result of test conditions rather than calorie differences. To that end, energy expenditure for exercise was estimated through the formulas outlined, but also verified by indirect calorimetry. Finally, the frequency at which ketone readings were obtained gave a more stable curve while minimally impacting sleep and life patterns.

Limitations

There are a few limitations that should be considered when interpreting the results of this study. First, while each participant abstained from food for four hours prior to presenting at the lab and were fed a standardized meal at the beginning of each fast, we did not control the food intake of the participants that took place early in the day. The amount and type of food and drink

consumed prior to the fast and standardized meal may have affected the metabolic state, especially when the participant is anticipating a subsequent 36-hour fast. However, we did ask participants to follow normal dietary patterns and to not overconsume at these meals. In addition, neither plasma insulin nor glucagon differed between conditions at baseline, suggesting that the participant entered the conditions in a similar metabolic state on both days.

Second, hydration was encouraged, but not directly monitored in this study and hydrations seems to have an impact on ketone production (Johnson, 1959). However, we did measure thirst and the only difference between conditions was immediately after exercise. In addition, overall thirst scores were very low.

Third, while the study was sufficiently powered to observe a significant difference in area under the BHB curve, we may have been underpowered to evaluate the difference in time to ketosis between conditions and between genders in each condition. This lack of significance was largely driven by one participant whose results were opposite that of the other participants, reaching nutritional ketosis more quickly on the nonexercise day compared to the exercise day. This difference in results may be explained, in part, by the fact that this subject had the highest BMI of all participants and was the only participant classified as obese. Obesity has an impact on ketone production and may explain the divergent observation (McGarry & Foster, 1977).

Finally, the exercise prescribed in this study was intense and for a relatively long duration. It may be difficult for many people to complete this type of exercise protocol. However, the results do suggest that exercise does have an impact on ketone production and it is likely that other exercise protocols that are less intense or of shorter duration may still influence this relationship but to a lesser extent.

Conclusions/Future Research

Being in nutritional ketosis, even irregularly, has been linked to a number of positive health outcomes. The findings of our study suggest that combining a fast with exercise increases BHB production but does not significantly decrease the time to nutritional ketosis. Consequently, combining a fast with exercise results in higher concentrations of BHB in roughly the same period of time compared to fasting. Future research might explore the effects of incorporating exercise at various times throughout a fast. Additionally, future studies should also evaluate how lower intensity exercise or high intensity interval training alter the production of BHB during a fast.

References

Abbasi, J. (2018, Jan 16). Interest in the Ketogenic Diet Grows for Weight Loss and Type 2 Diabetes. *The Journal of the American Medical Association, 319*(3), 215-217. https://doi.org/10.1001/jama.2017.20639

Ahmed, K., Tunaru, S., & Offermanns, S. (2009, Nov). GPR109A, GPR109B and GPR81, a family of hydroxy-carboxylic acid receptors. *Trends Pharmacol Sci, 30*(11), 557-562. https://doi.org/10.1016/j.tips.2009.09.001

Anson, R. M., Guo, Z. H., de Cabo, R., Iyun, T., Rios, M., Hagepanos, A., Ingram, D. K., Lane, M. A., & Mattson, M. P. (2003, May 13). Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from calorie intake. *Proceedings of the National Academy of Sciences of the United States of America, 100*(10), 6216-6220. https://doi.org/DOI 10.1073/pnas.1035720100

Anton, S. D., Moehl, K., Donahoo, W. T., Marosi, K., Lee, S. A., Mainous, A. G., 3rd, Leeuwenburgh, C., & Mattson, M. P. (2018, Feb). Flipping the Metabolic Switch: Understanding and Applying the Health Benefits of Fasting. *Obesity (Silver Spring), 26*(2), 254-268. https://doi.org/10.1002/oby.22065

Appleton, K. M., & Baker, S. (2015, Jun). Distraction, not hunger, is associated with lower mood and lower perceived work performance on fast compared to non-fast days during intermittent fasting. *Journal Of Health Psychology, 20*(6), 702-711. https://doi.org/10.1177/1359105315573430

Bailey, B. W., LeCheminant, G., Hope, T., Bell, M., & Tucker, L. A. (2018). A comparison of the agreement, internal consistency, and 2-day test stability of the InBody 720, GE iDXA, and BOD POD® gold standard for assessing body composition. *Measurement in Physical Education and Exercise Science, 22*(3), 231-238. https://doi.org/10.1080/1091367X.2017.1422129

Balasse, E. O., Fery, F., & Neef, M. A. (1978, Jan). Changes induced by exercise in rates of turnover and oxidation of ketone bodies in fasting man. *J Appl Physiol Respir Environ Exerc Physiol, 44*(1), 5-11. https://doi.org/10.1152/jappl.1978.44.1.5

Bergqvist, A. G. S., Joan I.; Gallagher, Paul R.;Cnaan, Avital; Stallings, Virginia A. (2005). Fasting versus Gradual Initiation of the Ketogenic Diet: A Prospective, Randomized Clinical Trial of Efficacy. *Epilepsia, 46*(11), 1810-1819. https://onlinelibrary.wiley.com/doi/full/10.1111/j.1528-1167.2005.00282.x

Browning, J. D., Baxter, J., Satapati, S., & Burgess, S. C. (2012, Mar). The effect of short-term fasting on liver and skeletal muscle lipid, glucose, and energy metabolism in healthy women and men. *Journal of Lipid Research, 53*(3), 577-586. https://doi.org/10.1194/jlr.P020867

Byrne, H. A. T., Kenneth L.; Hollis, Sally. (2000). Evaluation of an Electrochemical Sensor for Measuring Blood Ketones. *Diabetes Care, 23*(4), 500-503. https://care.diabetesjournals.org/content/diacare/23/4/500.full.pdf

Cahill, G. F. (1976). Starvation in Man. *Clinics in Endocrinology and Metabolism, 5*(2), 397-415. https://doi.org/Doi 10.1016/S0300-595x(76)80028-X

Cahill, G. F., Jr. (2006). Fuel metabolism in starvation. *Annu Rev Nutr, 26*, 1-22. https://doi.org/10.1146/annurev.nutr.26.061505.111258

Capozzi, M. E., Coch, R. W., Koech, J., Astapova, I. I., Wait, J. B., Encisco, S. E., Douros, J. D., El, K., Finan, B., Sloop, K. W., Herman, M. A., D'Alessio, D. A., & Campbell, J. E. (2020, May). The Limited Role of Glucagon for Ketogenesis During Fasting or in Response to SGLT2 Inhibition. *Diabetes, 69*(5), 882-892. https://doi.org/10.2337/db19-1216

Centers for Disease Control and Prevention. (2019). *Leading Causes of Death*. Retrieved June 30 from https://www.cdc.gov/nchs/fastats/leading-causes-of-death.htm

Finnell, J. S., Saul, B. C., Goldhamer, A. C., & Myers, T. R. (2018, Feb 20). Is fasting safe? A chart review of adverse events during medically supervised, water-only fasting. *BMC Complement Altern Med, 18*(1), 67. https://doi.org/10.1186/s12906-018-2136-6

Gibas, M. K., & Gibas, K. J. (2017, Nov). Induced and controlled dietary ketosis as a regulator of obesity and metabolic syndrome pathologies. *Diabetes Metab Syndr, 11 Suppl 1*, S385-S390. https://doi.org/10.1016/j.dsx.2017.03.022

Gibson, A. A., Seimon, R. V., Lee, C. M., Ayre, J., Franklin, J., Markovic, T. P., Caterson, I. D., & Sainsbury, A. (2015, Jan). Do ketogenic diets really suppress appetite? A systematic review and meta-analysis. *Obes Rev, 16*(1), 64-76. https://doi.org/10.1111/obr.12230

Goldhamer, A. C., Lisle, D. J., Sultana, P., Anderson, S. V., Parpia, B., Hughes, B., & Campbell, T. C. (2002, Oct). Medically supervised water-only fasting in the treatment of borderline hypertension. *J Altern Complement Med, 8*(5), 643-650. https://doi.org/10.1089/107555302320825165

Grajower, M. M., & Horne, B. D. (2019, Apr 18). Clinical Management of Intermittent Fasting in Patients with Diabetes Mellitus. *Nutrients, 11*(4). https://doi.org/10.3390/nu11040873

Guerci, B., Benichou, M., Floriot, M., Bohme, P., Fougnot, S., Franck, P., & Drouin, P. (2003, Apr). Accuracy of an electrochemical sensor for measuring capillary blood ketones by fingerstick samples during metabolic deterioration after continuous subcutaneous insulin infusion interruption in type 1 diabetic patients. *Diabetes Care, 26*(4), 1137-1141. https://www.ncbi.nlm.nih.gov/pubmed/12663586

Haces, M. L., Hernandez-Fonseca, K., Medina-Campos, O. N., Montiel, T., Pedraza-Chaverri, J., & Massieu, L. (2008, May). Antioxidant capacity contributes to protection of ketone bodies against oxidative damage induced during hypoglycemic conditions. *Exp Neurol, 211*(1), 85-96. https://doi.org/10.1016/j.expneurol.2007.12.029

Hall, K. D., Sacks, G., Chandramohan, D., Chow, C. C., Wang, Y. C., Gortmaker, S. L., & Swinburn, B. A. (2011, Aug 27). Quantification of the effect of energy imbalance on bodyweight. *Lancet, 378*(9793), 826-837. https://doi.org/10.1016/S0140-6736(11)60812-X

Harvey, C., Schofield, G. M., & Williden, M. (2018). The use of nutritional supplements to induce ketosis and reduce symptoms associated with keto-induction: a narrative review. *PeerJ, 6*, e4488. https://doi.org/10.7717/peerj.4488

Harvey, K. L., Holcomb, L. E., & Kolwicz, S. C., Jr. (2019, Sep 26). Ketogenic Diets and Exercise Performance. *Nutrients, 11*(10). https://doi.org/10.3390/nu11102296

Harvie, M., Wright, C., Pegington, M., McMullan, D., Mitchell, E., Martin, B., Cutler, R. G., Evans, G., Whiteside, S., Maudsley, S., Camandola, S., Wang, R., Carlson, O. D., Egan, J. M., Mattson, M. P., & Howell, A. (2013, Oct 28). The effect of intermittent energy and carbohydrate restriction v. daily energy restriction on weight loss and metabolic disease risk markers in overweight women. *British Journal of Nutrition, 110*(8), 1534-1547. https://doi.org/10.1017/S0007114513000792

Haymond, M. W., Karl, I. E., Clarke, W. L., Pagliara, A. S., & Santiago, J. V. (1982, Jan). Differences in circulating gluconeogenic substrates during short-term fasting in men, women, and children. *Metabolism, 31*(1), 33-42. https://www.ncbi.nlm.nih.gov/pubmed/7043160

Heilbronn, L. K., Smith, S. R., Martin, C. K., Anton, S. D., & Ravussin, E. (2005, Jan). Alternate-day fasting in nonobese subjects: effects on body weight, body composition, and energy metabolism. *American Journal of Clinical Nutrition, 81*(1), 69-73. https://doi.org/10.1093/ajcn/81.1.69

Hoffman, L. D., & Polich, J. (1998, Jun). EEG, ERPs and food consumption [Article]. *Biological Psychology, 48*(2), 139-151. https://doi.org/10.1016/s0301-0511(98)00010-6

Jette, M., Sidney, K., & Blumchen, G. (1990, Aug). Metabolic equivalents (METS) in exercise testing, exercise prescription, and evaluation of functional capacity. *Clin Cardiol, 13*(8), 555-565. https://doi.org/10.1002/clc.4960130809

Johnson, R. E. P., R. . (1959). Interrelations Among Post-Exercise Ketosis (Courtice-Douglas Effect), Hydration and Metabolic State. *Metabolism. Clinical and Experimental, 9*(5), 443-451.

Johnston, K. L., Clifford, M. N., & Morgan, L. M. (2003, Oct). Coffee acutely modifies gastrointestinal hormone secretion and glucose tolerance in humans: glycemic effects of chlorogenic acid and caffeine. *American Journal of Clinical Nutrition, 78*(4), 728-733. https://doi.org/10.1093/ajcn/78.4.728

Kang, H. C., Lee, Y. M., Kim, H. D., Lee, J. S., & Slama, A. (2007, Jan). Safe and effective use of the ketogenic diet in children with epilepsy and mitochondrial respiratory chain complex defects. *Epilepsia, 48*(1), 82-88. https://doi.org/10.1111/j.1528-1167.2006.00906.x

Kashiwaya, Y., Sato, K., Tsuchiya, N., Thomas, S., Fell, D. A., Veech, R. L., & Passonneau, J. V. (1994, Oct 14). Control of glucose utilization in working perfused rat heart. *J Biol Chem, 269*(41), 25502-25514. https://www.ncbi.nlm.nih.gov/pubmed/7929251

Kashiwaya, Y., Takeshima, T., Mori, N., Nakashima, K., Clarke, K., & Veech, R. L. (2000, May 9). D-beta-hydroxybutyrate protects neurons in models of Alzheimer's and Parkinson's disease. *Proc Natl Acad Sci U S A, 97*(10), 5440-5444. https://doi.org/10.1073/pnas.97.10.5440

Kerndt, P. R., Naughton, J. L., Driscoll, C. E., & Loxterkamp, D. A. (1982, Nov). Fasting: the history, pathophysiology and complications. *West J Med, 137*(5), 379-399. https://www.ncbi.nlm.nih.gov/pubmed/6758355

Longo, V. D., & Mattson, M. P. (2014, Feb 4). Fasting: molecular mechanisms and clinical applications. *Cell Metab, 19*(2), 181-192. https://doi.org/10.1016/j.cmet.2013.12.008

Maalouf, M., Sullivan, P. G., Davis, L., Kim, D. Y., & Rho, J. M. (2007, Mar 2). Ketones inhibit mitochondrial production of reactive oxygen species production following glutamate excitotoxicity by increasing NADH oxidation. *Neuroscience, 145*(1), 256-264. https://doi.org/10.1016/j.neuroscience.2006.11.065

Malinowski, B., Zalewska, K., Wesierska, A., Sokolowska, M. M., Socha, M., Liczner, G., Pawlak-Osinska, K., & Wicinski, M. (2019, Mar 20). Intermittent Fasting in Cardiovascular Disorders-An Overview. *Nutrients, 11*(3). https://doi.org/10.3390/nu11030673

Mattson, M. P., Longo, V. D., & Harvie, M. (2017, Oct). Impact of intermittent fasting on health and disease processes. *Ageing Research Reviews, 39*, 46-58. https://doi.org/10.1016/j.arr.2016.10.005

Mattson, M. P., Moehl, K., Ghena, N., Schmaedick, M., & Cheng, A. (2018, Feb). Intermittent metabolic switching, neuroplasticity and brain health. *Nat Rev Neurosci, 19*(2), 63-80. https://doi.org/10.1038/nrn.2017.156

McGarry, J. D., & Foster, D. W. (1977, Apr). Hormonal control of ketogenesis. Biochemical considerations. *Archives of Internal Medicine, 137*(4), 495-501. https://www.ncbi.nlm.nih.gov/pubmed/403870

Michalsen, A., Kuhlmann, M. K., Ludtke, R., Backer, M., Langhorst, J., & Dobos, G. J. (2006, Oct-Dec). Prolonged fasting in patients with chronic pain syndromes leads to late mood-enhancement not related to weight loss and fasting-induced leptin depletion. *Nutr Neurosci, 9*(5-6), 195-200. https://doi.org/10.1080/10284150600929656

Mohammad, A., De Lucia Rolfe, E., Sleigh, A., Kivisild, T., Behbehani, K., Wareham, N. J., Brage, S., & Mohammad, T. (2017, Jan 9). Validity of visceral adiposity estimates from DXA against MRI in Kuwaiti men and women. *Nutr Diabetes, 7*(1), e238. https://doi.org/10.1038/nutd.2016.38

Morrow, P. G., Marshall, W. P., Kim, H. J., & Kalkhoff, R. K. (1981, Mar). Metabolic response to starvation. II. Effects of sex steroid administration to pre- and postmenopausal women. *Metabolism, 30*(3), 274-278. https://doi.org/10.1016/0026-0495(81)90151-7

Muoio, D. M. (2014, Dec 4). Metabolic Inflexibility: When Mitochondrial Indecision Leads to Metabolic Gridlock. *Cell, 159*(6), 1253-1262. https://doi.org/10.1016/j.cell.2014.11.034

Muoio, D. M., & Newgard, C. B. (2008, Mar). Mechanisms of disease:Molecular and metabolic mechanisms of insulin resistance and beta-cell failure in type 2 diabetes. *Nat Rev Mol Cell Biol, 9*(3), 193-205. https://doi.org/10.1038/nrm2327

National Institutes of Health. (2019). *Healthy Weight Tools*. Retrieved August from https://www.nhlbi.nih.gov/health/educational/lose_wt/BMI/bmicalc.htm

Naude, C. E., Schoonees, A., Senekal, M., Young, T., Garner, P., & Volmink, J. (2014). Low carbohydrate versus isoenergetic balanced diets for reducing weight and cardiovascular risk: a systematic review and meta-analysis. *Public Library of Science One, 9*(7), e100652. https://doi.org/10.1371/journal.pone.0100652

Plumelle, D., Lombard, E., Nicolay, A., & Portugal, H. (2014, Jan). Influence of diet and sample collection time on 77 laboratory tests on healthy adults. *Clin Biochem, 47*(1-2), 31-37. https://doi.org/10.1016/j.clinbiochem.2013.11.002

Puchalska, P., & Crawford, P. A. (2017, Feb 7). Multi-dimensional Roles of Ketone Bodies in Fuel Metabolism, Signaling, and Therapeutics. *Cell Metab, 25*(2), 262-284. https://doi.org/10.1016/j.cmet.2016.12.022

Purdom, T., Kravitz, L., Dokladny, K., & Mermier, C. (2018, Jan 12). Understanding the factors that effect maximal fat oxidation. *Journal of the International Society of Sports Nutrition, 15*. https://doi.org/ARTN 310.1186/s12970-018-0207-1

Rohlfs, I., Rotta, T., Andrade, A., Terry, P., Krebs, R. and Carvalho, T. (2005). The Brunel of Mood Scale (BRUMS): Instrument for Detection of Modified Mood States in Adolescents and Adults Athletes and Non Athletes. . *FIEP Bulletin, 75*, 281-284.

Rothney, M. P., Xia, Y., Wacker, W. K., Martin, F. P., Beaumont, M., Rezzi, S., Giusti, V., & Ergun, D. L. (2013, Jan). Precision of a new tool to measure visceral adipose tissue (VAT) using dual-energy X-Ray absorptiometry (DXA). *Obesity (Silver Spring), 21*(1), E134-136. https://doi.org/10.1002/oby.20140

Roy, S., & McCrory, J. (2015). Validation of Maximal Heart Rate Prediction Equations Based on Sex and Physical Activity Status. *Int J Exerc Sci, 8*(4), 318-330. https://www.ncbi.nlm.nih.gov/pubmed/27182419

Schranner, D., Kastenmuller, G., Schonfelder, M., Romisch-Margl, W., & Wackerhage, H. (2020, Feb 10). Metabolite Concentration Changes in Humans After a Bout of Exercise: a Systematic Review of Exercise Metabolomics Studies. *Sports Med Open, 6*(1), 11. https://doi.org/10.1186/s40798-020-0238-4

Shimazu, T., Hirschey, M. D., Newman, J., He, W., Shirakawa, K., Le Moan, N., Grueter, C. A., Lim, H., Saunders, L. R., Stevens, R. D., Newgard, C. B., Farese, R. V., Jr., de Cabo, R., Ulrich, S., Akassoglou, K., & Verdin, E. (2013, Jan 11). Suppression of oxidative stress by beta-hydroxybutyrate, an endogenous histone deacetylase inhibitor. *Science, 339*(6116), 211-214. https://doi.org/10.1126/science.1227166

Solheim, T. J., Keller, B. G., & Fountaine, C. J. (2014). VO2 Reserve vs. Heart Rate Reserve During Moderate Intensity Treadmill Exercise. *Int J Exerc Sci, 7*(4), 311-317. https://www.ncbi.nlm.nih.gov/pubmed/27182409

Speakman, J., Booles, D., & Butterwick, R. (2001, 2001). Validation of dual energy X-ray absorptiometry (DXA) by comparison with chemical analysis of dogs and cats. *Int J Obes, 25*, 439-447. http://www.nature.com/ijo/journal/v25/n3/pdf/0801544a.pdf

Stubbs, R. J., Hughes, D. A., Johnstone, A. M., Rowley, E., Reid, C., Elia, M., Stratton, R., Delargy, H., King, N., & Blundell, J. E. (2000, Oct). The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. *British Journal of Nutrition, 84*(4), 405-415. https://doi.org/Doi 10.1017/S0007114500001719

Sumithran, P., Prendergast, L. A., Delbridge, E., Purcell, K., Shulkes, A., Kriketos, A., & Proietto, J. (2013, Jul). Ketosis and appetite-mediating nutrients and hormones after weight loss. *Eur J Clin Nutr, 67*(7), 759-764. https://doi.org/10.1038/ejcn.2013.90

Suresh, K. (2011, Jan). An overview of randomization techniques: An unbiased assessment of outcome in clinical research. *J Hum Reprod Sci, 4*(1), 8-11. https://doi.org/10.4103/0974-1208.82352

Sutton, E. F., Beyl, R., Early, K. S., Cefalu, W. T., Ravussin, E., & Peterson, C. M. (2018, Jun 5). Early Time-Restricted Feeding Improves Insulin Sensitivity, Blood Pressure, and Oxidative Stress Even without Weight Loss in Men with Prediabetes. *Cell Metab, 27*(6), 1212-1221 e1213. https://doi.org/10.1016/j.cmet.2018.04.010

Taggart, A. K., Kero, J., Gan, X., Cai, T. Q., Cheng, K., Ippolito, M., Ren, N., Kaplan, R., Wu, K., Wu, T. J., Jin, L., Liaw, C., Chen, R., Richman, J., Connolly, D., Offermanns, S., Wright, S. D., & Waters, M. G. (2005, Jul 22). (D)-beta-Hydroxybutyrate inhibits adipocyte lipolysis via the nicotinic acid receptor PUMA-G. *J Biol Chem, 280*(29), 26649-26652. https://doi.org/10.1074/jbc.C500213200

Tataranni, P. A., Pettitt, D. J., & Ravussin, E. (1996, 1996). Dual energy X-ray absorptiometry: Inter-machine variability. *Int.J.Obes., 20*, 1048-1050.

Tieu, K., Perier, C., Caspersen, C., Teismann, P., Wu, D. C., Yan, S. D., Naini, A., Vila, M., Jackson-Lewis, V., Ramasamy, R., & Przedborski, S. (2003, Sep). D-beta-hydroxybutyrate rescues mitochondrial respiration and mitigates features of Parkinson disease. *J Clin Invest, 112*(6), 892-901. https://doi.org/10.1172/JCI18797

Verhaegen, A. A., & Van Gaal, L. F. (2017, Nov). Drug-induced obesity and its metabolic consequences: a review with a focus on mechanisms and possible therapeutic options. *Journal of Endocrinological Investigation, 40*(11), 1165-1174. https://doi.org/10.1007/s40618-017-0719-6

Wegman, M. P., Guo, M. H., Bennion, D. M., Shankar, M. N., Chrzanowski, S. M., Goldberg, L. A., Xu, J., Williams, T. A., Lu, X., Hsu, S. I., Anton, S. D., Leeuwenburgh, C., & Brantly, M. L. (2015, Apr). Practicality of intermittent fasting in humans and its effect on oxidative stress and genes related to aging and metabolism. *Rejuvenation Res, 18*(2), 162-172. https://doi.org/10.1089/rej.2014.1624

Winters, N., Higgins, J., & Turner, K. (2017). *The Metabolic Approach to Cancer*. Chelsea Green Publishing.

World Health Organization. (1985). *Energy and Protein Requirements*. Retrieved May from https://pubmed.ncbi.nlm.nih.gov/3937340/

Wu, J. H., Foote, C., Blomster, J., Toyama, T., Perkovic, V., Sundstrom, J., & Neal, B. (2016, May). Effects of sodium-glucose cotransporter-2 inhibitors on cardiovascular events, death, and major safety outcomes in adults with type 2 diabetes: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol, 4*(5), 411-419. https://doi.org/10.1016/S2213-8587(16)00052-8

Yamamoto, H., Suzuki, J., & Yamauchi, Y. (1979). Psychophysiological study on fasting therapy. *Psychother Psychosom, 32*(1-4), 229-240. https://doi.org/10.1159/000287392

Youm, Y. H., Nguyen, K. Y., Grant, R. W., Goldberg, E. L., Bodogai, M., Kim, D., D'Agostino, D., Planavsky, N., Lupfer, C., Kanneganti, T. D., Kang, S., Horvath, T. L., Fahmy, T. M., Crawford, P. A., Biragyn, A., Alnemri, E., & Dixit, V. D. (2015, Mar). The ketone metabolite beta-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. *Nat Med, 21*(3), 263-269. https://doi.org/10.1038/nm.3804

Table 1. Demographic characteristics of participants

	Male (n = 11)		**Female (n = 9)**		**Cumulative (n = 20)**	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*
Age (years)	26.5	7.1	25.8	4.3	26.2	5.8
BMI (kg/m^2)	24.7	3.1	22.7	3.6	23.8	3.4
BF %	18.8	6.3	27.8	4.5	22.9	7.1
Visceral Adipose (g)	443.6	275.5	133.5	116.6	304.0	265.9
Ethnicity	***n***	***%***	***n***	***%***	***n***	***%***
Asian	1	9	2	22	3	15
Caucasian	8	73	7	78	15	75
Hawaiian/Pacific Islander	2	18	0	0	2	10

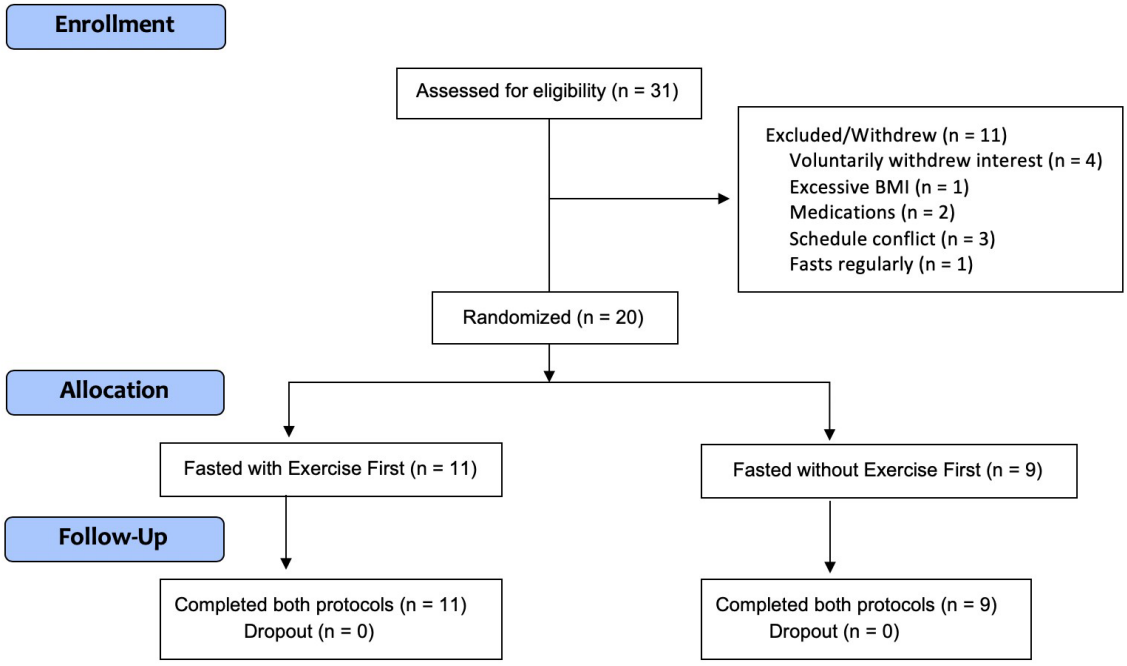

Figure 1. Participant Flow Diagram

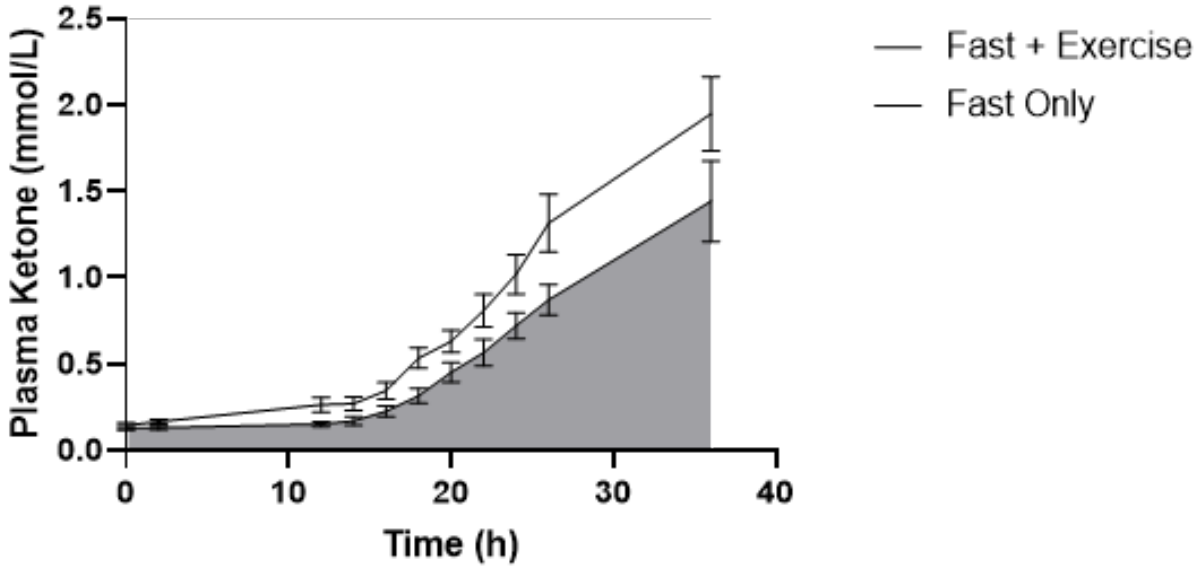

Figure 2. Area Under the Beta-Hydroxybutyrate Curve

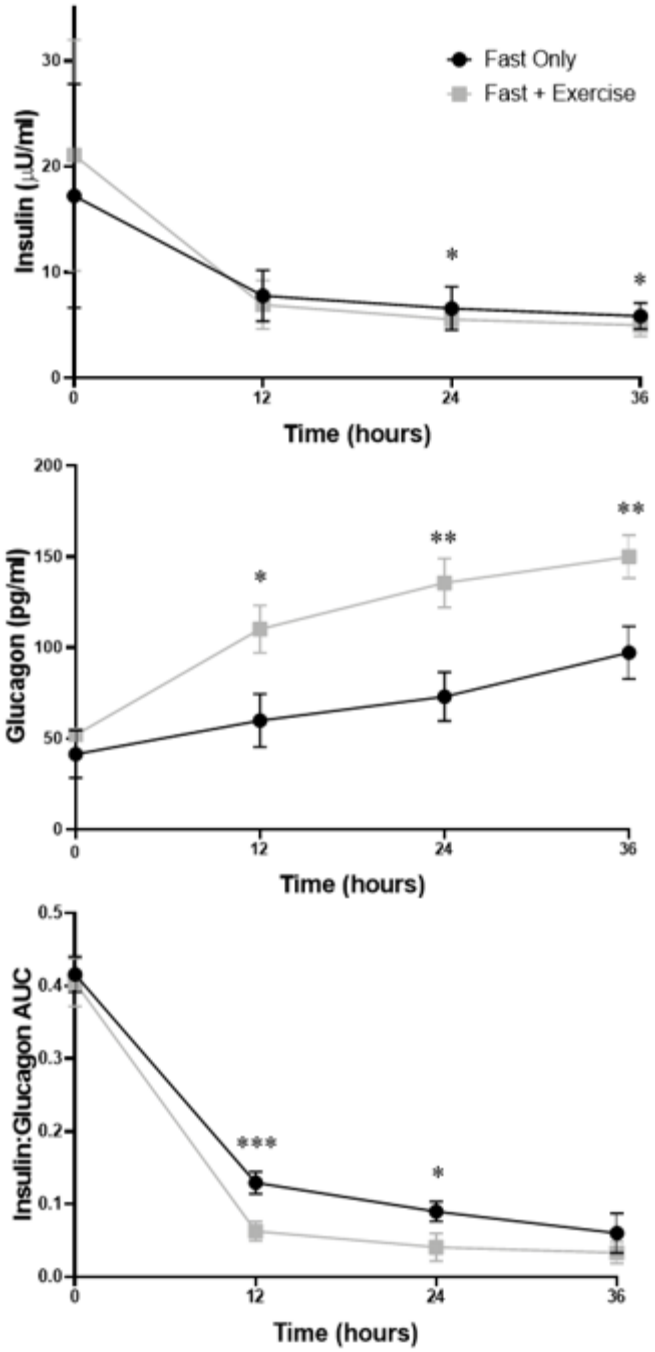

Figure 3. Plasma Insulin and Glucagon Trends

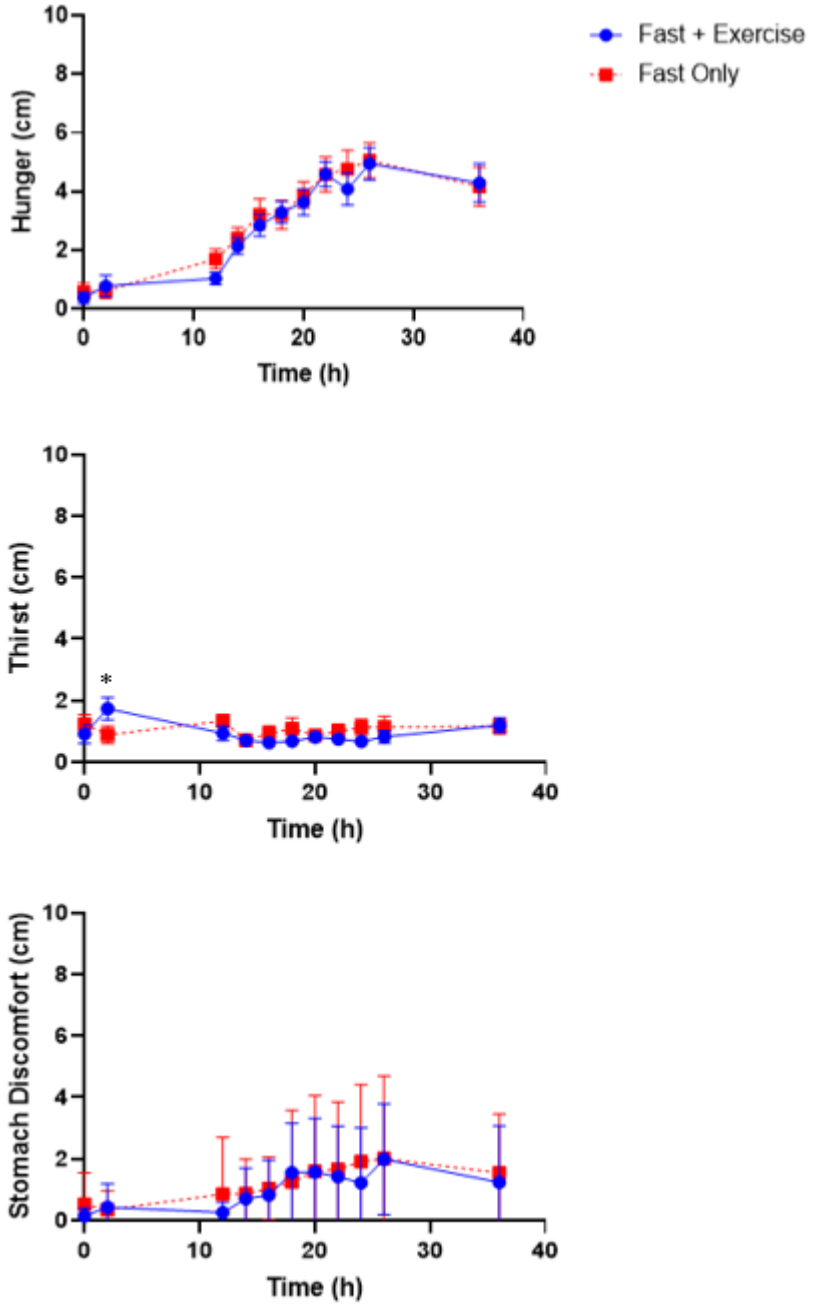

Figure 4. Hunger, Thirst and Stomach Discomfort Trends

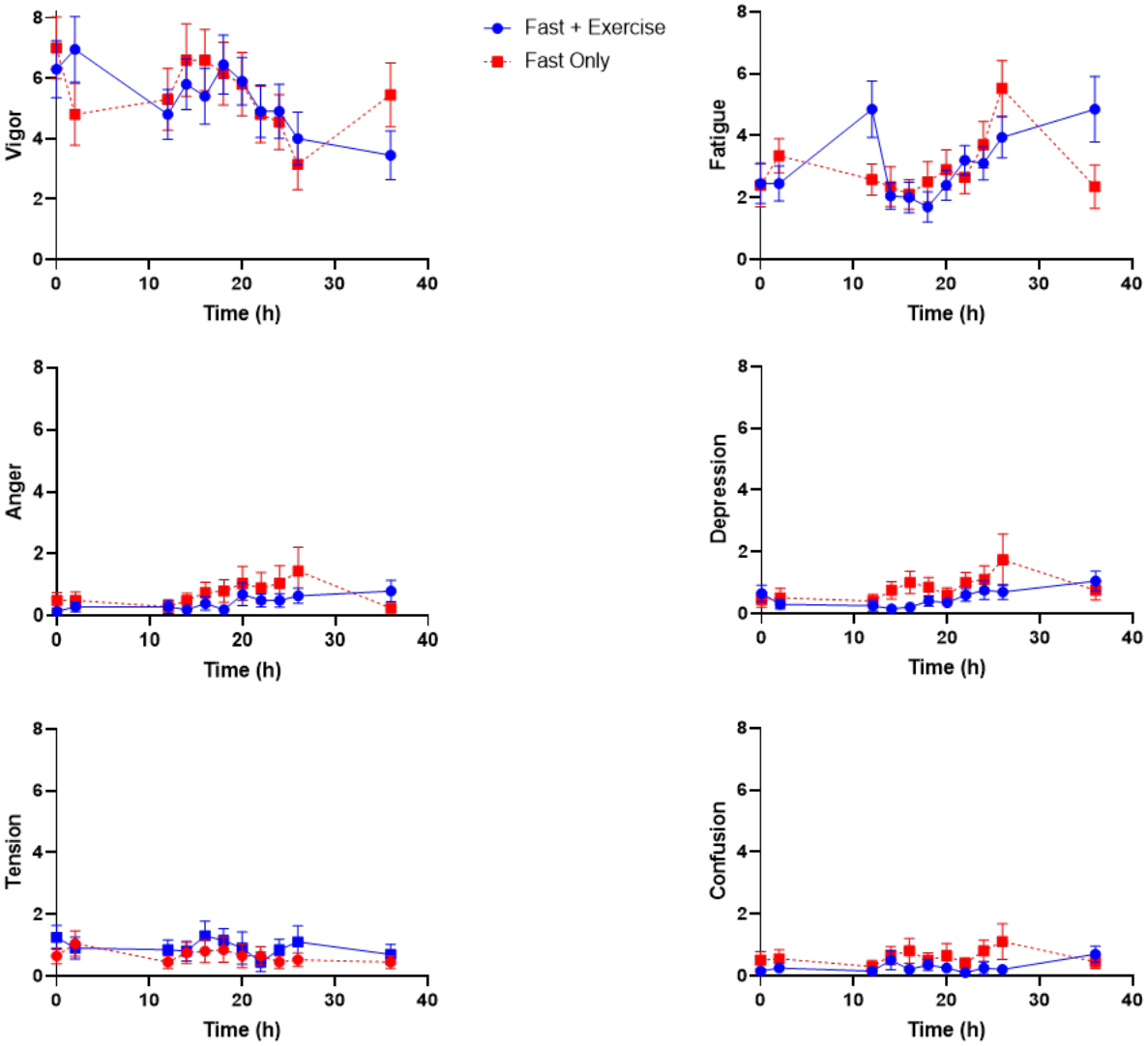

Figure 5. Mood Trends