International Journal of Obesity (2002), 870–872
 0307–0565/02 $25.00
www.nature.com/ijo

SHORT COMMUNICATION

The effect of (−)-hydroxycitrate on energy intake and satiety in overweight humans

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OBJECTIVE: Assessment of the effects of 2 weeks of daily administration of HCA on energy intake and satiety in overweight men and women.
DESIGN: A 6-week randomized placebo-controlled single-blinded cross-over trial. Subjects consumed three times daily for 2 weeks 100 ml tomato juice (placebo) and, separated by a 2 week wash-out period, 100 ml tomato juice with 300 mg HCA.
SUBJECTS: Twelve males and 12 females (body mass index $27.5 \pm 2.0\,kg/m^2$; age 37 ± 10 y).
MEASUREMENTS: After 2 weeks, 24 h energy intake (EI), appetite profile, hedonics, mood and possible change in dietary restraint were assessed in the laboratory restaurant. Prevention of degradation and bio-availability was documented.
RESULTS: Twenty-four-hour EI was decreased by 15–30% ($P < 0.05$) with HCA treatment compared to placebo, without changes in the appetite profile, dietary restraint, mood, taste perception and hedonics, while body weight tended to decrease ($P = 0.1$).
CONCLUSION: HCA treatment reduced 24 h EI in humans while satiety was sustained.
International Journal of Obesity (2002) **26,** 870–872. doi:10.1038/sj.ijo.0801979

Keywords: obesity; *Garcinia cambogia* (−)-hydroxycitrate; satiety; energy intake regulation; humans

Obesity is a medical condition associated with a relatively high rate of morbidity, if it remains untreated.[1] Weight control methods often produce short-term success, but sustained weight maintenance is difficult to attain.[2–4] Moreover, the increasing prevalence of obesity[5] requires preventive treatments of obesity. Substances that reduce energy intake without a major reduction in satiety would prove to be useful. We therefore investigated the potential of (−)-hydroxycitrate (HCA) in overweight men and women. HCA is an ingredient extracted from the rind of the fruit *Garcinia cambogia*, a native species from India, and is promoted as a weight loss agent. HCA is an inhibitor of ATP-citrate lyase, a cytosolic (extramitochondrial) enzyme that catalyses the cleavage of citrate to oxaloacetate and acetyl-CoA.[6–8] Limitation of acetyl-CoA for lipid synthesis during carbohydrate feeding results in increased hepatic glycogen synthesis, which might result in reduction of energy intake.[9,10] Results on the effects of HCA on appetite and body weight regulation in humans showed positive[11] and negative[12,13] outcomes. In this study we assessed the efficacy of HCA with respect to energy intake and satiety, hypothesizing that, when HCA is effective in reducing energy intake, satiety is sustained.

Twenty-four healthy, overweight, dietary unrestrained[14,15] subjects: 12 males and 12 females (body mass index (BMI) $27.5 \pm 2.0\,kg/m^2$; age 37 ± 10 y) were enrolled for the trial. The Medical Ethics Committee approved of the protocol, and all subjects signed informed consent forms. During the single-blinded, randomized and placebo-controlled study, subjects consumed HCA or placebo during 2 weeks as drinks, three times per day. Before and after 2 weeks they came for 24 h energy intake, satiety and body weight assessment to the laboratory.

The drinks consisted of either 100 ml tomato juice alone or 100 ml tomato juice with HCA—500 mg SuperCitrimax/100 ml tomato juice (ie 300 mg (−)-hydroxycitrate HCA from 500 mg SuperCitrimax HCA 600 SGX dissolved). The composition of Citrimax HCA 600 SGX is 58.7% HCA, 11% calcium, 16% potassium, 1% moisture; the rest is carbohydrate and fat (EuroChem Feinchemie GmbH, München, Germany). Thus the dosage was 900 mg HCA/day. day. Prevention of degradation was confirmed by an efficacy

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Received 13 August 2001; accepted 17 October 2001

PHYTOTHERAPY RESEARCH
Phytother. Res. (2013)
Published online in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/ptr.5085

SHORT COMMUNICATION

Lipolytic Activity of Svetol®, a Decaffeinated Green Coffee Bean Extract

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The beneficial health effects of chlorogenic acids (CGAs), major components of coffee beans, are well known and have been attributed to multiple mechanisms of action. However, the lipolytic activity of CGAs does not appear to have been reported. We studied the effects of varying concentrations of Svetol®, a decaffeinated green coffee bean extract enriched in CGAs, on the liberation of free fatty acids from human adipocytes following short-term (2 h) and long-term (192 h) exposure. The results showed that although lipolytic activity observed following short-term incubation could be tentatively linked to residual caffeine traces in the sample, longer-term exposure clearly showed the effects of Svetol® on release of free fatty acids, and this effect was not due to caffeine. The results of this study provide a further mechanism by which to explain the long-term health benefits of CGAs and Svetol®.

Keywords: chlorogenic acid; adipocyte; weight loss; diabetes.

INTRODUCTION

Chlorogenic acids (CGAs), phenolic compounds, which are esters of hydroxycinnamic and quinic acid, are widely dispersed in the plant kingdom and found at elevated levels in coffee beans (Clifford, 1999). Epidemiological studies have shown that consumption of coffee reduces the risk of diabetes development (van Dam *et al*., 2006), and this health benefit has been attributed to the CGA fraction of coffee. Human intervention studies have shown the efficacy of a standardized decaffeinated green coffee bean extract (DGCE) extract, Svetol®, to significantly reduce body weight (Dellalibera *et al*., 2006; Thom, 2007) and post-prandial blood glucose (Thom, 2007; Blum *et al*., 2007) in overweight and obese individuals.

Chlorogenic acids are known to be able to inhibit the glucose-6-phosphatase system (Henry-Vitrac *et al*., 2010) and amylolytic enzymes (Kamitani *et al*., 2009), which may explain improved glucose homeostasis following administration. However, these mechanisms of action do not fully explain body weight reduction and lean:fat mass ratio increases observed following long-term administration of Svetol®. Therefore, CGAs may affect adipocyte metabolism.

Activation of lipolytic activity in adipocyte tissue results in liberation of free fatty acids and glycerol with a concomitant reduction in adipocyte cell volume. Although the *in vivo* lipolytic activity of a green coffee bean extract rich in caffeine and CGAs was previously described (Tanaka *et al*., 2009), the lipolytic activity of CGAs *per se* does not appear to have been reported. Therefore, the objective of this study was to evaluate the lipolytic activity of Svetol®, a commercial DGCE, which contains a specific blend of CGAs and which has been previously demonstrated to aid with weight loss.

MATERIALS AND METHODS

Svetol® was supplied by Naturex Inc. (South Hackensack, NJ) and contained >45% CGAs and >10% 5-caffeoylquinic acid.

Adipose tissue explant preparation. Normal human adipocytes were freshly isolated from surgical samples of healthy abdominal skin (34-year-old woman) as described previously (Rodbell, 1964) and recently detailed by Dallas *et al*. (2008).

Acute evaluation of lipolytic activity. Stock solutions of Svetol® (10 mg/mL) and caffeine as a positive control (10 mM) were prepared immediately prior to the experiment and were added to the isolated adipocytes to obtain a final concentration of 0.04, 0.2 and 1 mg/mL for Svetol® and 1 mM for caffeine (0.194 mg/mL). After 2 h incubation at 37 °C, the concentration of free fatty acids in the supernatant was determined using a FFA-C kit (OXOID, Dardilly, France), an *in vitro* enzymatic colorimetric method assay for the quantitative determination of non-esterified fatty acids, which relies upon the acylation of coenzyme A by the fatty acids in the presence of added acyl-CoA synthetase. Results were expressed as micromoles of free fatty acids or percentage of the negative control. The absence of interference of the test substances on the free fatty acid assay was also determined.

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Received 16 August 2013
Revised 15 October 2013
Accepted 31 October 2013

Sub-chronic evaluation of lipolytic activity. Stock solutions of Svetol® (10 mg/mL) and caffeine as a positive control (10 mg/mL) were prepared immediately prior to the experiment and were added to the isolated adipocytes to obtain a final concentration of 0.01, 0.1 and 1 mg/mL for Svetol® and 1 mg/mL for caffeine. These additions were repeated after 48, 96 and 144 h. At each time point (48, 96 and 144 h) and at the end of the study (192 h), aliquots were taken from the supernatant and were combined prior to being stored at −20 °C for analysis of free fatty acids. Free fatty acids were determined as described earlier.

RESULTS AND DISCUSSION

Short-term treatment (2 h) of human-derived adipocytes with DGCE revealed a dose-dependent increase in free fatty acid liberation, with a free fatty acid content of 47 μM found at 1 mg/mL, a 67% increase over the control (Fig. 1). Caffeine, as expected, elicited a very strong lipolytic response at 1 mM. Although most of the caffeine have been removed from DGCE during industrial processing, up to 2% caffeine may remain. With this in mind, the results were re-analyzed and presented as liberation of free fatty acids expressed as a percentage of the maximum value (caffeine) for each sample based on its inherent caffeine content. The DGCE samples were calculated to potentially contain up to 0.8, 4.0 and 20.0 mg/L caffeine (corresponding to 4.1, 20.6 and 103.0 μM caffeine, respectively), and linear regression identified a relatively good fit between these values and the liberation of free fatty acids obtained with caffeine at a higher dose ($r^2 = 0.9863$; Fig. 1 insert).

There is a strong possibility that the lipolytic activity observed in previous *in vitro* studies of the lipolytic activity of natural plant extracts with residual caffeine content could be largely due to the activity of the inherent caffeine content of the samples, similar to the results obtained herein.

To further elucidate the effects of DGCE on metabolism of triglycerides in human-derived adipocytes, a study of longer duration was conducted using the same model. Analysis of aliquots taken at various time points following

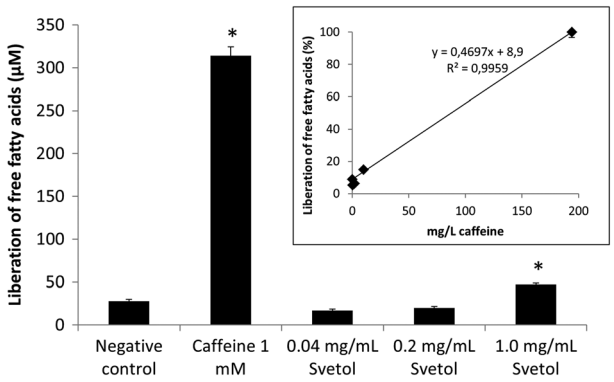

Figure 1. Lipolytic activity of varying concentrations of Svetol® (0.04, 0.2 and 1.0 mg/mL) on the liberation of free fatty acids (μM) from human adipocytes compared with negative and positive (194 mg/mL caffeine) controls following 2 h incubation. Insert: lipolytic activity of samples expressed by caffeine content. Error bars represent standard deviation, $n = 3$; $^*p < 0.05$.

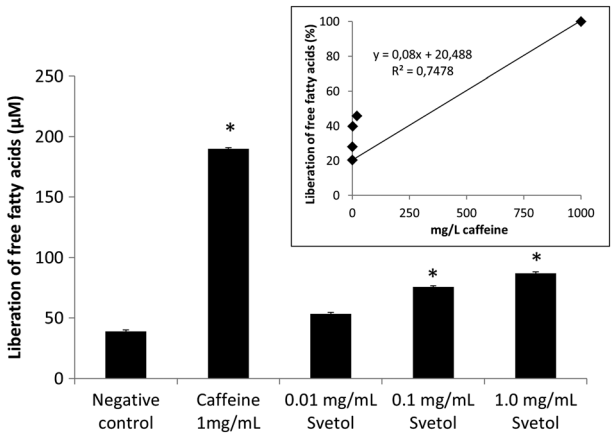

Figure 2. Lipolytic activity of varying concentrations of Svetol® (0.01, 0.1 and 1.0 mg/mL) on the liberation of free fatty acids (μM) from human adipocytes compared with negative and positive (100 mg/mL caffeine) controls from combined aliquots taken during 192 h incubation. Insert: lipolytic activity of samples expressed by caffeine content. Error bars represent standard deviation, $n = 3$; $^*p < 0.05$.

incubation of human-derived adipocytes with varying concentrations of DGCE confirmed the dose–response effect observed in the study of shorter duration (Fig. 2). Liberation of free fatty acids was found to be significantly greater than the control at 0.1 and 1.0 mg/mL DGCE. The results showed a poor correlation between liberation of free fatty acids and caffeine content, with an $r^2 = 0.7478$ (Fig. 2, insert).

Incubation of adipocytes with DGCE for a longer duration of time (192 h) permitted a clear differentiation between the effects of caffeine and other bioactive compounds present in the DGCE. It is also worth noting that the CGA content of the lowest concentration of DGCE reported in this study (0.01 mg/mL Svetol®; 20 μM CGAs) corresponds approximately to the maximum concentration of CGAs observed in plasma (total CGAs 14.8 μM) following single administration of 400 mg of Svetol® (Farah *et al.*, 2008). Thus, we can hypothesize on the biological relevance of the results obtained in the current study.

The demonstration of the lipolytic activity of Svetol® adds further knowledge to the mechanisms underpinning CGA-correlated weight loss, particularly those derived from DGCE (Thom, 2007; Dellalibera *et al.*, 2006). It can be postulated that Svetol® can have a generic effect on glucose control and body weight management, a hypothesis reinforced by the numerous epidemiological studies highlighting the health benefits of consumption of coffee and decaffeinated coffee rich in CGAs (van Dam *et al.*, 2006).

Acknowledgements

This work was funded by Naturex.

Conflict of Interest

Naturex is involved in the research/development and marketing/sales of Svetol® as an ingredient for the food, cosmetic and nutraceutical industries. Therefore, Naturex has a commercial interest in this publication.

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study in rodents, using HCA from the same batch, showing consistent positive results twice, also 3 months after our study was finished.[16] Water solubility and pH level mainly determine the bio-availability. A compound complexed with calcium and potassium is nearly 100% soluble and creates a pH level favourable for gastrointestinal absorption. Bio-availability of HCA in humans was checked by analysing the concentration of HCA in plasma.[17] HCA was analysed with a concentration gradient of NaOH, starting with 0.5 mM NaOH during 2.5 min, followed by subsequent linear concentration gradients of 0.5–5 mM NaOH during 3.5 min, and 5–38.25 mM NaOH during 12 min (Dionex DX 500 chromatography system; IonPac AS 11 column 4.6×250 mm, Dionex, Sunnyvale, California, USA; flow rate 2 ml/min). Ingestion of a single dose of HCA resulted in maximal plasma HCA concentration of 1.4%,[17] 60–90 min after ingestion, thereafter decreasing linearly to zero over at least 3 h.[17] Thus we instructed the subjects to take the drinks 1 h before lunch and dinner, and a drink 2 h after dinner, to prevent snacking in the evening.

After the 2 weeks, food intake was assessed in the laboratory restaurant, and analysed using the Dutch food composition table (Stichting Nederlands Voedingsstoffenbestand[18]) and the accessory computer program (Becel Nutrition Program[18]). Twenty-four-hour energy intake, body weight, scores on appetite profile, mood, taste perception, hedonics, and dietary restraint (mean ± s.d.) were tested for significant differences between the HCA and placebo treatments, using two-factor ANOVA repeated measures (STATVIEW + GRAPHICS; Abacus Concepts Inc., Berkeley, CA, USA); statistical significance was set at $P < 0.05$.

Twenty-four-hour energy intake on the test-day after 2 weeks of treatment with HCA was significantly reduced compared to placebo, mainly due to a significant decrease in snack intake (Table 1), with a tendency to a decrease in body weight of 0.5 kg ($P = 0.1$; Table 2). On the test-day, the appetite profile, changes in dietary restraint, taste perception, hedonics or mood did not differ with the HCA treatment compared to placebo. Reported compliance to the drinks was 90% on average, without complaints or negative effects.

In this randomized placebo-controlled crossover design, (−)-hydroxycitrate, consumed three times daily (300 mg dissolved in tomato juice) for 2 weeks by overweight males and females, showed a significant reduction in 24 h energy intake, while satiety was sustained. The fact that the main energy intake reduction took place between meals might indicate that HCA works by increasing fat oxidation (inhibiting malonyl-CoA synthesis, thus stimulating carnitine palmitoyl transferase activity) since fat is oxidized after protein and carbohydrate, thus later during the intermeal interval.[15] During this interval satiety might be sustained by increased fat oxidation and ketone body formation. We also observed a tendency toward reduction in body weight with HCA compared to placebo, but the treatment period may have been too short to result in a significant body weight loss. Our results are in line with results from a recent study by Mattes and Bormann,[11] reporting a larger body weight loss in subjects using HCA for 12 weeks, compared to placebo. Reasons for the controversial results reported on HCA, may be possible degradation and bio-availability of the HCA used, which we controlled for, and timing of HCA administration according to the peak level of HCA in plasma. Also the number of obese subjects, as in the study by Heymsfield *et al*[12] who report a negative result, may be a reason, since resistance to HCA in obese rats was shown.[19]

Summarizing, we showed that daily administration of a relatively low dosage of HCA (900 mg/day), during 2 weeks, reduced energy intake in overweight subjects, while satiety was sustained. HCA might not primarily be a weight loss agent, as indicated by the minor changes observed in body weight, but might be effective in preventing weight (re)gain in humans.[20]

Table 1 Twenty-four-hour energy intake (EI) in MJ on the test-day at the end of 2 weeks with 300 mg (−)-hydroxycitrate/100 ml tomato juice, three times daily; or 2 weeks with placebo (100 ml tomato juice, three times daily)[a]

	Placebo (n = 24)	*HCA (n = 24)*
24 h EI	9.4 ± 2.2	7.0 ± 2.1*
EI at breakfast	1.0 ± 0.6	0.8 ± 0.8
EI at lunch	3.1 ± 2.5	2.0 ± 1.9
EI at dinner	2.7 ± 1.2	2.5 ± 0.5
EI between meals	2.7 ± 1.9	1.6 ± 1.5*

[a] (Mean ± s.d.); $n = 24$ (12 men and 12 women).
* Significantly different from placebo; $P < 0.05$.

Table 2 Subject characteristics before and after 2 weeks of treatment with 300 mg (−)-hydroxycitrate/100 ml tomato juice, three times daily, or 2 weeks with placebo (100 ml tomato juice, three times daily)[a]

	Before treatment	*After treatment*
Age (y)	37 ± 11	
Placebo (n = 24)		
Body weight (kg)	85.2 ± 12	85.2 ± 11
Body mass index (kg/m^2)	27.2 ± 2.8	27.2 ± 2.8
Hydroxycitrate (n = 24)		
Body weight (kg)	84.2 ± 12	83.7 ± 11
Body mass index (kg/m^2)	26.9 ± 2.8	26.7 ± 2.7

[a] (Mean ± s.d.); $n = 24$ (12 men and 12 women).

Acknowledgements

This study was financially supported by Novartis Consumer Health Ltd (Nyon, Switzerland) and by Novartis Pharma (Basel, Switzerland, and Summit, NJ, USA).

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Effect of Raspberry Ketone (Razpberi-K®) and Acute Resistance Exercise on Post-exercise Caloric Energy Expenditure

Final Statistical Report

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December 21, 2006

SUMMARY

Purpose: The main purpose of this study was to determine if a single dose of raspberry ketone (RK) magnified the post-exercise increase in metabolic rate and/or fat oxidation (EPOC). A secondary purpose was to assess the safety profile of this ingredient after 30-days of continuous use. **Methods**: After giving informed consent and being cleared for participation, ten healthy subjects (eight men, two women) performed two EPOC trials in counterbalanced order. During the EPOC trials, subjects performed 3 sets of 6 exercises (squat, bench, stiff-legged dead-lift, bent-over row, shoulder press, and upright row) at a 10-12 repetition maximum weight load. Rest periods and exercise duration were standardized during both trials. Metabolic rate (via indirect calorimetry) and substrate oxidation (via respiratory exchange ratios) were measured twice during a pre-exercise baseline period (from 0-10 minutes before RK or placebo ingestion and from 20-30 minutes after RK or placebo ingestion), as well as 0-30 minutes post-exercise. After both EPOC trials were complete, all 10 subjects continued supplementing with 200 mg RK per day (in an open-label format) for 30-days. At the end of the 30-day period, subjects had a final blood draw to determine the effect of RK on standard clinical chemistry profiles. **Results**: As expected, resistance exercise significantly increased metabolic rate and carbohydrate oxidation relative to baseline, (via ANOVA, Figures 1 and 2) however, ingestion of RK did not appear to augment these responses compared to placebo. One possible exception is the trend towards an increase in fat oxidation ($P<0.15$) that occurred 30-minutes post exercise during the RK trial (via ANCOVA, Figure 3). No significant changes in blood chemistry were noted after 30-days of RK supplementation, and all values remained within normal clinical limits (via Wilcoxon, Table 2). **Discussion**: These preliminary data indicate that: 1) a 200 mg dose of RK, when taken 30 minutes prior to whole-body resistance exercise, does not affect post-exercise metabolic rate; 2) a 200 mg dose of RK, when taken 30 minutes prior to whole-body resistance exercise, may enhance fat oxidation late into the recover period (i.e., 30 minutes or after); and 3) daily supplementation with 200 mg RK does not adversely affect clinical chemistry profiles. Although promising, we recommend follow-up studies using larger sample sizes, a higher dose of RK, and a longer timeframe between ingestion and the initiation of exercise (i.e., at least 60 minutes) in order to more fully comprehend the effects of this unique ingredient on human physiology.

Table 1. Descriptive Statistics

Raz-K Trial (A)	Valid N	Mean	Min	Max	SD	SE
RMR Base	10	0.3360	0.2900	0.4500	0.05232	0.01655
RMR 30 post ingestion	10	0.3390	0.2900	0.4600	0.05724	0.01810
RMR 10 min post exercise	10	0.4160	0.3100	0.5700	0.09823	0.03106
RMR 20 min post exercise	10	0.3470	0.2600	0.4600	0.07227	0.02285
RMR 30 min post exercise	10	0.3410	0.2400	0.4700	0.08279	0.02618
RER Base	10	0.7990	0.6500	0.8700	0.06437	0.02036
RER 30 post ingestion	10	0.7810	0.6400	0.8500	0.06190	0.01958
RER 10 min post exercise	10	0.9110	0.7800	1.0400	0.08412	0.02660
RER 20 min post exercise	10	0.7600	0.6200	0.8300	0.05617	0.01776
RER 30 min post exercise	10	0.7470	0.5600	0.8100	0.06977	0.02206
Placebo Trial (B)						
RMR Base	10	0.327000	0.260000	0.370000	0.033015	0.010440
RMR 30 post ingestion	10	0.330000	0.270000	0.420000	0.049216	0.015563
RMR 10 min post exercise	10	0.407000	0.320000	0.530000	0.065498	0.020712
RMR 20 min post exercise	10	0.374000	0.270000	0.450000	0.065862	0.020827
RMR 30 min post exercise	10	0.341000	0.250000	0.420000	0.060083	0.019000
RER Base	10	0.797000	0.660000	0.860000	0.053135	0.016803
RER 30 post ingestion	10	0.785000	0.630000	0.820000	0.057975	0.018333
RER 10 min post exercise	10	0.907000	0.820000	1.030000	0.064644	0.020442
RER 20 min post exercise	10	0.795000	0.670000	0.880000	0.057203	0.018089
RER 30 min post exercise	10	0.765000	0.600000	0.830000	0.064850	0.020507
Safety Data						
Glucose (pre)	10	89.2000	79.0000	99.0000	6.39097	2.02100
Glucose (post)	10	87.6000	77.0000	105.0000	8.26236	2.61279
BUN (pre)	10	17.3000	13.0000	24.0000	3.59166	1.13578
BUN (post)	10	17.9000	13.0000	24.0000	3.75500	1.18743
Creatinine (pre)	10	1.0000	0.8000	1.3000	0.18856	0.05963
Creatinine (post)	10	1.0100	0.7000	1.2000	0.19692	0.06227
BUN/Creat ratio (Pre)	10	17.9000	13.0000	30.0000	5.34270	1.68951
BUN/Creat ratio (Post)	10	18.3000	12.0000	24.0000	3.94546	1.24766
Sodium (Pre)	10	139.4000	137.0000	142.0000	1.50555	0.47610
Sodium (Post)	10	140.4000	139.0000	144.0000	1.64655	0.52068
Potassium (Pre)	10	4.3000	3.7000	4.7000	0.27889	0.08819
Potassium (post)	10	14.2400	3.6000	105.0000	31.89159	10.08501
Chloride (pre)	10	102.4000	98.0000	106.0000	2.50333	0.79162
Chloride (post)	10	94.6000	21.0000	106.0000	25.94952	8.20596

CO2 (pre)	10	24.8000	22.0000	28.0000	2.34758	0.74237
CO2 (post)	10	23.1900	9.9000	28.0000	4.85626	1.53568
Calcium (pre)	10	9.5800	9.2000	9.9000	0.22010	0.06960
Calcium (post)	10	9.4300	7.5000	10.3000	0.79449	0.25124
Protein (Pre)	10	7.0800	6.6000	7.5000	0.34254	0.10832
Protein (post)	10	7.2200	6.2000	7.8000	0.49844	0.15762
Albumin (Pre)	10	4.3300	4.1000	4.6000	0.15670	0.04955
Albumin (post)	10	4.4600	4.1000	4.8000	0.24129	0.07630
Globulin (Pre)	10	2.7500	2.2000	3.1000	0.32404	0.10247
Globulin (post)	10	2.7600	2.1000	3.2000	0.32387	0.10242
A/G Ratio (pre)	10	1.6000	1.4000	2.0000	0.21082	0.06667
A/G Ratio (post)	10	1.6300	1.4000	2.0000	0.18886	0.05972
Bilirubin (pre)	10	0.4900	0.2000	0.8000	0.23781	0.07520
Bilirubin (post)	10	0.4500	0.2000	0.7000	0.18409	0.05821
Alkaline Phos (pre)	10	69.8000	45.0000	114.0000	19.75292	6.24642
Alkaline Phos (post)	10	69.2000	38.0000	102.0000	19.87628	6.28543
AST (Pre)	10	27.8000	16.0000	58.0000	11.98888	3.79122
AST (Post)	10	36.3000	16.0000	98.0000	22.75009	7.19421
ALT (Pre)	10	28.2000	15.0000	61.0000	14.14842	4.47412
ALT (Post)	10	28.8000	13.0000	57.0000	12.54149	3.96597
Chol (Pre)	10	169.3000	113.0000	213.0000	30.32802	9.59056
Chol (post)	10	182.5000	119.0000	236.0000	33.88625	10.71577
TAG (Pre)	10	77.6000	57.0000	122.0000	18.87503	5.96881
TAG (post)	10	97.6000	40.0000	142.0000	38.48290	12.16936
HDL (Pre)	10	59.7000	49.0000	100.0000	15.37711	4.86267
HDL (Post)	10	56.9000	38.0000	102.0000	18.66935	5.90377
VLDL (pre)	10	15.5000	11.0000	24.0000	3.80789	1.20416
VLDL (post)	10	19.5000	8.0000	28.0000	7.56086	2.39096
LDL (Pre)	10	94.1000	43.0000	130.0000	26.21895	8.29116
LDL (Post)	10	106.1000	49.0000	160.0000	34.50749	10.91223

Figure 1. Comparison of Raspberry Ketone (Trial A) vs. Placebo (Trial B) on Resting Metabolic Rate (RMR)

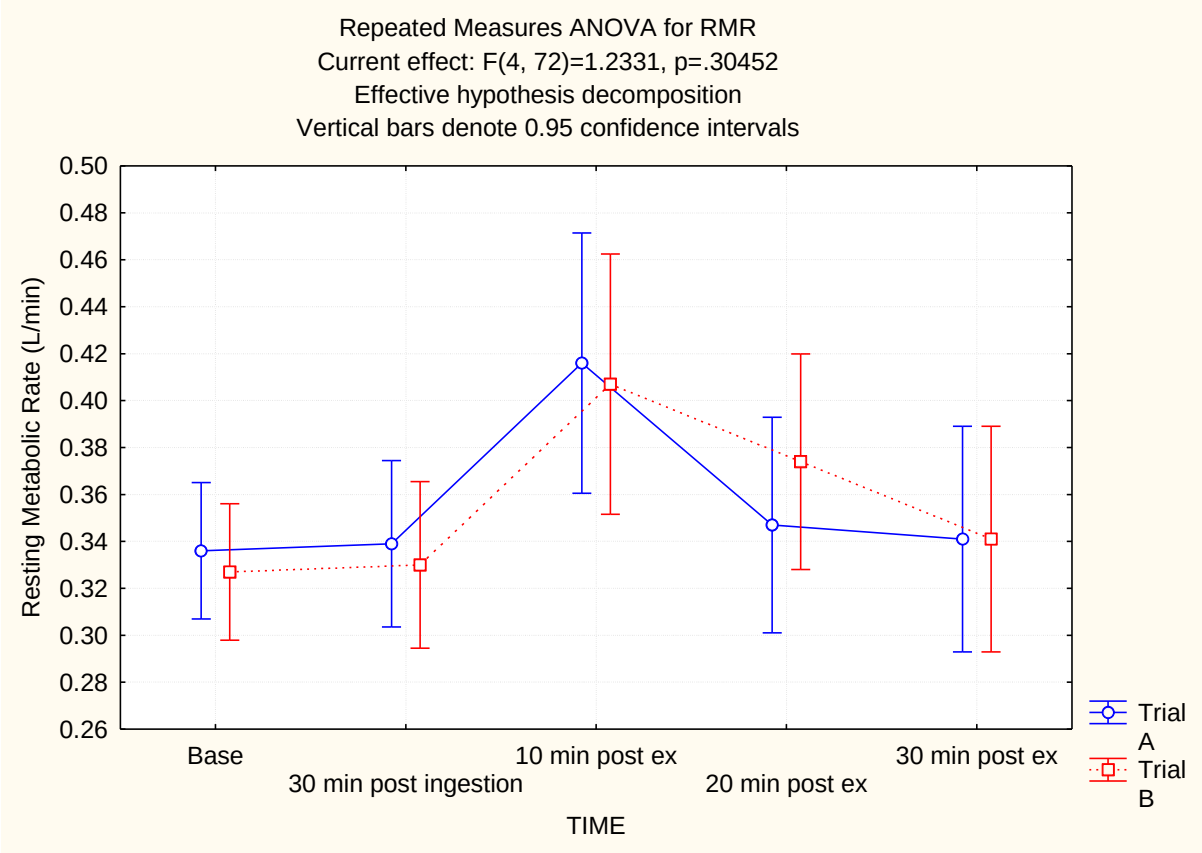


Figure 2. Comparison of Raspberry Ketone (Trial A) vs. Placebo (Trial B) on Substrate Oxidation (RER) at All Time Points

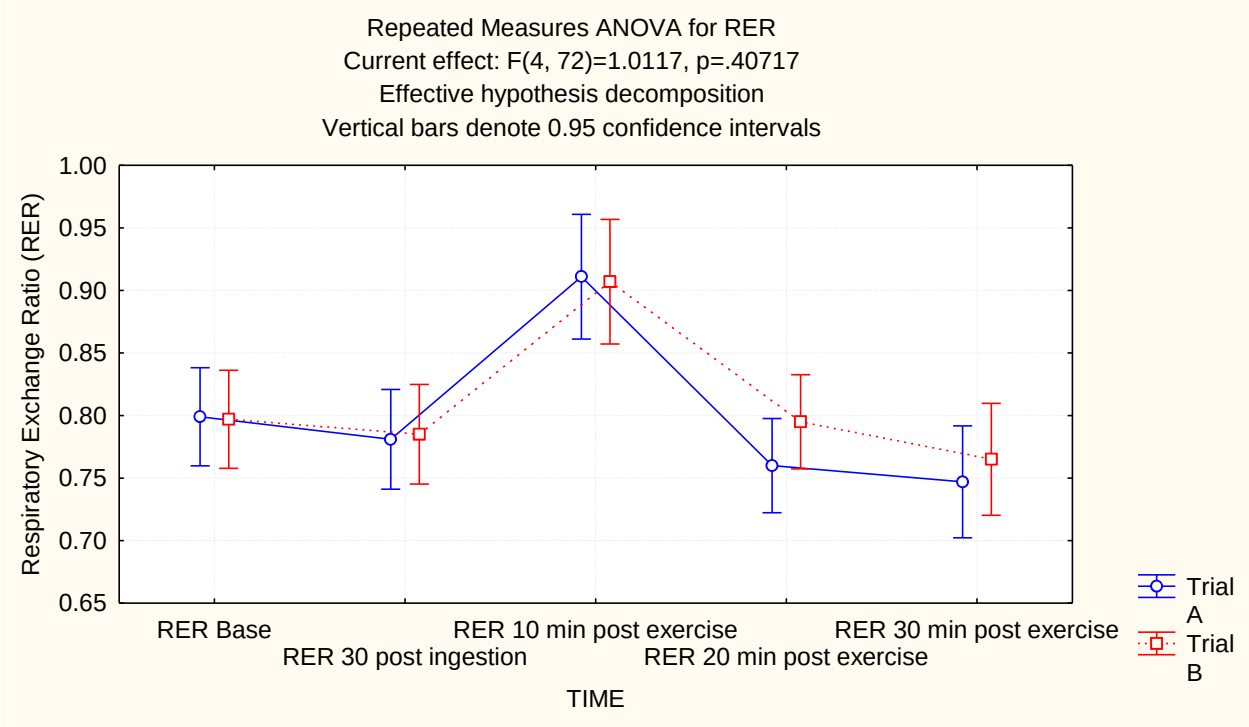
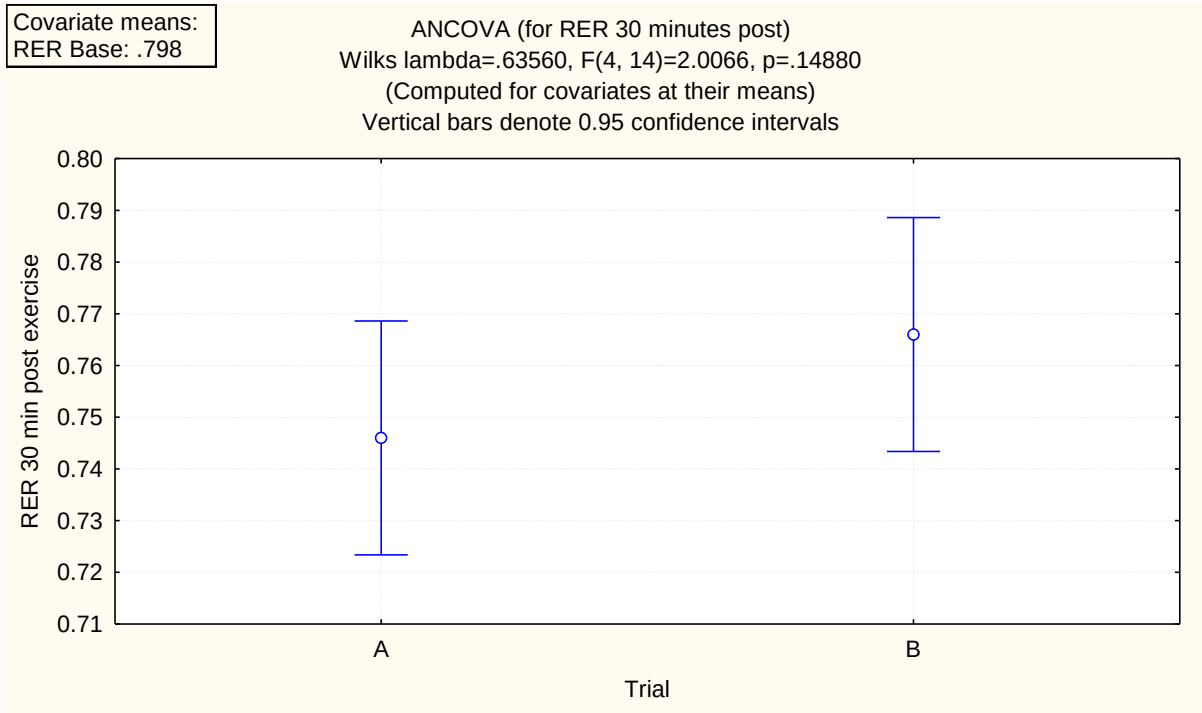


Figure 3. Comparison of Raspberry Ketone (Trial A) vs. Placebo (Trial B) on Substrate Oxidation (RER) at 30 min post exercise



*** Lower RER reflects a greater reliance on fat as the metabolic fuel.**

Table 2. Pre vs. Post Differences for Phase II (30-day safety)

Results: No changes from pre to post in any variable were noted.

Wilcoxon Matched Pairs Test (Razz data (final)

	Valid	T	Z	p-level
Glucose (pre) & Glucose (post)	10	21.50000	0.611577	0.540818
BUN (pre) & BUN (post)	10	20.00000	0.296174	0.767097
Creatinine (pre) & Creatinine (post)	10	13.50000	0.630126	0.528613
BUN/Creat ratio (Pre) & BUN/Creat ratio (Post)	10	25.50000	0.203859	0.838464
Sodium (Pre) & Sodium (Post)	10	7.00000	1.540308	0.123486
Potassium (Pre) & Potassium (post)	10	18.50000	0.917365	0.358952
Chloride (pre) & Chloride (post)	10	19.00000	0.414644	0.678403
CO2 (pre) & CO2 (post)	10	24.50000	0.305788	0.759766
Calcium (pre) & Calcium (post)	10	20.00000	0.296174	0.767097
Protein (Pre) & Protein (post)	10	13.50000	1.066228	0.286321
Albumin (Pre) & Albumin (post)	10	13.00000	1.477977	0.139415
Globulin (Pre) & Globulin (post)	10	18.00000	0.000000	1.000000
A/G Ratio (pre) & A/G Ratio (post)	10	9.50000	0.760639	0.446873
Bilirubin (pre) & Bilirubin (post)	10	10.00000	0.676123	0.498963
Alkaline Phos (pre) & Alkaline Phos (post)	10	27.00000	0.050965	0.959354
AST (Pre) & AST (Post)	10	8.00000	1.717812	0.085832
ALT (Pre) & ALT (Post)	10	26.50000	0.101929	0.918813
Chol (Pre) & Chol (post)	10	12.00000	1.579906	0.114129
TAG (Pre) & TAG (post)	10	16.00000	1.172189	0.241122
HDL (Pre) & HDL (Post)	10	19.00000	0.866400	0.386271
VLDL (pre) & VLDL (post)	10	11.00000	1.362402	0.173072
LDL (Pre) & LDL (Post)	10	15.50000	1.223153	0.221273

Note: p-values (via dependent t-test) for Sodium = 0.12; Albumin = 0.13; AST = 0.28; Chol = 0.18. These trends are difficult to interpret because there was no placebo group for comparative purposes (i.e., they could reflect normal variability). Nonetheless, all differences were small and all values remained well within normal clinical limits.