

RESEARCH ARTICLE

Greater lactate accumulation following an acute bout of high-intensity exercise in males suppresses acylated ghrelin and appetite postexercise

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Vanderheyden LW, McKie GL, Howe GJ, Hazell TJ. Greater lactate accumulation following an acute bout of high-intensity exercise in males suppresses acylated ghrelin and appetite postexercise. *J Appl Physiol* 128: 1321–1328, 2020. First published April 2, 2020; doi:10.1152/jappphysiol.00081.2020.—High-intensity exercise inhibits appetite, in part, via alterations in the peripheral concentrations of the appetite-regulating hormones acylated ghrelin, active glucagon-like peptide-1 (GLP-1), and active peptide tyrosine-tyrosine (PYY). Given lactate may mediate these effects, we used sodium bicarbonate (NaHCO_3) supplementation in a double-blind, placebo-controlled, crossover design to investigate lactate's purported role in exercise-induced appetite suppression. Eleven males completed two identical high-intensity interval training sessions (10×1 min cycling bouts at $\sim 90\%$ heart rate maximum interspersed with 1-min recovery), where they ingested either NaHCO_3 (BICARB) or sodium chloride (NaCl) as a placebo (PLACEBO) preexercise. Blood lactate, acylated ghrelin, GLP-1, and PYY concentrations, as well as overall appetite were assessed preexercise and 0, 30, 60, and 90 min postexercise. Blood lactate was greater immediately ($P < 0.001$) and 30 min postexercise ($P = 0.049$) in the BICARB session with an increased ($P = 0.009$) area under the curve (AUC). The BICARB session had lower acylated ghrelin at 60 ($P = 0.014$) and 90 min postexercise ($P = 0.016$), with a decreased AUC ($P = 0.039$). The BICARB session had increased PYY ($P = 0.034$) with an increased AUC ($P = 0.031$). The BICARB session also tended ($P = 0.060$) to have increased GLP-1 at 30 ($P = 0.003$) and 60 min postexercise ($P < 0.001$), with an increased AUC ($P = 0.030$). The BICARB session tended ($P = 0.059$) to reduce overall appetite, although there was no difference in AUC ($P = 0.149$). These findings support a potential role for lactate in the high-intensity exercise-induced appetite-suppression.

NEW & NOTEWORTHY We used sodium bicarbonate to increase lactate accumulation or sodium chloride as a placebo. Our findings further implicate lactate as a mediator of exercise-induced appetite suppression, given exercise-induced increases in lactate during the sodium bicarbonate session altered peripheral concentrations of appetite-regulating hormones, culminating in a reduction of appetite. This supports a lactate-dependent mechanism of appetite suppression following high-intensity exercise and highlights the potential of using lactate as a means of inducing a caloric deficit.

anorexigenic; appetite regulation; gut peptides; high-intensity interval training; orexigenic

INTRODUCTION

The physiological regulation of energy intake involves a complex interplay between key brain regions, peripheral organs, and tissues that secrete hormones involved in regulating

energy homeostasis (26). These hormones can either be orexigenic (appetite-stimulating) or anorexigenic (appetite-inhibiting) in nature and can act on specific brain regions to influence our perceptions of hunger and satiety (22). During periods of energy deficit, ghrelin, the only known orexigenic peptide, is released from gastric mucosal cells lining the stomach (33). Ghrelin is octanoylated at Ser-3 by ghrelin *O*-acyl transferase (GOAT), a posttranslational modification that confers to ghrelin the ability to signal via the growth hormone secretagogue receptor and stimulate appetite (57), among a variety of other metabolic effects (58). There are also several anorexigenic hormones that act in opposition to acylated ghrelin, including active peptide tyrosine-tyrosine_{3–36} (PYY) and active glucagon-like peptide-1_{7–36,7–37} (GLP-1), both of which are secreted from enteroendocrine L-cells (26) and exist in multiple isoforms (2, 53). These peptides are highly responsive to nutrient composition and effectively act to increase perceptions of satiety (26).

Though perceptions of appetite and food intake behaviors are regulated through complex interactions between peripheral and central signals (9, 26), accumulating evidence suggests that acute (30–90-min duration), high-intensity ($\geq 70\%$ maximal oxygen consumption, $\dot{V}\text{O}_{2\text{max}}$) aerobic exercise induces a suppression of appetite that involves decreases in circulating acylated ghrelin and increases in circulating PYY and GLP-1 (3, 6, 7, 10, 11, 21, 23, 24, 31, 32, 34, 36, 40, 45, 46, 48, 54). However, the potential mechanisms involved in this aerobic exercise-induced appetite suppression are not currently well understood (24). Recently, we demonstrated that postexercise appetite suppression occurs in an intensity-dependent manner associated with decreases in circulating plasma concentrations of acylated ghrelin and increases in total PYY and active GLP-1 (27). Importantly, these transient effects of aerobic exercise on the appetite-regulating hormone responses translated into a meaningful suppression of appetite in the days following exercise, highlighted by a reduction in free-living energy intake (27). Moreover, these effects were all strongly associated with exercise-induced increases in circulating blood lactate concentrations. This, combined with work from others (19, 48), strongly suggests that lactate may be involved in mediating the effects of aerobic exercise on appetite in a manner that favors reductions in energy intake.

Although our previous work (27) and that of others (48) supports the potential involvement of lactate in the exercise-induced suppression of appetite, a direct/causal relationship between lactate and appetite-related parameters has yet to be fully established. Interestingly, the G_i -coupled protein recep-

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tor, GPR81, is specific to lactate and was recently found to be highly enriched on the surface of the ghrelin-producing gastric mucosal cells (15, 16). Moreover, direct evidence suggests that treating primary gastric mucosal cells with physiologically relevant concentrations of lactate *ex vivo* induces a dose-dependent inhibition of both acylated and total ghrelin secretion (15). Recently, lactate's role as a signaling molecule during intense exercise has expanded our view of what was once deemed an inert by-product of anaerobic glycolysis (5, 39, 56). Furthermore, our recent data linking lactate accumulation to appetite suppression postexercise further posits lactate as a key exercise-inducible factor involved in regulating appetite (27).

Experimentally manipulating blood lactate concentrations during aerobic exercise without inducing alterations in exercise performance or tolerance is commonly achieved via the use of sodium bicarbonate (NaHCO_3) supplementation. NaHCO_3 is an ergogenic aid that can be used to decrease blood pH, improve muscle-buffering capacity, and, thereby, delay intramuscular acidosis, resulting in an increased work capacity and improved performance (8, 47). As such, we reasoned that combining NaHCO_3 supplementation with a work-matched exercise protocol consisting of high-intensity interval training (HIIT), should allow for a sufficiently large increase in blood lactate accumulation, such that the effects of lactate on appetite could be investigated without manipulating exercise intensity (13, 41). The particular strength of this design, in addition to allowing lactate to accumulate (41), is the ability to minimize alterations in other proposed mechanisms of appetite suppression (24) between exercise sessions.

Therefore, the primary purpose of this study was to investigate the role of lactate as a mechanism of appetite suppression. We hypothesized that, relative to a placebo-controlled session, exercise-induced increases in blood lactate following HIIT with NaHCO_3 would decrease circulating plasma concentrations of acylated ghrelin, leading to reductions in appetite.

MATERIALS AND METHODS

Participants. Eleven active males (18–35 yr) volunteered to participate in this study. This sample size was determined on the basis of expected changes in blood lactate concentrations observed in a previous investigation (41). All participants engaged in vigorous exercise at least 3 times per week (assessed by the Godin leisure-time exercise

questionnaire) and were comfortable performing high-intensity exercise bouts (20). All participants provided written and informed consent before participation in this study and passed the Physical Activity Readiness Questionnaire (PAR-Q+) health survey (55). Participants were healthy, nonsmokers, and were not taking any medications or dietary supplements during the study. This study was approved by the Research Ethics Board at Wilfrid Laurier University, in accordance with the 1964 Declaration of Helsinki.

Study design. All participants completed two experimental sessions (~3 h each) in a randomized, double-blinded, counterbalanced order separated by at least 1 wk (Fig. 1). Experimental sessions consisted of a HIIT exercise session with NaHCO_3 supplementation (BICARB) or sodium chloride (NaCl) as the placebo (PLACEBO). Blood samples and subjective appetite measures were obtained at several time points during each session. Participants were instructed to refrain from alcohol, caffeine, and physical activity for >24 h before any laboratory visits.

Preexperimental procedures. Participants completed two familiarization sessions before the experimental sessions. During the first familiarization session participants were acclimated to the exercise equipment, as well as the types and degrees of effort required during the exercise protocols. Height was measured to the nearest 0.5 cm, and body mass was measured to the nearest 0.1 kg with a physician beam scale (Health-o-meter Professional, Sunbeam Products, Boca Raton, FL). Participants were assessed for body fat percentage using a seven-site skinfold caliper method (28), and the same researcher performed all of the measurements. Participants then completed an incremental exercise test to volitional exhaustion to determine $\dot{V}\text{O}_{2\text{max}}$ and maximum work rate (W_{max}) on a Velotron electronically braked cycle ergometer (RacerMate, Seattle, WA). Respiratory gas collection was measured using an online breath-by-breath gas collection system (MAX II, AEI Technologies, Pittsburgh, PA), and heart rate (HR) was recorded throughout the test using an integrated HR monitor (Polar Electro, New York). Before testing, gas analyzers were calibrated using gases of known concentrations and a 3-liter syringe for flow. After a 5-min warm-up at 50 Watts (W) on the cycle ergometer, work rate increased by 1 W every 4 s from 50 W until the participant could no longer maintain a pace greater than 50 revolutions per minute (RPM), or they reached volitional exhaustion. $\dot{V}\text{O}_{2\text{max}}$ was defined as the greatest 30-s average of $\dot{V}\text{O}_2$ plateaus ($<1.35 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) despite increases in workload, or two of the following criteria: 1) respiratory exchange ratio >1.10 , 2) maximal HR [within 10 bpm of age-predicted maximum ($220-\text{age}$)], and/or 3) volitional exhaustion. To verify that the participant achieved their $\dot{V}\text{O}_{2\text{max}}$, a verification phase was completed, as recommended by (42), after a rest period of 20 min following the completion of the incremental exercise test. A workload 10% greater than W_{max} was selected, and participants were asked to pedal at 70–90 RPM until volitional exhaustion (~3–5 min)

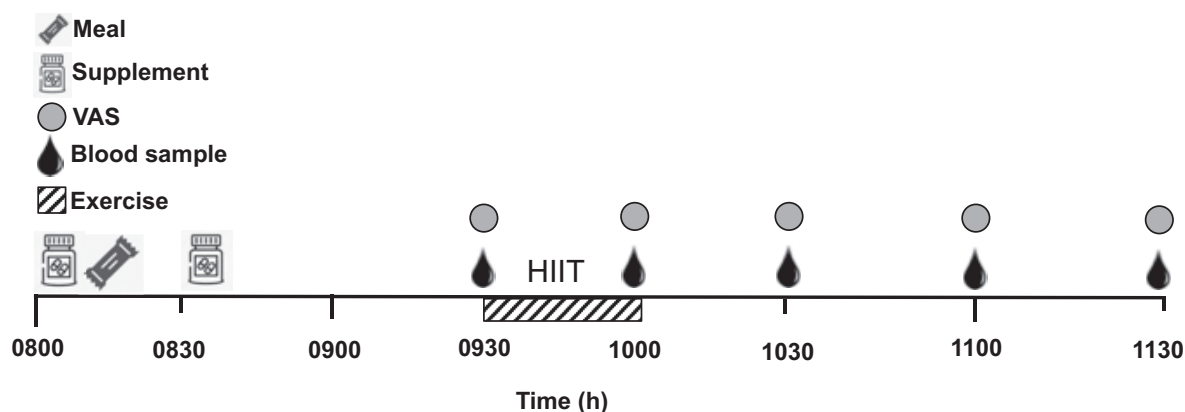


Fig. 1. Experimental session timeline. HIIT, high-intensity interval training; VAS, visual analog scale.

or they could no longer maintain 50 RPM. If there was a plateau in $\dot{V}O_2$ during this verification phase and the values did not differ by $>1.35 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, the test was deemed a valid $\dot{V}O_{2\text{max}}$ test (42).

The second familiarization session (separate day) allowed the participants to become accustomed to an abbreviated version of the experimental protocol and to validate that the selected workload elicited $\sim 90\%$ HR_{max} . Participants completed four \times 60-s cycling efforts, thereby, mimicking the experimental HIIT protocol (see *Exercise protocol* for further details). The power output at $\dot{V}O_{2\text{max}}$ (W_{max}) and the maximum HR (HR_{max}) attained were used to determine interval workloads for the subsequent experimental sessions.

Experimental sessions. To maintain plasma volume while fasted, participants were instructed to consume $\sim 250 \text{ mL}$ of water before bed and upon waking before arriving at the laboratory. Participants arrived at the laboratory at 0800 after an overnight fast (no food after 2000) and remained there for the next $\sim 3.5 \text{ h}$ (Fig. 1). Upon arrival, participants consumed their first round of supplements (either NaHCO_3 or NaCl) and a standardized breakfast consisting of 7 kcal/kg body mass Chocolate Chip Clif Bar (CLIF Bar, Emeryville, CA; 68% carbohydrate, 17% fat, and 15% protein) similar to our previous work (27). For the BICARB session, participants ingested NaHCO_3 (cat. no. S5761, MilliporeSigma, Oakville, Ontario, Canada; 0.2 g/kg body mass) at 90 and 60 min before exercise (total dose of 0.4 g/kg), while the PLACEBO session had participants ingest an equimolar amount of NaCl (cat. no. S9888; MilliporeSigma). All supplements were administered orally via gelatin capsules (size 00 clear gelatin capsules; CapsulCN International, Wenzhou City, China) that were pressed in house, and participants, as well as lead investigators, were blinded to the condition. This dosing protocol has been used previously to ensure treatment differences, minimize gastrointestinal distress, and allow for the largest difference in lactate levels between conditions (4, 13, 41). Participants then relaxed, sitting quietly in the laboratory (doing homework devoid of food cues) until it was time to consume their second round of supplementation at 0830, after which they continued to relax quietly (reading or using a laptop) for another 60 min. The exercise session commenced at 0930, and upon completion of exercise (1000), participants rested quietly for an additional 90 min. Venous blood samples were obtained at 0930 (no. 1; preexercise), 1000 (no. 2; 0 min postexercise), 1030 (no. 3; 30 min postexercise), 1100 (no. 4; 60 min postexercise), and 1130 (no. 5; 90 min postexercise). Appetite perceptions and gastrointestinal distress were assessed immediately before all blood samples. Identical procedures were used in both sessions, as the only difference between the experimental sessions was the supplement ingested before exercise. Both sessions were expected to increase lactate; however, the BICARB session was expected to generate a greater lactate accumulation (41).

Exercise protocol. The HIIT sessions were performed on the Velotron electronically braked cycle ergometer (RacerMate, Seattle, WA), which provided the ability for work-matched cycling sessions. Participants completed a 3-min warm-up at 50 W followed by the HIIT protocol, involving $10 \times 1\text{-min}$ cycling bouts interspersed with 1-min recovery, as previously described (35), followed by a 2-min cool down at 50 W for a total exercise session duration of 25 min (5-min buffer was provided for the first preexercise blood draw). Participants were asked to pedal at a cadence of 80–100 RPM during interval bouts, where a workload known to elicit $\sim 90\%$ HR_{max} ($\sim 75\%$ peak power output) was selected from familiarization sessions, and the peak HR achieved was recorded. During the 1-min recovery, participants were instructed to perform active recovery by pedaling slowly ($\sim 50 \text{ RPM}$) against 50 W of resistance.

Blood processing and analysis. Blood samples were collected for the measurement of acylated ghrelin, GLP-1 (GLP-1_{7–36} and GLP-1_{7–37}), PYY (PYY_{3–36}), and blood lactate by venipuncture from the antecubital vein, while participants were in a supine position similar to our previous work (27). At each time point, two samples (3 mL of whole blood each) were collected into separate prechilled Vacutainer

tubes coated with 5.4 mg of $\text{K}_2 \text{ EDTA}$. A droplet of blood was taken from one of the Vacutainer tubes immediately and placed on a lactate strip for the measurement of blood lactate using a handheld analyzer (Lactate Plus, Nova Biomedical), which was calibrated according to manufacturer's specifications and has shown high levels of accuracy and reliability when compared with standard radiometers (51). The remaining blood was treated appropriately for the preservation of the appetite-regulating hormones, as we have done previously (27). All plasma supernatant was stored at -80°C before analyzing. Commercially available enzyme-linked immunosorbent assay kits were used to determine plasma concentrations of acylated ghrelin (EMD Millipore, Danvers, MA), GLP-1 (EMD Millipore), and PYY (Phoenix Pharmaceuticals, San Diego, CA), according to manufacturer's instructions. All samples were run in duplicate and batch analyzed for each participant to eliminate interassay variation. Respective coefficients of variation were all within manufacturers' specifications; acylated ghrelin = $4.1 \pm 3.1\%$, PYY = $8.9 \pm 5.4\%$, and GLP-1 = $8.8 \pm 4.8\%$.

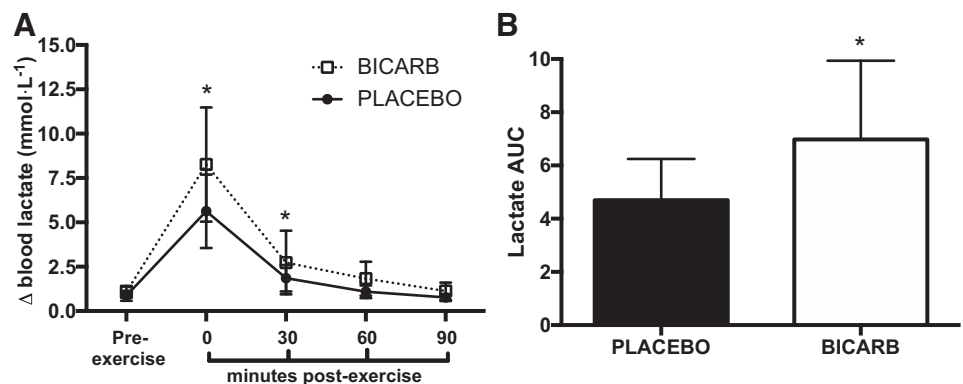
Overall appetite. Appetite perceptions were assessed similar to previous work in our laboratory (27) where perceptions of hunger ("How hungry do you feel?"), satisfaction ("How satisfied do you feel?"), fullness ("How full do you feel?"), and prospective energy intake ("How much do you think you can eat?") were measured using a visual analog scale with a 100-mm line anchored at each end with contrasting statements (18). This has been previously validated and used frequently to study appetite (49). The mean values of the four appetite perceptions were used to calculate an overall appetite score after inverting the values for satisfaction and fullness (49). At this same time point, participants were also asked to rate their current gastrointestinal distress ("How nauseous are you?") on a visual analog scale.

Statistical analyses. All data were analyzed using GraphPad PRISM version 6.0 (GraphPad Software, La Jolla, CA). A two-way repeated-measures ANOVA (session \times time) was used to compare blood lactate concentrations, appetite-regulating hormone concentrations, overall appetite, and gastrointestinal distress. Tukey's highly significant difference (HSD) tests were used for post hoc analyses when necessary. Partial eta-squared (η_p^2) values were calculated to estimate the effect sizes (small: 0.04, medium: 0.25, and large: 0.64) for main effects and interactions where necessary. All area under the curve (AUC) calculations for blood-related parameters and appetite perceptions were calculated using the trapezoid method and analyzed with a paired t test to compare between sessions. Cohen's d was calculated to estimate effect size (small 0.2, medium 0.5, large 0.8, very large 1.3) for the AUC analyses. Statistical significance was accepted as $P < 0.05$, and $P < 0.1$ was interpreted as "approaching significance" or "trending". All data are presented as means \pm SD.

RESULTS

Participant characteristics. Eleven recreationally active male participants were recruited for this study with a mean $\dot{V}O_{2\text{max}}$ of $44.06 \pm 6.73 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and the following physical characteristics: height: $176.0 \pm 5.7 \text{ cm}$; body mass: $72.9 \pm 9.0 \text{ kg}$; body mass index (BMI): $23.6 \pm 2.9 \text{ kg}\cdot\text{m}^{-2}$; and body fat: $13.0 \pm 5.4\%$. All completed the study. With regard to gastrointestinal distress (data not shown), there was a significant interaction ($P = 0.028$; $\eta_p^2 = 0.254$) though no differences in the subsequent post hoc comparisons ($P > 0.105$) and no difference ($P = 0.685$; $d = 0.13$) in session AUC (PLACEBO: $20.1 \pm 20.0 \text{ mm}$; BICARB: 25.3 ± 40.4). Only 4 of 11 participants answered correctly when asked if they thought they had received NaHCO_3 , which suggests they were correctly blinded to the sessions. The exercise protocols elicited 86 ± 8 and $87 \pm 10\%$ of HR_{max} during the PLACEBO and BICARB sessions, respectively ($P = 0.267$), whereas

Fig. 2. A: changes in blood lactate across all time points in each experimental session. B: area under the curve (AUC) values for each experimental session. *Significantly different between sessions: A: $*P < 0.05$ and *B: $P < 0.05$.



exercise intervals were performed at a workload corresponding to 226.4 ± 37.0 W (identical for both sessions). All participants completed all intervals during both exercise sessions with no adverse events, such as fainting, gastrointestinal distress, or muscle injury.

Blood lactate. There was a significant interaction between sessions across time ($P < 0.001$; $\eta_p^2 = 0.477$; Fig. 2A). There was no difference between sessions preexercise ($P = 0.971$). The exercise-induced increase in blood lactate was greater in the BICARB session compared with the PLACEBO session at 0 min ($P < 0.001$) and 30 min postexercise ($P = 0.049$), though blood lactate was not different between sessions at 60 min ($P = 0.138$) or 90 min postexercise ($P = 0.794$). The BICARB session AUC was significantly greater ($P = 0.009$; $d = 0.939$; Fig. 2B) compared with the PLACEBO session.

Acyated ghrelin. There was a significant session by time interaction between sessions across time ($P = 0.010$; $\eta_p^2 = 0.276$; Fig. 3A). There was no difference between sessions pre- ($P = 0.995$), 0 min ($P = 0.879$), or 30 min postexercise ($P = 0.416$). The exercise-induced decrease in acyated ghrelin concentrations was greater in the BICARB session compared with PLACEBO at 60 ($P = 0.014$) and 90 min postexercise ($P = 0.016$). The BICARB session AUC was decreased ($P = 0.039$; $d = 0.517$; Fig. 3B) compared with the PLACEBO session.

PYY. There was no session by time interaction ($P = 0.774$; $\eta_p^2 = 0.042$; Fig. 4A) and no main effect of time ($P = 0.178$; $\eta_p^2 = 0.143$). However, there was a main effect of session ($P = 0.034$; $\eta_p^2 = 0.368$), where BICARB was increased compared with PLACEBO. The BICARB session AUC was greater ($P = 0.031$; $d = 0.727$; Fig. 4B) compared with PLACEBO.

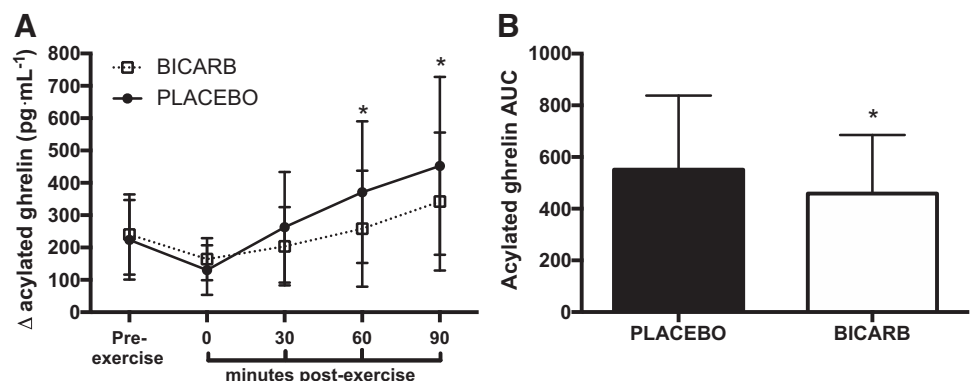
GLP-1. There was a session by time interaction approaching significance ($P = 0.060$; $\eta_p^2 = 0.199$; Fig. 5A). There was no difference between sessions preexercise ($P = 0.137$) or 0 min postexercise ($P = 0.130$); however, GLP-1 concentrations in the BICARB session were increased relative to the PLACEBO session at 30 min ($P = 0.003$) and 60 min postexercise ($P < 0.001$), but not at 90 min postexercise ($P = 0.621$). There was a main effect of session ($P = 0.030$; $\eta_p^2 = 0.532$), where the BICARB session was increased compared with the PLACEBO, whereas the main effect of time approached significance ($P = 0.051$; $\eta_p^2 = 0.762$), although there were no differences in the subsequent post hoc comparisons ($P > 0.119$). The BICARB session AUC was greater ($P = 0.030$; $d = 0.750$; Fig. 5B) compared with the PLACEBO session.

Overall appetite. There was no session by time interaction ($P = 0.453$; $\eta_p^2 = 0.086$; Fig. 6A). There was a near-significant main effect of session ($P = 0.059$; $\eta_p^2 = 0.307$), where overall appetite tended to be increased in the PLACEBO session compared with the BICARB session. There was also a main effect of time ($P < 0.001$; $\eta_p^2 = 0.804$), where overall appetite was increased at 60 and 90 min postexercise compared with preexercise ($P < 0.001$) and 0 min postexercise ($P < 0.001$), with 90 min also being greater than 30 min postexercise ($P < 0.001$). There were no other differences between time points ($P > 0.184$). There was no difference in the AUC ($P = 0.149$; $d = 0.471$; Fig. 6B) between sessions.

DISCUSSION

Our group recently provided support for lactate as a potential mechanism mediating the suppression of appetite following

Fig. 3. A: changes in acyated ghrelin across all time points in each experimental session. B: area under the curve (AUC) values for each experimental session. *Significantly different between sessions: A: $*P < 0.05$ and *B: $P < 0.05$.



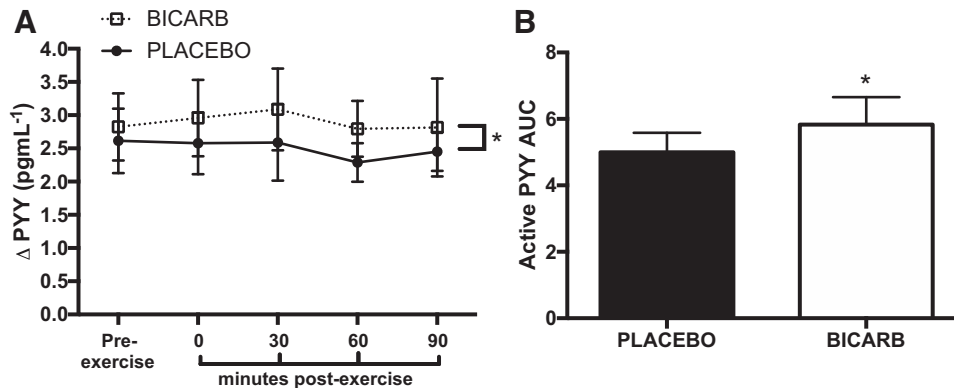


Fig. 4. A: changes in PYY across all time points in each experimental session. B: area under the curve (AUC) values for each experimental session. *Significantly different between sessions: A: $P < 0.05$ and B: $P < 0.05$.

intense exercise (27), and to our knowledge, this is the first study to further that line of inquiry. This study was purposefully designed using a work-matched HIIT paradigm with and without NaHCO_3 supplementation to allow for a manipulation of blood lactate concentrations independent of alterations between sessions in mechanisms thought to mediate appetite (24). Previous HIIT exercise studies have demonstrated exercise-induced appetite suppression (11, 34, 40, 48); however, we are the first to report on the potential mechanism by which this likely occurs. The exercise-induced increase in lactate accumulation observed with HIIT plus NaHCO_3 altered the peripheral concentrations of appetite-regulating hormones to a greater degree than did the same exercise but with NaCl . Although we failed to observe a statistically significant difference in overall appetite between sessions, overall appetite tended to decrease (medium estimated effect size) with increasing concentrations of lactate in the BICARB session. Thus, when taken together, this not only explains prior observations of appetite suppression with HIIT (11, 34, 40, 48), but highlights a key role for lactate in mediating the regulation of appetite postexercise.

Blood lactate. Our study confirmed that NaHCO_3 supplementation is effective at increasing blood lactate concentrations, as evidenced by the values immediately (BICARB: 8.3 vs. PLACEBO: 5.6 mmol/L) and 30 min postexercise (BICARB: 2.7 vs. PLACEBO: 1.9 mmol/L). Furthermore, while the absolute concentrations of lactate generated in our HIIT sessions appear lower than previous research using NaHCO_3 supplementation and the same HIIT protocol (41), the magnitude of the difference between the BICARB and PLACEBO sessions was similar (2.7 vs. 2.3 mmol/L). In

addition, while the magnitude of change in blood lactate following HIIT was similar to previous research (17, 48), the absolute increase was lower than previous work using HIIT (1) and sprint interval training (SIT) (36, 38, 41), including our previous study (27) that observed increases greater than 10 mmol/L immediately postexercise.

Acylated ghrelin. The BICARB session (with greater lactate accumulation) had lower acylated ghrelin concentrations. This is in line with our previous work (27) and aligns with several others demonstrating HIIT and SIT elicits an exercise-induced decrease in acylated ghrelin postexercise (10, 11, 34, 36, 40, 48). While the greatest suppression in acylated ghrelin did not temporally coincide with the greatest levels of blood lactate immediately postexercise, it is possible that there is a time lag between peak levels of blood lactate, secreted from working skeletal muscle and white adipose tissue (44), and the ensuing signaling required to downregulate acylated ghrelin secretion from gastric mucosal cells. Nevertheless, the decreased acylated ghrelin (at 60 and 90 min postexercise) in the BICARB session further supports that blood lactate is involved in the suppression of circulating acylated ghrelin postexercise. Given that ghrelin-producing gastric mucosal cells are highly enriched with the lactate-specific GPR81 receptor (15, 16) and that ex vivo administration of lactate to primary gastric mucosal cells at physiologically relevant doses (1 and 10 mmol/L) inhibits acylated and total ghrelin secretion in a dose-dependent manner (15), it would stand to reason that the exercise-induced accumulation of lactate in our BICARB session led to the subsequent reductions observed in circulating acylated ghrelin. Although future work is certainly warranted, this

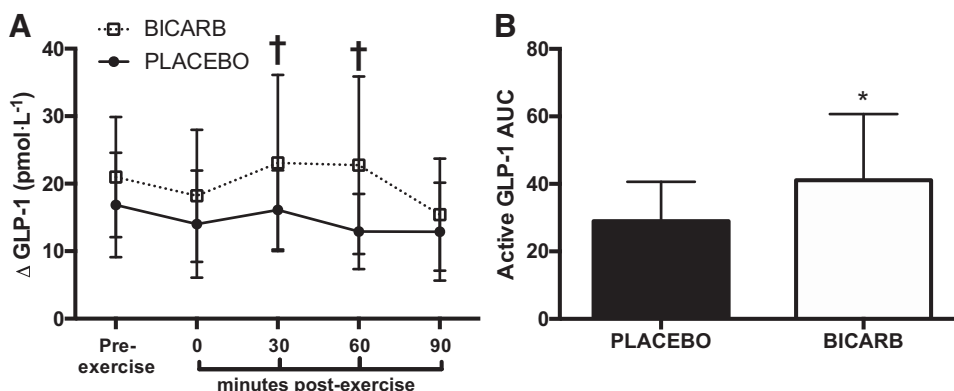
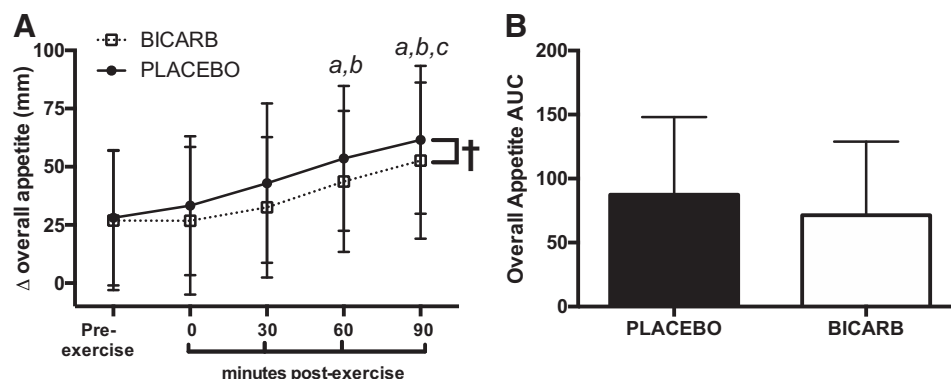


Fig. 5. A: changes in GLP-1 across all time points in each experimental session. B: area under the curve (AUC) values for each experimental session. *Significantly different between sessions: B: $P < 0.05$. †Significant difference approached between sessions.

Fig. 6. A: changes in overall appetite across all time points in each experimental session. B: area under the curve (AUC) values for each experimental session. *Significant increase compared with preexercise, $P < 0.001$. ^bSignificant increase compared with immediately postexercise, $P < 0.001$. ^cSignificant increase compared with 30 min postexercise, $P < 0.001$. †Significant difference approached between sessions.



provides strong *in vivo* evidence to support a lactate-dependent mechanism of appetite suppression postexercise.

GLP-1 and PYY. GLP-1 and PYY concentrations were greater during the BICARB session relative to PLACEBO, indicating that NaHCO_3 and thus lactate may have an effect on the secretion of these anorectic peptides. This anorectic response to the BICARB session was unexpected, as previous investigations failed to observe a relationship between blood lactate and these peptides (27). Furthermore, we are aware of no direct mechanistic link between lactate and the regulation of these peptides; in fact, the majority of research suggests no effect of exercise intensity on GLP-1 (3, 21, 23, 27, 54). It would, however, be expected that circulating PYY and GLP-1 concentrations decrease over time, as the duration since the participants' last meal increases. In support of this contention, our previous work using a no-exercise control session demonstrated a continual decline in GLP-1 and PYY in the postprandial state (27). Certainly, research focused on how exercise can alter these hormones is warranted.

Overall appetite. Appetite increased similarly over time during both sessions, becoming significantly different from preexercise values at 60 min postexercise and remaining elevated at 90 min. However, the main effect of the session demonstrated that BICARB had a lower overall appetite compared with the PLACEBO session, although this was not supported by a statistically significant difference in the AUC data. Although the increased lactate accumulation in the BICARB session was not sufficient to statistically lower appetite, it is worth noting again that the absolute increase in blood lactate in this study was lower than that which has been previously reported (27, 41). Therefore, it is likely that a significant reduction in appetite would have been apparent had a greater accumulation of lactate been observed. Future investigations should attempt to confirm this hypothesis.

Strengths and limitations. To our knowledge, this is the first study to use NaHCO_3 as a means of manipulating blood lactate concentrations, such that the effects of lactate on appetite regulation could be studied. Overall, we were successful in manipulating blood lactate levels using NaHCO_3 supplementation. We also purposefully chose a matched-work HIIT exercise session in an attempt to minimize any potential mechanisms involved in exercise-induced appetite suppression between sessions. While some previous results demonstrate NaHCO_3 supplementation induces gastrointestinal distress (29), several have demonstrated no issues (4, 13, 41). Still, a potential limitation of the current study is that we cannot

discount the fact that NaHCO_3 may have had independent effects on appetite via gastrointestinal distress. With that being said, there were no differences in appetite hormones or perceptions preexercise during either session, nor were there differences in the AUC for gastrointestinal distress between sessions (data not shown), suggesting the consumption of NaHCO_3 preexercise had negligible confounding effects, if any. Nonetheless, future work should consider recent developments in NaHCO_3 delivery through topical creams (30, 37), which may allow manipulation of blood lactate levels independent of gastrointestinal side effects. Another potential limitation is that we only controlled for physical activity in the 24 h before data collection, and while previous work suggests that this should occur for at least 72 h before to ensure residual effects of exercise on appetite have diminished (43), we failed to observe baseline differences between exercise sessions, suggesting this had little measurable impact. Although the present study does not include energy intake data, we did measure energy intake over a 3-day period (day before, day of, and day after exercise) in line with our prior investigation (27) but felt the data were not reliable enough to be disseminated in the literature due to issues with noncompliance in reporting, and as such, omitted this data. Future work should consider subsequent effects on energy intake through more robust assessments of energy intake (12). Finally, considering energy balance involves both energy intake and energy expenditure, future work should also consider objective measures of physical activity before and following the experimental sessions. Although we did not confirm energy expenditure was the exact same between exercise sessions, both sessions were work-matched meaning potential differences would be minimal.

Conclusion. The present study provides further evidence for the role of lactate in aerobic exercise-induced appetite suppression. The current results demonstrate that blood lactate accumulation with intense intermittent aerobic exercise mediates changes in circulating levels of acylated ghrelin, PYY, and GLP-1 postexercise that are associated with reductions in overall appetite. Many questions remain unanswered with regard to the role of lactate and acylated ghrelin, including whether gastric blood flow redistribution brought about via intense aerobic exercise (24) promotes the secretion of lactate, as well as the autocrine inhibition of ghrelin from gastric mucosal cells (50). Importantly, accumulating evidence supports the existence of a lactate-ghrelin signaling axis, and future work to tease apart the underlying mechanisms is certainly warranted. Moreover, recent work suggests that lactate

may be an integral signal in the release of IL-6 from skeletal muscle (25), and given that IL-6 is known to regulate the secretion of GLP-1 from enteroendocrine cells (14), further work should investigate the relationship between lactate and IL-6 in the context of GLP-1 and PYY release. Importantly, this study extends previous work in animal/cell models providing further evidence of the involvement of lactate in exercise-induced appetite suppression. As such, the current findings suggest that the ability of regularly performed HIIT to induce improvements in body composition may be explained, at least in part, by a postexercise suppression of appetite mediated by exercise-induced increases in lactate accumulation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

L.W.V., G.L.M., G.J.H., and T.J.H. conceived and designed research; L.W.V., G.L.M., and G.J.H. performed experiments; L.W.V., G.L.M., and T.J.H. analyzed data; L.W.V., G.L.M., and T.J.H. interpreted results of experiments; L.W.V. and T.J.H. prepared figures; L.W.V. drafted manuscript; L.W.V., G.L.M., G.J.H., and T.J.H. edited and revised manuscript; L.W.V., G.L.M., G.J.H., and T.J.H. approved final version of manuscript.

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