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The role of IL-6 in exercise-induced anorexia in normal-weight boys.

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Title: The role of IL-6 in exercise-induced anorexia in normal-weight boys.

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Abstract

Background: Our previous study showed that Interleukin-6 (IL-6) is associated with a suppression of appetite after high-intensity exercise (HIEX), but an independent role within food intake (FI) was not defined.

Hypothesis: IL-6 after HIEX (75%VO₂peak) is independent of appetite-hormones in suppressing appetite and FI in normal-weight (NW) boys.

Objective: To investigate the effect of HIEX, with and without the inflammation inhibitor, ibuprofen (IBU), on IL-6 and selective biomarkers of inflammation and appetite on FI and ratings of appetite in NW boys.

Design: Fifteen NW boys (aged 13-18y) were randomly assigned in a crossover design to four sessions: 1)Water+Rest; 2)Rest+IBU; 3)Water+HIEX; 4)HIEX+IBU. HIEX consisted of three 10min bouts of HIEX at 75% VO₂max with 1:30min active rest interposed. IBU was given in a 300mg liquid solution. EI, ratings and plasma biomarkers of appetite, inflammation, stress and glucose control were measured.

Results: FI was not affected by HIEX or IBU. Appetite increased over TIME ($p=0.002$) but was lower after HIEX ($p<0.001$) with no effect of IBU. HIEX, but not IBU, also resulted in higher levels of IL-6 ($p<0.001$) and cortisol ($P<0.001$), and lower active ghrelin ($P<0.001$). IL-6 correlated with active ghrelin ($r=0.37$; $p=0.036$) and Cortisol ($r=0.26$; $p=0.049$).

Conclusion: HIEX reduces subjective appetite but not EI, accompanied by an increase of IL-6 and cortisol and a decrease of active ghrelin and blood glucose. An independent role for IL-6 in appetite suppression was not supported. However, IL-6 was associated with active ghrelin and cortisol, thus potentially mediating appetite via these interactions.

Keywords: Exercise, Appetite, Food Intake, IL-6, Children

Introduction:

Information regarding energy status, hunger and satiety are communicated between the brain and the gut via appetite hormones. These episodic signals arise in response to macronutrients in the intestinal system and are thought to be involved in appetite regulation during and after exercise. Although high levels of prolonged activity should initiate hunger, exercise at high intensities suppresses appetite and food intake (FI) (King et al. 1994; Blundell and King 2000; Sim et al. 2014; Blundell et al. 2015).

This high-intensity exercise (HIE) induced anorexia is dependent on exercise modality (Panissa et al. 2016), duration (Bozinovski et al. 2009), and the time between the cessation of exercise and initiation of FI (King et al. 1994). However, the mechanisms are not well understood. Appetite hormones have been reported to be responsible for the post-exercise reduction in appetite in some (Anderson et al. 2016; Hazell et al. 2016) but not other studies (Unick et al. 2010; Larson-Meyer et al. 2012). Biomarkers from the stress and inflammatory system also respond to HIE. Interleukin-6 (IL-6) has been associated with suppression of appetite after HIE in adults (Almada et al. 2013) and boys (Hunschede et al. 2017). In our previous study, IL-6 correlated with reduced subjective appetite and fullness VAS, but active ghrelin also correlated with fullness VAS (Hunschede et al. 2017) making an independent role for IL-6 uncertain.

IL-6 is an inflammatory cytokine and its increase in plasma is believed to be related to muscle micro-damage (Bruunsgaard et al. 1997). It is secreted by the muscle, confirming its role as a myokine (Pedersen et al. 1998; Pedersen et al. 2004; Petersen and Pedersen, 2006). The increase of systemic IL-6 after HIE has been a consistent finding, and its appearance in plasma precedes other inflammatory cytokines (Pedersen et al. 1998). The peak of IL-6 induced by HIE is reached shortly after the end of exercise (Pedersen et al. 2001) and has been associated with decreased

appetite (Almada et al. 2013; Hunschedev et al. 2017). Several animal studies have also shown decreased appetite after high levels of IL-6 were induced by lipopolysaccharide (LPS) (Boelen et al. 1995) or direct IL-6 (Wallenius et al. 2002) and TNF- α injections (Langhans et al. 1999). Conversely, IL-6 deficient mice display obesity as early as ten weeks of age, due to increased FI but not EE (Chida et al. 2006).

Further evidence for a potential role of cytokines in appetite control is provided by their known transport across the blood-brain barrier, where they interact with the luminal surface of brain endothelial cells to release substances that affect appetite (Banks 2001). They inhibit appetite directly by stimulating hypothalamic neurons sensitive to glucose in the lateral hypothalamic area (Plata-Salaman 1996) by suppression of neuropeptide systems such as corticotrophin-releasing factor or neuropeptide-Y (Plata-Salaman 1991; Laviano et al. 1996).

Ibuprofen or isobutylphenylpropanoic acid (IBU) is a nonsteroidal anti-inflammatory drug (NSAID) used for treating pain, fever by reducing the acute inflammatory response. Athletes and recreational exercisers often take IBU to decrease muscle soreness, cell injury and the inflammatory response after exercise (Mahler 2001). An oral dose of IBU decreases circulating levels of cytokines such as IL-6, TNF- α and CRP within the 30-45 minutes after ingestion (Gallelli et al. 2013; Barnes et al. 2014). However, its effect on appetite and FI has not been reported.

Therefore, the objective of this study was to determine the effect HIEEX, with and without IBU consumption, on IL-6, FI regulatory hormones, appetite, and FI in normal-weight (NW) boys.

Participants

Fifteen NW (BMI for age percentile: 15th-85th) boys aged 13-18yrs completed the study. Sample size was estimated using appetite VAS scores from our previous studies, assuming that differences in VAS scores will translate to differences in FI. A telephone questionnaire was employed to determine eligibility for this study. Boys who answered “yes” to one of the questions of the physical activity readiness questionnaire, displayed a form of haematophobia, dieters, had been diagnosed with diabetes or other metabolic diseases, and those scoring ≥ 11 on an Eating Habit Questionnaire were excluded from the study. All experimental procedures were approved by the University of Toronto Health Sciences Research Ethics Board, and informed consent was obtained from all adult participants, parents of the children as well as assent from the children themselves. Originally 21 participants were recruited through local advertisement at the University of Toronto and Toronto subway stations. However, 6 participants did not complete the study, due to either a mild form of vasovagal syncope likely due to the IV-catheter insertion, or difficulties scheduling the sessions.

Fig. 1.:

Participant Assessment

Participants were asked to come to the University of Toronto - Goldring Centre for High-Performance Sport for the initial screening. Age, height, body-weight, BMI, BMI for age percentile and percent body-fat were determined. Physical fitness (PF) was assessed by indirect calorimetry during continuous incremental cycling protocol on a Kettler RE7 recumbent bicycle (Kettler, Enneparsit, NRW, Germany). Participants cycled for 3 min at 25 Watts, then the intensity was increased every minute by 20 watts. Ventilatory gases were collected using a Moxus metabolic cart (AEI

Technologies Inc., Pittsburgh, PA, USA), a facemask, and a 2-way non-rebreathing valve (Hans Rudolph, Inc., Shawnee, KS, USA). Inspiratory ventilation was measured with a pneumotachometer, the O₂ and CO₂ contents of mixed expired gas with an S-3A Oxygen Analyzer, and CO₂ content with a CD-3A 251 Carbon Dioxide Analyzer (AEI Technologies Inc., Pittsburgh, PA, USA). Known gas concentrations of 16.04% O₂ and 4.06% CO₂ and 20% O₂ and 0.03% CO₂ were used to calibrate the metabolic cart, prior to each test. The Moxus metabolic cart has been validated over a wide measurement range using two sensors for ventilation against the Douglas bag method (Rosdahl et al. 2013). VO_{2peak} was determined using the highest six consecutive breaths (Hunschede et al. 2015). Participants were included in the study if they exhibited normal body-weight (15th-85th BMI for age percentile) according to the Center for Disease Control and Prevention (2000) growth charts (Flegal et al. 2002). Bioelectrical impedance analysis was used to estimate body fat mass and fat-free mass (RJL Systems, Inc., Clinton Township, MI, USA) based on the Horlick equation (Horlick et al. 2002). All anthropometric measurements during the assessment are displayed in table 1.

Study design, Experimental Protocol and Sample Size

Sample size for subjective appetite response to HIEG was estimated at N=14, using a within-subject design with $\alpha = 0.05$ ($Z = -0.025 = 1.96$); $\beta = 0.20$ ($Z = 0.80 = 0.84$); $\sigma = 20.7$ mm and $\Delta = 8.7$ mm in appetite VAS observed between control and treatment based on a previous study (Hunschede et al. 2016). σ represents standard deviation, Δ represents the minimal difference, N is the number of subjects needed.

$$N = 2 \times \left[\frac{(Z\alpha - Z\beta) \times \sigma}{\Delta} \right]^2$$

All participants completed four sessions in a randomized order: (i) rest and water (ii) or exercise water, 30 min of HIEX at 75% VO_2peak or (iii) rest and IBU, 300 mg of Motrin IBU solution for children (Johnson and Johnson, New Brunswick, NJ, United States) or (iv) exercise and IBU, 30 min of HIEX at 75% VO_2peak and 300 mg of Motrin IBU solution. Both water control and IBU solution contained 0.8 g of Sucralose (Heartland Food Products Group, Amsterdam, HP, Netherlands) and 1.2 g of orange flavoured Kool-Aid (Kraft Foods, Northfield, IL, United States) to mask the taste of the Motrin solution. To match sweetness and calorie content of the IBU Motrin drink, the water control also contained 6 mL high fructose corn syrup (ACH Food Companies, Mississauga, ON, Canada). To achieve peak IBU plasma levels during the HIEX session, the IBU treatment was given 20 min before the initiation of the HIEX session. Previous research has shown that IBU reaches its peak plasma levels in circulation after 30-40 min (Scott et al. 1999), thus being fully absorbed in the bloodstream halfway through the HIEX session.

Sessions were conducted on weekends between 9 and 10 am after a 12-h overnight fast. Parents were asked to encourage their children to drink water up to 1-h before the scheduled session, to refrain from physical activity, and to maintain the same dietary patterns the evening before each test. All baseline values

Visual Analog Scales and Food Intake

Visual Analog Scales (VAS) were employed to assess subjective appetite based on the 100 mm lines anchored by extreme statements: "Determination to Eat" (DTE), "Hunger", "Fullness" and "Prospective Food Consumption" (PFC). Physical Comfort, Nausea and Thirst were also measured with VAS, again anchored with extreme statements to determine possible confounders for the appetite scores as previously described (Hunschede et al. 2015). Furthermore, Nausea has been

shown to be associated with exercise-induced anorexia (Kondo et al. 2001). Participants were instructed to read each question and place an “X” along a 100-mm line depending on how they felt (e.g, not hungry to as hungry as I have ever been) at the current moment. Visual analogue scale questionnaires were administered at baseline (0 min), 10, 30, 65, 80, 95 and 120 min after the start of each session.

Participants were provided with *ad libitum* lunch meal, consisting of rice (Uncle Ben’s, Bolton, ON, Canada), beef meatballs (President’s Choice, Brampton, ON, Canada) and tomato sauce (Ragu, Mount Prospect, IL, USA) at 100 – 120 min. The meatballs were cut into small and uniform pieces and were mixed homogeneously with the other ingredients in a bowl. Each bowl contained a 479.5 g portion which provided 827.5 kcal, 30.8 g fat, 104.8 g carbohydrate and 30.2 g protein based on the compositional information provided by the manufacturers. Two portions were served in 10 min intervals and FI from the meal was calculated based on the weight consumed during the lunch. Participants were instructed to eat until comfortably full and stay seated for the duration of the meal. A 500ml bottle of spring water (Danone Crystal Springs, Quebec City, QC, Canada) was provided during the HIE session and with the meal, and additional bottles were supplied if requested. Water consumption was measured by the weight (g) consumed. All baseline VAS measurements are displayed in table 1.

Blood Collection

Blood samples were collected into pre-chilled 10 mL BD Vacutainer™ (BD Diagnostics, Sparks, MD, USA) at baseline (0 min), 65 min and 95 min. Blood collection tubes contained spray dried K₂EDTA anticoagulant, and a proprietary cocktail of protease inhibitors [e.g. DPP-IV (R-3-Amino-1-{3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazol[4,3-a]pyrazin-7-yl}-4-(2,4,5-

trifluorophenyl)butan-1-on), AEBSF (4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride) and aprotinin (Trasylo) to prevent the proteolytic breakdown of hormones. Immediately after collection, plasma was separated by centrifugation for 15 min at 2000 RCF at 4 °C, then aliquoted into 2 mL Eppendorf (Eppendorf, Hamburg, Germany) tubes and stored at -80 °C for later analyses. In addition, to enhance the active ghrelin stability in blood samples, 200 µL 1 N HCl was added to every 1 ml of plasma collected for ghrelin analysis. All baseline blood measurements are displayed in table 1.

Biochemical measurements

Plasma levels of glucose, insulin, CRP and cortisol were analyzed by the clinical laboratory at Mount Sinai Hospital (Mount Sinai, Toronto, ON, Canada). All other analyses were performed in the Department of Nutritional Sciences at the University of Toronto. Leptin, total PYY [i.e. PYY (1-36 amide) and PYY (3-36)] and the biological active form of ghrelin and GLP-1 [i.e. GLP-1 (7-36 amide)] were analyzed using commercial ELISA kits (Millipore, Billerica, MA, USA): (Leptin cat. #EZHL-80SK, Sensitivity: 0.5 ng/mL, Range: 0.5–100 ng/mL; active ghrelin cat. #EZGRA-88K, Sensitivity: 8pg/mL, Range: 25-2000 pg/mL; GLP-1 cat. #EGLP-35K, Sensitivity: 2pM, Range: 2-100 pM; PYY cat. #EZHPYYT66K, Sensitivity: 6.5 pg/mL, Range: 14-1800 pg/mL). Interleukin-6 and TNF-α were analyzed using sandwich ELISA kits from R&D systems (R&D Systems, Minneapolis, MN, USA); (IL-6 cat. #HS600B, Sensitivity: 0.11 pg/mL, Range: 0.156 - 10 pg/mL; TNF-α cat. #HSTA00D, Sensitivity: 0.191 pg/mL, Range: 0.5 - 32 pg/mL). For all assays, intra-CV was <4%, and Inter-CV was <8%.

Statistical Analysis

Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, N.C., USA). The data was tested for normality using the SAS PROC UNIVARIATE procedure. The randomization schedule to treatments was generated using the SAS PROC PLAN procedure (SAS Institute v. 9.4, Cary, NC, USA). Baseline characteristics are presented as absolute mean $\pm$ SEM. Visual analog scale and blood biomarker changes over time from baseline were analyzed using a 3-factor ANCOVA by SAS PROC MIXED procedure followed by Tukey–Kramer’s post hoc test, with ACT (HIEX vs. rest), DRK (IBU vs. Water) and TIME as independent variables, and baseline (0 min) as covariate variable. VAS and blood biomarkers over time are presented as analyzed, as mean $\pm$ SEM change from baseline until 95 min. Pre- and Post (95 – 120 min) lunch VAS scores were presented separately. Pearson correlation analysis was conducted between mean raw data of each biomarker, each of the mean raw data in VAS scores and FI. For clarity, the data is presented for each treatment arm. Statistical significance was declared at $P < 0.05$.

Results

Food Intake and Water Consumption:

FI (kcal/kg) was not affected by HIEX or IBU. Water consumption (mL/Kg) was increased by HIEX ($p < 0.001$), but not affected by IBU. No other main effects or interactions were found.

Fig. 2.

222 **Post-exercise Appetite Scores**

223 No differences in pre- and post meal VAS scores among treatments were found for appetite,
224 PFC, DTE, hunger or fullness.

225 **Fig. 3.**

227 **Appetite Visual Analog Scales:**

228 HIEX lowered appetite ($p = 0.04$), DTE ($p = 0.024$) and PFC ($p = 0.021$). IBU had no effect on
229 VAS scores. Over TIME, APP ($p = 0.002$), DTE ($p < 0.001$) and hunger ($p < 0.001$) increased but
230 neither fullness nor PFC were changed. TIME and HIEX interactions were found due to increase in
231 appetite ($p = 0.026$), DTE ($p = 0.009$), hunger ($p = 0.025$) and PFC ($p = 0.05$) over time. No other
232 main effects or interactions were found.

233 **Fig 4.**

235 **Thirst, Physical Comfort, Nausea and Palatability Visual Analog**
236 **Scales:**

237 Thirst was increased by HIEX ($p = 0.016$) and decreased by IBU ($p = 0.03$) and TIME ($p <$
238 0.001). Neither palatability ratings between DRK nor of the *ad libitum* lunch meal were affected by
239 HIEX or IBU. Nausea and physical comfort rating were also not affected by HIEX or IBU. No other
240 main effects or interactions were found.

Appetite Biomarkers:

HIEX decreased Active Ghrelin ($p < 0.001$), but did not affect GLP-1, PYY, Insulin or Blood Glucose. IBU increased GLP-1 ($p = 0.01$) but had no effect on Active Ghrelin, PYY, GLP-1, Blood Glucose or Insulin. Over TIME, Active Ghrelin ($p < 0.001$), Blood Glucose ($p < 0.001$) and Insulin ($p < 0.001$) steadily decreased while GLP-1 and PYY were unchanged. Active Ghrelin was lowest at 65 min and then increased to baseline levels at 95 min, explaining the TIME by HIEX interaction ($p < 0.001$). The ACT*DRK interaction ($p = 0.007$) and TIME*ACT interaction ($p = 0.014$) were due to the increased active ghrelin after HIEX and IBU compared with rest or water, respectively.

Fig. 5.

Inflammatory Biomarkers:

HIEX increased IL-6 ($p < 0.001$) and Cortisol ($p < 0.001$), but did not affect TNF- α or CRP compared with the rest condition. IBU had no effect on IL-6, TNF- α , Cortisol or CRP compared with the water control. Over TIME, IL-6 ($p < 0.001$) and Cortisol ($p = 0.021$) increased, but CRP and TNF- α were not affected. TIME*ACT interactions were found for IL-6 ($p = 0.001$) showing an increase to 95 min and Cortisol ($p = 0.002$) to 65 min with HIEX when compared to rest. ACT*DRK interactions were found for CRP ($p = 0.04$) and TNF- α ($p = 0.001$). CRP was lower at 65 min, but rebounded to 95 min in the rest condition, whereas it was highest at 65 min and decreased to 95 min, after HIEX. IBU dampened the effect of HIEX at 65 min. Similarly, at 65 min the effect of DRK on TNF- α reversed the effect of the two ACT treatments.

Fig. 6.

Correlations

Ghrelin was correlated with Cortisol ($r = -0.31$; $p = 0.018$) and Insulin ($r = 0.39$; $p = 0.002$). GLP-1 was correlated with PYY ($r = 0.47$; $p = 0.002$). Insulin was correlated with Glucose ($r = 0.39$; $p = 0.022$), Appetite ($r = 0.43$; $p < 0.001$) and DTE ($r = -0.43$; $p < 0.001$). IL-6 was correlated with Ghrelin ($r = 0.37$; $p = 0.036$), TNF- α ($r = -0.26$; $p = 0.045$) and Cortisol ($r = 0.26$; $p = 0.049$).

Discussion:

IBU did not affect FI or appetite at rest or with exercise. The IL-6 response to HIEEX was not significantly affected by IBU administration. However, this study suggests IL-6 may affect appetite in a correlated response with active ghrelin and cortisol. The study confirms previous reports showing that HIEEX leads to suppression in appetite (Blundell and King 2000; Sim et al. 2013), but in addition, we show that FI was not affected. Furthermore, IBU did not affect appetite or FI, although it demonstrated a significant improvement in inflammatory markers, appetite scores and bodyweight, in patients with gastrointestinal cancer (McMillan et al. 1997; McMillan et al. 1999).

Many studies have shown that HIEEX increases IL-6 which is produced as a myokine by the muscle (Pedersen and Fischer 2007) but has no effect on TNF- α which is produced by macrophages (Croft et al. 2009). However, the lack of effect of IBU on IL-6 was surprising. IBU is known to reduce IL-6 by inhibiting the cyclooxygenase enzymes (COX)-1 and COX-2 (Davies 1998), thus preventing the formation of various prostaglandins (PGE), including PGE-2, that increase IL-6 production in macrophages in response to acute inflammation. However, we found IBU reversed the effect of ACT on CRP and TNF- α , thus confirming that the dose of 300mg IBU was sufficient to alter some inflammatory responses, but those responses did not correlate with appetite.

Although the present study does not show an independent role for inflammatory biomarkers contributing to a suppression of appetite, several potential mechanisms support continued investigation of cytokines role in appetite and FI regulation. During states of an increased inflammatory response, cytokines are transported across the blood-brain barrier and exert their effects on the luminal surface of endothelial brain cells causing secretion of substances that affect appetite (Banks et al. 2011). TNF- α and IL-1 receptors in the hypothalamic area of the brain which regulate FI (Hellerstein et al. 1989) and intraperitoneal injections of human TNF- α receptor antagonist increase FI in tumor-bearing rats (Torelli et al. 1999) indicating a potential role in FI and appetite regulation.

In the present study, IBU reduced the TNF- α response to exercise but did not affect appetite, suggesting TNF- α did not play a role but perhaps because responses in these inflammatory biomarkers were many times lower than the ones observed in the tumour bearing rats. Finally, it has also been shown in rats that HIEX increases the permeability of the blood-brain barrier (BBB) (Sharma et al. 1991), potentially increasing transport of appetite-regulating components that usually do not affect appetite and FI.

Appetite-regulating hormones are proposed to play a role in the control of appetite during and after exercise (Anderson et al. 2016), but not one hormone has been shown to be a consistent factor in the response. For example, several studies show that active ghrelin is decreased (Broom et al. 2007; King et al. 2010; King et al. 2011; Becker et al. 2012; Wasse et al. 2012; Wasse et al. 2013), while GLP-1 (Ueda et al. 2009) and PYY (Broom et al. 2009; Wasse et al. 2012) are often increased after HIEX. However, others reported no effect of HIEX on GLP-1 (Larson-Meyer et al. 2012) and PYY (Kelly et al. 2012; Larson-Meyer et al. 2012) or even decreased levels of GLP-1 (Unick et al. 2010).

This inconsistent association of appetite hormones from the gut with HIEX induced anorexia may also be explained by decreased splanchnic blood flow after HIEX compared with an increase in intestinal blood flow after FI. During and after eating, splanchnic blood flow is increased to aid digestion, releasing appetite-regulating hormones to signal nutrient availability (Austin and Marks 2009). Blood flow in the celiac artery peaks rapidly at a 38–60% increase from fasting levels (Qamar et al. 1985; Someya et al. 2008), while the blood flow in the superior mesenteric artery increases 1.5- to 3.5-fold, 5–60 min after a meal (Qamar and Read, 1988; Sidery et al. 1991; Someya et al. 2008). In contrast, during HIEX splanchnic blood flow is decreased and redistributed from the gut to contracting skeletal muscle to increase oxygen supply (Eriksen et al. 1994). HIEX at 70% VO_2max decreased blood flow in the portal vein by 80% after 60 min of cycling at (Rehrer et al. 2001), and by 43% after in the superior mesenteric artery after 30 min of treadmill running (Qamar and Read 1987). This contrast in blood flow after HIEX supports a continued exploration of alternative explanations for the effects of HIEX on appetite regulation.

This study has several limitations. First, the study may be underpowered for measures of FI, but other studies have found this sample size is sufficient for identifying treatment effects on FI (Sim et al. 2014). However, the time of measuring FI may have been too late. Our previous study showed a potential peak suppression of appetite at 30 min post-HIEX, based on measurements of VAS appetite scores and, appetite and inflammatory biomarkers (Hunschede et al. 2017). In the present study, VAS ratings of appetite and hunger were suppressed at 65 min, but recovered quickly from 65 to 95 mins after HIEX, at the time of FI measures. Second, females were not included in this study due to financial limitations but this needs to be addressed because several studies have been shown differences in males (Sim et al. 2014) vs. females (Pomerleau et al. 2004) in appetite behaviour after HIEX. Third, the limited number of blood samples taken in this study

may have contributed to failure to detect treatment effects on PYY and GLP-1. We were not able to take blood samples more frequently because the University of Toronto Research Ethics board allows for only 40 mL of blood to be sampled over a 2-hr period in an adolescent study population.

Conclusion

In conclusion, HIEX reduces subjective appetite but not EI, accompanied by an increase of IL-6 and cortisol and a decrease of active ghrelin and blood glucose. An independent role for IL-6 in appetite suppression was not supported. However, IL-6 was associated with active ghrelin and cortisol, thus potentially mediating appetite via these interactions.

Conflict of Interest

The authors have no conflicts of interest to report.

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Tables:

Table 1.:

Baseline Characteristics:

Participant Characteristics			
Measurement	NW	±	SEM
Anthropometric Measurements			
Age (y)	16.1	±	0.5
Weight (Kg)	66.2	±	2.9
Height (cm)	175.5	±	2.4
BMI (Kg·m ⁻²)	21.5	±	0.7
BMI percentile	58.8	±	6.1
VO ₂ peak (mL·Kg ⁻¹ ·min ⁻¹)	44.3	±	1.5
Body fat (%)	23.4	±	2.4
Lean mass (Kg)	50.5	±	2.3
Tanner stage	3.7	±	0.3
Appetite Visual Analog Scores			
Appetite (mm)	55.9	±	2.9
DTE (mm)	47.1	±	3.7
Hunger (mm)	45.8	±	3.8
Fullness (mm)	32.9	±	3.3
PFC (mm)	63.8	±	3.1
Thirst (mm)	47.1	±	2.8
Plasma Biomarkers			
Ghrelin (pg/mL)	457.3	±	39.5
PYY (pg/mL)	66.9	±	3.2
GLP-1 (pM)	4.4	±	0.6
Cortisol (nmol/L)	243.6	±	9.5
CRP (mg/L)	1.02	±	0.2
Glucose (mmol/L)	5.3	±	0.03
Insulin (pmol/L)	67.8	±	5.6
TNF-α (pg/mL)	0.7	±	0.06
IL-6 (pg/mL)	0.96	±	0.08
Note: DTE, Determination to eat; PFC, Prospective food consumption; PYY, Peptide Tyrosine Tyrosine; GLP-1, Glucagon-like-peptide-1; CRP, C-reactive protein; IL-6, Interleukin-6; TNF-α, Tumor necrosis factor alpha. Values are means ± SEM; n = 15 NW.			

540 Figure Captions

541 **Fig.1:** Study protocol flow diagram. Note: VAS = Visual analog scale; light grey area = 10 min HIEX

Fig. 2 Food Intake (A; 100 – 120 min) and Water (B; 0 - 120 min). Letters denote values that are significantly different from control at each time point (3-way ANOVA, Tukey–Kramer post hoc test, $p < 0.05$). Values are Mean $\pm$ SEM, $n=15$ NW.

Fig. 3 *Change from baseline* in Appetite (A), Prospective Food Consumption (B), Determination to Eat (C), Hunger (D) and Fullness (E) in response to ACT and DRK at 0, 65 and 95 min. Values are Mean $\pm$ SEM, $n=15$ NW. Letters denote values that are significantly different from control at each time point (3-way ANOVA, Tukey–Kramer post hoc test, $p < 0.05$). Striped blocks represent each 10 min HIEX bout. WARE = Water and Rest; IBRE = Ibuprofen and Rest; WAEX = Water and High Intensity Exercise; IBEX = Ibuprofen and High Intensity Exercise.

Fig. 4 *Change from baseline VAS Delta (95-120 min)* Appetite (A), PFC (B), DTE (C), Hunger (D) and Fullness (E) scores. Values are Mean $\pm$ SEM, $n=15$ NW, 3-way ANOVA, Tukey–Kramer post hoc test, $p < 0.05$.

Fig 5. *Change from baseline plasma levels of* Active Ghrelin (A), Peptide YY (B), GLP-1 (C), Blood Glucose (D) and Insulin (E) in response to ACT and DRK at 0, 65 and 95 min. Values are Mean $\pm$ SEM, $n=15$ NW. Letters denote values that are significantly different from control at each time point (3-way ANOVA, Tukey–Kramer post hoc test, $p < 0.05$). Striped blocks represent each 10 min HIEX bout. WARE = Water and Rest; IBRE = Ibuprofen and Rest; WAEX = Water and High Intensity Exercise; IBEX = Ibuprofen and High Intensity Exercise.

Fig.6 *Change from baseline plasma levels of* Interleukin-6 (A), Tumor Necrosis Factor- α (B), Cortisol (C) and C-reactive protein (D) in response to ACT and DRK at 0, 65 and 95 min. Values are Mean $\pm$ SEM, $n=15$ NW. Letters denote values that are significantly different from control at each time point (3-way ANOVA, Tukey–Kramer post hoc test, $p < 0.05$) WARE = Water and Rest; IBRE = Ibuprofen and Rest; WAEX = Water and High Intensity Exercise; IBEX = Ibuprofen and High Intensity Exercise.

542 bout; dark grey area = 1:30 min rest; blood drop = time point for each blood draw.

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Draft

Study protocol flow diagram. Note: VAS = Visual analog scale; light grey area = 10 min HIEX bout; dark grey area = 1:30 min rest; blood drop = time point for each blood draw.

150x79mm (300 x 300 DPI)

Food Intake (A; 100 – 120 min) and Water (B; 0 - 120 min). Letters denote values that are significantly different from control at each time point (3-way ANOVA, Tukey-Kramer post hoc test, $p < 0.05$). Values are Mean $\pm$ SEM, $n=15$ NW.

72x29mm (300 x 300 DPI)

Change from baseline in Appetite (A), Prospective Food Consumption (B), Determination to Eat (C), Hunger (D) and Fullness (E) in response to ACT and DRK at 0, 65 and 95 min. Values are Mean $\pm$ SEM, n=15 NW. Letters denote values that are significantly different from control at each time point (3-way ANOVA, Tukey-Kramer post hoc test, $p < 0.05$). Striped blocks represent each 10 min HIEX bout. WARE = Water and Rest; IBRE = Ibuprofen and Rest; WAEX = Water and High Intensity Exercise; IBEX = Ibuprofen and High Intensity Exercise.

210x244mm (300 x 300 DPI)

Change from baseline VAS Delta (95-120 min) Appetite (A), PFC (B), DTE (C), Hunger (D) and Fullness (E) scores. Values are Mean $\pm$ SEM, $n=15$ NW, 3-way ANOVA, Tukey-Kramer post hoc test, $p < 0.05$.

189x198mm (300 x 300 DPI)

Change from baseline plasma levels of Active Ghrelin (A), Peptide YY (B), GLP-1 (C), Blood Glucose (D) and Insulin (E) in response to ACT and DRK at 0, 65 and 95 min. Values are Mean $\pm$ SEM, n=15 NW. Letters denote values that are significantly different from control at each time point (3-way ANOVA, Tukey–Kramer post hoc test, $p < 0.05$). Striped blocks represent each 10 min HIEX bout. WARE = Water and Rest; IBRE = Ibuprofen and Rest; WAEX = Water and High Intensity Exercise; IBEX = Ibuprofen and High Intensity Exercise.

206x235mm (300 x 300 DPI)

Change from baseline plasma levels of Interleukin-6 (A), Tumor Necrosis Factor-α (B), Cortisol (C) and C-reactive protein (D) in response to ACT and DRK at 0, 65 and 95 min. Values are Mean $\pm$ SEM, $n=15$ NW. Letters denote values that are significantly different from control at each time point (3-way ANOVA, Tukey-Kramer post hoc test, $p < 0.05$) WARE = Water and Rest; IBRE = Ibuprofen and Rest; WAEX = Water and High Intensity Exercise; IBEX = Ibuprofen and High Intensity Exercise.

146x117mm (300 x 300 DPI)