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Associations between physical activity, adiposity, appetite, and metabolic health in adolescent males

Robin P. Shook[1, 2, 3], Seung-Lark Lim[4], Amanda S. Bruce[2,5], Tarin C. Philips[6], Kelsee L. Halpin[3,7], Ann M. Davis[2,5], Joseph E. Donnelly[8], John E Blundell[9], John P. Thyfault[2, 10, 11, 12]

[1]Children's Mercy, Department of Pediatrics

[2]Center for Children's Healthy Lifestyles and Nutrition

[3]University of Missouri-Kansas City, School of Medicine

[4]University of Missouri-Kansas City, Department of Psychology and Counseling,

[5]University of Kansas Medical Center, Department of Pediatrics

[6]Navy Undersea Medical Institute

[7]Children's Mercy, Department of Pediatrics, Division of Pediatric Endocrinology and Diabetes

[8]University of Kansas Medical Center, Department of Internal Medicine, Division of Physical Activity & Weight Management

[9]University of Leeds Appetite Control and Energy Balance Research Group, School of Psychology, Faculty of Medicine and Health, Leeds, UK

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[10]University of Kansas Medical Center, Departments of Cell Biology and Physiology and Internal Medicine

[11]KU Diabetes Institute

[12]Kansas Center for Metabolism and Obesity Research

Corresponding author: Robin P. Shook 610 E 22nd St, Kansas City, MO 64108, USA rpshook@cmh.edu 816-234-9443 (office)

List of abbreviations:

AUC: Area under the curve

BMI: Body mass index

DXA: Dual energy X-ray absorptiometer

FFM: Fat free mass

FM: Fat mass

HighACT: High Active

LowACT: Low Active

NW: Normal Weight

OW, OB: Overweight, Obese

Abstract

Regular physical activity for adults is associated with optimal appetite regulation, though little work has been performed in adolescents. To address this gap in the literature, we conducted a study examining appetite across a range of physical activity and adiposity levels in adolescent males. Healthy males (N=46, 14-18 years old) were recruited across four body weight and activity categories: normal weight/high active (n=11), normal weight/low active (n=13), overweight, obese/high active (n=14), overweight, obese/low active (n=8). Participants from each group completed a six-hour appetite assessment session on Day 0, followed immediately by a 14-day free-living physical activity and dietary assessment period on Days 1-14, and a fitness test session occurring between Days 15-18. Subjective and objective assessment of appetite, resting energy expenditure, body composition using dual energy absorptiometry`, and thermic effect of feeding was conducted on Day 0. Physiological variables in the normal weight low active group that were different than their peers included lower fat-free mass, cardiorespiratory fitness, glucose/fullness response to a standardized meal, thermic effect of feeding in response to a standardized meal, lower self-rated fullness and satiety, and higher self-rated hunger to a standardized meal. Conversely, the overweight, obese high active group displayed better subjective appetite responses, but higher insulin responses to a standardized meal. Taken together, these results suggest that physical inactivity during adolescence has a negative impact on metabolic health and appetite control which may contribute to future weight gain.

Keywords: Physical activity, obesity, adolescents, appetite

Introduction

Childhood obesity is arguably the most serious public health problem of the 21st century, given the prevalence, global reach, and widespread health, economic, and social consequences. Childhood obesity prevalence rates appeared to have slowed among certain age ranges,[1] but have increased once again in recent years across all groups.[2] While weight gain at the most basic level is due to a chronic energy surplus, a host of known and unknown systems involving complex relationships between biological, physiological, psychosocial, and environmental factors[3; 4] influence the components of energy balance.[5; 6]

In adults, there is evidence that regular physical activity is associated with optimal appetite regulation. Jean Mayer and others [7; 8] explored the topic in a series of studies in the 1950s, observing that energy intake only increased proportionally with energy expenditure within a certain range of physical activity, described by Mayer as the 'normal activity range'[9; 10; 11] and by Blundell et al. as the 'zone of regulation.'[12] Conversely, when activity levels are low (termed 'sedentary' zone or 'non-regulated zone' by Mayer and Blundell), the relationship between intake and expenditure is uncoupled, resulting in energy imbalance.

Despite a growing body of evidence in adults that physical activity plays a role in mediating appetite control, little work has been performed in adolescents. Most studies in adolescents consist of appetite measures performed as secondary outcomes in studies with possible confounding factors (e.g., underlying disease risk).[13; 14; 15] Observational adolescent data suggest higher levels of obesity are associated with dysregulated appetite as defined by satiety and responses to food cues[16] and higher energy intake despite lower levels of physical activity.[17] A smaller number of clinical trials have found that exercise in adolescents improves biomarkers of appetite control (ghrelin and unacylated ghrelin), though corresponding weight loss confounds any associations with exercise itself.[18] To identify the independent effects of exercise and adiposity, we conducted a cross-sectional study examining appetite across a wide range of physical activity and adiposity levels in adolescent males.

Methods

Participants.

Participants were healthy males, aged 14-18 years, Tanner Stage III-IV identified by self-report.[19] This project included only males to reduce variability in appetite variables due to gonadal development[20; 21; 22; 23; 24; 25] and menstrual cycle phase[26; 27; 28; 29] that have been noted to occur. Inclusion criteria included self-reported stable body weight (±5%) over the previous three months and being healthy for physical activity as assessed using a physical activity readiness scale based on the 2014 PAR-Q+.[30] Exclusion criteria included the presence of cardiovascular or metabolic disease, use of thyroid medications, beta blockers, or other stimulants, or any other medical conditions or medication use known to effect metabolism. All study procedures were approved by the Children's Mercy Institutional Review and all participants provided consent, or in the case of minors, assent in addition to consent by a legal guardian. The ClinicalTrials.gov Identifier is NCT03157063.

Participants were recruited across four categories based on physical activity level (high active, low active) and body weight status (normal weight, overweight/obese). For recruitment purposes, physical activity status was determined via self-reported activity ('*On a typical day, how many minutes does your child spend in active play/exercise (breathing harder or sweating)*?'), with those reporting <60 min/day categorized initially as low active and >=60 min/day categorized initially as high active in accordance with current US Centers for Disease Control and Prevention guidelines.[31] For data analysis, physical activity was subsequently measured objectively during a 14-day free-living period using accelerometry (see below). The vector magnitude average count median split was calculated for all participants, and those below this threshold were categorized as low active (LowACT) and above as active (HighACT). Body weight status was categorized using standard criteria for body mass index (BMI) age- and sex-specific percentiles[32], with normal weight (NW) defined as the 5^{th} percentile to <85^{th} percentile and overweight (OW) defined as >85^{th} percentile to <95^{th} percentile. Obese (OB) was defined as >= 95^{th} percentile; we did not include participants >99^{th} percentile to avoid severe obesity and potential co-morbid conditions.[33] Participants were grouped by activity level and body weight

status, resulting in four groups: NW/HighACT, NW/LowACT, OW,OB/HighACT, OW,OB/LowACT.

Visit 1- Appetite, body composition, resting energy expenditure.

Participants completed a six-hour appetite assessment session on Day 0, followed immediately by a 14-day free-living physical activity and dietary assessment period on Days 1-14, and a fitness test session occurring between Days 15-18. The appetite assessment session occurred in the morning before 9:00am following a 12-hour dietary fast. Participants changed into scrubs, were measured for height and weight, and completed a whole-body dual-energy x-ray absorptiometry (DXA) scan for body composition. Resting energy expenditure (REE) was then assessed using a standard protocol using indirect calorimetry (TrueOne 2400 ventilated hood system, ParvoMedics, Parvo, UT, USA).[34; 35; 36] Briefly, the initial 10 minutes of the test were discarded while flow through a ventilated hood was adjusted to result in expired CO2 normalized at approximately 1.1%. Data collection continued for an additional 30 minutes and REE was calculated as the 10-minute average with the lowest coefficient of variation. A research nurse then inserted an indwelling-catheter into the antecubital vein. Following a short acclimatization period, baseline assessments were made for the following: hormonal regulators of appetite (insulin, leptin, ghrelin, glucagon-like peptide-1 [GLP-1], and polypeptide YY [PYY]); subjective ratings of hunger and satiety using visual analog scales (VAS);[37] the Three-Factor Eating Questionnaire to assess dietary restraint, disinhibition, and hunger;[38] and the Control of Eating questionnaire to assess food craving, dietary restraint, and mood.[39]

Participants were then provided a breakfast sandwich, chocolate milk, and 236 mL (8 ounces) water. The sandwich and chocolate milk amounts varied by participants to match approximately 40% of measured REE, while maintaining a macronutrient composition of 50% of kcals from carbohydrates, 30% from fat, and 20% from protein resulting in a food quotient of 0.883.[40; 41] Participants were instructed to eat the entire meal within 15 minutes. Blood and VAS assessments were repeated post meal at 15, 45, 60, 90, 120, 150, and 180 minutes. Blood was collected in 4 mL vacutainers and placed immediately on ice and transported to the biochemistry lab and 40 µL of aprotinin was added to the vacutainer, inverted, and placed back on ice. Within 30 minutes samples were centrifuged at 3000 rpm at 4°celsius for 15 minutes. Plasma was

aliquoted and stored in -80°celsious freezer for later analysis. At the completion of the study, all samples were processed by an outside laboratory (Preclinical Models Core, University of Kansas Medical Center) using a human metabolic multiplex assay. The coefficient of variation across all samples are as follows: ghrelin (active) 11.9%, GLP-1 (total) 9.7%, insulin 7.0%, leptin 8.3%, and PYY (total) 4.9%. Thermic effect of feeding (TEF) assessments were collected on a subset of participants from 15-45 minutes, 60-90 minutes, and 120-150 minutes. TEF assessments and analysis was made using the same protocol as REE, except data was collected for 30 minutes rather than 40 minutes during REE. Participants completing the TEF assessments (n=20) were similar to those not (n=26) in terms of group assignment (with TEF: NW/HighACT= 5/11 participants, NW/LowACT= 6/13 participants, OW, OB/HighACT= 7/14 participants) except for the OW, OB/LowACT group (2/8 participants).

At 3.5 hours following the breakfast meal, participants were given access to an *ad libitum* cheese pizza buffet, served individually in a quiet room. The pizza (58% carbohydrate, 25% fat, 17% protein) was served in 435 kcal portions, cut into six non-uniform pieces to make it difficult for the participant to determine amount consumed, along with 236 mL (8 ounces) of water. Participants were instructed they could eat as much as they would like until they were comfortably full. Research associates observed the meal from a window outside of the room and provided a new plate of pizza when the participant began eating the last piece on the plate. The meal was terminated when five minutes passed without the participant eating, and a final VAS assessment was made. Plate weight was measured before and after the meal, and total kcals consumed were calculated.

Free living energy balance.

At the conclusion of the appetite assessment session, participants began a 14-day free-living assessment period for physical activity and dietary behavior. Participants were provided a wrist-worn Actigraph GT9x accelerometer (Actigraph, Inc, Pensacola, Florida, USA) and thigh-worn ActivPAL3 (PAL Technologies Ltd., Glasgow, UK). Actigraphs were initialized using a 30hz sampling rate and worn on the non-dominant hand. Participants were instructed to wear the activity monitors at all times over the next 14 days, including when sleeping and showering. Any non-wear time was self-recorded in a log, along with daily time to bed. Dietician-administered

dietary recalls conducted via phone were completed on three randomly selected days (two week days, one weekend day) by a registered dietician using a multi-pass approach.[42; 43] Participants were given a 15-minute tutorial at the start of the assessment period on how to assess portion sizes using a food portion visual handout, which contains a variety of shapes and images (measuring cups, glasses, plates, etc.) to be used during dietary recall. The first day of the 14-day free-living assessment period was excluded for both physical activity monitoring and dietary recalls given participation in the appetite assessment session during that day.

Visit 2- Fitness testing.

Between 15-18 days following the appetite assessment session, the participants returned for a fitness test. The participants returned their activity monitors and non-wear log, and conducted another assessment of height, weight, and body composition following the protocols previously described. Participants then changed into exercise clothes and completed submaximal and maximal exercise tests. The submaximal exercise test consisted of walking at two speeds for five minutes each corresponding to approximately 60% and 70% of age-predicted maximal heart rate.[44] Following a five-minute break, the maximal test was conducted using the Bruce protocol.[44] During both the submaximal and maximal tests, heart rate was measured via monitor (Polar) and gas exchanged was measured using indirect calorimetry (TrueOne, ParvoMedics, Parvo, UT, USA). Delta efficiency was calculated using the steady-state submaximal exercise test (RER <1.0) as an indicator of the body's ability to transfer energy into work.[45] Work rate was estimated for each stage based on American College of Sports Medicine walking equation using speed and grade of the treadmill[44] as described by Scheadler and Devor[46] and energy expenditure was based on measured oxygen consumption. Delta efficiency was calculated as the reciprocal of the slope for the plot of work rate and energy expenditure.[47] Grip strength was also assessed according to the protocol put forth in the American College of Sports Medicine Guidelines for Exercise Testing and Prescription, 11th edition, using a handgrip dynamometer.[48] The grip bar was adjusted so the second joint of the fingers fits over the handle and the dynamometer is adjusted to zero. The participant stood with their feet slightly apart and held the dynamometer in line with the forearm at the level of the thigh, away from the body. The participant squeezed the dynamometer as hard as possible with neither the hand or the

dynamometer touching the body. Each hand is tested twice with the maximal value for each hand recorded.

Statistical approach.

Participant characteristics were based on demographic and physiological measurements and reported using means and standard deviations for continuous variables and percentages for categorical variables. Actigraph data was analyzed using ActiLife 6 (Actigraph, LLC), using Choi 2011 to calculate wear time. ActivPAL data was analyzed using PALbatch (version 8.11.1.63, PAL Technologies, LLC). Area under the curve (AUC) was calculated for appetite variables using the trapezoidal rule; incremental AUC (iAUC) was calculated in a similar manner, with each timepoint value subtracted from baseline values. All hormones and peptide analyses included data from the following timepoints: fasted and post-meal 15, 45, 60, 90, 120, 150, and 180 minutes; glucose and insulin AUC and iAUC were calculated using fasted and post-meal 15, 45, 60 minute post meal timepoints.[49] Hormones and peptides related to appetite were log transformed to improve normality. Statistical significance for comparison between groups was tested using analysis of variance (ANOVA) for continuous variables and chi-square tests for categorical variables, with adjusted for multiple comparisons using the Tukey-Kramer procedure. All computations were performed using SAS® 9.4 (Cary, N.C.) with the exception of the submaximal exercise testing linear regression analysis which was completed in Graphpad Prism 9.0.0. A post hoc power analysis was completed using G*Power (version 3.1.9.7).

Results

Participant characteristics

Participant descriptive characteristics by group are presented in Table 1. By design, the participants differed in body weight and physical activity metrics based on grouping. The NW/HighACT and NW/LowACT groups were significantly different from the OW, OB/HighACT and OW, OB/LowACT groups by body weight, BMI, BMI percentile, and BMI z-score. Likewise, the NW/HighACT and OW, OB/HighACT groups differed from the NW/LowACT and OW, OB/LowACT by vector magnitude average daily counts and steps per day. Time spent in sedentary behavior (minutes per day spent sedentary >1 hour but <2 hours) differed between the NW/HighACT, NW/LowACT, OW, OB/LowACT. In addition to expected

differences by group for body composition (normal weight groups differing from overweight, obese groups), OW, OB/ LowACT had higher amounts of fat mass, trunk mass, and percent body fat compared to the OW, OB/ HighACT group. The only significant difference in fat-free mass was between the NW/ LowACT group (50.5±4.4 kg) and the OW, OB/ HighACT group (61.5±8.6 kg). There was no difference between groups for age, height, percent Non-Hispanic White, or self-reported puberty status (genital or pubic hair). Average activity monitor wear time was 17hr and 53 min per day and average number of diet recalls completed was 2.9 out of a possible 3.

Resting metabolic rate was lowest in the NW/ LowACT group (1502±134 kcal/day) and was statistically different from the OW, OB/ HighACT (1964±293) and OW, OB/ LowACT group (1829±232). When adjusted for differences in body weight, NW/ LowACT (1594±105) REE remained lower than OW, OB/ HighACT (1860±206, P=0.0017) but not NW/HighACT (1736±140, P=0.21) or OW, OB/ LowACT (1790±244, P=0.08). Peak oxygen consumption in mL/kg/min during the exercise test was statistically highest (P<0.05) in the NW/ HighACT group (57.2±6.8 mL/kg/min) compared to each of the other groups, with OW, OB/ LowACT the lowest (37.8±7.3 mL/kg/min). When peak oxygen consumption was examined in L/min or normalized to fat-free mass, the NW/ LowACT had values (2.83±0.56 L/min, 56.0±10.0 mL/FFMkg/min) lower than the NW/ HighACT (3.70±0.46 L/min, 66.3±6.5 mL/FFMkg/min) and OW, OB/ACT (4.01±0.79 L/min, 64.5±6.5 mL/FFMkg/min) groups. The relationship between heart rate and oxygen consumption response during submaximal and maximal exercise tests are plotted in **Figure 1, panel A**. The intercepts of the line for this relationship differed between groups (P=0.007), with the slope of the lines not different than zero for the NW/ LowACT and OW, OB/ LowACT groups. The relationship between work rate and energy expenditure during submaximal exercise is plotted in **Figure 1, panel B**, along with delta efficiency. These findings indicate that LowACT groups required more energy to generate the same amount of work during exercise.

Appetite responses

The mean breakfast meal was 576 kcal and did not vary by group (P=0.26). Self-rated AUC appetite responses are presented in **Figure 2**. The NW/ LowACT group differed from the OW, OB/ LowACT group for hunger (8908.125±772 score*min vs. 5354±1010 score*min),

fullness (9024±773 score*min vs. 13039±985 score*min), satiety (9398±596 score*min vs. 13042±759 score*min), and prospective consumption (10964±810 score*min vs 5605±1033 score*min). The OW, OB/ HighACT group also differed with the OW, OB/ LowACT group for fullness, satiety, and prospective consumption. **Table 2** displays the appetite hormone and peptide values. *Post hoc* power analysis identified the following Cohen's *d* effect sizes and power levels; Fasting Log_Insulin: *d*= 0.66, power= 0.48; Insulin iAUC *d*= 1.9, power= 0.95; Fasting Glucose: *d*= 0.54; power= 0.29; Glucose iAUC: *d*= 0.48, power= 0.30; Fasting GLP: *d*= 0.86, power= 0.36; GLP-1 iAUC (log): *d*= 0.89, power= 0.27; Fasting Ghrelin: *d*= 0.58; power= 0.35; Ghrelin iAUC (log): *d*= 0.58, power= 0.3; Fasting Leptin: *d*= 1.87, power= 0.97; Leptin iAUC (log): *d*= 0.86, power= 0.33; Fasting PYY: *d*= 0.83, power= 0.42; PYY iAUC (log): *d*= 0.66, power= 0.39. Fasting insulin (log transformed) was higher in the NW/ HighACT group vs. the NW/ LowACT and OW, OB/ HighACT groups, and the post-meal insulin AUC was higher compared to the OW, OB/ LowACT group. The OW, OB/ LowACT had higher leptin values (log transformed) than all other groups. There were no differences *ad libitum* energy intake measured using plate waste during the lunch buffet during the measurement session or energy intake during the 14-day free living assessed via dietary recalls. However, the ratio between energy intake and REE for all groups except NW/HighACT were less than 1.35, suggesting underreporting as defined by Goldberg et al.[50] Overall, free living energy intake was correlated with REE (ρ=0.38, $p<0.05$) and FFM (ρ=0.46, $p<0.001$).

We observed significant negative correlations between insulin and hunger AUC (-0.41) and between glucose and hunger AUC (-0.34) overall, but not by group (**Table 3**). We observed significant positive correlations between insulin and fullness AUC (0.44) and glucose and fullness AUC (0.37) overall, with a higher correlation (0.58) for the NW, LowACT group (**Table 4**). Additional significant correlations were found between insulin and satiety (0.41) and insulin and prospective consumption (-0.33) overall, but not by group (**Tables 5 & 6**). The NW, LowACT group had a lower thermic effect of feeding (adjusted for group differences in fat-free mass) AUC compared to the OW/OB, HighACT group (50224 kcal*min vs. 90903 kcal*min, P<0.05) (**Figure 3**); there no differences in TEF between groups in unadjusted models (NW/HighACT= 49072±19490 kcal*min, NW, LowACT= 53496±7230 kcal*min, OW, OB/ HighACT= 49729±17794 kcal*min, OW, OB/ LowACT= 76276±15210 kcal*min).

Discussion

The primary finding from this study is that normal weight low active adolescent males possess a physiological phenotype that may be indicative of subclinical metabolic dysfunction. Physiological variables in the NW/ LowACT group that were different than their peers include lower fat-free mass, cardiorespiratory fitness, glucose/fullness response to a standardized meal, thermic effect of feeding in response to a standardized meal, and lower self-rated fullness and satiety and higher self-rated hunger to a standardized meal. Conversely, the OW, OB/ HighACT group displayed better subjective appetite responses, but higher insulin responses to a standardized meal. Taken together, these results suggest that physical inactivity during adolescence has a negative impact on metabolic health, independent of adiposity.

The NW/ LowACT group had the lowest fat-free mass of any group which was associated with the lowest resting energy expenditure (Table 1). Additionally, they also possessed the lowest maximal oxygen consumption in L/min and mL/FFMkg/min during exercise testing. Even after statistical adjustment for differences in fat-free mass, they also had the lower thermic effect of feeding response (Figure 3). When these findings from the NW/ LowACT group of low fat-free mass, low oxygen consumption relative to fat-free mass, and low thermic effect of feeding statistically adjusted for differences in fat-free mass, are considered alongside the lower self-rated fullness and satiety and higher self-rated hunger to an standardized meal, it extends previous findings suggesting inactivity creates a metabolic phenotype characterized by reduced skeletal muscle mass accompanied with oxidative dysfunction resulting in dysregulated appetite.[51; 52; 53]

It has been proposed[54; 55; 56; 57; 58] that overall appetite is regulated through three components: 1) a tonic (i.e. constant) drive for food driven by energy demands; 2) tonic inhibition from signals of energy storage (e.g. leptin); 3) episodic (i.e. response-initiated) signals from the periphery in response to food. This approach emphasizes the contribution of physical activity and energy expenditure on both the tonic excitatory drive to eat and episodic regulation of fasting hormones (ghrelin) and satiety hormones (GLP-1 and PYY) [56; 59; 60]. Importantly, this new formulation recognizes the role of resting energy expenditure[12; 61] and fat free mass on appetite control.

Within this perspective, in the present study we did not observe any differences between groups in terms of hormones or peptides associated with appetite (ghrelin, PYY, GLP-1) in either the fasting state or in response to a meal (i.e., episodic signals). This was not the case with variables associated with the tonic drive to eat or tonic inhibition of energy intake. For example, the NW/LowACT group possessed low FFM and resultant low REE, and also lower self-rated fullness and satiety and higher self-rated hunger to a standardized meal (i.e., lower drive to eat). Conversely, the OW, OB/HighACT group possess the highest amount of FM and resultant fasting leptin, and also the highest fullness and satiety and lowest self-rated hunger in response to a standardized meal (i.e., inhibition of energy intake).

The strengths of this study include a diverse set of assessments that provide a detailed physiological and metabolic profile of healthy adolescent males. These diverse assessments combined with participants recruited across four categories based on physical activity level (high active, low active) and body weight status (normal weight, overweight/obese) for a comprehensive examination of metabolic phenotypes. This design affords the opportunity to identify specific contributions of each with minimal statistical adjustments.

However, it is also true that *a priori* grouping of participants reduces statistical power to detect differences between groups, especially when the differences may be small. We attempted to minimize this impact by only including adolescent males in this project, which of course results in additional, separate limitations in terms of generalizability. We acknowledge these limitations and believe future research should extend the finding here by expanding the sample sizes in all categories and include female participants where early life obesity and metabolic dysfunction are also a crucial problem. For biochemical analysis we used a serine protease inhibitor, aprotinin, but not a DPP-4 inhibitor, which may have resulted in degradation of gut hormones. When combined with the limited group samples sizes as described above, this may explain lack of significant findings for ghrelin, leptin, GLP-1, and PYY, especially in response to a meal. There are no specific criteria that encompasses a complete ‘metabolic’ or ‘physiological’ profile, and certain relevant variables associated with each (insulin sensitivity via hyperinsulinemic euglycemic clamp, skeletal muscle substrate oxidative capacity, hepatic metabolism and lipid storage, etc.) were not included in this project. Future work should include these variables to inform a complete understanding of adolescent metabolic and physiologic

health. Finally, given cross-sectional nature of the study design we cannot determine causality, nor can we be certain that any results are the result of chronic physical activity or merely a response to the acute activity accumulated in the data collection period.

In conclusion, we observed that adolescent males who are normal weight but inactive possess a physiological phenotype that may be indicative of subclinical metabolic dysfunction. Conversely, the active overweight and obese adolescents had better subjective appetite responses, but higher insulin responses to a standardized meal. These findings are consistent with research in adults and extend those findings to a novel population of adolescents. Taken together, these results suggest that physical inactivity likely harms metabolic health during adolescence, and poor appetite control may contribute to future weight gain.

Author roles:

RPS: Conceptualization, formal analysis, funding acquisition, writing- original draft

SLL, ASB: Conceptualization, writing- review and edit

TCP, KLH: Writing- review and edit

AMD, JED, JEB, JPT: Conceptualization, supervision, writing- review and edit

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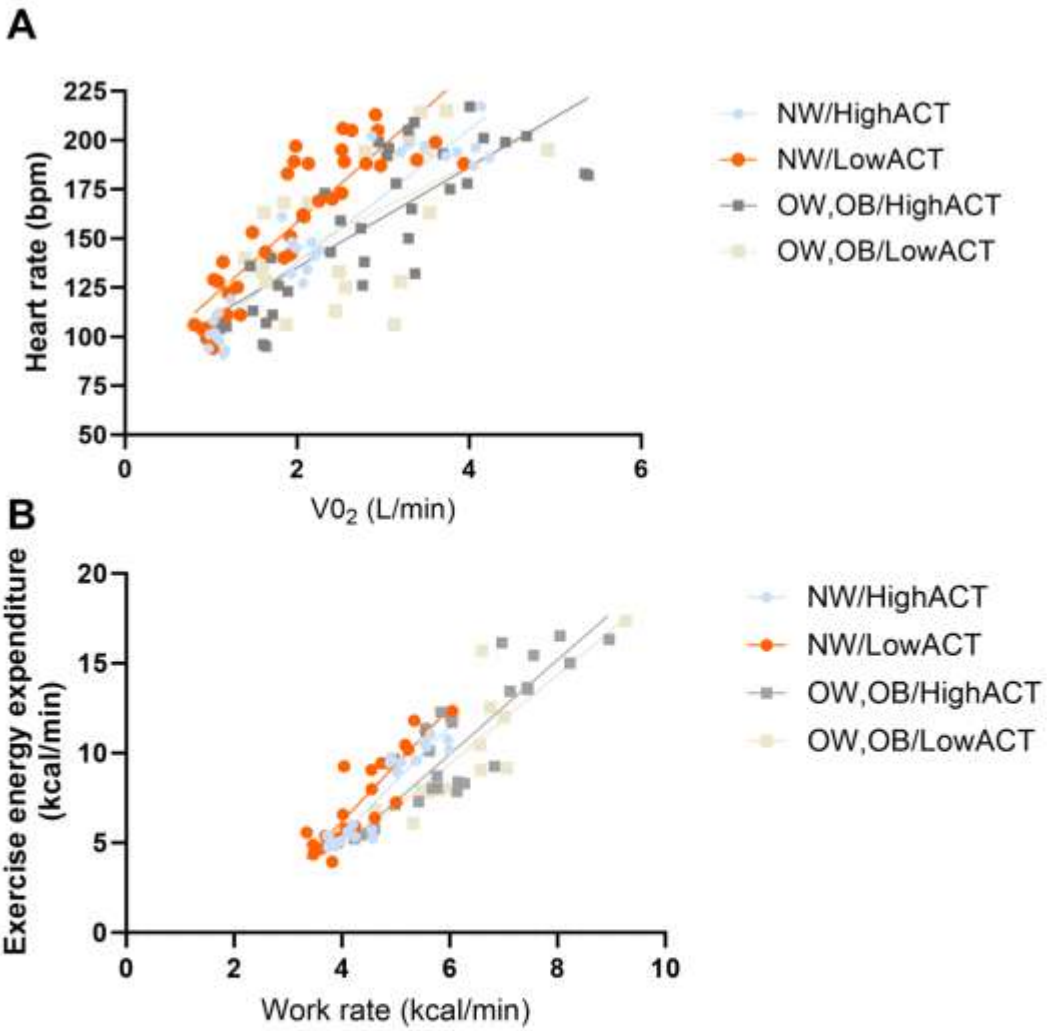

	NW/ HighACT	NW/ LowACT	OW, OB/ HighACT	OW, OB/ LowACT
Work rate, SS1 (kcal/min)	4.08±0.31	3.85±0.35	5.4±0.85	5.79±0.75
Ex. EE SS1 (kcal/min)	5.30±0.40	5.29±0.76	7.3±1.34	7.86±1.02
Work rate, SS2 (kcal/min)	5.40±0.41	5.01±0.53	6.99±1.10	7.04±1.13
Ex. EE SS2 (kcal/min)	10.02±0.68	9.83±1.46	13.65±2.10	13.37±2.60
Delta efficiency (%)	32.9	31.6	38.0	40.5

Figure 1. Heart rate and oxygen consumption responses during submaximal and maximal exercise testing.

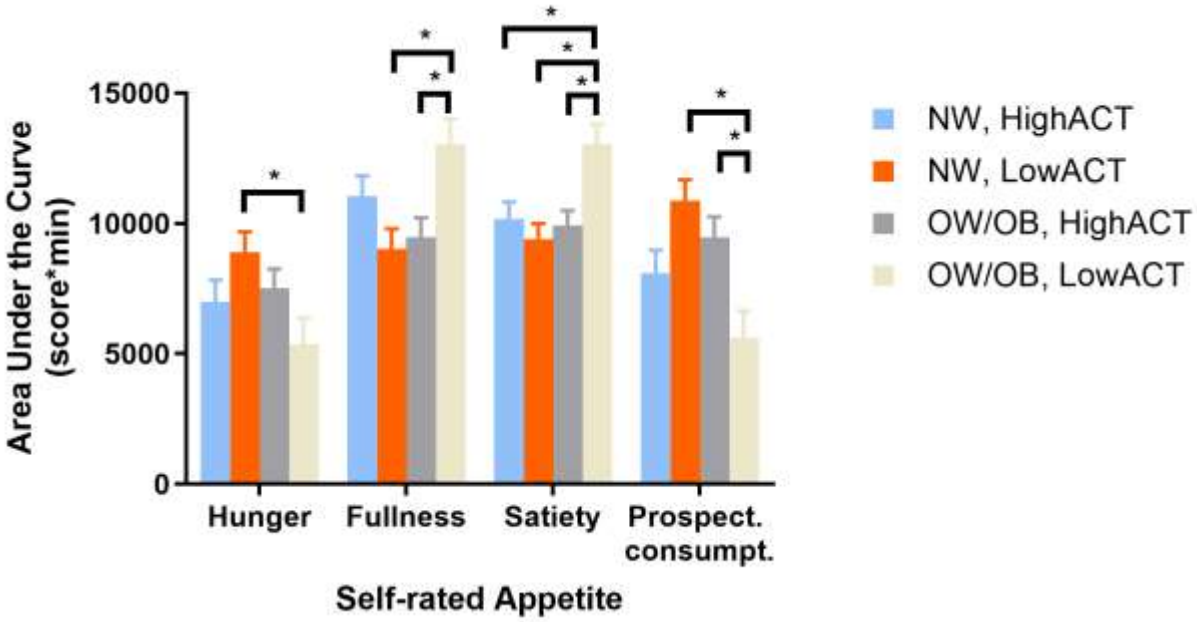

Figure 2. Self-rated area under the curve appetite responses (score*min) following a meal by group ($P<0.05$).

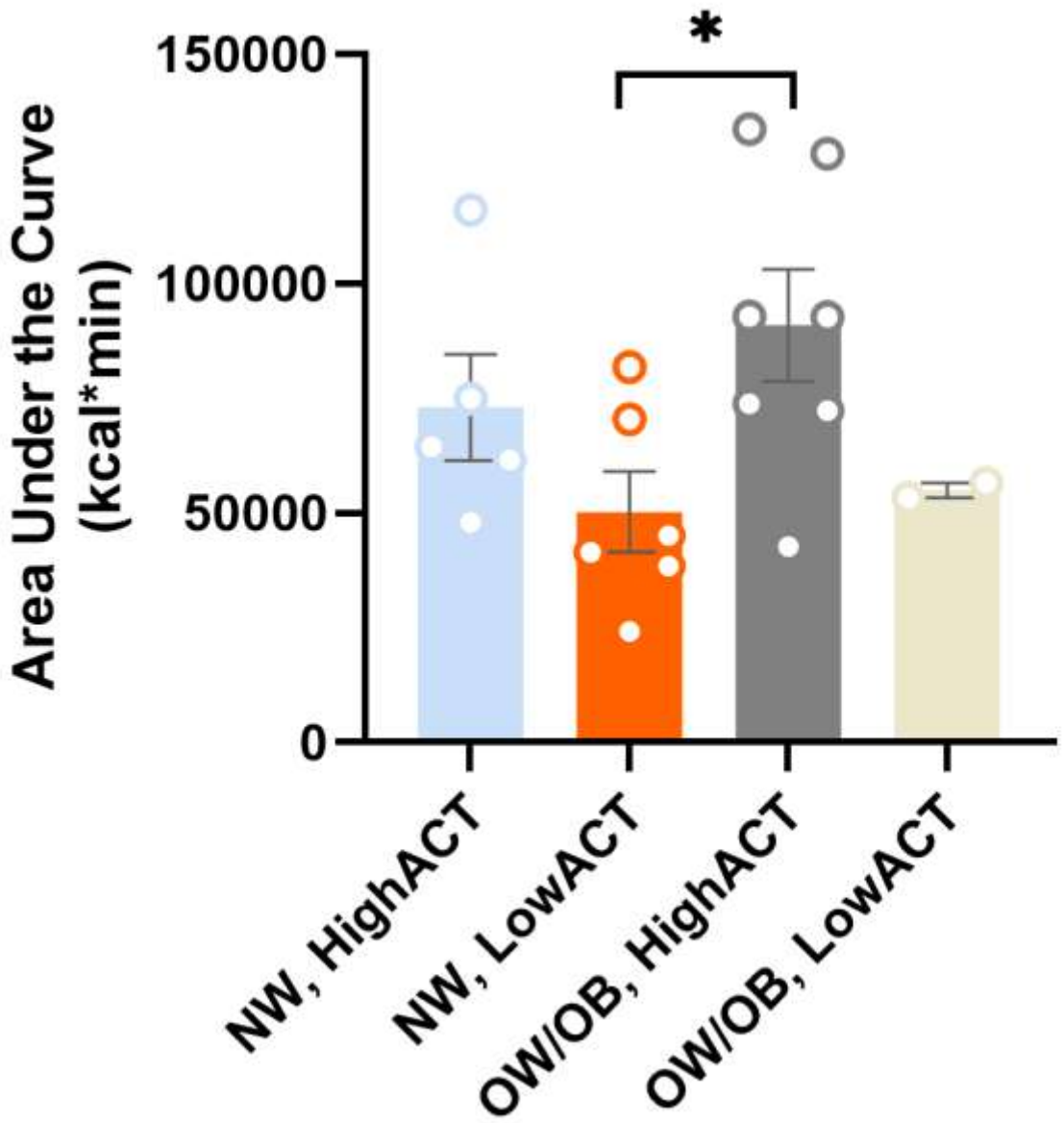

Figure 3. Thermic effect of feeding, adjusted for fat-free mass, incremental AUC (kcal*min, $P<0.05$)

Table 1. Participant characteristics and group differences for key variables.

	NW/ HighACT[1] N=11	NW/ LowACT[2] N=13	OW, OB/ HighACT[3] N=14	OW, OB/ LowACT[4] N=8	Group differences*
Age	16.0±1.2	16.0±1.1	15.6±1.0	15.9±0.6	NS
% Non-Hispanic White	72.7	76.9	78.6	50.0	NS
Genital stage	3.6±0.8	3.8±0.9	4.0±0.7	3.9±0.8	NS
Pubic hair stage	3.8±1.0	4.2±1.1	4.2±0.8	4.0±1.1	NS
Height (cm)	176.4±7.0	173.9±6.1	177.7±9.6	176.7±6.4	NS
Weight (kg)	65.7±6.2	60.8±5.5	85.6±12.9	93.2±11.9	1,2 vs. 3, 4
BMI	21.1±1.5	20.4±1.8	27.0±3.1	29.8±2.2	1,2 vs. 3, 4
BMI%	56.3±18.7	45.3±18.8	92.2±5.2	96.8±2.1	1,2 vs. 3, 4
BMIz	0.2±0.5	-0.1±0.5	1.5±0.5	1.9±0.3	1,2 vs. 3, 4
Fat mass (kg)	9.8±2.5	10.8±3.4	24.3±8.3	34.1±8.4	1,2 vs. 3, 4 3 vs. 4
Trunk fat mass (kg)	3.7±1.2	4.4±1.6	11.9±5.0	17.3±4.7	1,2 vs. 3, 4 3 vs. 4
Fat-free mass (kg)	56.4±5.8	50.5±4.4	61.5±8.6	58.6±10.1	2 vs. 3
Fat mass (%)	14.9±0.04	17.6±0.05	28.0±0.07	36.5±0.07	1,2 vs. 3, 4 3 vs. 4
REE (kcals/day)	1675±204	1502±134	1964±293	1829±232	1 vs. 3 2 vs. 3, 4
VO_{2PEAK} (mL/kg/min)	57.2±6.8	46.1±8.7	46.8±6.9	37.8±7.3	1 vs. 2, 3, 4 2 vs. 4
VO_{2PEAK} (L/min)	3.70±0.46	2.83±0.56	4.01±0.79	3.49±0.65	1 vs. 2 2 vs. 3
VO_{2PEAK} (mL/FFMkg/min)	66.3±6.5	56.0±10.0	64.5±6.5	59.6±5.9	1 vs. 2 2 vs. 3
Vector magnitude average counts	2141±284	1590±200	2181±314	1345±268	1, 3 vs. 2, 4
Steps (steps/day)	11,923±5111	6828±2347	9530±2480	4914±2356	1, 3 vs. 2, 4
Sedentary time (min/day)	73.8±31.7	132.9±43.4	94.6±76.2	154.6±57.7	1 vs. 2, 4 3 vs. 4
Energy intake (kcals/day)	2536±789	1800±656	2377±654	1891±753	NS
Peak grip strength (kg)	38.1±6.5	39.1±8.6	43.1±10.3	42.2±12.1	NS

NW= normal weight; OW, OB= overweight or obese; ACT= active; BMI%= BMI percentile based on CDC guidelines; BMIz= BMI z-score based on CDC guidelines

*P<0.05

Table 2. Fasting and incremental area under the curve responses to a meal for hormones and peptides related to appetite.

	All Mean±SD	**NW/ HighACT [1] N=11 Mean±SE**	**NW/ LowACT [2] N=13**	**OW, OB/ HighACT [3] N=14**	**OW, OB/ LowACT [4] N=8**	**Group differences**
Fasting Log_Insulin	6.20±0.77	6.91±0.25	5.99±0.20	5.98±0.19	6.14±0.32	1 vs. 2, 3
Insulin iAUC (mg/dL*min)	88404±44973	65056±11498	85913±11838	85377±11373	139610±18339	1 vs. 4
Fasting Glucose (mg/dL)	91.5±8.0	90.5±2.7	89.9±2.2	91.9±2.2	95.6±3.3	NS
Glucose iAUC (mg/dL*min)	743±760	904±257	823±214	511±206	872±315	NS
Fasting GLP (pg/mL)	33.2±29.0	32.5±10.9	44.5±8.3	24.8±9.1	17.9±16.7	NS
GLP-1 iAUC (log) (pg/mL*min)	17.3±124.0	53.2±52.7	9.5±48.8	18.3±48.8	-66.8±91.4	NS
Fasting Ghrelin (pg/mL)	67.5±23.8	65.9±8.0	67.2±7.0	63.1±6.7	79.9±9.3	NS
Ghrelin iAUC (log) (pg/mL*min)	791±50	46.3±19.1	63.6±17.1	48.5±15.0	33.3±24.2	NS
Fasting Leptin (pg/mL)	2083.1±2761	1073.8±772	722.1±605.7	2443.1±605.7	6300.5±976.7	1, 2, 3 vs. 4
Leptin iAUC (log) (pg/mL*min)	1179±238	44.2±13.6	54.5±11.1	20.9±10.6	16.1±22.1	NS
Fasting PYY (pg/mL)	63.1±33.4	72.9±12.1	66.5±9.9	59.6±9.2	51.6±14.0	NS
PYY iAUC (log) (pg/mL*min)	741±78	9.8±15.0	9.3±12.3	21.0±11.8	31.2±19.0	NS

*P<0.05

Table 3. Correlation between hunger, insulin, and glucose area-under-the-curve responses to a breakfast meal, overall and by physical/adiposity group

Group	Insulin * Hunger	Glucose * Hunger
All	**-0.41 (-0.65 to -0.08)***	**-0.34 (-0.59 to -0.02)***
NW/ HighACT	-0.37 (-0.87 to 0.55)	-0.11 (-0.75 to 0.65)
NW/ LowACT	-0.23 (-0.73 to 0.44)	-0.35 (-0.76 to 0.29)
OW, OB/ HighACT	-0.33 (-0.75 to 0.31)	-0.27 (-0.71 to 0.34)
OW, OB/ LowACT	-0.79 (-0.99 to 0.77)	-0.66 (-0.97 to 0.59)

*P<0.05

Table 4. Correlation between fullness, insulin, and glucose area-under-the-curve responses to a breakfast meal, overall and by physical/adiposity group

Group	Insulin * Fullness	Glucose * Fullness
All	**0.44 (0.13 to 0.67)***	**0.37 (0.07 to 61)***
NW/HighACT	0.24 (-0.64 to 0.83)	0.38 (-0.46 to 0.85)
NW/LowACT	0.20 (-0.43 to 0.69)	**0.58 (0.14 to 0.85)***
OW,OB/HighACT	0.34 (-0.27 to 0.74)	0.37 (-0.22 to 0.75)
OW,OB/LowACT	0.51 (-0.71 to 0.96)	-0.16 (-0.86 to 0.75)

*P<0.05

Table 5. Correlation between satiety, insulin, and glucose area-under-the-curve responses to a breakfast meal, overall and by physical/adiposity group

Group	Insulin * Satiety	Glucose * Satiety
All	**0.41 (0.09 to 0.64)***	0.18 (-0.14 to 0.46)
NW/ HighACT	0.60 (-0.23 to 0.91)	-0.42 (-0.84 to 0.36)
NW/ LowACT	0.01 (-0.56 to 0.58)	0.38 (-0.23 to 0.76)
OW,OB/ HighACT	0.33 (-0.28 to 0.74)	0.40 (-0.18 to 0.76)
OW,OB/ LowACT	0.36 (-0.79 to 0.94)	-0.22 (-0.87 to 0.73)

*P<0.05

Table 6. Correlation between prospective consumption, insulin, and glucose area-under-the-curve responses to a breakfast meal, overall and by physical/adiposity group

Prospective consumption	Insulin * Prospective consumption	Glucose * Prospective consumption
All	**-0.33 (-0.59 to -0.01)***	-0.08 (-0.37 to 0.23)
NW/ HighACT	-0.35 (-0.84 to 0.49)	0.21 (-0.54 to 0.76)
NW/ LowACT	0.11 (-0.50 to 0.64)	-0.17 (-0.66 to 0.42)
OW,OB/ HighACT	-0.20 (-0.67 to 0.41)	-0.14 (-0.62 to 0.43)
OW,OB/ LowACT	-0.32 (-0.93 to 0.80)	0.31 (-0.69 to 0.89)

*P<0.05