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Title: Influence of netball-based exercise on energy intake, subjective appetite and plasma acylated ghrelin in adolescent girls.

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Abstract

This study explored 5-d regulation of exercise-induced energy expenditure, energy intake, and hormonal appetite, via acylated ghrelin, after acute exercise. Using a randomized crossover design, 10 female adolescents (13-15 y) completed two 7-d treatment weeks (2-d maintenance; 1-d treatment; 4-d follow-up), interspersed with a 1-wk period. On day 3, 47-mins of netball-based exercise or sedentary activity was imposed with a test meal 1-h later. Measures of energy expenditure, subjective appetite, test meal energy intake, plasma acylated ghrelin, insulin, and glucose were taken during this period. Energy intake compensation for the exercise period was calculated. 4-day follow-up measures were daily subjective appetite, energy intake, energy expenditure and energy balance. Girls felt more full 20-min during the netball-based exercise bout compared with sedentary (87±15v75±24 mm). An energy intake compensation of 27% was identified for the netball-based exercise. Compared with immediately before exercise/sedentary, plasma acylated ghrelin was elevated 45-min after netball (103.8±56.9v85.7±26.9 pg·mL^{-1};n=7) and sedentary (98.2±27.1v60.8±33.5 pg·mL^{-1};n=7) but not different between treatments. Adolescent girls (13-15 y) only partially compensated for the netball-based exercise-induced energy expenditure. The effect of exercise on appetite needs to be further explored in adolescents, whereby nutritional behaviour is tracked for >1-wk to investigate full compensation for acute exercise.

Key words

Adolescents, netball-based exercise, subjective appetite, energy intake, acylated ghrelin, energy balance

Introduction

Findings regarding the acute effects of exercise on energy intake (EI) in children and adolescents are equivocal, with several authors reporting no differences after exercise (Bozinovski et al. 2009, Dodd et al. 2008, Moore et al. 2004, Rumbold and Dodd 2007) and others describing a change (Rumbold et al. 2011a, Thivel et al. 2011a, Thivel et al. 2011b). With regards to subjective appetite and hunger, most studies have observed increases in appetite and/or hunger in girls after exercise (Bellissimo et al. 2007, Bozinovski et al. 2009, Dodd et al. 2008, Rumbold et al. 2011a), whilst one study has observed the opposite in young boys and girls, respectively (Bozinovski et al. 2009, Moore et al. 2004). Indeed, we have previously alluded to the possibility that the subjective nature of the visual analogue scale (VAS) questions might be a contributory factor towards the identification of such varied results (Rumbold et al. 2011a).

Given that the majority of paediatric studies have identified an increase in subjective hunger/appetite, examination of the appetite stimulating hormone, ghrelin, could provide insights into these energy and appetite interactions, since this is the only known orexigenic hormone (Cummings 2006), stimulating neuropeptide Y and agouti-related peptide and therefore feeding (Wren et al. 2001). Acylated ghrelin is the most active form, possessing the pituitary and pancreatic endocrine associated activities important for appetite regulation (Broglio et al. 2003). Several studies in adults have been conducted exploring the influence of exercise on acylated ghrelin, the majority of which have identified a suppression after exercise (Broom et al. 2007, Broom et al. 2009, King et al. 2010, Marzullo et al. 2008, Ueda et al. 2009, Unick et al. 2010).

To date, one study has investigated the acute effects of exercise on total ghrelin in healthy 10-18 y boys (Pomerants et al. 2006) and only one has specifically examined acylated ghrelin in healthy adolescent girls (Sauseng et al. 2010). The latter study (Sauseng et al.

2010) identified that plasma acylated ghrelin levels were higher in a group of adolescent girls who completed a short bout of exhaustive cycling exercise compared with a control group. These data provide a foundation for further exploration of acylated ghrelin responses to exercise in an adolescent population. However, these studies did not employ a within-groups experimental design, in addition to the exercise bouts being unrepresentative of young people's habitual physical activity. In English Schools, the National Curriculum for Physical Education anchors teachers to deliver sporadic game-based activities such as netball, which we have successfully used in one of our previous studies to serve as a bout of representative exercise for a group of adolescent girls (Rumbold et al. 2011a). Indeed, we identified an elevation in total-daily EI (TDEI) over the 48-h period after the netball-based exercise in the group of female adolescent netball players (Rumbold et al. 2011a). In order to provide a holistic view of energy and appetite regulation in young people, both the acute EI and appetite response to representative exercise should be explored, in conjunction with TDEI, total-daily energy expenditure (TDEE), energy balance and subjective appetite responses over a follow-up period of several days.

Consequently, the present study proposed is the first to assess short term acylated ghrelin and test meal EI responses to acute netball-based exercise and a corresponding sedentary period, in a female adolescent group. In addition, TDEI, TDEE, energy balance and subjective appetite was explored over four follow up days.

Materials and methods

Design

A within-participants, randomised cross over design was used to compare the EI and appetite of 13–15 y girls over 7-d (2-d maintenance, 1-d treatment, 4-d follow-up). The study was approved by the University of Northumbria, School of Life Sciences Ethics Committee.

Prior to data collection written informed consent was obtained from both the children and parents or guardians. They were notified that the study was to investigate general aspects of diet and exercise, rather than specific EI and appetite following an exercise bout, to ensure no alteration to eating habits as a result of knowledge of the study methodology.

Participants

Ten girls from a local secondary school in the North East of England took part in the study [age (mean±SD) 14.6±1.1 y; stature 1.66±0.10 m; body mass 52.7±6.8 kg; body fat 18.3±3.0% and BMI 19.0±1.8 kg/m^2]. All girls were classified as being normal body mass, as determined using age and gender-specific classification centiles for the United Kingdom, where BMI scores which fell between the 9th centile and the 91st centile were accepted as an indicator of a healthy BMI (Cole et al. 2000). The maturity offset was 2.1±1.0 y from peak height velocity, indicating that all the girls had a positive maturity offset and were of a similar maturation status. In addition, all girls were classified as unrestrained eaters according to the Dutch Eating Behaviour Questionnaire (van Strien et al. 1986), with the mean (±SD) dietary restraint score (1.93±0.93) falling into the average category for high school females (1.85±2.55). All girls were habitually active, participating in physical activity two to three times per week, including at least one netball-based session per week as part of the National Curriculum. For logistical reasons, the girls were tested in two groups of five.

Preliminary testing

On the first visit to the laboratory, the girls were familiarised with the food weighing and recording process, food diaries (Livingstone et al. 1992b) and VAS. During this session the participants completed a full familiarisation of the netball-based exercise session, heart rate (HR) monitors and receivers (Polar RS-400 and S610i, Heart Rate Monitors, Polar, O.Y.

Finland) and Actigraph™ GT1M monitors, used for assessing physical activity counts. Secondly, anthropometric data (stature, seated height, body mass and percentage body fat) were collected as previously described (Rumbold et al. 2011a). To ensure that the girls were of a similar maturation status, maturity offset was calculated using sex-specific multiple regression equations, to predict years from peak height velocity, using the growth patterns of height, seated height and leg length (Mirwald et al. 2002). Finally, with participants at least 2-h post-prandial and rested for 20–30 min, individual 'FLEX' HR [defined by Ceesay et al. (1989) 'as the mean of the highest HR during rest and the lowest HR during the lightest imposed exercise'] and physical activity count calibrations were conducted for each participant, to establish a regression line for HR and physical activity counts against oxygen uptake (Ceesay et al. 1989). Heart rate, physical activity counts and oxygen uptake (breath-by-breath then averaged over 60 s) were measured simultaneously during 4 min stages of lying supine, sitting, standing and over the last 2 min of levels 1–4 of an adapted incremental netball exercise fitness test (Rumbold 2010). Each girl then continued to complete the fitness test with intensity increasing every 2 min to identify peak oxygen uptake ($\dot{V}O_{2\ peak}$). The criteria used to determine when $\dot{V}O_{2\ peak}$ had been reached were based on those proposed by Armstrong and Welsman (2001). These included voluntary exhaustion, despite continued verbal encouragement (nonstandardised), subjective endpoints such as facial flushing, sweating, hyperpnoea and an unsteady gait, and either heart rate levelling off or a respiratory exchange ratio ≥ 1.00. Oxygen uptake was monitored using a facemask, attached via breathing tubes to a Metamax 3B portable gas analyser (Cortex, Leipzig, Germany). Consequently, individual FLEX HR [as described in one of our previous studies (Rumbold et al. 2011a)], and physical activity thresholds were calculated for each girl to differentiate between sedentary and exercise-induced EE.

The next day, resting metabolic rate of each participant was assessed in the laboratory after a 12-h period of fasting and rest. Resting metabolic rate was later used to contribute towards the calculation of TDEE. A 5-10 min rest period preceded a 5-10 min 'settling in' period which was then followed by a 12-16 min measurement period (Ventham and Reilly 1999). The Quark b^2 breath-by-breath pulmonary gas exchange system (Quark b^2, Cosmed, Rome, Italy), with the canopy attachment was used.

Finally, on the Monday preceding the netball and control weeks, the girls were asked to complete a self-reported weighed food record of their lunchtime EI. On this day the girls chose to consume white pasta in a tomato and herb sauce with grated mild cheddar cheese, thus such food items were used to later comprise the test meal.

Netball-based exercise and sedentary weeks

Each week was separated by a 1-wk 'wash-out' period. Participants were randomly allocated to each week (condition) and acted as their own controls, thus ultimately taking part in both the exercise and sedentary periods.

Saturday and Sunday of each week were maintenance days and researchers liaised with parents to facilitate this. Participants were asked to replicate their food and fluid intake and portion sizes between weeks, using photocopies of the first week's self-reported, weighed food diaries. Participants were also asked to refrain from physical activity. Heart rate and Actigraph™ GT1M monitors were worn during waking hours to confirm that participants refrained from physical activity in the 2 days prior to the treatment day.

On the Monday (treatment day) of each week, participants were asked to follow their normal morning breakfast routine at home. The same breakfast was consumed, on average, at 07:19 before the netball-based exercise and equivalent sedentary period. Energy intake and macronutrient composition of breakfast was not different between the netball-based exercise and sedentary period [226 ± 141 versus 264 ± 162 kcal respectively, $p = 0.540$; 10 ± 5 versus 9 ± 4 % protein respectively, $p = 0.887$; 64 ± 26 versus 58 ± 24 % carbohydrate respectively, $p = 0.645$; 12 ± 14 versus 19 ± 18 % fat respectively, $p = 0.232$]. At 08:15 the girls were collected from school and were transported to the university. At 09:00 a qualified medical doctor inserted a cannula into an antecubital vein, whilst each participant was in a semi-supine position, out of view of the other participants. If a participant felt nauseous and/or dizzy, or a blood sample could not be obtained at the required time point, this procedure was discontinued. At 10:00 half of the group completed the 47-min netball-based exercise and half participated in sedentary activities. Details regarding the structure and organisation of the 47-min netball-based exercise have been provided previously (Rumbold et al. 2011a). In brief, the session simulated netball exercise, comprising of movements such as walking, jogging and running forwards and backwards, turning, jumping, lunging, sidestepping, foot-specific agility and a choice reaction task, completed without a netball (Gasston and Simpson 2004). Heart rate and physical activity counts (Actigraph^{TM} GT1M) were recorded over 60 s epochs during the netball-based exercise and sedentary period. Visual analogue scales (100 mm) and the Pictorial Children's Effort Rating Table (P-CERT) (Yelling et al. 2002), were administered immediately before and after the netball-based exercise and sedentary period, and at 10, 20 and 30 min during each. These measures provided an indication of subjective appetite (hunger, prospective food consumption and fullness) and ratings of perceived exertion, respectively. The child-specific P-CERT was used as opposed to the Borg scale, as it has been suggested that young people find the numerical values (6-20) of the Borg scale difficult to interpret (Eston and Lamb 2000). After the completion of the netball-based exercise or sedentary period at approximately 10:47, the participants rested for 1-h (sitting reading, watching DVDs or completing school work) and were then offered a test meal at

11:47. The participants had 1-h to consume the test meal before being returned to school by the research team.

Test meal

The test meal consisted of white pasta in a tomato and herb sauce with grated mild cheddar cheese. These food items were provided in excess, equating to 503 g cooked pasta, 112 g of tomato and herb sauce and 30 g of cheese (4.07 MJ). The macronutrient content of the meal provided was 76.5% carbohydrate, 16.5% protein and 7.0% fat. The girls consumed the test meal as a group. Food was covertly weighed and recorded before and after the test meal by the research team. Nutrient information was derived from food packaging in order to estimate test meal EI.

Blood sampling

Venous blood samples were collected in precooled 4.9-mL EDTA monovettes, immediately before and immediately after, and at 15, 30, 45 and 60 min after the netball-based exercise and corresponding sedentary period. The cannula was kept patent using saline solution after each blood collection. All blood samples were collected whilst the participants were in a semi-supine position. The EDTA monovettes were then spun at 1,681 g (4,000 revs·min^{-1}) for 10 min in a refrigerated centrifuge at 4^{o}C. The plasma supernatant was then aliquoted into Eppendorf tubes and then stored at -80^{o}C for analysis of glucose and insulin.

In separate 4.9-mL monovettes, additional blood samples were drawn at the same time points, with the exception of the 60 min post treatment blood sample (due to logistical problems), in order to determine plasma acylated ghrelin concentrations. These monovettes contained EDTA and p-hydroxymercuribenzoic acid to prevent degradation of the acylated ghrelin by protease. They were then spun at 1,287 g (3,500 revs·min^{-1}) for 10 min in a refrigerated centrifuge at 4°C. The plasma supernatants were then aliquoted into Eppendorf tubes and 100 μl of 1M HCl was then added per ml of plasma. Samples were then spun at 1,287 g (3,500 revs·min^{-1}) for a further 5 min. These were then stored at -80°C.

Follow-up days

In both conditions, the girls attended school as normal on Tuesday, Wednesday, Thursday and Friday (4-d follow up). The researchers liaised with parents and staff at the school to ensure participants continued with normal, free-living daily food and fluid intake and curriculum activities, whilst avoiding any physical activities.

Energy expenditure

Heart rate was measured as previously described (Rumbold et al. 2011a) to contribute to the estimation of TDEE. Alongside the monitoring of HR, physical activity counts were concurrently recorded at epochs of 60 s, using an Actigraph™ GT1M monitor. The participants were required to wear the Actigraph™ GT1M monitor over the right hip for the waking hours of the seven study days. Data was retrieved using a USB connector cable via the Actilife Data Analysis Software (Version 3.5.0, Actigraph™, LLC, Pensacola, Florida, USA). Energy expenditure was estimated as 20.5 kJ/L of oxygen consumed/min (Weir 1949) using the pre-determined individual FLEX HR and physical activity count calibrations, calculated as part of the preliminary testing. The simultaneous interpretation of HR and physical activity count data in young people, allows TDEE to be predicted more accurately, as opposed to using HR data alone (Eston et al. 1998). Thus, the HR data was interpreted with regards to the physical activity count threshold value. Therefore, when there was an elevation in HR without an associated increase in physical activity count above the physical activity count threshold value, EE was calculated as the equivalent of the mean oxygen consumption of the resting activities (lying supine, sitting and standing) (Ceesay et al. 1989). This scenario indicated that extraneous variables other than exercise were likely to have caused the elevation in HR to occur, which is not necessarily associated with an elevation in oxygen uptake and thus EE (Livingstone et al. 2000). Consequently, for HR and physical activity count values above the respective thresholds, energy expenditure was determined based on the linear regression between HR, physical activity count and $\dot{V}O_2$ during exercise (Livingstone et al. 1992a).

Energy intake

Total-daily EI, was estimated using a combined self-reported, weighed food diary and 24-h recall interview technique (Rumbold et al. 2011b). The reporting accuracy of this combined technique has been demonstrated to be an effective method to use to collect dietary information in active adolescent girls (confidence interval for bias ranging from 0.00 to 0.92 $MJ \cdot d^{-1}$) (Rumbold et al. 2011b). Total-daily EI was then calculated using a hierarchical method described in one of our previous studies (Rumbold et al. 2011a). Firstly, an online source (http://www.tesco.com/superstore) was accessed to obtain food label information, followed by the actual interpretation of food labels in supermarkets if foods were not available on this site. Finally, some nutrient values were reported according to McCance and Widdowson (Holland et al. 1991).

In addition, EI compensation for the netball exercise-induced EE was calculated as:

[EI on Monday (treatment day) in the netball week (8.94 MJ) – EI on Monday (treatment day) of the sedentary week (8.62 MJ)]/cost of the netball exercise (1.20 MJ)].

The purpose of this information was solely to provide an indication of energy intake, relative to the energy expended during the netball-based exercise bout on the intervention day. This calculation has successfully been used in adult-based energy compensation studies (Stubbs et al. 2002) to provide the same information.

Subjective appetite

During all seven study days (2-d maintenance, 1-d treatment and 4-d follow up) 100 mm VAS were used to assess hunger [‘How hungry do you feel now?’ anchored by *very hungry (100)* and *not at all hungry (0)*], prospective food consumption [‘How much would you like to eat now?’ anchored by *a lot (100)* and *nothing at all (0)*] and fullness [‘How full do you feel now?’ anchored by *very full (100)* and *not full at all (0)*] upon wakening, before and after each meal and immediately before bed.

Biochemical analysis

Plasma acylated ghrelin concentrations were determined by enzyme immunoassay (SPI BIO, Montigny le Breton-neux, France; supplied by Immuno Diagnostic Systems). Plasma glucose concentrations were determined using enzymatic and colorimetric methods (Biosen, EFK, Diagnostics), whilst plasma insulin concentrations were determined using solid-phase Enzyme Amplified Sensitivity Immunoassay performed on microtiter plates (BioSource, INS-EASIA, Europe S.A). Samples from each participant were analyzed in the same run, to eliminate interassay variation. The within-batch coefficients of variation for the two acylated ghrelin assays were 9.1% and 8.1%, respectively and 5.3% for the insulin assay. After the blood samples had been analysed, they were autoclaved then disposed of in clinical waste.

Statistical analysis

The statistical package SPSS (SPSS Inc., Version 16.0. Chicago, IL) was used for data analyses. Means ± SD were calculated for all data. For Saturday and Sunday (maintenance days), TDEE, TDEI, energy balance, and subjective appetite (hunger, prospective food consumption and fullness) were each analysed for main effects and interactions using a 2 (treatment) x 2 (day) ANOVA and for Monday, Tuesday, Wednesday, Thursday and Friday (treatment day and four follow-up days) using a 2 (treatment) x 5 (day) ANOVA. Mean subjective appetite (hunger, prospective food consumption and fullness) were also calculated immediately before, 10, 20 and 30 min during, immediately after, 15, 30, 45 and 60 min after the 47-min netball-based exercise session and equivalent sedentary period and analysed using a 2 (treatment) x 9 (time) ANOVA. In addition, for seven participants mean plasma insulin and plasma glucose values were calculated before, after and 15, 30, 45 and 60 min following the 47-min netball exercise session and equivalent sedentary period and analysed using a 2 (treatment) x 6 (time) ANOVA. Due to missing plasma acylated ghrelin samples at 60 min, a 2 (treatment) x 5 (time) ANOVA was used to analyse this data. Mean ± SD time averaged area under the curve (AUC) data was calculated for acylated ghrelin (pre intervention-45 min post; 92 min), in addition to a paired t-test being conducted to explore any differences. Mean EE during the netball-based exercise and corresponding sedentary period and subsequent test meal EI, were analysed using separate one way repeated measures ANOVA. Where significant differences were identified, post hoc Tukey tests were conducted as appropriate. Cohen's d effect size for one-way ANOVA was also calculated and interpreted against the effect size categories of ≤ 0.1 = small effect, ~ 0.25 = moderate effect and ≥ 0.4 = large effect (Cohen 1992).

Statistical significance was accepted at $p < 0.05$ for all analyses.

Results

Energy expenditure

Total-daily EE on Saturday and Sunday (maintenance days) was affected by week day (higher on the Saturday in both weeks) ($p = 0.005$) but not by treatment ($p = 0.569$) and there were no significant interactions ($p = 0.863$).

For the treatment and follow up days, there was a significant treatment x day interaction ($p < 0.001$), whereby TDEE was elevated on the Monday (treatment day) in the netball week compared with the corresponding day in the sedentary week [(11.14 ± 1.17 versus 10.09 ± 1.07 $MJ \cdot d^{-1}$, ($p < 0.001$, effect size = 3.55)]. In the netball week, post-hoc Tukey tests also confirmed an elevation in TDEE on Monday (treatment day) compared with the follow up days Tuesday, Wednesday, Thursday and Friday [11.14 ± 1.17 versus 9.93 ± 1.09; 9.95 ± 1.10; 9.98 ± 1.04; 9.99 ± 1.08 $MJ \cdot d^{-1}$ respectively, ($p < 0.01$)].

Average exercise-induced EE was higher for the 47-min netball-based exercise compared with the corresponding 47-min sedentary period (1.20 ± 0.22 versus 0.34 ± 0.05 MJ) ($p < 0.001$, effect size = 5.74).

Energy intake

There was no main effect of treatment ($p = 0.223$) or week day ($p = 0.123$) or interaction effects ($p = 0.440$) for TDEI on the Saturday and Sunday (maintenance days).

However, for the treatment and follow up days there was a main effect of week day ($p = 0.006$). Post-hoc Tukey tests identified that TDEI was higher on Monday (treatment day) compared with Friday in both weeks ($p < 0.01$) (Figure 1).

A partial compensation of 27% for the exercise-induced EE was identified on the Monday of the netball week.

Energy intake (MJ) at the test meal was not affected by treatment [netball: 3.25 ± 0.78 versus sedentary: 3.33 ± 0.92 MJ) (p = 0.647)].

Energy balance

Energy balance on Saturday and Sunday (maintenance days) was not affected by treatment (p = 0.248) or week day (p = 0.195) and there were no significant interactions (p = 0.350). Similarly, the treatment day and follow up days were not affected by treatment (p = 0.742) or week day (p = 0.075) and there were no significant interactions (p = 0.199) (Table 1).

Subjective appetite

Daily hunger, prospective food consumption and fullness for Saturday and Sunday (maintenance days) were not affected by treatment (p = 0.538; p = 0.604; p = 0.288, respectively) or week day (p = 0.929; p = 0.695; p = 0.711, respectively) and there were no significant interactions (p = 0.749; p = 0.842; p = 0.732, respectively). Similarly, hunger, prospective food consumption and fullness during the follow up days were not affected by treatment (p = 0.086; p = 0.297; p = 0.459, respectively) or week day (p = 0.478; p = 0.461; p = 0.796, respectively) and there were no significant interactions (p = 0.500; p = 0.305; p = 0.471, respectively).

There was a significant treatment x time point interaction (p = 0.015) for fullness, whereby the girls felt significantly more full 20 min into the 47-min netball-based exercise compared with the corresponding time point during the sedentary period (87 ± 15 versus 75 ± 24 mm) (p = 0.026, effect size = 1.25) (Figure 2).

Acylated ghrelin, insulin and glucose

Plasma acylated ghrelin was not affected by treatment ($p = 0.764$) and there was no time x treatment interaction ($p = 0.138$) but there was a main effect of time ($p = 0.013$) (Figure 2), whereby acylated ghrelin concentrations were elevated at 45 min after the netball-based exercise and sedentary sessions compared to pre-treatment ($p < 0.01$). There was no significant difference in acylated ghrelin ($pg \cdot mL^{-1}$) (AUCx92 min) between the netball-based exercise and sedentary period 89.1 ± 29.3 versus 83.8 ± 29.7 $pg \cdot mL^{-1}$, respectively ($p = 0.612$).

Plasma insulin was not affected by treatment ($p = 0.085$) and there was no time x treatment interaction ($p = 0.352$), but there was a main effect of time ($p = 0.0002$), whereby insulin was decreased in response to netball-based exercise and at 15 min, 30 min, 45 min ($p < 0.05$) and 60 min ($p < 0.01$), after each session compared to pre-treatment.

For plasma glucose there was a treatment x time interaction ($p = 0.0003$) whereby glucose concentrations were increased immediately after the netball-based exercise (4.67 ± 0.83 $mmol \cdot L^{-1}$) compared with pre treatment (4.07 ± 0.53 $mmol \cdot L^{-1}$) ($p < 0.05$) (Figure 3).

Exercise intensity

The average exercise intensity induced by the self-paced netball-based exercise was 72 ± 6% of $\dot{V}O_{2\ peak}$ and the girls were working on average at 81 ± 6% of their maximum HR. Heart rate and RPE data for each time point in relation to the netball exercise session are presented in table 2.

There was a significant main effect of time for both RPE ($p < 0.001$) and HR ($p < 0.001$), whereby as time increased so did RPE and HR in comparison to baseline (see table 2).

Discussion

To the best of our knowledge, the present study is the first to explore 5-d free-living TDEI, TDEE, energy balance and subjective appetite, after a single bout of netball-based exercise in 13-15 y adolescent girls. This is also the first time acute plasma acylated ghrelin responses to a single bout of netball-based exercise have been examined using a randomised crossover design.

The partial EI compensation value of 27% is similar to a previous finding where a partial EI compensation value of ~30% for cycling exercise was identified in adult females (Stubbs et al. 2002). However, the present EI compensation value of 27% is lower compared with that identified in our previous work (53%) (Rumbold et al. 2011a). A plausible explanation for the partial EI compensation value of only 27% for the netball-based exercise-induced EE and energy balance values in the present study, may be explained by the lack of an elevation in EI after the netball-based exercise, which we have previously identified (Rumbold et al. 2011a). Indeed, we speculated in our previous study that in order for exercise to have a sensitising effect on appetite control (more sensitive eating behaviour in response to exercise), an important feature maybe that it needs to be sport-specific, or at least representative of the test group's normal daily activities (i.e. netball typically promoted in English Schools as part of the National Curriculum for Physical Education). Considered together, these EI compensation values indicate that despite the population group and exercise characteristics (type, duration, frequency, intensity), over periods of ≤5-d we are only likely to observe a partial EI compensation for any exercise induced energy deficits. It has been suggested that this lack of total EI compensation (average EI compensation of 30%), and thus negative energy balance can transpire for as long as 14-d in adults (Whybrow et al. 2008). Thus, as was the case in the present study, it is not surprising that acute energy regulation studies in adolescents are repeatedly identifying participants to be in a negative energy balance state over several days (Rumbold et al. 2011a).

The present study also identified that the girls felt fuller 20 min into the netball-based exercise compared with the corresponding time point in the sedentary period. This finding is similar to two other paediatric studies whereby 9-10 y lean young girls had a ‘desire to eat less’ during high intensity cycling (Moore et al. 2004), and older boys (9-14 y) experienced a reduction in average appetite, hunger and desire to eat during 15 min of walking exercise (Bozinovski et al. 2009). Unfortunately, in the present study, blood sampling during the netball-based exercise bout was not logistically feasible and indeed this has been an obstacle in other paediatric studies exploring ghrelin (as opposed to acylated ghrelin) responses to exercise (Pomerants et al. 2006, Sauseng et al. 2010). However, the elevation in fullness during the netball-based exercise compared to the corresponding time point in the sedentary session could, according to the glucostatic theory of appetite regulation (Mayer 1953), be speculatively linked to the profiles of plasma glucose in the present study. These findings may reflect an increased release of glucose into the bloodstream by the liver as a result of the netball-based exercise bout, whereby the brain is then informed of nutrients in the circulation and consequently, appetite regulatory messages are disseminated concerning overall fuel status (Stubbs 1999).

We acknowledge however, that the increase in plasma glucose concentrations after the netball-based exercise was not associated with any change or reduction in plasma acylated ghrelin. This lack of change in plasma acylated ghrelin concentration agrees to some extent, with previous paediatric findings (Pomerants et al. 2006), which found no change in total ghrelin immediately after a 30 min bout of cycling exercise (at 95% of ventilatory threshold), in a group of adolescent boys (10-18 y). Despite this agreement, our findings contradict that of other authors (Sauseng et al. 2010), who demonstrated that compared with a control group, acylated ghrelin concentrations were significantly elevated in a group of female adolescents after cycling exercise to voluntary exhaustion. Indeed, pertinent methodological differences must be noted, with the former study (Pomerants et al. 2006) exploring total ghrelin as opposed to acylated ghrelin and not imposing a corresponding control condition, and the latter study (Sauseng et al. 2010) employing a between participants design.

However, several limitations must be acknowledged in the present study, despite the attempts to employ a robust study design along with rigorous data collection techniques. Firstly, the sample size of 10 (seven for the blood parameters) is small (therefore we cannot make a definite conclusion from a non-significant effect), however we have provided initial information regarding plasma acylated ghrelin values and responses to exercise in active girls undertaking free-living exercise. Therefore, we believe this study to have been exploratory in nature and we encourage researchers to use the paediatric acylated ghrelin responses to exercise presented in this manuscript to help power future studies. Secondly, although an established method for estimating free-living EE, it is acknowledged that the simultaneous FLEX HR and physical activity count threshold technique may still slightly overestimate EE, and thus may be a potential contributor to the negative energy balance values identified on the maintenance days. Finally, blood collection was not feasible during the netball-based exercise. Thus a definite conclusion regarding plasma acylated ghrelin, is that this orexigenic hormone requires further study in a young population, but with researchers employing a robust study design. Given the difficulties of blood sampling in young people, researchers should seek to explore the potential to measure acylated ghrelin and other appetite hormones from saliva samples and arterialized finger prick blood samples. More specifically, paediatric energy regulation studies should explore a more varied array of appetite hormones, including those of an anorexigenic nature.

This study demonstrates that 13-15 y active adolescent girls only partially compensated (27%) for the energy expended during a bout of high intensity (72% of $\dot{V}O_{2\ peak}$), intermittent netball-based exercise. The effect of exercise on subjective appetite and hormonal indicators of appetite, needs to be further explored in adolescents, whereby nutritional and appetitive behaviour is tracked for >1-wk to investigate full compensation for acute exercise.

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Tables

Table 1. Mean ±SD energy balance ($MJ\cdot d^{-1}$) for each study day.

	Netball		Sedentary	
	Mean	**SD**	**Mean**	**SD**
Saturday ($MJ\cdot d^{-1}$)	-0.37	3.23	-0.69	3.17
Sunday ($MJ\cdot d^{-1}$)	-1.49	2.31	-2.33	2.37
Monday ($MJ\cdot d^{-1}$)	-2.20	2.72	-1.46	1.62
Tuesday ($MJ\cdot d^{-1}$)	-2.17	1.92	-2.04	2.62
Wednesday ($MJ\cdot d^{-1}$)	-2.91	2.26	-1.71	1.93
Thursday ($MJ\cdot d^{-1}$)	-1.09	2.34	-2.60	2.12
Friday ($MJ\cdot d^{-1}$)	-3.24	1.94	-3.14	2.30

Table 2. Mean ± SD RPE and HR ($b \cdot min^{-1}$), for all participants (n=10).

	P-CERT		Heart rate ($b \cdot min^{-1}$)	
	Mean	SD	Mean	SD
Immediately before netball-based exercise	1	0*	85	11§
10 minutes into netball-based exercise	4	1†	157	20
20 minutes into netball-based exercise	6	1‡	158	15
30 minutes into netball-based exercise	7	1	163	15
Immediately after netball-based exercise	8	1	162	15

*Significantly lower compared to 10 minutes, 20 minutes, 30 minutes and immediately after the netball exercise ($p < 0.01$). †Significantly lower compared to 20 minutes into, 30 minutes into and immediately after the netball exercise ($p < 0.01$). ‡ Significantly lower compared to immediately after the netball exercise ($p < 0.01$).

§Significantly lower compared to 10 minutes into, 20 minutes into, 30 minutes into and immediately after the netball exercise ($p < 0.01$).

Figure captions

Figure 1. Mean ± SD daily 24-hour energy intake ($MJ \cdot d^{-1}$) for each study day. *Total-daily energy intake significantly elevated on the treatment day (Monday) compared with the last follow up day (Friday) in both weeks ($p < 0.01$).

Figure 2. Mean ± SD fullness (mm). Netball-based exercise (dashed line) and sedentary period (solid line). *Girls felt significantly more full 20 min during the netball-based exercise compared with the corresponding time point during the sedentary period (p= 0.026, effect size = 1.25).

Figure 3. Mean ± SD (a) plasma acylated ghrelin concentrations ($pg \cdot mL^{-1}$), (b) plasma insulin concentrations (pmol/L) and (c) plasma glucose (mmol/L) for a sample of participants (n=7). Netball-based exercise (dashed line) and sedentary period (solid line). *Significantly higher in both the netball-based exercise and sedentary sessions compared to pre-treatment values ($p < 0.01$). †Significantly decreased in response to netball-based exercise and at 15 min, 30 min, 45 min ($p < 0.05$) and 60 min ($p < 0.01$), after each session compared to pre-treatment. ‡Significantly higher in the netball-based exercise session compared to pre-treatment ($p < 0.05$).