

Relationship between salivary androstenedione levels, body composition and physical activity levels in young girls

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Abstract

Background: High androgenic activity in adolescent girls and adult women is associated with adiposity and metabolic disturbances. This study examined the relationship between salivary androstenedione levels, body composition, and physical activity levels in young girls.

Method: Twenty-three girls (8.4 ± 0.9 years), nine of normal weight, and 14, overweight or obese, according to Centers for Disease Control and Prevention body mass index (BMI) percentile ranking, wore an ActiGraph® accelerometer for five days. Skin folds were measured for percentage body fat and fat-free mass calculation. Saliva samples were collected at home, three times in one day (upon waking, 30 minutes post-waking and in the evening). Previously published cut-points estimated the minutes of sedentary behaviour, light, moderate, moderate-to-vigorous, vigorous, and very vigorous physical activity. Meeting physical activity recommendations was defined as accumulating 60 minutes/day of moderate-to-vigorous physical activity. T-tests, correlation analysis, and analysis of variance, examined the relationships among the variables ($\alpha = p\text{-value} \leq 0.05$).

Results: Thirty-five per cent of the girls met current physical activity recommendations. There were no differences between the normal weight and overweight or obese categories in terms of meeting physical activity recommendations, or salivary androstenedione levels. Evening salivary androstenedione levels were higher among girls who did not meet the physical activity recommendations (116.9 ± 15.3 pg/ml vs. 80.45 ± 8.5 pg/ml, $p\text{-value} = 0.04$). Salivary androstenedione levels at 30 minutes post-waking were positively correlated with BMI ($r = 0.45$, $p\text{-value} = 0.03$) and percentage body fat ($r = 0.40$, $p\text{-value} = 0.05$).

Conclusion: These research results were similar to those of circulating androgen research performed in adolescent girls and adult women, in that salivary androstenedione levels were positively correlated with BMI and percentage body fat in young girls. Meeting physical activity recommendations was associated with the maintenance of the normal diurnal rhythm of salivary androstenedione secretion.

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Introduction

Androstenedione is produced in the adrenal gland and gonads, has a weak intrinsic androgenic activity (~ 20% of testosterone), and is a prohormone for androgens (testosterone) and estrogens.¹ High levels of serum androstenedione may confer androgenic or estrogenic risk to women and men, respectively.^{2,3}

Children and adolescents are particularly vulnerable to the effects of androstenedione conversion to active sex steroids. The conversion of androstenedione to estrogens can cause feminisation of boys. In both boys and girls, the combined effects of excessive androgens and estrogens can induce premature puberty, and significantly compromise adult stature, by causing early closure of the growth plates of the long bones.^{2,3} High-circulating androstenedione levels are indicated

in virilising congenital adrenal hyperplasia, polycystic ovarian syndrome, and other causes of hirsutism in women. Specifically, high levels may cause disruption of normal sexual development, severe acne, excessive body hair, disruption of the menstrual cycle, and infertility in girls.^{2,3} Elevated androstenedione levels may occur as a result of adrenal or ovarian tumours.^{2,3}

Increased androgen production, or a high androgenic activity, has also been shown to be associated with obesity or adiposity, metabolic disturbances, and development of metabolic syndrome in adult women.^{4,5} Specifically, obese women with increased androgens are more prone to metabolic disturbances, such as hyperinsulinaemia, type 2 diabetes mellitus, lipid abnormalities, and hypertension. Therefore, they may be at particular risk of developing atherosclerotic complications.^{6,7}

In children and adolescents, high androgenic activity has been associated with polycystic ovarian syndrome, precocious puberty, and accelerated bone age, with relatively tall stature.^{8,9} All of these clinical features occur more frequently in obese children, than in children of normal weight.^{9,10} Furthermore, children with premature adrenarche may tend to obesity.¹¹ While an association between androgens, obesity, and metabolic syndrome, is well established in obese women, studies concerning this relationship are scarce in obese preadolescent and adolescent girls.¹² In terms of a reduction in androgen levels, studies of obese women and obese adolescents, after menarche, have shown a normalisation of hyperandrogenaemia and metabolic disturbances after weight loss.^{13,14} Although these studies incorporated either moderate or vigorous physical activity as part of the weight loss intervention, the relationship between salivary androgen levels (specifically androstenedione), body composition and physical activity levels has yet to be determined.

The strong serum-saliva correlation for androstenedione ($r = 0.77$)¹⁵ suggests that serum androstenedione levels may be accurately estimated using saliva as a non-invasive alternative specimen for examining androgenic risk.^{16,17} The aim of this study was to determine whether salivary androstenedione levels were associated with body composition indices in young girls. In addition, the relationship between physical levels and salivary androstenedione levels was determined.

Method

Participant selection

Participants were recruited via local after-school programmes, churches, junior and senior primary schools, and advertising in the newspaper.

Inclusion in the study entailed the following. Girls had to be between the ages of seven and 10 years old, and both the parents and the child provided written consent and assent, respectively, in accordance with the University of KwaZulu-Natal's institutional review board guidelines. Children could not participate if they had known cardiovascular disease, diabetes (type 1 or 2), or any condition that limited their ability to perform physical activity. Participation was not restricted by physical activity levels, race or socio-economic status.

Study design

Twenty-five girls completed two study visits that were seven days apart.

The first visit included the following procedures:

- Informed consent and assent
- Resting blood pressure measurement

- Height and weight measurement
- Blood draw (this study formed part of a larger, more comprehensive work, examining the existence of metabolic syndrome in young girls using blood markers. These blood results are not presented here).
- Completion of questionnaires (demographics and physical activity)
- Tanner staging (parent proxy)
- Distribution and explanation of activity monitors, physical activity log books and saliva collection kits.

The second visit occurred seven days later, and included the following procedures:

- Resting blood pressure measurement
- Return of activity monitors, physical activity log books and saliva collection kits
- Skin folds measurement.

Upon completion of the study, participants and one parent each received a pedometer. For this study, only height, weight, skin fold, saliva, and physical activity (from activity monitors) data were used.

Sexual maturity

Sexual maturity was determined by Tanner staging via parent proxy report, and the parents were also asked whether their daughters had started menarche. The parents of the participants viewed sketches of stages of sexual maturation, identified their children's personal development, and then placed the sheets in an envelope, and sealed them before returning them to the investigator. For Tanner stages I and II, this method has been shown to be as valid as Tanner staging by a physician, without the increased burden placed on the child.¹⁸

Anthropometric data

Height was measured to the nearest 0.1 cm, using a wall-mounted stadiometer (Perspective Enterprises, Portage, Michigan, USA). Weight was measured to the nearest 0.1 kg, using a portable electronic scale (Model number 68987, Belfour, Saukville, Wisconsin, USA). Both height and weight were measured in duplicate, with shoes off, but wearing lightweight clothing. Age- and gender-adjusted body mass index (BMI) was calculated as kg/m^2 according to the Centers for Disease Control and Prevention guidelines.¹⁹

Participants were grouped into one of the following three BMI categories:

- Normal (< 85th percentile)
- Overweight (85th-94th percentile)
- Obese (\geq 95th percentile).

Given the low number of participants who were considered overweight ($n = 4$), the participants were placed into two BMI groups for the statistical analysis, namely normal weight ($n = 8$, 35%) and overweight or obese ($n = 15$, 65%).

Total percentage body fat was determined by skin fold measurements from the triceps and calf (CRE100 Lange®, Beta Technology, Sana Cruz, California, USA). Trained personnel took the measurements in duplicate, using procedures outlined by the American College of Sports Medicine.²⁰ Age- and gender-appropriate equations were used to calculate percentage body fat. Once body fat percentage was determined, the amount of fat and fat-free mass was then calculated.

Physical activity assessment

Participants wore an ActiGraph® accelerometer (GT1M, ActiGraph LLC, Pensacola, Florida, USA) for five days, including two weekend days. The activity monitor was worn during waking hours around the waist. To increase compliance, participants called the laboratory each morning, and indicated that they were wearing the activity monitor. Study staff followed up with children who did not call the laboratory each day. Data from the ActiGraph® determined the amount of time spent on being sedentary, and on light, moderate, moderate-to-vigorous, vigorous and very vigorous physical activity per day.

Data reduction

Epoch length was set at one-minute intervals. Data were included if the ActiGraph® was worn for at least eight hours and four days. The amount of time spent being sedentary as well as on light, moderate and vigorous physical activity was determined using Freedson's cut-points.²¹ Light activity was classified as ≥ 1.5 METs and < 4 METs. Moderate physical activity was classified as 4-6.9 metabolic equivalent of tasks (METs), vigorous was 7-8.9 METs and very vigorous ≥ 9 METs. Moderate-to-vigorous physical activity was defined as activities ≥ 4 METs. Participants were classified as meeting physical activity recommendations if they accumulated 60 minutes of moderate-to-vigorous physical activity per day.²²

Saliva collection

The children, together with a parent or guardian, were trained in the saliva collection procedure. They were requested to adhere as closely as possible to the following precautions.

The children had to:

- Avoid eating a major meal within 60 minutes of sample collection.
- Avoid consuming dairy products for 20 minutes before sample collection.

- Avoid eating foods with high sugar, acidity, or caffeine content, immediately before sample collection (since these might compromise the assay by lowering saliva pH, and increasing bacterial growth).
- Rinse the mouth with water to remove food residue before sample collection, and swallow to increase hydration.
- Wait at least 10 minutes after rinsing before collecting saliva, to avoid sample dilution.

Saliva samples were collected at home, three times in one day [upon waking, 30 minutes post-waking, and in the evening (~8 pm)]. Saliva samples were collected via unstimulated, passive drool, over a time period of three minutes. The children were asked to lean slightly forward while seated, and tilt their heads down and accumulate saliva in the floor of the mouth for a minute. At the end of that time, the saliva was swallowed, and they then had to accumulate saliva for a further three minutes. During those three minutes, they could dribble the saliva through a 5 cm plastic straw, into a pre-weighed polypropylene cryovial (2 ml capacity) at any time. Care was taken to allow the saliva to dribble into the collecting tubes, with minimal orofacial movement. Samples were refrigerated immediately after collection in home freezers (-20 °C), and kept frozen until reaching the laboratory, after which they were stored at -70°C until analysis.

Salivary androstenedione

Salivary androstenedione was measured using an enzyme immunoassay (Salimetrics®, State College, Pennsylvania, USA). All samples were analysed in duplicate, with the inter- and intra-assay coefficients of variation averaging 4.8% and 5.5%, respectively.

Statistical analysis

Of the 25 girls who participated in the study, 23 provided complete data that were used in the analysis. Descriptive statistics were calculated for the total sample. Data were presented as mean \pm standard deviation (SD). Student's t-tests were run to examine differences in the demographic characteristics of the two groups. Chi-square analysis determined differences in meeting physical activity recommendations by BMI category. Linear regression was performed controlling for Tanner stage. Tanner stage was shown not to have an impact on the salivary androstenedione, body composition, or physical activity measurements (results not presented). Pearson correlations examined the relationship between salivary androstenedione variables, physical activity (moderate, vigorous, very vigorous and moderate-to-vigorous), fat-free mass, body fat percentage, and BMI. A 3 x 2 repeated measures analysis of variance

(ANOVA) was used to examine differences in salivary androstenedione levels (upon waking, 30 minutes post-waking and in the evening), and BMI group, and salivary androstenedione levels (upon waking, 30 minutes post-waking and in the evening), and meeting physical activity recommendations. A 3 x 2 repeated measures ANOVA was used to examine differences in salivary androstenedione levels (waking, 30 minutes post waking and evening) and BMI group. Tukey's post-hoc analysis was completed when appropriate. All statistical analyses were conducted using SAS® (version 9.2, Research Triangle, North Carolina, USA) and statistical significance was set at p-value < 0.05.

Results

With regard to the sexual maturity of the girls, the number in each of the Tanner stages was as follows:

- Breast stage development: 11 in Tanner stage I, and 12 in Tanner stage II
- Pubic hair development: 18 in Tanner stage I, and 5 in Tanner stage II.

In addition, none of the girls indicated that they had started menarche. The descriptive physical, anthropometric, physical activity, and daily salivary androstenedione data (morning, 30 minutes post-waking, and in the evening) for the girls (n = 23) are presented in Table I. In general, the girls of normal

Table I: Descriptive data of young girls, including group anthropometry, physical activity, and salivary androstenedione levels

Variable	Normal weight (n = 8) Mean ± SD	Overweight or obese (n = 15) Mean ± SD	All (n = 23) Mean ± SD
Age (years)	7.88 ± 0.83 ^a	8.73 ± 0.96	8.43 ± 0.99
Height (cm)	135.01 ± 6.15	140.82 ± 8.93	138.80 ± 8.42
Weight (kg)	28.98 ± 2.77 ^a	47.05 ± 13.01	40.76 ± 13.70
Body fat (%)	19.72 ± 4.18 ^a	34.01 ± 9.86	29.04 ± 10.76
Fat mass (kg)	5.74 ± 1.37 ^a	17.03 ± 10.02	13.10 ± 9.73
Fat-free mass (kg)	23.24 ± 2.27 ^a	30.02 ± 4.18	27.66 ± 4.87
Physical activity (minutes/day)			
Sedentary	865.20 ± 241.84	928.43 ± 60.82	908.31 ± 142.01
Light	338.65 ± 62.72	363.14 ± 51.97	355.35 ± 55.33
Moderate	53.99 ± 23.59	51.37 ± 14.20	52.20 ± 17.17
Moderate to vigorous	64.87 ± 31.55	54.66 ± 14.44	57.91 ± 21.15
Vigorous	10.88 ± 11.17	3.29 ± 2.43	5.70 ± 7.26
Very vigorous	1.03 ± 1.56	0.06 ± 0.17	0.46 ± 1.09
Salivary androstenedione (pg/ml)			
Upon waking	80.93 ± 38.90	85.02 ± 56.52	83.60 ± 50.18
30 minutes post-waking	79.17 ± 39.94	105 ± 73.61	96.57 ± 64.22
In the evening	97.70 ± 52.31	105.30 ± 50.55	102.66 ± 50.11

a = significance p-value < 0.05

Table II: Correlations between sedentary behaviour or physical activity, and body composition indices

Physical activity level	Fat-free mass	Body fat percentage	Body mass index
Sedentary	0.10	0.13	0.13
Light	0.20	0.11	0.12
Moderate	0.12	-0.20	-0.19
Moderate to vigorous	0.04	-0.33	-0.31
Vigorous	-0.15	-0.50 ^a	-0.43 ^a
Very vigorous	-0.27	-0.46 ^a	-0.43 ^a

a = significance p-value ≤ 0.05

Table III: Correlations between body composition indices and daily salivary androstenedione values

Adiposity indices	Upon waking	30 minutes post-waking	In the evening
Fat-free mass	0.31	0.35	-0.04
Body fat percentage	-0.05	0.40 ^a	0.07
Body mass index	0.02	0.45 ^a	-0.00

a = significance p-value ≤ 0.05

weight were younger and lighter, and had less body fat, fat-free mass, and fat mass. The amount of time spent on different physical activity intensities, and salivary androstenedione levels, were similar between the two groups.

The chi-square analysis revealed no difference in meeting physical activity recommendations by BMI category (two levels, normal vs. overweight or obese). In the normal-weight group, 50% of the girls met the physical activity recommendations, while 50% did not. In the overweight or obese group, 33% met the physical activity recommendations, while 66% did not.

Table II provides the results for the correlations between time spent on sedentary behaviour, or different levels of physical activity and fat-free mass, percentage body fat, and BMI. There were significant negative correlations between time spent on vigorous physical activity and body fat percentage (r = -0.50, p-value = 0.02) and BMI (r = -0.43, p-value = 0.04), and time spent on very vigorous physical activity and body fat percentage (r = -0.46, p-value = 0.03) and BMI (r = -0.43, p-value = 0.04).

Table III provides the results for the correlations between body composition indices (fat-free mass, percentage body fat, and BMI) and the salivary androstenedione values upon waking, at 30 minutes post-waking, and in the evening. There were significant positive correlations between salivary androstenedione at 30 minutes post-waking and body fat percentage (r = 0.40, p-value = 0.05), as well as BMI (r = 0.45, p-value = 0.03).

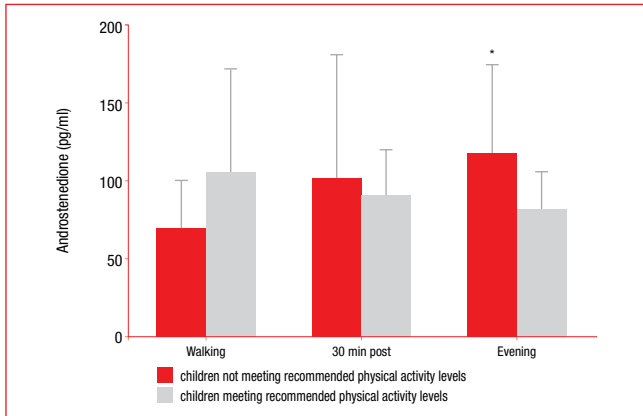


Figure 1: Daily salivary androstenedione values (mean ± standard deviation) according to physical activity level status
 * = significant interaction effect between evening salivary androstenedione levels; p-value = 0.04.

Table IV: Differences in daily salivary androstenedione levels (upon waking, 30 minutes post-waking and in the evening) by meeting physical activity recommendation and body mass index and racial category

Salivary androstenedione (pg/ml)	n	Mean	SD	95% CI	p-value
Upon waking					
Normal weight	8	80.9	25.7	48.4, 113.5	0.8
Overweight or obese	15	85.0	41.4	53.7, 116.3	
30 minutes post-waking					
Normal weight	8	79.2	39.9	45.8, 112.6	0.3
Overweight or obese	15	105.9	73.6	65.1, 146.6	
In the evening					
Normal weight	8	97.7	52.3	53.9, 141.4	0.7
Overweight or obese	15	105.3	50.6	77.3, 133.3	

Figure 1 and Table IV provide the results for the ANOVA that was used to examine differences in salivary androstenedione levels, and meeting physical activity recommendations, as well as salivary androstenedione levels and BMI category (normal, or overweight or obese). The mean ± SD and 95% CI are provided for salivary androstenedione for the BMI classification. There were no interaction, group or time effects for salivary androstenedione levels between the normal and overweight or obese categories (see Table IV). However, there was a significant interaction effect (p-value = 0.04) for salivary androstenedione levels in the evening for the physical activity recommendations analysis. Specifically, post hoc testing demonstrated that the evening salivary androstenedione levels were significantly higher (+ 45%) for those girls who did not meet daily physical activity recommendations, compared to girls who did (see Figure 1). Interestingly, the girls meeting physical activity recommendations demonstrated the expected diurnal rhythm for androstenedione (higher in the morning, than in the evening), with the evening value being 23% lower

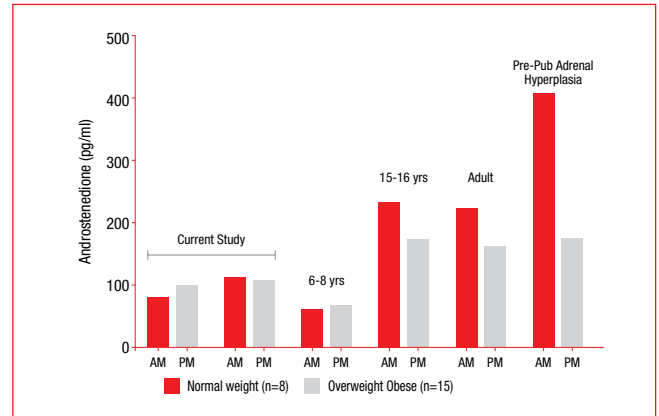


Figure 2: Salivary androstenedione levels in different female populations^{15,23} compared with values in the normal-weight and overweight or obese girls in the current study

than that of the waking value (see Figure 1). Also, the girls who did meet physical activity recommendations demonstrated a higher evening, compared to waking, level (+ 67%)

Discussion

This is the first study comparing salivary androstenedione concentrations between normal-weight and overweight or obese young girls. Importantly, it also analyses the association between their salivary androstenedione and physical activity levels.

Figure 2 displays a cross-section of expected salivary androstenedione levels for healthy women, from prepuberty to adulthood, as well as for prepubescent adrenal hyperplasia with significant hyperandrogenism.^{15,23} Specifically, for healthy six-to eight-year-old girls (n = 6), salivary androstenedione levels have been reported to be 59.78 ± 18.63 pg/ml (am) and 66.64 ± 47.02 pg/ml (pm). By ages 15-16 (n = 8), the values have increased to 233.16 ± 102.97 pg/ml (am), and 171.94 ± 43.59 pg/ml (pm).¹⁵ In contrast, in prepubescent girls with hyperandrogenism, salivary androstenedione levels are as high as 400 pg/ml in the morning, and similar to levels for adult women in the evening.²³ Based on these values, the salivary androstenedione levels (see Table I) in the current study seem to fall within the expected range for young girls. However, the lack of normative data, relating to salivary androstenedione levels in healthy and unhealthy young girls, makes this statement speculative.

In our cohort, overweight or obese girls did not demonstrate hyperandrogenaemia (elevated salivary androstenedione levels), compared with normal-weight girls. However, there were significant positive correlations between salivary androstenedione at 30 minutes post-waking and indices of adiposity (body fat percentage and BMI). This finding is consistent

with the literature, that has demonstrated that excessive adiposity promotes hyperandrogenaemia in adolescent girls and adult women,^{4,5} and contributes to the limited information available on androgens in overweight or obese children, specifically prepubertal and adolescent girls.

Currently, the literature is conflicting regarding androgen levels in obesity in young girls. Hyperandrogenaemia has been suggested to only occur after menarche,²⁴ while lower testosterone levels have been reported in prepubertal obese girls, compared with normal-weight girls.²⁵ In contrast, research has demonstrated similar testosterone concentrations in obese and normal-weight prepubertal girls,^{26,27} whereas studies on obese female adolescents showed higher testosterone levels, compared with lean adolescents.^{14,28} In addition, testosterone and dehydroepiandrosterone (DHEA) sulfate levels have been shown to increase in obese prepubertal children,^{8,26,27} obese pubertal girls, and obese female adolescents,^{14,28} compared with normal-weight children of the same age, gender and pubertal stage.

It has been suggested that hormonal, adipokine, and pro-inflammatory mechanisms, are responsible for the elevation of androgens in obesity. Corticotrophin-releasing hormone (CRH), which is activated with increased adiposity, has been found to increase adrenal androgen secretion directly.²⁹ The adipose cell-derived hormone, leptin, has also been shown to promote the formation of adrenal androgens,³⁰ and to regulate the onset of puberty,^{31,32} while a low level of adipose cell-derived adiponectin is associated with hyperandrogenism in adolescent girls.³³ Recently, polymorphisms in the pro-inflammatory interleukin (IL)-6 receptor complex have been associated with hyperandrogenism.³⁴ Finally, a correlation between insulin levels and androgen secretion has been reported,³⁵ specifically between insulin and testosterone in obese children,³⁶ and insulin and DHEA sulfate during puberty.^{37,38} Whether any of these mechanisms are associated with regulating salivary androstenedione levels in overweight or obesity is presently unknown, and should be the focus of future research.

The finding that time spent on vigorous physical activity is associated with reduced adiposity, and the fact that girls classified as meeting physical activity recommendations had reduced evening salivary androstenedione levels, suggest an important role of physical activity in the regulation of androgenic risk in young girls. The literature indicates that androstenedione follows a diurnal rhythm, with higher levels in the morning, and lower levels in the afternoon or evening, similar to that of cortisol.³⁹ Although the

ANOVA only found differences in the evening salivary androstenedione, and not at waking, or 30 minutes post-waking, the results suggest that the diurnal rhythm of those girls not meeting physical activity recommendations was altered. A similar alteration of diurnal rhythm has been demonstrated for salivary cortisol, which was associated with a less favourable metabolic profile.⁴⁰

Previous research has demonstrated that weight loss, in response to a behavioural, exercise, and nutrition intervention, decreased serum testosterone in prepubertal children and pubertal girls.⁴ Reductions in testosterone, in response to weight loss interventions, have also been demonstrated in obese women and female adolescents, after menarche.⁴ These findings point to a reversible increase of androgens, due to obesity. Although the present study examined the relationship between physical activity levels and salivary androstenedione, and not the impact of a physical activity intervention, the result suggests that performing 60 minutes of moderate-to-vigorous physical activity per day may be responsible for maintaining appropriate daily androgenic activity in young girls.

Limitations

This study is not without limitations. While the physical activity levels were measured through the use of an accelerometer to obtain a more accurate measure of physical activity compared to self-report, the epoch length was set at one-minute intervals. Children's physical activity patterns are intermittent by nature, and vary in intensity.^{41,42} By setting the epoch length at one minute, it is likely that short bursts of either moderate, or vigorous, physical activity, were not detected. Further, the accelerometer does not measure specific activities, such as swimming and bike riding, which are activities that young children often partake in. These limitations could result in undercalculating the amount of time spent on moderate and vigorous physical activity. However, McClain et al⁴³ reported that when comparing direct observation with different epoch lengths in 10-year-old boys and girls, similar amounts of time spent on moderate-to-vigorous physical activity were measured, regardless of the epoch length. Further, when the relationship between short (≥ 4 seconds) and long (≥ 5 minutes) physical activity bouts and health outcomes was examined in eight-to ten-year-old boys, the long bouts resulted in similar strength correlations as the short ones.⁴⁴

Conclusion

While the present study demonstrated a relationship between adiposity and salivary androstenedione in young girls, the results suggest an additional physical activity-related mechanism that regulates their androgen levels. This mechanism may operate independently of reduced adiposity in optimising androgen levels in young girls. Further research is required to determine whether an appropriate androgen level, or diurnal rhythm, is associated with improved CRH, and/or insulin, and/or adipokine/inflammatory profiles in young girls, in response to increased moderate-to-vigorous physical activity.

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