Relationship between Caloric Intake, Body Composition, and Physical Activity to Leptin, Thyroid Hormones, and Cortisol in Adolescents

A. C. HACKNEY, R. G. MCMURRAY, D. A. JUDELSON, and J. S. HARRELL*

Applied Physiology Laboratory, Department of Exercise and Sport Science, and * School of Nursing, University of North Carolina, Chapel Hill, NC, 27599 U.S.A.

Abstract: This study examined the interrelationships between (1) the hormones leptin, TSH, free T3, and cortisol, and (2) caloric intake, body composition, and physical activity to these select hormones in adolescent boys and girls ($n=125$, age= 13.4 ± 0.9 years). The data indicate that adolescent boys and girls have some common

hormonal interrelationships. However, certain hormonal relationships are not in agreement with reports for adults, suggesting that researchers should not assume that all endocrine findings in adults are automatically applicable to adolescents. [The Japanese Journal of Physiology 53: 475–479, 2003]

Key words: children, endocrines, exercise.

The energy balance of a person is determined by energy intake (i.e., dietary caloric intake) and energy expenditure, which can be influenced by physical activity level. When the energy intake exceeds the energy expenditure, the energy stores of the body increase, resulting in an elevated body weight and potentially increased adiposity. Unfortunately, overweight and obesity rates in American adults and youth (children and adolescents) are reaching near epidemic proportion and are becoming a major public health concern [1]. Obesity is, however, a complex, multifactorial medical problem. It is linked to disturbances in hormonal status, heredity-genetic factors, and behavioral attributes (such as, food-type selection [i.e., high fat foods], food quantity [i.e., overeating], and physical inactivity) [1]. Nevertheless, it is a problem that is not well understood and is in need of substantial research.

Leptin is a hormone primarily secreted by the adipose tissue in humans. It is associated with several physiological functions; one of which involves a role in the management of energy balance. Specifically, leptin has been implicated in the regulation of body fat content and in the regulation of energy expenditure [2]. Some of these actions are thought to be the direct influences of this hormone. Some aspects of leptin ac-

tion, however, are thought to be indirect because of the influence of leptin interacting with other endocrine hormones in the body (e.g., insulin, thyroid hormones, glucocorticoids, and others) [3–5].

Few research reports focus on the interactions of dietary caloric intake, energy expenditure, and hormones in adolescents. The adolescence period of growth and maturation is critical in physiological development and is one in which boys and girls follow varying pubertal timelines. It is also a period of prime importance to public health researchers, since the onset of body weight and obesity problems for adulthood may be developing at this time [1, 6].

This study was conducted to address these issues. Specifically the purpose of this study was twofold: (1) to examine the interrelationship between select metabolic hormones—leptin, thyroid stimulating hormone, free T3, and cortisol; and (2) to examine the interrelationship between dietary caloric intake, body composition, and physical activity to these select metabolic hormones in adolescent boys and girls. These hormones were selected for study because of the lack of substantial research literature dealing with them in adolescent boys and girls.

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Correspondence should be addressed to: Anthony C. Hackney, CB # 8700-UNC-CH, Chapel Hill, NC 27599, Tel: +1-919-962-0334, Fax: +1-919-962-0489, E-mail: thackney@med.unc.edu

Methods and Procedures

Subjects. The subjects were part of an ongoing epidemiological study to evaluate cardiovascular disease risk factors in children (CHIC Study [7]). The sample consisted of 63 girls and 62 boys 12–16 years of age. There were 39 African-Americans, 81 Caucasians, and 5 other races in the sample. All subjects and parents gave written informed consent prior to participating in the study in accordance with institutional review board procedures of the University of North Carolina at Chapel Hill. The demographic characteristics of the subjects appear in Table 1.

Procedures. All physiologic measurements were collected “on-site” at the subjects’ schools. All physical tests and research procedures were completed in the same order, which was (1) resting blood collection, (2) questionnaires, (3) height, (4) weight, (5) skinfold measurements, and (6) PWC¹⁹⁵ cycle ergometry test.

All blood samples were obtained after an overnight fast (8 h) by using venipuncture techniques performed by a trained phlebotomist. Specimens were collected into EDTA treated tubes, placed on ice immediately, and later centrifuged to separate plasma. Plasma samples were put into cyrofreeze tubes and placed on dry ice, then transported to our laboratory where they were stored at -80°C until hormonal analysis could take place.

Height was determined by using a stadiometer (± 0.1 cm, without shoes), and weight was determined by using a calibrated balance-beam scale (± 0.1 kg, in only shorts and shirt). These latter measures were used to calculate body mass index (BMI). Skinfolds were measured with calibrated Lange skinfold calipers (the sites measured were triceps and subscapular [8]). Skinfold values were based on the average value of three measurements. Blood pressure was measured by auscultation. The PWC¹⁹⁵ test was performed by using a calibrated Monarch mechanically braked cycle ergometer [7]. Heart rates were obtained during cycling by using Polar heart monitors. Cycle ergometry workload and heart rate responses were used to estimate maximal aerobic capacity ($\dot{V}\text{O}_{2\text{max}}$) in the subjects. The details of these procedures and protocols are reported elsewhere [7, 9].

The adolescents completed three questionnaires. First, estimates of physical activity level (PA score) were obtained from a 7-d activity recall questionnaire [10]. Pilot testing of this questionnaire instrument indicated good internal consistency (Cronbach’s $\alpha = 0.74$) and test-retest reliability ($r = 0.70$) [6, 9]. Second, pubertal development stage (PDS) was assessed by using the “PDS questionnaire” developed by Petersen

Table 1. Physical characteristics and resting hormonal levels of the 125 subjects used in the study (mean $\pm$ SD).

	Male ($n=62$)	Female ($n=63$)
Age (year)	13.4 $\pm$ 1.0	13.3 $\pm$ 0.8
Height (cm)	165.3 $\pm$ 8.4	160.5 $\pm$ 7.0
Weight (kg)	63.8 $\pm$ 17.8	60.2 $\pm$ 14.2
BMI (kg/m^2)	23.1 $\pm$ 5.3	23.3 $\pm$ 5.0
Pubertal stage (Tanner)	3.2* $\pm$ 1.0	4.0 $\pm$ 1.0
Sum skinfolds (mm)	29.0 [†] $\pm$ 17.5	34.5 $\pm$ 15.5
PA score	188.2* $\pm$ 93.9	171.7 $\pm$ 80.2
Caloric intake (kcal/d)	1,951* $\pm$ 1,159	1,542 $\pm$ 774
$\dot{V}\text{O}_{2\text{max}}$ ($\text{ml}/\text{kg}/\text{min}$)	37.8* $\pm$ 10.5	31.5 $\pm$ 7.5
Leptin (ng/ml)	9.8* $\pm$ 9.7	15.6 $\pm$ 10.2
Cortisol ($\mu\text{g}/\text{dl}$)	14.4* $\pm$ 4.6	16.9 $\pm$ 5.9
TSH ($\mu\text{IU}/\text{ml}$)	2.08 $\pm$ 1.13	2.19 $\pm$ 1.76
Free T3 (pg/dl)	248.4 $\pm$ 103.3	263.5 $\pm$ 100.9

* Denotes $p < 0.01$ for boy vs. girl. [†] Denotes $p < 0.05$ for boy vs. girl.

et al. [11] to approximate Tanner Stage (details concerning this instrument are also reported elsewhere [6, 9, 11]). The intraclass correlation between PDS rating and physician’s ratings of pubertal development (Tanner Stage) have been found to be good ($r = +0.68$ to $+0.83$) [11]. Third, the adolescents completed the “Eating Habits” questionnaire to estimate caloric intake. The questionnaire was an adaptation of the one used in the Bogalusa Heart Study [12].

Blood biochemical assays. Plasma leptin, cortisol, thyroid stimulating hormone (TSH), and free triiodothyronine (T3) concentrations were assessed in duplicate determination by using commercially available highly specific radioimmunoassay procedures. The reagents for the leptin assays were obtained from Linco Inc. (St Charles, MO, USA), and all other hormonal reagents were provided by Diagnostic Products Corporation Inc. (Los Angeles, CA, USA). Assay sensitivity for leptin, cortisol, TSH, and free T3 were as follows: 0.5 ng/ml, 0.7 $\mu\text{g}/\text{dl}$, 0.3 $\mu\text{IU}/\text{l}$, and 5.0 pg/dl, respectively. All assay inter- and intracoefficients of variation were less than 10.0%.

Statistical analysis. The mean and standard deviations were computed for all research variables by gender. Pearson Product Moment correlations were calculated and used to determine the relationship between physiologic measurements. ANOVA analyses were also conducted to determine if gender differences existed among variables. The alpha level for statistical significance was set at ≤ 0.05 .

Results

Gender comparison results for all measurements are

displayed in Table 1. There were no significant gender differences in age, height, weight, or BMI. The pubertal stage of the girl adolescents was significantly more advanced than the boys ($p < 0.001$), being approximately one Tanner Stage ahead. However, all five Tanner stages were not represented within each gender; therefore, we did not complete any inferential analysis using the pubertal status as a grouping factor. The girls also had a significantly larger sum of skinfolds than the boys ($p < 0.05$). The boys had significantly higher PA scores, daily caloric intake, and $\dot{V}O_{2\max}$ than the girls did ($p < 0.01$). The circulating hormonal concentrations of leptin and cortisol were significantly lower in the boys than in the girls ($p < 0.01$), and no significant gender differences were seen for the TSH or free T3 hormones.

Only the significant ($p \leq 0.05$) correlation relationships are given in this report. In boys and girls, three of the significant relationships were held in common:

(1) Dietary caloric intake vs. PA score: [boy, $r = +0.389$, $r^2 = 0.151$, $p = 0.002$; girl, $r = +0.296$, $r^2 = 0.087$, $p = 0.018$]; (2) sum of skinfolds vs. leptin: [boy, $r = +0.864$, $r^2 = 0.746$, $p < 0.001$; girl, $r = +0.853$, $r^2 = 0.728$, $p < 0.001$]; (3) TSH vs. free T3: [boy, $r = +0.526$, $r^2 = 0.276$, $p < 0.001$; girl, $r = +0.522$, $r^2 = 0.273$, $p < 0.001$].

Significance tests (Hotelling statistic [14]) were conducted to determine if the magnitude of the correlation coefficients for each of these common relationships differed between the genders. No gender significant differences were noted for these coefficients; thus the strength of the relationships appears of the same magnitude.

Additionally, significant separate correlation coefficients were found within in each of the genders. In the boys, TSH and cortisol were significantly related ($r = +0.365$, $r^2 = 0.133$, $p = 0.003$); and in the girls free T3 and PA scores were significantly related ($r = -0.357$, $r^2 = 0.127$, $p = 0.003$).

It should be noted that significant relationships were also noted in the boys and girls for leptin vs. $\dot{V}O_{2\max}$ (boy, $r = +0.597$, $r^2 = 0.356$, $p < 0.001$; girl, $r = +0.649$, $r^2 = 0.421$, $p < 0.001$), and leptin vs. BMI (boy, $r = +0.812$, $r^2 = 0.666$, $p < 0.001$; girl, $r = +0.845$, $r^2 = 0.714$, $p < 0.001$). These findings have been published elsewhere and discussed in detail; thus they are not addressed in this report [9, 13].

Discussion

The purpose of this study was to examine the inter-relationship between select hormones and between dietary caloric intake, body composition, and physical activity to select metabolic hormones in adolescent

boys and girls. In general, the hormonal and other physiological data found for the subjects were within customary biological ranges and agreed with values previously reported for adolescent children or young adults [6, 9, 13, 15]. Thus the data does not seem to be affected by outliers and appears normative in nature.

Gender displayed an influence on several of the hormonal concentrations measured in this study. Specifically, the hormonal concentrations of leptin and cortisol were significantly lower in the boys than in girls. This is in agreement with previous published research and most likely reflects the advanced pubertal maturation found in our girl subjects and a specific gender influence on these hormones [6, 9, 16, 17].

No significant gender differences, however, were seen for TSH or free T3 concentrations. Research by others suggests that a slight gender-based effect may exist for the thyroid hormones in adults (females typically have lower levels than males [17, 18]). This gender difference seems to be a result of menstrual-related fluctuations in the hormonal milieu of females [17]. Our girl subjects were more mature (i.e., greater Tanner Stage) than our boys. However, not all of our girls were menstrual or were completely eumenorrheic in their menstrual function at the time of the study. Unfortunately, we do not know the phase of our menstruating subjects' cycles during which blood collection occurred. We acknowledge that this is a limitation in our study. These factors may account our TSH and free T3 findings not being in total agreement with previously published research based on primarily adult subjects.

In examining the correlation results, there was a great deal of similarity in the level of the relationship seen among several of the variables, even through there was a maturation difference between our boy and girl subjects. Specifically, caloric intake vs. PA score, SSF vs. leptin, and TSH vs. free T3 where all associated to essentially the same degree in the boy and girl subjects. Only TSH vs. cortisol (seen in boys only) and free T3 vs. PA score (seen in girls only) were found to be gender-related differences in relationships. Collectively, this might suggest that these adolescents had more in common than was different in their physiology and endocrinology profiles. Nevertheless, the difference in the relationship between TSH vs. cortisol and free T3 vs. PA scores suggest some developmental and, or behavioral constructs are influencing adolescent boys and girls differently. The limitations of the current study do not allow us to speculate what these constructs are, but most emphatically they point to the need for future research in this

area.

It is interesting to note that in neither gender no significant association was seen between leptin and cortisol or thyroid concentrations. This novel finding is apparently different from what is found in adults. That is, Zakrewske *et al.* [3] propose that glucocorticoids serve as counterregulatory hormones for leptin production and are thus hormonally interrelated. Similarly, several researchers propose [4, 5, 19] a thyroid-leptin connection in counter-regulatory function. Our lack of finding any relationship among these hormones may represent that developmentally the adrenal and thyroid regulatory axes and the leptin regulatory axis have not begun to fully “cross-talk” as to the energy flux status within the body of adolescents. Obviously this is speculation on our part, but we feel this issue is most compelling and warrants much further investigation.

Dietary caloric intake was significantly greater in the boys than in the girls. This finding is in agreement with previously published research on North American adolescents and most likely is a function of the greater body mass in the boys than in the girls [6, 9]. It also seems plausible that the caloric intake was higher in the boys because of their greater level of physical activity, which is born out by the significant association between PA scores and caloric intake.

Diet interestingly showed no direct correlation with any of the hormones measured. However, it is arguable that the body fatness level of the adolescents is influenced by their dietary habits (as well as their physical activity level). The sum of skinfolds (SSF) was assessed to examine body fat, and the SSF responses were strongly associated with leptin concentrations in both the boys and girls. This relationship has been seen in many research studies using adults [3] and would support that this hormonal construct is displaying mature functions even in the early teen years.

As noted, the physical activity (as measured by PA scores) level was related to dietary caloric intake; and PA scores were significantly lower in girls than in boys. Furthermore, the PA scores were not associated to circulating leptin levels. All these findings are in accord with other researchers who have examined similar populations [6, 7, 9, 13]. Moreover, a correlation analysis showed a negative relationship between PA scores and free T3 in the girl adolescents in a similar fashion to those seen in adults (i.e., negative association) [18]. As individuals become more physically active, there is a greater increase in the turnover of thyroid hormones and sometimes in reduced circulating levels [20]. However, in the boys no relationship

was seen between PA scores and free T3, which does seem to exist in adult males [17]. One could argue that perhaps the maturation difference in the thyroid axis between the genders accounts for this difference between our adolescents. However, similar correlation coefficients for TSH vs. free T3 were seen in both the boys and girls; suggesting comparable levels of thyroid axis functioning. Furthermore, no differences were observed in the circulating thyroid concentrations between genders. At this point it is difficult to explain this occurrence in our finding, and causality must await further study in order to elucidate a possible mechanism.

In conclusion, the findings from the study offer several major points. First, there is the indication that adolescent boys and girls have different resting levels of the hormones leptin and cortisol, but not for thyroid hormones. Second, diet as assessed by total caloric intake has no discernable relationship with the resting hormone levels we measured. Third, physical activity level affects thyroid hormonal status in girls, but not in adolescent boys. Whether this last finding is the function of a difference in pubertal maturation is uncertain. Finally, in adults significant counter-regulatory relationships have been reported within the leptin, thyroid, and cortisol hormones. A correlation analysis revealed no such findings in either the boy or the girl adolescents within this study. The lack of any interrelationship in these hormones suggests that not all endocrine findings in adults should be automatically assumed applicable to boy and girl adolescents.

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