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Research paper

Plasma levels of irisin in children with idiopathic premature adrenarche

Vanessa O. Furino [1]*, Fernanda O. Furino [1], Lucimar R. S. Avó [2], Débora G. Melo [1, 2], Carla M. R. Germano [1, 2]

[1] Postgraduate Program in Nursing - Federal University of Sao Carlos, São Paulo, Brazil
[2] Department of Medicine - Federal University of Sao Carlos, São Paulo, Brazil
**Corresponding author E-mail: vafurino@hotmail.com*

Abstract

Background: Premature adrenarche (PA) is characterized by presence of isolated pubic and/or axillary hair, acne and body odor before age 8(9) in girls (boys). These individuals are at increased risk of developing insulin resistance, metabolic syndrome and polycistic ovarian syndrome. Irisin seems to have an active role in the metabolism of carbohydrates and lipids, however little is known about this hormone in PA.
Objective: To analyze irisin levels in children diagnosed with PA and its relationship with their body composition.
Methodology: Exploratory cross-sectional study that evaluated 15 children with PA and 15 matched controls (C). Anthropometric data were measured: height, weight, waist circumference (WC) and triceps skinfold. Fasting blood glucose(G), insulin(I), 17OHP, total cholesterol, LDL, HDL, triglycerides, DHEA-S, 25(OH)D and irisin levels were determined.
Results: The levels (mean±SEM) of triglycerides [99±14, 8mg/dl (C); 68±9, 1mg/dl (AP)] and 25(OH) D [26±0, 9ng/ml(C); 30.2±1.6ng/ml (AP)] were significantly different between the groups. WC above p90 and G/ I<7 were found in 6.7% of the C group versus 33.3% and 20% of the PA group, respectively.
Conclusions: PA children presented a lower G/I and a higher waist WC compared to the C group, suggesting an increased risk of metabolic disease. Irisin levels were not different between the groups. The significantly higher levels of TGs from the C group may be related to their reduced levels of 25(OH) D, which may also have masked differences in irisin levels. This study suggests that determination of vitamin D levels may be necessary to evaluate metabolic data, based on the significant frequency of this hormone insufficiency/deficiency in the pediatric population.

Keywords: *Body Composition; Children; Irisin; Premature Adrenarche; Vitamin D.*

1. Introduction

1.1. Premature adrenarche

Premature adrenarche (PA) results in appearance of pubic hair and/or axillary, acne and body odor before 8 years of age in girls and before 9 in boys. The condition is determined by early activation of the adrenal gland and is biochemically characterized by serum concentration of dehydroepiandrosterone sulfate (DHEA-S) ≥40μg/dl (1μmol/L) (Ibáñez et al. 2000, Oron et al. 2012). The clinical expression of PA is classified as isolated or idiopathic when premature development is not accompanied by clinical or laboratory signs of gonadal axis activation (Korth-Schutz et al. 1976).
In the past, idiopathic PA was considered a benign condition, a normal variation in the time of puberty. In subsequent studies, however, researchers demonstrated that PA may be a sign of increased risk for metabolic disorders (Oron et al. 2012), such as dyslipidemia (Ferran et al. 2011) and insulin resistance (IR) (Sopher et al. 2011), and also for the development of diabetes mellitus 2 (DM2), (Sopher et al. 2011, Utriainen et al. 2015) metabolic syndrome (MetS) (Idkowiak et al. 2011, Utriainen et al. 2015) and polycystic ovary syndrome (PCOS) (Idkowiak et al. 2011).

1.2. Irisin

Irisin was identified in 2012 as a 112 aminoacid peptide secreted mainly by muscle and adipose tissue. Its structure consists of a N-terminal fibronectin III (FNIII)-like domain attached to a flexible C-terminal tail (Schumacher et al. 2013), from the cleavage of the transmembrane protein fibronectin type-III domain containing protein 5 (FNDC5). This myokine appears to have important autocrine and endocrine functions, mediating the beneficial systemic effects of exercise on metabolism (Boström et al. 2012, Hecksteden et al. 2013).
Irisin levels are regulated and directly related to physical activity. The increase in energy demand leads to a series of mitochondrial reactions that result in an increased expression of FNDC5 membrane protein in skeletal muscle. After its cleavage, with the removal of the signal sequence, FNDC5 originates the myokine irisin, which is secreted into the bloodstream (Boström et al. 2012). Elevation of irisin in the white adipose tissue stimulates mitochondria activity, increases energy expenditure and induces adipocyte browning (Hecksteden et al. 2013, Schumacher et al. 2013). Recent studies have shown that irisin can affect insulin action, suggesting its protective role in the development of obesity and diabetes mellitus (Yang et al. 2015). Other studies have shown an inhibitory action of irisin in liver fat accumulation, hypothesizing that irisin supplementation could result in health metabolic effects

(Xiong et al. 2015, Tang et al. 2016). More recently, an aging-related effect of irisin was demonstrated through its action on mononuclear cell telomere extension (Rana et al. 2014) and neurogenesis stimulation (Moon et al. 2013), suggesting a preventive role for this hormone in neurodegenerative diseases.
Therefore, this study aims to analyze irisin levels in children diagnosed with PA and the relationship of the peptide with their body composition.

2. Methods

A cross-sectional study that evaluated 15 children diagnosed with PA and 15 control children of the same age and sex, matched for BMI and pubertal stage, with no chronic diseases was conducted. The children were recruited from public pediatric endocrinology and primary care units from the city of Sao Carlos, SP, Brazil.
This study was approved by the Human Research Ethics Committee at the institution (CEP- UFSCar - nº 712,011). Children's parents (or legal guardians) agreed to participate and signed the informed consent.

2.1. Subjects

The PA group consisted of 15 children with idiopathic PA, i.e., they had pubic and/or axillary hair and odor before the age of 8 years in girls and 9 years in boys, absence of clinical and laboratory signs of gonadal axis activation (Tanner stage B1P1 or G1P1), DHEA-S serum levels ≥40μg/dl and exclusion of other pathologies.
The control group (C) consisted of 15 prepubertal children selected for not presenting clinical features consistent with the diagnosis of adrenarche or puberty (B1P1 or G1P1) and serum levels of DHEA-S <40mg/dl. The groups were matched by BMI (body mass index) and age (with ≤ 1 year variation between groups).

2.2. Anthropometric and clinical data

Patients were evaluated for weight, height, blood pressure and BMI (calculated by weight (kg)/height2 (m)). Their weight and height were measured using a certified anthropometric scale. The waist circumference (WC) was determined by an inelastic measuring tape on the midpoint between the edge of the last rib and the anterior superior iliac crest. WC was analyzed using specific references for gender and age. Triceps skinfold (TSF) was measured using a scientific adipometer LANGE®.

2.3. Biochemical analysis

The plasma was collected in EDTA tubes, centrifuged at 4°C, 3000 rpm for 10 minutes and stored at -20ºC pending analysis.
All participants underwent fasting glucose (G) (enzymatic colorimetric assay), fasting insulin (I) (chemiluminescence), total cholesterol (enzymatic colorimetric assay), HDL (enzymatic colorimetric assay), LDL (enzymatic colorimetric assay), triglycerides (TG) (enzymatic colorimetric assay), DHEA-S (chemiluminescence), 17OHP (ELISA) and vitamin D (25(OH)D) (chemiluminescence) determination. Plasma irisin was determined by the Enzyme Immunoassay Kit (EK-067-29, Phoenix Pharmaceuticals, Inc.), with a detection range of 0.1-1000 ng/ml.

2.4. Statistical analysis

Data analysis was performed using the GraphPad Prism 5®. Data were presented as mean ± standard error of the mean (SEM). The Wilcoxon paired test was used to evaluate the differences between the means and the Spearman test was used for correlation analysis, at 5% significance level.

3. Results

This study evaluated a total of 30 individuals, of which 40% were boys (n = 12) and 60% were girls (n = 18). Twenty six point seven percent (n = 4) of the individuals in the C and PA groups were classified as obese. The ethnic distribution showed that in the C group 53.3% (n = 8) of children were white and 46.7% (n = 7) were african american, and in the PA group 46.7 % (n = 7) were white and 53.3% (n = 8) were african american.

Table 1: Clinical and Anthropometric Data of the Control (C) and Premature Adrenarche (PA) Groups.

Clinical and anthropometric data	Control group (C) (n=15)	Premature adrenarche group (PA) (n=15)	P value
Age (months)	95.1 ± 5.1	100.1 ± 5.5	0.0432*
Weight (kg)	30.2 ± 2.3	32.0 ± 2.3	0.3028
Height (m)	1.30 ± 0.02	1.33 ± 0.02	0.1470
Body mass index (kg/m2)	17.4 ± 0.8	17.7 ± 0.9	0.4431
Waist circumference (cm)	63 ± 1.9	66 ± 3.4	0.2928
Thickness skinfold (mm)	16 ± 1.8	16 ± 1.9	0.4286
Bone age (months)	93 ± 8	105 ± 16	0.2500
Systolic blood pressure (mmHg)	97 ± 2.5	96 ± 2.4	0.7150
Diastolic blood pressure (mmHg)	66 ± 2.1	70 ± 2.0	0.1200

After individual value analysis, it was noted that 6.7% (n = 1) of the C group and 33.3% (n = 5) of the PA group had WC above the 90th percentile for age and height. None of the individuals in both groups presented systolic blood pressure (SBP) and diastolic blood pressure (DBP) levels above the 90th percentile.
Table 2 shows the lipid, glycemic and hormonal profile of the C and PA groups. The C group showed significant higher TGs (p = 0.0233) and lower vitamin D (p = 0.0307) levels compared with the PA group. DHEA-S levels on the other hand, were significantly higher in the PA group as expected by the group selection criteria described in the methodology. The age difference between groups was significantly different, but lower than one year, as described in the methodology. The other laboratory data were not different between groups.

Table 2: Biochemical and Hormonal Data of the Control (C) and Premature Adrenarche (PA) Groups

Biochemical and hormonal data	Control group (C) (n=15)	Premature adrenarche group (PA) (n=15)	P value
Glucose (mg/dl)	80 ± 1.7	79 ± 2.3	0.7293
Insulin (μUI/mL)	5.7 ± 1.1	8.2 ± 1.8	0.2939
Glucose/insulin ratio	18 ± 2.0	18 ± 4.2	0.7197
Total cholesterol (mg/dl)	158 ± 7.4	144 ± 6.3	0.1876
LDL (mg/dl)	87 ± 8.0	79 ± 4.5	0.4101
HDL (mg/dl)	46 ± 1.9	48 ± 1.5	0.4768
Triglycerides (mg/dl)	99 ± 14.8	68 ± 9.1	0.0233*
17OHP (ng/ml)	0.72 ± 0.12	1.02 ± 0.13	0.1354
DHEA-S (μg/dl)	24.1 ± 6.0	78.3 ± 5.9	0.0002*
25(OH)D (ng/ml)	26.0 ± 0.9	30.2 ± 1.6	0.0307*
Irisin (mg/dl)	16.1 ± 1.3	17.3 ± 1.7	0.5880

LDL: low fraction of lipoprotein; HDL: high fraction of lipoprotein; 17OHP: 17-hydroxyprogesterone; DHEA-S: dehydroepiandrosterone.

After individual value analysis, it was noted that 6.7% (n = 1) of the C group and 20% (n = 3) of the PA group had insulin >15 μUI/ml and glucose/insulin ratio (G/I) <7. None of the individuals had blood glucose levels above 100 mg/dl.

In the C group, the variable that was significantly correlated with irisin was 25 (OH) D ($r = – 0.9358$, $p < 0.001$); in the PA group, it was the LDL cholesterol ($r = 0.5620$, $p = 0.0456$), 17OHP ($r = 0.6014$, $p = 0.0386$), TG ($r = 0.6434$, $p = 0.024$), insulin ($r = 0.5998$, $p = 0.0302$) and G/I ($r = – 0.7033$, $p = 0.0073$).

4. Discussion

This study did not come across any differences in the glycemic profile and anthropometric data between the C and PA groups, however a higher percentage of children in the PA group had WC above the 90th percentile, insulin levels higher than 15 μUI/ ml, and G/I ratio below 7. These results suggest that the PA group may have a risk in developing MetS, DM2 and cardiovascular disease (CVD). The expression of these diseases may not be evident in the prepubertal period, but can develop during or after the development of puberty (Santos, 2011). Therefore, these children should be monitored to detect adverse health effects early on throughout their growth and development.

Vitamin D deficiency is a worldwide epidemic unrecognized among children and adults (Holick et al. 2011). The deficiency is represented by levels of 25(OH)D (25-hydroxyvitamin D) less than 20ng/mL (50 nmol/L) and insufficiency by levels between 20 and 30 ng/ml (Holick et al. 2011). Ethnicity, sex and sun exposure appears to modulate 25(OH)D serum concentrations (Valtueña et al. 2011). Nowadays, children spend more time on indoor than outdoor activities protecting themselves with clothes and sunscreen, which may limit vitamin D skin production (Peters et al. 2013). The decrease in vitamin D status has been suggested as a predisposing factor for a large number of diseases, including respiratory infections, cardiovascular disease, dyslipidemia, diabetes and prostate cancer (Holick et al. 2011). The similarity between the action of irisin and vitamin D in the regulation of energy metabolism, through the peroxisome proliferator molecule expression, may point to a link between these two substances in metabolic processes (Matsuda & Kitagishi et al. 2013, Chen et al. 2016, Park et al. 2016).

In this study, there was no difference in irisin concentration between the PA and C groups. This may be due to the absence of metabolic dysfunction in the PA group or a difference may have been masked by the low concentration of vitamin D in the C group. Scientific studies have shown that patients with a metabolic profile similar to that of children with PA had higher levels of irisin when compared to controls without Mets (Park et al. 2013) and PCOS (Chang et al. 2014). These data suggested that irisin could function as a protective agent against an excessive influx of energy as it increases energy expenditure. Other authors suggested that the increased irisin levels were secondary to a mechanism similar to the leptin increased levels, i.e., the body increases basal levels of this hormone in response to its resistance (Xiong et al. 2015). When Boström et al. (2012) published the first results on irisin and carbohydrate metabolism, hormone levels were associated with beneficial effects on health, such as reduced body weight and increased insulin sensitivity. The hypothesis was reinforced by the findings of Kurdiova et al. (2014) that reported higher levels of circulating irisin in lean and healthy subjects compared to subjects with DM2 (Kurdiova et al. 2014). Other studies, however, showed that people with unfavorable glycemic profile expressed higher levels of irisin, especially individuals with DM2 (García-Fontana et al. 2015), MetS (Park et al. 2013) and obesity (Stengel et al. 2013, Hou et al. 2015). These more recent data suggested that the increased irisin levels might be explained by the hormone role in the modulation of glucose and lipid metabolism and energy expenditure (Huh et al. 2012, Park et al. 2013) in a situation of resistance to its action. This study's data reaffirms the link of irisin with insulin levels (positive correlation) and resistance index (negative correlation with G/I). Garcia-Fontana et al. (2015) reported that irisin could increase in response to an unfavorable metabolic state, increasing glucose uptake and the number of beige adipocytes, and activate energy metabolism and thermogenesis, especially in diabetic individuals. Furthermore, an unfavorable metabolic profile could determine oxidative stress, releasing inflammatory elements that increase PGC1-α and FNDC5/irisin expression in muscles, stimulating the production of brown adipocytes, inducing thermogenesis and reducing the IR (Sanchis-Gomar et al. 2014). More studies are needed to further investigate this relationship.

An innovative result of this study was the demonstration of a strong negative correlation between irisin and serum levels of 25 (OH) D in the C group. This data suggests that the reduced levels of vitamin D may have contributed to the unfavorable lipid profile and irisin levels could be the consequence of a compensatory response. The significant correlation of irisin with insulin and its sensitivity may represent a physiological correlation between these two hormones, as this correlation was evident in the PA group, as well as in the C group, although weaker (G/I: $r = – 0.5915$, $p = 0.0717$). The highest correlation of irisin with lipids in the PA group could be secondary to the role of irisin as a predictor of adverse outcomes related to metabolic dysfunction (Gamas et al. 2015). As PA may induce increased cardiovascular and metabolic risk, this association merits further investigation.

5. Conclusions

This study has shown high WC and lower G/I ratio in PA children and these parameters are important markers of IR, MetS, DM2 and CVD. The metabolic effects of PA are the subject of many studies, but the information does not seem to reach the community, which was perceived by the lack of knowledge of parents and guardians regarding the disease and its diagnosis. Therefore, further efforts are needed in order to detect PA early on and perform a proper clinical follow-up, so that future health impacts from this clinical situation could be better recognized and managed.

5.1. Study limitations

The number of individuals in the sample may have been insufficient to reveal the differences between the groups, but other exploratory studies on irisin had a number of participants close to this (Cavalier et al. 2014, Huh et al. 2015).

The decreased levels of vitamin D in the control group may have affected the comparison of lipids and irisin levels between the two groups.

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