Research Article

Possible crosstalk between leptin and insulin resistance in sedentary obese boys at different stages of puberty

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Abstract

Puberty with interactive growth in tissues is a sensitive period in life that can be more affected by obesity. Also the increase of leptin and insulin resistant independent of obesity have been seen in puberty. The aim of our study was to investigate the role of puberty on changes in leptin, testosterone, and insulin resistance in sedentary obese boy with considering possible crosstalk between leptin and insulin resistance. 58 sedentary obese boys (14.10 + 1.37 years) participated in this study. Tanner stage scales were used to measure puberty by self-reporting. Initially anthropometric characteristics and then, fasting serum's glucose, insulin, leptin and testosterone, were measured. With increasing mature from TS2 to TS5, the increasing of testosterone, body mass and lean body mass and the reduction of body fat percentage were significantly ($p \leq 0.05$). but the changes in leptin and HOMA-IR were not significant. However, the decreased leptin after adjusting for BMI between TS3 and TS4 were significant ($p \leq 0.05$). During puberty, rapid growth in muscle tissue were associated with decreased body fat percent, serum leptin and insulin resistance. Among the possible reasons is a 15-fold increase in serum testosterone from TS2 to TS5. These changes reflect the cross talk between muscle and adipose tissue by hormonal mediators.

Key Words: **Obese boys, Leptin, Testosterone, HOMA-IR, Pubertal stages**

Introduction

At any age, obesity is worrisome, because of the risk metabolic syndrome include insulin resistance increase blood lipids and blood pressure (Gesteiro et al., 2021). Puberty is a critical period due to the flexible nature that is more influenced by obesity and metabolic consequences (Huang, Reinehr, & Roth, 2020). Changes in leptin and insulin levels and resistance to themes are common issues in obesity and puberty in many studies (Ackel-D'Elia et al., 2014; Ahmed et al., 1999; Bray, 1997; Buyken, Karaolis-Danckert, & Remer, 2009; Gueorguiev, Góth, & Korbonits, 2001; Han, Lawlor, & Kimm, 2010). Leptin plays an important role in controlling food intake, metabolic rate and body weight. Also the influence on pancreatic β-cells and insulin receptors has a key role in glucose homeostasis (Marroqui et al., 2012). Also it is stated that leptin and insulin regulate a common set of hypothalamic neurons, the overlapping function of these two inhibitors has the potential to produce significant crosstalk between the signaling pathways, particularly during diet-induced obesity as hyperinsulinemia and hyperleptinemia develop. Previous studies have suggested that obesity is associated with increased levels of leptin and insulin resistance (Carnier et al., 2012; Labayen et al., 2013; Prado et al., 2011; Vatier, Gautier, & Vigouroux, 2012). However, both changes during puberty, independent of obesity has been seen (Guercio, Rivarola, Chaler, Maceiras, & Belgorosky, 2003; Jeffery et al., 2012; Nasreddine et al., 2012; Rutters et al., 2009). Puberty phase is transition from childhood to adulthood that is associated with the development of reproductive system.

Testosterone, the main male sex hormone, increases rapidly during adolescence and it is associated with somatic growth and development of secondary sex characteristics (Ahmed, Ong, & Dunger, 2009). Although it was previously thought based on the Frisch hypothesis a critical threshold of fat mass is necessary to begin the process of puberty, it was later discovered there were many potential mechanisms by which fat stores may affect the timing of puberty. One of them is the direct action of leptin that signals to the CNS about the body's energy stores (Biro, Greenspan, & Galvez, 2012). In fact, when adequate energy stores as subcutaneous fat tissue exist, leptin allows activation of gonadotropin-releasing horm-

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-one (Odle et al., 2018).

Leptin and its receptor are involved in at least two perspectives on the evolution of maturity: (1) sexual evolution and (2) changes in body composition. However, leptin changes during puberty is associated with a decrease in body fat percentage for boys but increased it for girls (Damaso et al., 2011). Horlick study on 102 boys and girls aged 6 to 19 years old showed that leptin concentrations at all stages of puberty in both sexes, had a significant relationship with body fat. Between early (TS1) and end of puberty (TS5), there was significant differences between leptin levels. But with advanced stages of puberty serum leptin increases in girls and decreases in boys (Horlick et al., 2000). Rotter's study showed in the early stages of puberty (TS2) in boys, leptin levels reduced (Rutters et al., 2009) However Biro have noted this decline in mid-puberty (TS3) (Biro et al., 2012). Another physiological changes during puberty is increase in insulin resistance.

Insulin resistance that is associated with increased insulin secretion, selectively affect glucose metabolism, not proteins. This mechanism is associated with anabolic effects of insulin and GH during puberty (Caprio et al., 1994). Amiel first reported in 1986 that insulin resistance increased during puberty. He showed the disappearance of glucose in children who were at TS3 and TS4 were less than 30% of prepubertal children and even adults (Amiel, Sherwin, Simonson, Lauritano, & Tamborlane, 1986). Although Hoffman after adjusting for BMI, did not observe differences in insulin resistance between Tanner stages (Hoffman, Vicini, Sivitz, & Cobelli, 2000). But later studies showed the greatest decrease in insulin sensitivity happened in TS3. Where with reaching adolescent to TS5 returned to the previous level (Aguilar-Gomez, Bueno-Lozano, Leis, Aguilera, & Gil-Campos, 2020). Furthermore, many studies have shown that obesity increases insulin resistance (Lee, Kim, Park, Lee, & Park, 2016; Nyambuya, Dludla, Mxinwa, & Nkambule, 2019). However, in the second decade of life and during puberty, insulin resistance due to decreased tissue sensitivity to insulin the biological activity increases Goran's long-term study on children who were the same age, but their puberty was in TS1 (control group) or TS3 and TS4 (experimental groups) compared with each other. Thereby the effect of age was adjusted. The results showed that in the control group, even though some of the subjects were obese, insulin resistance was not observed. This indicates that insulin resistance independent of obesity in adolescence can occur in both sexes (Goran & Gower, 2001). However, many obese people wish to remain slim. Studies show that people respond to weight loss program is not the same. Serums leptin and insulin and resistance to them are two physiological factors that can affect weight loss programs (Boutcher & Dunn, 2009; King et al., 2007; Myers, Cowley, & Munzberg, 2008; Roth, Kratz, Ralston, & Reinehr, 2011). It seems in pubertal phase the changes in insulin resistance and leptin is more evident. So in this sensitive period of life, described various aspects for the treatm-ent and prevention of obesity and its consequences seem necessary.

Materials and Methods

Subject and puberty measurements

Statistical population consisted of obese students aged 12-17 all from Rasht, Iran. Available samples from the two schools were selected. Initial screening for selected obese subjects based on the CDC (Oyama et al., 2010) Chart of 95 percentages by measuring height, weight and BMI were performed. After receiving permission from the Department of province Education Research Administration, necessity of the research and methods were explained in separate sessions for school administrators, subjects and their parents. After written informed consent by the subjects and their parents, Medical- Sport History Questionnaire was used to identify eligible subjects. The criteria include: having a healthy and regular sleep, not taking any medication, lack of family history of diseases, lack of participation in a regular exercise program or diet especially for weight loss. Of the 98 obese children aged 12 to 17 identified in the initial screening, 67 people were announced to participate in research. Among them, only 58 people were qualified to be selected as subjects.

Pubertal stage was assigned by self-report methods, according to the Tanner scale image (Marshall & Tanner, 1970). To ensure the accuracy of the diagnosis of puberty by the subjects, the serum testosterone levels were measured (Wu, 2006). Lean mass, fat mass and body fat percent were measured by using a body composition assessment (In body 3.0 produced by BIOSPACE companies in South Korea) Via Bioelectric resistance method.

Biochemical measurement

Before blood sampling, important points with date, time and location of laboratory were given in writing form to the subjects and their parents. The important points include 12-hour fast, comfortable sleep, not eating High-fat meal the day before the test, not taking any medication, lack of exercise and no change in diet.

5 ml of blood was taken from the brachial vein of each subject. Blood sampling was performed under medical supervision in a school laboratory. Germany LDN kit for measurement of serum leptin was used by ELISA method, America MONOBIND kit to measure serum insulin levels was used by ELISA method. Belgium BIOSOURCE kit to measure serum Testosterone was used by Radioimmunoassay method. And Iran PARS AZMON kits to measure serum glucose was used by Photometric method.

In order to detect insulin resistance homeostasis model assessment (HOMA) indices were calculated as follows: fasting insulin concentration (mU/mL) × fasting glucose concentration (mmol)/22.5

(Conwell, Trost, Brown, & Batch, 2004; Gungor, Saad, Janosky, & Arslanian, 2004).

Statistical analysis

Kalmvgraf- Smirnov test was used to check the normality of data distribution. One-way analysis of variance (ANOVA) with Scheffe post hoc test (in case of of significant F) were used to compare groups. Statistical analysis was done by using SPSS software version 16. Also EXCEL software for the Microsoft Office 2013 was used for drawing graphs.

Results

Characteristics of subjects were presented in Table 1. As is evident, the age and sexually mature increased from TS2 to TS5. As well as the subjects' height, body mass and lean body mass, significantly increased ($P < 0.05$). The body fat mass and body mass index did not change significantly but body fat percent decreased from TS2 to TS5.

Measurement of biochemical variables showed that leptin, insulin, Glucose and insulin resistance index had no significant difference between subjects at different stages of puberty. Nevertheless, testosterone is defined as in Figure 1, with sexually mature from TS2 to TS5 increased significantly ($P < 0.05$).

In Figure 2, serum leptin was reduced from TS2 to TS5, but its decline is more pronounced between TS3 and TS4 although it was not significant statistically. After adjustment for, serum leptin levels by BMI, the difference between these two stages of puberty (TS3 and TS4), as is evident in figure 3, was significant ($P < 0.05$).

According to the Figure 4, the insulin resistance index from TS2 to TS3 TS4 increased then declined to TS4 and the next stage of puberty (TS5) did not change. Also after adjustment for insulin resistance index values based on body mass index, there was no difference between the different stages of puberty (Figure 5).

In Figure 6, the significant relationship between serum levels of leptin and testosterone was shown.

Discussion

Development of obesity and overweight in the past century, was a motivation to many researchers to look at this issue from different

Table 1. Characteristics of obese children in various stages of puberty (mean ± SD).

Tanner Stage	TS2(N=8)	TS3(N=21)	TS4(N=21)	TS5(N=8)
Age (yr)	13.12±0.35	13.61±1.11	14.28±1.18	16.14±1.21
Height (cm)	158.38±7.48	164.86±6.92	170.26±7.99	174.57±6.47
Body mass (kg)	76.15±18.9	80.06±15.33	88.42±16.81	95.57±11.95
BMI (kg/m^2)	30.01±4.52	29.34±4.82	30.32±4.11	31.24±2.06
Lean body mass (kg)	45.83±8.07	49.62±6.81	57.09±12.37	66.81±11.16
Fat mass (kg)	27.93±10.55	27.96±10.06	27.37±7.46	26.21±3.59
Body fat (%)	35.87±4.77	34.22±6.15	31.31±5.37	27.65±4.35
Leptin (ng/ml)	49.87±23.88	50.66±18.98	39.19±18.97	39.71±14.22
Testosterone (ng/ml)	0.36±0.14	2.05±0.55	3.39±0.48	5.55±0.46
Glucose (mg/dl)	92.25±6.62	89.28±8.38	90.42±4.99	85.42±5.71
Insulin (µu/ml)	21.87±6.46	23.47±7.01	20.19±5.21	21.57±5.65
HOMA-IR	4.98±1.45	5.24±1.85	4.52±1.27	4.56±1.30

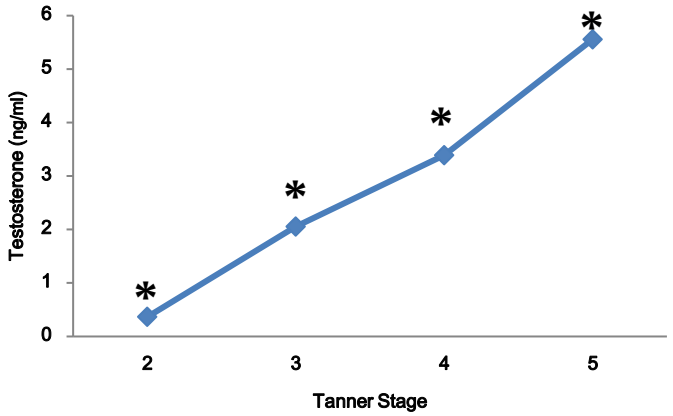

Figure 1. Mean Serum testosterone in obese boys at different stages of puberty. *P< 0.05: Significant difference compared to the previous Tanner stage

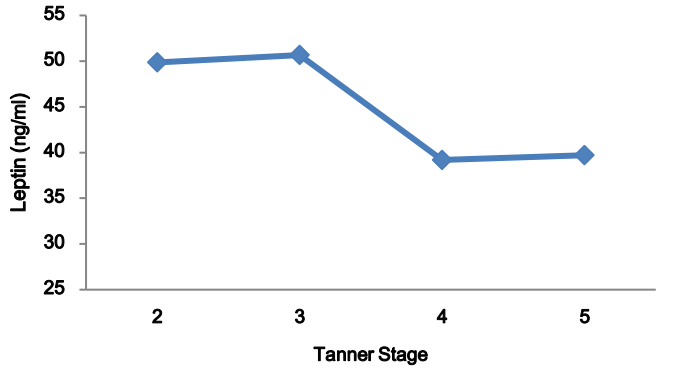

Figure 2. Mean Serum leptin in obese boys at different stages of puberty.

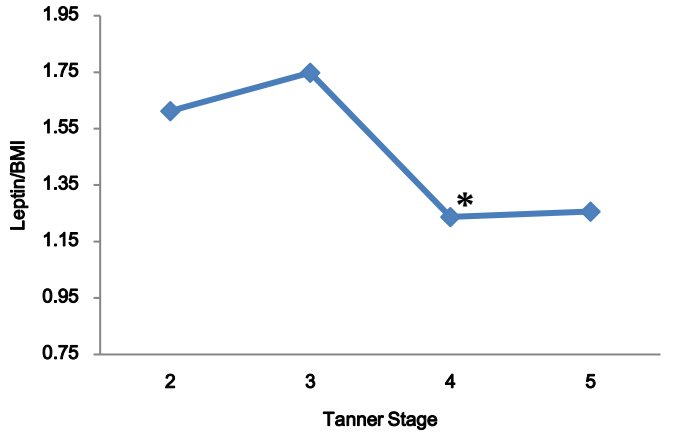

Figure 3. Mean Leptin/BMI in obese boys at different stages of puberty. *P< 0.05: Significant difference compared to the previous Tanner stage

angles to identify the physiological causes, consequences, treatment and prevention. (Boutcher & Dunn, 2009; King et al., 2007) Among the physiological factors can be mentioned to serums leptin and insulin resistance which naturally changes during puberty. Because fewer researches have examined the association between obesity and different stages of puberty. Consistent with other studies, our findings showed that high levels of leptin in obese. That can possibly lead to increased resistance to leptin (Labayen et al., 2013). Martinez and his colleagues in their study showed a high correlation between body fat and leptin in adolescents 13-17 years in both sexes (Martinez-Gomez et al., 2012)

As Chart 2 shows, the serum leptin changes during puberty stages. Argente and colleagues also reported similar changes in leptin levels during puberty (Argente, Barrios, Chowen, Sinha, & Considine, 1997). Mantzoros and colleagues in a long-term study on eight boys showed that at the beginning of puberty leptin levels increased, but, after that reduced (Mantzoros, Flier, & Rogol, 1997). The Rutters and his colleagues Findings showed that levels of leptin in the early stages of puberty (TS2) began to decline in boys (Rutters et al., 2009) However, as the current study, Biro and colleagues in 2012 reported a decrease in TS3 (Biro et al., 2012) Also Rogol and colleaguesfound in stage 4 and 5 of puberty in boys, leptin levels were lower in stage 1 (Rogol, 2010). Differences in leptin changes between the various reports and our study could be the result of different sample sizes, different methods of puberty assessment (Self-evaluation by the subjects or their parents, physician diagnosis or measuring sex hormone levels). The subject weight is another factor. Unlike other researches, in this study, all subjects were obese. The limitations of the present study were the unavailability of subjects in TS1. According to some researchers, the increase in leptin levels occurs at this stage (prepubertal) Thus leptin levels as a marker of fat tissue activates hypothalamic-pituitary-gonadal axis So It can be a facilitator role in beginning of maturation process (Burt Solorzano & McCartney, 2010). After adjustment of leptin based on BMI, the difference between TS3 and TS4 became more apparent (Figure 3). Similar to the present study, Blum reported that blood levels of leptin which was adjusted based on BMI, fell to a minimum for boys While reached to maximum values in TS5 for girls (Blum et al., 1997). Moran's research showed significant changes occur in adolescence. Sometimes these changes are independent of changes in body composition that may be related to sex hormones (Jeffery et al., 2012; Moran et al., 2008).

As Figure 1 shows, with the increase in maturity from TS2 to TS5, serum testosterone significantly increases. Wabitsch and colleagues reported that there is an inverse relationship between serum testosterone and leptin ratio to BMI in boys (Wabitsch et al., 1997). Horlick reported that blood levels of leptin that adjusted according to fat mass, declined at the TS5 in boys. However, in our study, after adjustment of leptin according to fat mass, no significant difference was observed between blood levels of leptin in different stages of

puberty. Maybe one of the reasons is that our subjects were obese that naturally had higher leptin levels. Arslanian and colleagues did not find any difference between serum leptin concentrations relative to fat mass in boys and girls before and during puberty. The reasons were the lack of subjects at the end of puberty (TS5) and lack of separation of the groups according to Tanner scale in 5 stage (Arslanian et al., 1998).

In pre-pubertal, differences in body composition between boys and girls is fairly low (Rogol, 2010) but with increasing age and maturity, the rate of growth in lean mass is greater than fat mass in boys (Table 1). in the end of puberty, increased leptin for boys and its decrease for girls is as a result of further subcutaneous fat stores in girls (Roemmich et al., 1998), differences in sex hormones (steroids Gonads, LH, FSH), and probably. Gender differences in metabolic dependent on some variables, such as insulin, GH, or distribution of body fat or even gender differences in X and Y gene expression (Horlick et al., 2000). Consistent with results of some studies (Horlick et al., 2000; Wabitsch et al., 1997) In this study, a negative correlation between testosterone and leptin were observed in Obese boys (Figure 6) which were accompanied by reducing in body fat percent, fat mass and increasing in lean body mass from TS2 to TS5 (table1). Such relationship was seen in male and female newborns at birth (Amiel et al.). Ertl and colleagues reported that There are associated between testosterone and leptin concentrations in cord blood Which can reflect the genetic determinants of blood concentrations of androgens by parents (Ertl et al., 1999). The presence of leptin receptor in testicular cells indicates the role of leptin in the onset of puberty, sperm production and testosterone secretion. However, excessive leptin secretion in obese men has negative effects on this process (Sengupta, Bhattacharya, & Dutta, 2019). Also decrease in leptin concentrations during late puberty in boys may be due to the suppression of mRNA expression in adipose tissue by androgens (Horlick et al., 2000).

One of the variables that was investigated in our study was insulin resistant. Although many studies have shown that obesity is associated with insulin resistance (Fernandez-Real & Ricart, 2003; Rubin et al., 2008; Smith & Ravussin, 2002) However, insulin resistance increases independent of obesity in the beginning of adolescence which is attributed to puberty (Amiel et al., 1986; Burt Solorzano & McCartney, 2010; Guercio et al., 2003; Sinaiko et al., 2001)The findings of Jeffery and colleagues showed that insulin resistance appears before the increase in LH Which is initiator of the puberty and its physical changes (Jeffery et al., 2012). Increased insulin resistance in prepuberty, is somewhat associated with an increase in fat cells (Park et al., 2012). Although in the present study prepubertal subjects (TS1) are not available, However, no significant increase in insulin resistance index from TS2 to TS3 and then reduction of it in later stages can be seen (Figure 4). Amiel, Goran and Guercio showed that the insulin resistance reaches a peak in TS

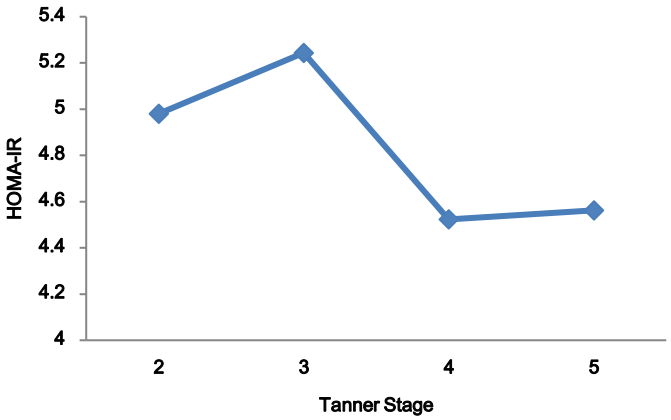

Figure 4. Mean HOMA-IR in obese boys at different stages of puberty.

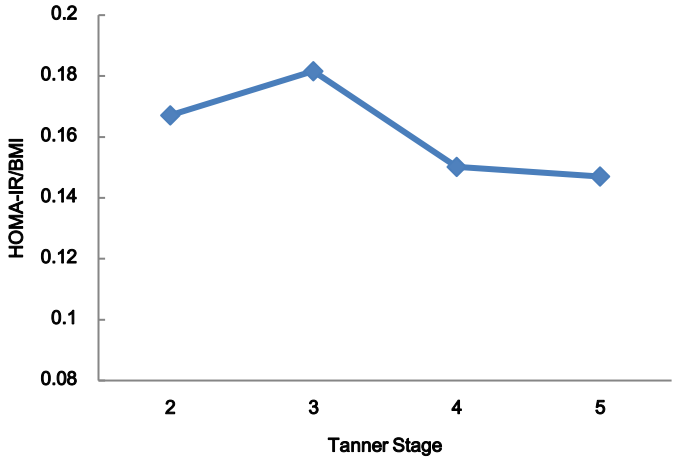

Figure 5. Mean HOMA-IR/BMI in obese boys at different stages of puberty.

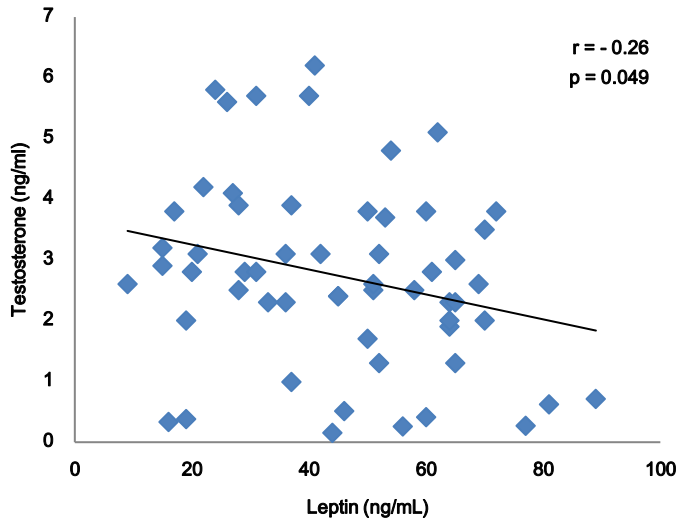

Figure 6. Association between serum testosterone with serum leptin in obese boys at different stages of puberty

3 and TS4. Consistent with our results (Figure 5), Hoffman and colleagues, after adjustment for insulin resistance index based on BMI, did not observe any difference between the pubertal stages (Hoffman et al., 2000). Insulin increases before the onset of puberty which is associated with increasing in adipocyte (Jeffery et al., 2012).

Fat is known, as a reducing agent for insulin acts. Also adipose tissue is associated with increased insulin resistance which leads to compensatory hyperinsulinemia, these conditions lead to increased LPL activity in adipose tissue and lead to more storage of fat. Hyperinsulinemia decreased sex hormone binding globulin (SHBG), which increases the availability of sex steroids. Thus, the process of puberty can be expedited (Biro et al., 2012) In addition to insulin resistance during puberty is associated with increased activity of GH-IGF-1 axis (Rajpathak et al., 2009). Leptin activates signaling pathways which are common with insulin (Dardeno et al., 2010). With increase in age, insulin resistance and leptin levels increased in children is also associated with weight gain (Park et al., 2012). Consistent with our results (Table 1) increase in leptin levels, especially in TS3 can be associated with increase in insulin resistance and fat mass (Morales et al., 2004; Qasim et al., 2008). Oyama and his colleagues reported that leptin resistance is associated with insulin resistance (Oyama et al., 2010). It seems that leptin is involved in the control of insulin sensitivity via different mechanisms (Mantzoros et al., 2011). Activation of leptin receptors in pancreatic β-cells inhibits directly the secretion of insulin and in the long term, especially in obese inhibits insulin gene expression (Marroqui et al., 2012). Also Leptin inhibits lipogenesis and stimulates lipolysis in muscle, liver and fat cells then improves insulin sensitivity (Harwood, 2012).

Conclusion

Generally, this study showed that, differences in serum leptin levels in obese boys at different stages of puberty, were independent of BMI. In the early stages of puberty higher levels of leptin, which indicates higher energy stores in adipose tissue, are essential to facilitate the onset of puberty and the secretion of testosterone. Serum testosterone that from TS2 to TS5 increased more than 15 times leads to rapid growth in muscle tissue were associated with decreased body fat percent, serum leptin and insulin resistance. These changes reflect the cross talk between muscle and adipose tissue by hormonal mediators.

What is already known on this subject?

In previous studies, the role of leptin and insulin and resistance in metabolic disease was investigated.

What this study adds?

In our study, it was shown that puberty can be effective in crosstalk between leptin, insulin and insulin resistance. However, it is suggested that in future studies, the role of exercise training during puberty be measured with these indicators

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All ethic was performed in this study.

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: E.A., H.M.; Methodology: M.R.F.C.; Software: E.A.; Validation: F.R.N.; Formal analysis: M.R.F.C.; Investigation: E.A., M.R.F.C.; Resources: M.R.F.C.; Data curation: E.A., M.R.F.C.; Writing - original draft: F.R.N.; Writing - review & editing: H.M., M.R.F.C.; Visualization: M.R.F.C.; Supervision: E.A.; Project administration: M.R.F.C.; Funding acquisition: E.A., M.R.F.C.

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