

## Serum Leptin Levels In Type-1 Diabetic Children and Adolescents versus Healthy Controls: Relationship to Age, Gender, Body Mass Index, Gonadal Hormones and Pubertal Stages

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### ABSTRACT

**Background:** leptin, the product of the obese (*ob*) gene is produced mainly by adipose tissues. Thus, it is thought to play a key role in the regulation of body fat mass. It appears to be an integral component of various hypothalamo-pituitary endocrine feedback loops. Impaired growth and pubertal delay had been observed in children with type -1 diabetes mellitus. Also they are more prone to develop obesity.

**Objectives:** Since childhood and puberty are periods of major metabolic and endocrine changes, the present study was conducted to: (1) Evaluate developmental changes of serum leptin levels in children and adolescents with type -1 diabetes mellitus in comparison with matched healthy controls in respect with chronological age and pubertal stages (2) Evaluate if leptin concentration would be related to obesity observed in children and adolescents with type-1 diabetes during puberty.

The study included 60 children (32 girls and 28 boys) with age range between 6-16 years with a mean age of  $11.7 \pm 4.3$  diagnosed as type -1 diabetes mellitus by the criteria of American Diabetes Association (ADA) as well as 48 healthy children (24 girls and 24 boys) with matched age and sex with diabetic patients. The patient and control children were grouped according to their chronological age into 4 groups (6-7yr, 8-10yr, 11-13yr and 14-16yr) and according to stages of puberty into 3 groups: prepuberty  $P_1$ , early puberty  $P_2$  and overt puberty  $P_3$ . Serum leptin levels and BMI were measured to all patients and controls. Also, serum testosterone in boys and serum estradiol in girls were measured by ELISA method.

The results of the study showed that Serum leptin levels significantly increased parallel with age and with pubertal stages both in control and diabetic girls. The maximum levels were observed at 14-16yr age group and at overt stage of puberty. Serum leptin levels were significantly higher in diabetic girls than controls at all studied groups. In control boys, leptin levels were significantly higher at 8-10yr and during  $P_1$  stage then a significant decline occurred thereafter. In contrast, the diabetic boys showed no such decline either with age or with pubertal staging. Diabetic boys had significantly higher leptin levels than control boys at all studied groups. Serum leptin levels in girls were significantly higher than boys either in control or diabetic children. Diabetic children (girls and boys) were significantly older than controls during  $P_2$  and  $P_3$  stage. BMI was significantly increased in diabetic children (girls and boys) than controls during  $P_1, P_2$  and  $P_3$  stage whereas

serum estradiol in diabetic girls and testosterone in diabetic boys were significantly lower than controls during  $P_2$  and  $P_3$  stages. Significant positive correlations were observed between serum leptin levels versus age, BMI and estradiol hormone in control girls ( $r=0.75$ ,  $P<0.001$ ,  $r=0.64$ ,  $P<0.001$  and  $r=0.77$ ,  $P<0.001$  respectively). Also significant positive correlations were found in diabetic girls between serum leptin levels and each of age, BMI and estradiol hormone ( $r=0.64$ ,  $P<0.001$ ,  $r=0.72$ ,  $P<0.001$  and  $r=0.66$ ,  $P<0.001$  respectively). In control boys significant negative correlations were observed between serum leptin level and each of age ( $r=-0.4$ ,  $P<0.05$ ) and testosterone hormone ( $r=-0.62$ ,  $P<0.001$ ) whereas non significant with BMI. Significant positive correlations were found in diabetic boys between serum leptin levels and each of age ( $r=0.67$ ,  $P<0.001$ ) BMI ( $r=0.57$ ,  $P<0.01$ ), while the correlation with testosterone was non significant.

**In conclusion** leptin appears to participate in various endocrinological and physiological process in human body. Among the more notable are obesity and pubertal delay-associated diabetes. Thus, it may be involved in regulation of body weight and signaling the onset of puberty and maintenance of reproductive function thereafter.

## INTRODUCTION

Puberty is the process of sexual maturation by which reproductive competence is attained. The changes in physical appearance and behavior that characterize puberty are the direct result of rising concentrations of gonadal steroids (testosterone in boys and estradiol in girls), their production is driven by increased levels of pituitary gonadotropins which are in turn regulated by the release of gonadotropin releasing hormone (GnRH) from hypothalamic neurons. However, why this process occurs at a particular age and not before or after is unknown<sup>(1)</sup>

It is widely accepted that a minimum level of body mass or a certain amount of body fat is necessary for initiation of puberty but what metabolic factors act to signal the reproductive axis that sexual development may proceed or the means by which this signal is transmitted to this axis has not been

fully delineated. A logical candidate to convey such information from the adipose tissues to the hypothalamus is the obese (ob) gene product, leptin.<sup>(2)</sup>

Leptin is derived from the Greek word "Leptos" means thin. It is a member of cytokine family which is mainly produced by the adipocytes and acts predominantly by binding to a receptor site on ventromedial nucleus of hypothalamic cells<sup>(3)</sup>. It has been shown that the soluble leptin receptor (sOB-R) is the major leptin binding protein in human circulation. It occurs in two different N-glycosylated isoforms, which circulate as dimers and oligomers in human blood. Its physiological function in human circulation has not yet been fully elucidated, presumably partially due to molecular diversity and to technical problems in the specific determination of sOB-R<sup>(4)</sup>

The biological effects of leptin include suppression of food intake and increase of energy expenditure and consequently the regulation of body

weight<sup>(5)</sup>. In addition, leptin has been proposed as physiological link between nutritional status and reproductive maturation and function and may potentially serve as a trigger or metabolic gate for sexual development and puberty<sup>(6,7)</sup>. The most important variable that determine circulating leptin levels is body fat mass. Obviously, under conditions of regular eating cycles, leptin reflects the proportion of adipose tissues<sup>(8,9)</sup>. In addition, some epidemiological studies had suggested that, independent of body composition, leptin concentration may be increased by environmental factors such as high carbohydrate diet and a high level of physical activity<sup>(10)</sup>. Leptin secretion is also affected by both insulin and cortisol which are potent and possibly the physiological regulators of leptin expression in human adipose tissue<sup>(11)</sup>.

Patients with complete deficiency of leptin due to mutations in leptin gene express a phenotype of congenital obesity and show numerous endocrinological abnormalities including insulin resistance and hypogonadotropic hypogonadism<sup>(12)</sup>. Also, low leptin levels caused by deficiency or destruction of adipocytes are associated with abnormalities such hypertriglyceridemia and severe insulin resistance. Therefore, leptin could be an integral component of various metabolic and endocrine feedback loops<sup>(13)</sup>.

Impaired growth and pubertal delay had been observed in children with type-1 diabetes<sup>(14,15)</sup>. These children are also more inclined to develop obesity even if they are

treated with modern insulin therapy. The mechanism of weight gain in subjects with type 1 diabetes mellitus has not been clarified. However, there are suggestions of an association between weight gain and hyperinsulinemia, which is reflected by correlations between the insulin dose and the frequency of insulin injections<sup>(16)</sup>. A putative relationship between leptin and obesity contributing to the pathophysiology of diabetes has been proposed. A potential contribution of leptin is supported by findings showing leptin to have a direct effect on insulin activity and regulating total body sensitivity to insulin and triglyceride levels in lipodystrophic syndromes<sup>(17)</sup>.

**The aim** of this of this study was to (1) Evaluate the developmental changes of serum leptin levels in children and adolescents with type-1 diabetes mellitus in comparison with matched healthy controls in respect with chronological age and pubertal stages. (2) Evaluate if leptin concentrations would be related to obesity observed in children and adolescents with type I diabetes during puberty.

## **SUBJECTS & METHODS**

The study included 60 children (32 girls and 28 boys) with age range between 6-16 years with a mean age of  $11.7 \pm 4.3$  diagnosed as type -1 diabetes mellitus by the criteria of the American Diabetes Association (ADA)<sup>(18)</sup> as well as 48 healthy children (24 girls and 24 boys) with matched age and sex with diabetic patients. The studied groups were recruited from Assiut Pediatric

University Hospital during the period from March to June 2007.

All patients had diabetes duration more than 2 years and were receiving conventional insulin therapy (two daily injections of mixed intermediate and rapidly acting insulins). Apart from insulin, no other permanent medications were used. Non of the patients had clinical symptoms or signs of either neuropathy, retinopathy or nephropathy. A thorough history and full clinical examination as well as anthropometric data were recorded on a structured data sheet. Body mass index (BMI) defined as weight in kilograms divided by the square of height in meters ( $\text{kg}/\text{m}^2$ ) was calculated.

- To assess the changes in serum leptin levels in relation to age: the children (patients and controls) were grouped according to their chronological age into 4 groups (6-7 years, 8-10 years, 11- 13 years and 14-16 years)
  - To assess the changes in serum leptin levels in relation to pubertal development: the children (patients and controls) were grouped according to their stage of pubertal development. Puberty stages were established based on pubic hair and breast development according to Tanner<sup>(19)</sup> and testosterone levels in boys and estradiol ( $\text{E}_2$ ) in girls according to Garcia-Mayor et al.<sup>(20)</sup>.
1.  $\text{P}_1$ = prepuberty:  $\text{E}_2$  levels <9.96 pg/ml and testosterone levels <1.74 nmol/L.
  2.  $\text{P}_2$ = early puberty:  $\text{E}_2$  levels ranging from 9.96-24.98 pg/ml and

testosterone levels ranging from 1.74-5.21 nmol/L.

3.  $\text{P}_3$ = overt puberty:  $\text{E}_2$  levels > 24.98 pg/ml and testosterone levels > 5.21 nmol/L.

The study was approved by the University Ethics committee, and written informed consent was obtained from parents of both diabetic and control children.

Blood samples (5c.c) were aspirated in the morning 08-09 O'clock after overnight fasting, serum was separated by centrifugation and stored at  $-20\text{C}^\circ$  until assay for serum leptin, testosterone in boys and estradiol hormone in girls. In pubertal girls, blood samples were obtained in the follicular phase of menstrual cycles,

Serum leptin level was measured by DRG diagnostics leptin ELISA kit. Serum testosterone were measured by ELISA test for the quantitative determination of testosterone kit. Serum estradiol was measured by FERTIGENIX- $\text{E}_2$  EASIA kit.

**Statistical analysis:** the data were presented as means values  $\pm$  SE. Differences between groups were determined using Student's Newman-Keuls- t-test. Pearson correlation was also used. The level of significance was accepted with  $\text{P}<0.05$ . Prism computer program (graph pad version 3.0) was used to draw diagrams<sup>(21)</sup>.

## RESULTS

**Table (1)** showed the mean values  $\pm$  SE of serum leptin levels (ng/ml) at different age groups in controls and diabetic children. It was found that there was a parallel significant increase of serum leptin

levels with age in control girls ( $P < 0.05$ ,  $P < 0.001$ , and  $P < 0.001$  at age groups 8-10yr, 11-13yr and 14-16yr respectively in comparison with age group 6-7yr) and in diabetic girls ( $P < 0.01$ ,  $P < 0.001$  and  $P < 0.001$  at age groups 8-10yr, 11-13yr and 14-16yr respectively in comparison with age group 6-7yr). At all age groups the levels were significantly higher in diabetic girls as compared to controls ( $P < 0.01$  at age groups 6-7yr, 8-10yr and 11-13yr and  $P < 0.001$  at age group 14-16yr) as shown in **table 2**.

In contrast, it was found that in control boys a significant rise of the levels occurred at age group 8-10yr and 11-13yr ( $P < 0.001$  and  $P < 0.05$ ) then the level declined at age group 14-16yr becoming non significantly varied in comparison with age group 6-7yr. The evolution of leptin levels in diabetic boys with age showed significant elevation at all age groups ( $P < 0.05$ ,  $P < 0.001$  and  $P < 0.001$ ) and no decline as compared to age group 6-7yr (table 1). In comparison to controls, the levels in diabetic boys were significantly higher at all age groups ( $P < 0.05$ ,  $P < 0.05$ ,  $P < 0.001$  and  $P < 0.001$  respectively) as shown at **table 2**.

As regard gender, table (2) showed that at all age groups the levels in control girls were significantly higher than control boys ( $P < 0.01$ ,  $P < 0.05$ ,  $P < 0.001$ ,  $P < 0.001$  respectively). Also, in diabetic girls than diabetic boys ( $P < 0.01$ ,  $P < 0.01$ ,  $P < 0.01$  and  $P < 0.001$  respectively).

**Table (3)** serum leptin levels were tabled according to pubertal stages (prepubertal  $P_1$ , early puberty  $P_2$  and overt puberty  $P_3$ ). It was observed that the levels both in

control and diabetic girls at  $P_2$  and  $P_3$  stages were significantly higher than  $P_1$  stage ( $P < 0.05$  and  $P < 0.01$  for controls and  $P < 0.01$  and  $P < 0.001$  for diabetics respectively). In comparison to control girls levels in diabetic girls were significantly higher at all pubertal stages ( $P < 0.05$ ,  $P < 0.001$  and  $P < 0.001$  respectively). Regarding control boys, the levels in  $P_2$  and  $P_3$  stages were significantly lower than  $P_1$  stage ( $P < 0.05$  and  $P < 0.001$  respectively), while in diabetic boys the levels were significantly higher at  $P_2$  and  $P_3$  stages in comparison to  $P_1$  stage ( $P < 0.05$  and  $P < 0.01$  respectively). On comparing diabetic and control boys, the levels were significantly higher in diabetic than controls at all pubertal stages ( $P < 0.05$ ,  $P < 0.001$  and  $P < 0.001$  respectively).

**Table (4)** showed the age, BMI and serum levels of estradiol (means  $\pm$ SE) in control and diabetic girls at different pubertal stages. As regard age, diabetic girls were older than controls at  $P_2$  and  $P_3$  stage ( $P < 0.05$  and  $P < 0.01$  respectively) whereas at  $P_1$  stage the age was non significantly varied. The BMI was significantly increased at all pubertal stages of diabetic girls in comparison to controls ( $P < 0.01$ ,  $P < 0.001$  and  $P < 0.001$  respectively). On comparing the hormonal pattern, estradiol levels were significantly lower in diabetic girls than control during  $P_2$  and  $P_3$  stages ( $P < 0.001$  and  $P < 0.001$  respectively) while the levels were non significantly different during  $P_1$  stage.

**Table (5)** showed the age, BMI, serum testosterone levels (means  $\pm$ SE) in control and diabetic boys in different pubertal stages. In was

observed that the diabetic boys were older than controls at P<sub>2</sub> and P<sub>3</sub> stage (P<0.05 and P<0.05 respectively) while the age was similar at P<sub>1</sub> stage. Regarding BMI, the values in diabetic boys were significantly higher than controls at all pubertal stages (P<0.01, P<0.001 and P<0.001 respectively). Serum levels of testosterone hormone were significantly decreased during P<sub>2</sub> and P<sub>3</sub> stages in diabetic boys than controls (P<0.001, and P<0.001 respectively), whereas the levels were non significantly varied at P<sub>1</sub> stage.

**Figures** (1A, B and C): showed the correlation coefficient between serum leptin levels versus age, BMI and estradiol hormone in control girls. Significant positive correlations were observed (r=0.75, P<0.001, r=0.64, P<0.001 and r=0.77, P<0.001 respectively). In figure (2 A, B and

C), also significant positive correlations were found in diabetic girls between serum leptin levels and each of age, BMI and estradiol hormone (r = 0.64, P<0.001, r = 0.72, P<0.001 and r = 0.66, P<0.001 respectively)

In control boys significant negative correlations were observed between serum leptin level and each of age (r = -0.4, P<0.05) and testosterone hormone (r = -0.62, P<0.001) whereas non significant with BMI (figure 3A, B and C). In figure (4 A, B and C), significant positive correlations were found in diabetic boys between serum leptin levels and each of age (r = 0.67, P<0.001) BMI (r = 0.57, P<0.01), while the correlation with testosterone was non significant.

**Table (1): Comparison of serum leptin levels, ng/ml (means ± SE) in control and diabetic children according to their chronological age.**

Age group (years)		Girls		Boys	
		Controls	Diabetics	Controls	Diabetics
I	6-7	4.5±0.5	6.77±0.53	2.11±0.35	4.21±0.65
II	8-10	6.57±0.58	9.78±0.7	4.92±0.48	6.72±0.65
III	11-13	8.31±0.64	12.3±0.87	3.46±0.43	8.2±0.55
IV	14-16	9.21±0.62	14.7±1.2	2.53±0.35	8.6±0.75
P- value on comparing:					
I and II		<0.05	<0.01	<0.001	<0.05
I and III		<0.001	<0.001	<0.05	<0.001
I and IV		<0.001	<0.001	NS	<0.001

**Table (2): Comparison between serum leptin levels, ng/ml (means ± SE) in controls and diabetic children in the different age groups as well as gender.**

		6-7 years	8-10 years	11-13 years	14-16 years
Girls	Controls	4.5 ± 0.5	6.57 ± 0.58	8.31 ± 0.64	9.21 ± 0.62
	Diabetics	6.77 ± 0.53	9.78 ± 0.7	12.3 ± 0.87	14.7 ± 1.2
	p-value	<0.01	<0.01	<0.01	<0.001
Boys	Controls	2.11 ± 0.35	4.92 ± 0.48	3.46 ± 0.43	2.53 ± 0.35
	Diabetics	4.21 ± 0.65	6.72 ± 0.65	8.2 ± 0.55	8.6 ± 0.75
	p-value	<0.05	<0.05	<0.001	<0.001
Comparison between control girls & boys		<0.01	<0.05	<0.001	<0.001
Comparison between diabetic girls & boys		<0.01	<0.01	<0.01	<0.001

**Table (3): Comparison between serum leptin levels, ng/ml (means ± SE) in controls and diabetic children in different pubertal stages.**

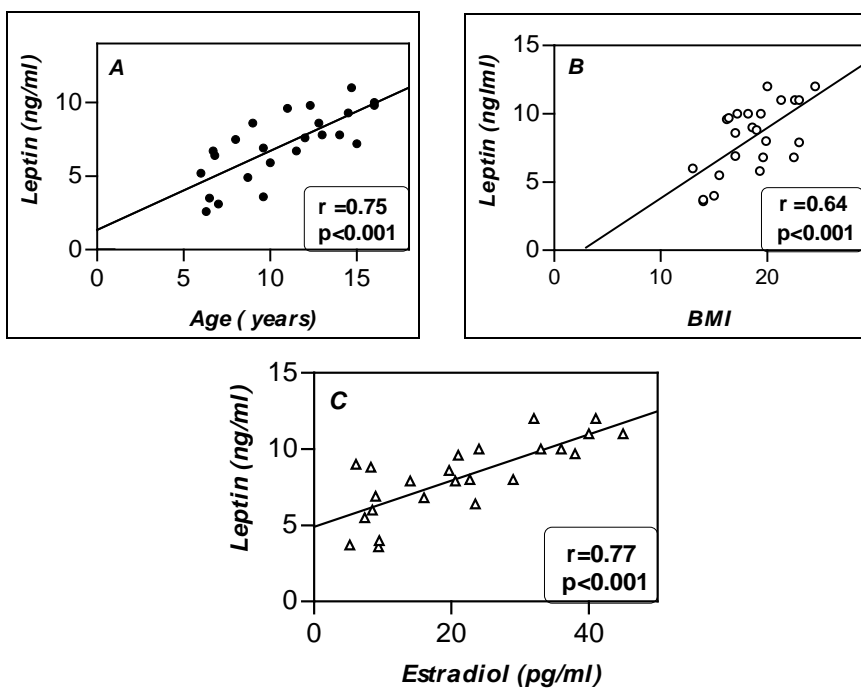
	Girls			Boys		
	Control	Diabetic	P-value	Control	Diabetic	P-value
Prepubertal (P <sub>1</sub> )	5.99 ± 0.64	8.47 ± 0.61	<0.05	4.36 ± 0.42	5.96 ± 0.47	<0.05
Early puberty (P <sub>2</sub> )	7.95 ± 0.66	11.6 ± 0.73	<0.001	3.23 ± 0.33	8.1 ± 0.51	<0.001
Overt puberty (P <sub>3</sub> )	9.41 ± 0.59	15.3 ± 0.81	<0.001	2.21 ± 0.26	8.8 ± 0.63	<0.001
P- Value on Comparing:						
P <sub>2</sub> versus P <sub>1</sub>	<0.05	<0.01		<0.05	<0.05	
P <sub>3</sub> versus P <sub>1</sub>	<0.01	<0.001		<0.001	<0.01	

**Table: (4) Age, BMI, serum estradiol levels (means ± SE) in control and diabetic girls in different pubertal stages.**

	Prepubertal (P <sub>1</sub> )		Early puberty (P <sub>2</sub> )		Overt puberty (P <sub>3</sub> )	
	Controls	Diabetics	Controls	diabetics	Controls	Diabetics
Age (years)	9.47 ± 0.35	10.32 ± 0.33	11.41 ± 0.66	13.63 ± 0.56	13.95 ± 0.53	16.4 ± 0.43
P- value		NS		<0.05		<0.01
BMI (kg /m <sup>2</sup> )	17.3 ± 0.5	22.1 ± 0.75	19.6 ± 0.76	25.2 ± 0.72	20.9 ± 0.86	27.3 ± 0.83
P- value		<0.01		<0.001		<0.001
Estradiol (pg/ml)	7.99 ± 0.61	6.93 ± 0.51	22.57 ± 1.8	15.8 ± 1.6	35.2 ± 1.9	27.6 ± 1.7
P- value		NS		<0.001		<0.001

**Table (5): Age, BMI, serum testosterone levels (means ± SE) in control and diabetic boys in different pubertal stages**

	Prepubertal (P <sub>1</sub> )		Early puberty (P <sub>2</sub> )		Overt puberty (P <sub>3</sub> )	
	Controls	Diabetic	Controls	Diabetic	Controls	Diabetic
Age (years)	9.92±0.41	10.66±0.35	12.7±0.59	14.9±0.64	14.6±0.56	16.22±0.44
P- value		NS		<0.05		<0.05
BMI (kg/m <sup>2</sup> )	16.6±0.6	20.6±0.66	19.3±0.63	24.6±0.76	21.7±0.73	26.8±0.87
P- value		<0.01		<0.001		<0.001
Testosterone (nmol/L)	0.88±0.03	0.72±0.02	4.86±0.2	2.11±0.09	10.2±1.2	7.1±0.53
P- value		NS		<0.001		<0.001



**Figure (1): The correlation coefficient between serum leptin level and age (A), BMI (B) and estradiol hormone (C) in control girls.**



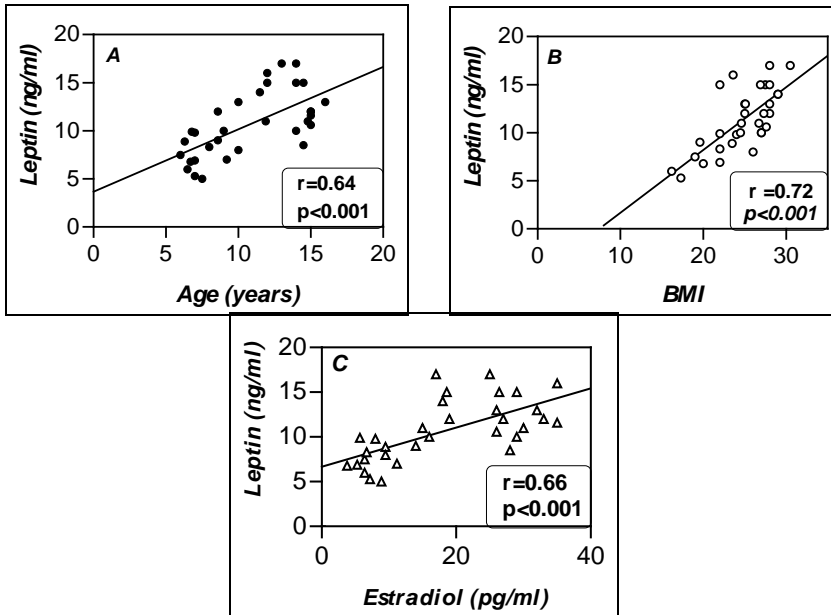


Figure (2): The correlation coefficient between serum leptin level and age (A), BMI (B) and estradiol hormone (C) in diabetic girls.

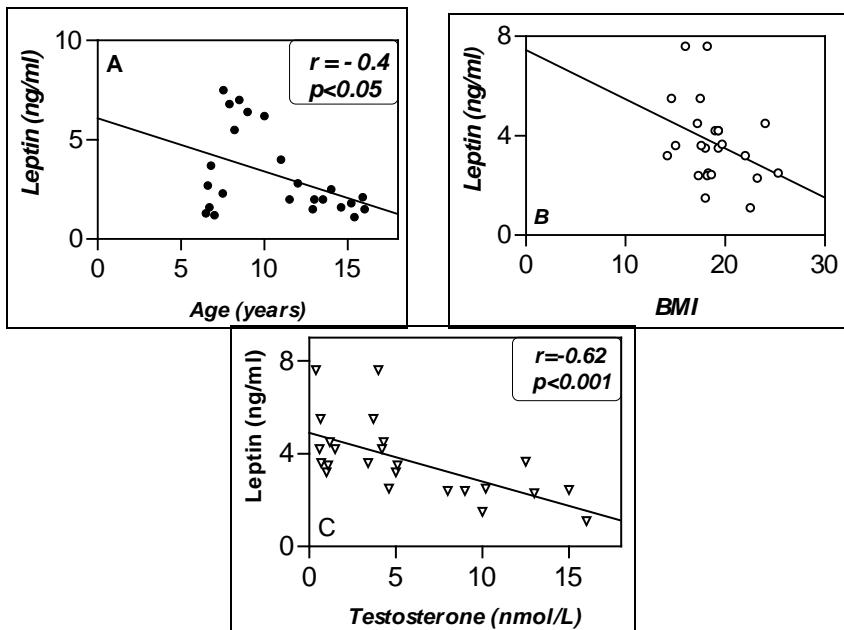


Figure (3): The correlation coefficient between serum leptin level and age (A), BMI (B) and testosterone hormone (C) in control boys.

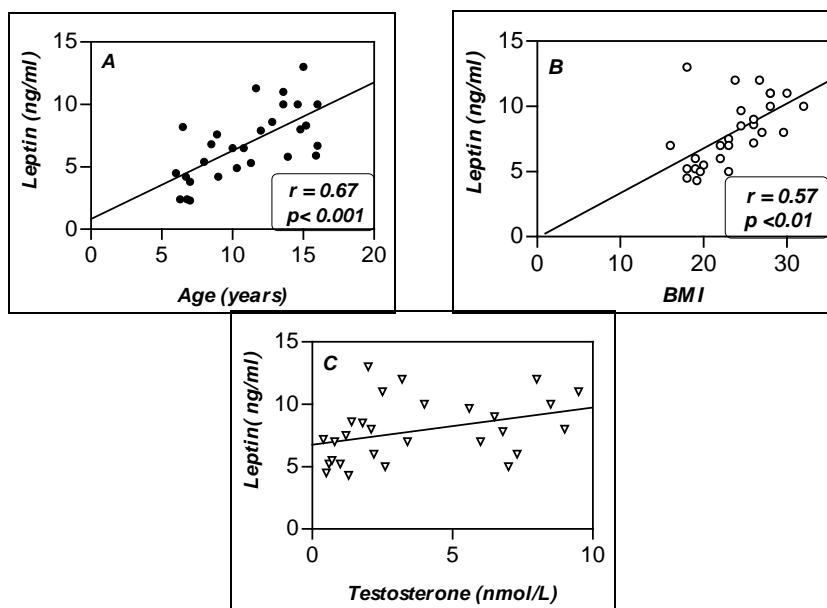


Figure (4): The correlation coefficient between serum leptin level and age (A), BMI (B) and testosterone hormone (C) in diabetic boys.

## DISCUSSION

The onset of puberty requires changes in hypothalamic GnRH secretion that entrain alterations in the secretion of pituitary gonadotropins and thereby augmented secretion of gonadal steroids. The identity of factors that act on the GnRH neuron to initiate puberty remains unknown<sup>(22)</sup>. The critical weight theory of puberty, although attractive on several counts, has not itself provided a molecular link between weight or adiposity and the central control of reproduction. Based on several lines of evidence, leptin is the plausible candidate for such a link<sup>(23)</sup>. This background was the basis for the present work in which the circulating

levels of leptin, were assessed in healthy and type-1 diabetic children under two different frames: chronological variation and stages of pubertal development.

In the present study, it was found that serum leptin level in healthy children and adolescents exhibited two divergent patterns depending on gender in relation to chronological age as well as to pubertal stage. In control girls, serum leptin levels showed a significant increase parallel with age reaching a maximum level at age group 14-16 yr and with pubertal development reaching its highest level during overt stage of puberty. On the other hand, in boys the significant highest levels of serum leptin were observed at 8-10yr and during

prepubertal stage, then a significant decline of the levels were observed during 14-16yr as well as during pubertal and overt puberty stage (table 1 and 3). Our results are in agreement with Garcia-Mayor et al.<sup>(20)</sup> who reported that, in girls, leptin levels in the circulation rise progressively from 5 to 15years of age, whereas in boys, levels increased between 5 and 10years, of age and declined thereafter through 13years of age. Hassink et al.<sup>(24)</sup> found that there is a developmental increase in leptin during childhood and early adolescence that precedes the initiation of major pubertal events and as adolescents approach the end of puberty, indicated by their passing through the different Tanner stages, leptin levels decline in boys but not in girls. In another study, leptin values did not change with pubertal stage in boys, nor was there a transient increase in leptin before the onset of puberty<sup>(25)</sup>.

Results of the present study showed a significant positive correlation between serum leptin and age in control girls ( $r=0.75$ ,  $P<0.001$ ), fig (1A) but negative correlation ( $r=-0.4$ ,  $p<0.05$ ), fig (3A) in control boys. These results are similar to those reported by Blum et al.,<sup>(26)</sup> and Mann et al.,<sup>(27)</sup> who found that leptin levels in girls were positively correlated with age but negatively in boys. They added that in girls an analysis according to pubertal stage showed a steady increase of the levels from Tanner stage 1 to Tanner stage 5. In boys leptin levels were highest at Tanner stage 2 and declined thereafter at Tanner stage 5.

Results of the current study showed that in type-1 diabetic girls serum leptin levels significantly increased parallel with age and with pubertal stages from pre to overt stage. In diabetic boys, a significant increase was observed from age group 8-10yr to 14-16yr and in early and overt puberty. Unlike healthy boys, no decline of the levels of leptin was observed in diabetic boys (table 1 and 3). Both diabetic girls and boys showed significant higher leptin level than healthy groups of age and sex-matched normal children. This could be observed according to age-related or pubertal stage analysis (table 2 and 3). In addition, based on gender-difference, serum leptin levels were significantly higher in girls than boys either in controls or diabetic children (table2). Significant positive correlations between serum leptin levels and age were observed in diabetic girls ( $r= 0.64$ ,  $p<0.001$ ), fig (2A) and in diabetic boys ( $r=0.67$ ,  $p<0.001$ ), fig (4A). Luna et al.<sup>(14)</sup>, reported that serum leptin concentration in diabetic girls were parallel to the values of the normal although as a group, they were significantly higher than in normal controls. Diabetic boys had lower leptin levels than girls and, in contrast with normal boys, did not show a drop after the 10 year period. They also, added that when serum leptin were analyzed according to pubertal stage, leptin levels in pre and overt puberty were higher in diabetic girls and diabetic boys than healthy matched control. Also, Kiess et al.<sup>(28)</sup> reported that serum leptin concentration in treated diabetic children at Tanner stage 5 were significantly higher than

those in the reference population when adjusted for sex, pubertal stage and BMI. In addition, Kratzsch et al.<sup>(29)</sup> found that in diabetic girls serum leptin concentrations increased during pubertal development. In boys, leptin levels increased only until Tanner stage 2 and decreased until the end of puberty. They also reported that leptin levels were significantly higher in girls with diabetes than in boys with the exception of patients with Tanner stage 2. On the other hand, other studies reported that serum leptin concentrations of the prepubertal type-1 diabetic children did not differ from those of the control subjects of similar age, sex and body composition<sup>(15,30)</sup>. Also, Karaguzel, et al.<sup>(31)</sup> found that neither serum leptin levels nor body composition was significantly altered in children and adolescents with type-1 diabetes managed with intensive insulin therapy.

Dencker et al.<sup>(32)</sup> reported that leptin expression and circulating levels increase in parallel to the amount of adipose stores. The relationship between leptin levels and fat mass is curvilinear, rather than linear implying that leptin secretion increases with increasing fat mass. The adipocytes size *per se* appears to be another major determinant of leptin mRNA expression. In addition, to total fat mass and the size of adipocytes, the type of adipose tissue distribution (i.e. visceral or subcutaneous) may also be related to leptin levels. Leptin mRNA expression is higher in subcutaneous than in visceral fat depots.

In the present study, an increased BMI was observed among pre, early

and overt pubertal children with type-1 diabetes mellitus (girls and boys) than controls (table 4 and 5). When leptin levels were related to BMI, significant positive correlations were observed in control girls ( $r=0.64$ ,  $P<0.001$ ), fig (1B), diabetic girls ( $r=0.72$ ,  $P<0.001$ ), fig (2B) and diabetic boys ( $r=0.57$ ,  $P<0.01$ ), fig (4B) but not in control boys fig (3B). Similar to these data. Sinha et al.<sup>(33)</sup> and Ostlund et al.<sup>(34)</sup> showed that serum leptin is strongly correlated with BMI in healthy and diabetic children. Christos et al.<sup>(35)</sup> reported that in healthy boys, both the increase in leptin concentrations before and at the onset of puberty and the subsequent decrease in leptin concentrations to baseline in the period after puberty occurred despite constantly increasing BMI. Ahmed et al.<sup>(36)</sup> reported that when the female diabetic patients were grouped with regard to pubertal status, there was a progressive increase in both BMI and serum leptin levels with progress of puberty, whereas leptin levels of males did not increase between pubertal and post pubertal stages despite the fact that BMI values increased during puberty and early adulthood in the male patients. The most likely explanation for this discrepancy is that in females the increase in BMI during and after puberty is mainly associated with an increase in adipose tissues, whereas in males the increase in BMI is due to an increase in the muscle mass<sup>(37)</sup>. Beside this sex difference in body composition is the direct influences of rising androgen levels which may themselves inhibit peripheral leptin secretion<sup>(38)</sup>. Also, Szadkowska et al.<sup>(39)</sup> reported that serum leptin levels

were positively correlated with BMI in children with type-1 diabetes and the observed relationship between leptin concentration and insulin resistance in these patients is mainly due to body fat composition. On the other hand, Luna et al.,<sup>(14)</sup> found that the significant correlation between leptin and BMI was found only in healthy girls and not in healthy boys or diabetic boys and girls.

Another factor that has been found to predict leptin levels is gender. Even after adjustment for BMI<sup>(40)</sup>, percentage of body fat and total fat mass<sup>(41)</sup> or skin fold thickness and age<sup>(34)</sup>, females appear to have higher leptin levels than males. Data of the present study revealed that serum leptin level is significantly higher in girls than boys either in controls or diabetics. In boys, leptin levels were always lower than in the girls, which was evident from the first time period studied (age 6-7yr). Because no sex-related hormonal changes are present at such early life period, a gender-based factor would explain such differences. These results are consistent with many previous studies<sup>(42,14,43,29)</sup>. In another study they found that the sexual dimorphism in leptin levels could be due to either the inducing effects of estrogen or progesterone in females and the suppressive effect of androgens on leptin production in males. Estrogens not only have been shown to increase leptin production but also to alter the balance between the long and short leptin receptor isoforms, thus increasing the tissue sensitivity to leptin<sup>(44)</sup>. Another reason for the observed sexual dimorphism in leptin levels might be the excess

subcutaneous fat in females which produce more leptin mRNA<sup>(41)</sup>. Also, female adipose tissue may be more sensitive to hormones such as insulin, glucocorticoid or other substances that stimulate leptin production<sup>(45)</sup>.

Kieffer and Habener<sup>(46)</sup> introduced the notion of the adipoinsular axis- a dual hormonal feed-back loop involving the hormones insulin and leptin. Insulin is an adipogenic hormone favoring anabolism and increased body mass. Leptin on the other hand is a hormone that confine the storage of triglyceride to the adipocytes, while limiting triglyceride storage in non-adipocytes, thus protecting them from lipotoxicity. It also, act centrally to suppress food intake and increase energy expenditure. Ahren et al.<sup>(47)</sup> reported that plasma leptin and insulin levels correlate with each other, possibly because insulin stimulates leptin synthesis and release.

Results of the current study showed that serum leptin levels were significantly higher in diabetic girls and boys than those of age and sex-matched controls. There are several possible explanations for this finding; one of these explanation is that, it is conceivable that body composition of IDDM patients is altered with the progressive use of multiple insulin injection in the late puberty shifting body composition towards increased fat mass with increasing leptin production<sup>(48)</sup>. Transient but chronically and repetitively occurring hyperinsulinemia might stimulate leptin expression. In fact, since exogenous insulin requirements increase during puberty due to reduced insulin sensitivity, higher

insulin doses administered by the adolescent patients might lead to hyperinsulinemia. This hyperinsulinemia acts to increase adipogenesis, exacerbating the existing obesity and increasing leptin production even further. This positive feedback loop may be a contributing factor to the pathogenesis of obesity-associated diabetes mellitus<sup>(9)</sup>. Supporting these explanations, Soliman et al.<sup>(49)</sup> found that, in newly diagnosed children with type-1 diabetes prior to initiation of insulin therapy, circulating levels of leptin were significantly lower compared with 5 days after insulin therapy. This indicates that treatment with insulin and increased food intake to avoid recurrent hypoglycemic episodes, stimulate fat synthesis and subsequently BMI and contribute to increased leptin secretion. This may explain the higher leptin levels in poorly controlled diabetic children who were over-treated with insulin.

Furthermore, it had been shown in obese children, the ratio of leptin in the cerebrospinal fluid (CSF) to circulating leptin levels is decreased, suggesting that the capacity for leptin transport via CSF into the brain is reduced in obese subjects despite having higher leptin in their plasma than those found in lean individuals. This mechanism may provide an explanation for leptin resistance observed in obese children<sup>(50,51)</sup>. This suggested that obese individuals who may not respond to their own high endogenous leptin levels may respond to a leptin analogue which is able to penetrate into the central nervous system<sup>(9)</sup>. Ebihara et al.<sup>(52)</sup> found that leptin-replacement therapy

ameliorated macro-and micro-albuminuria and showed no deterioration of neuropathy and retinopathy of diabetic patients. Therefore, leptin-replacement therapy may be beneficial to diabetic complications and lipodystrophic ones.

Additional explanation for increased serum leptin levels in diabetic than controls is the increased soluble leptin receptor (sOB-R) in diabetes which is the major leptin binding protein in human circulation. A possible explanation of the increased sOB-R secretion observed in diabetic patients may be the well-known induction of protein kinase C (PKC) activity in the diabetic state. PKC can induce A disintegrin and metalloprotease (ADAM) 17, which is able to shed the extracellular domain of cytokine-like hormone receptors, such as growth hormone-receptor and sOB-R leading to increased levels of the soluble receptors in diabetic patients. The biologic consequences of increased sOB-R levels in diabetes are unclear so far. But as the averaged binding affinity of the sOB-R for leptin is in the same range as the binding affinity of leptin for its membrane receptor, the soluble form is capable of modulating leptin's action<sup>(53)</sup>. This modulation may occur via the mechanism that is the excess of sOB-R may inhibit leptin binding to membrane receptors by competing directly with its ligand, thus suppressing leptin bioactivity and contribute to leptin insensitivity, with subsequent weight gain and decrease energy consumption in diabetic patients<sup>(4,54)</sup>.

In this study leptin levels were correlated with gonadal hormones. Overall, in the control and diabetic girls, a parallel increase in both hormones: leptin and estradiol was observed where rising of leptin levels coincided with rising of estradiol levels during P<sub>2</sub> stage, with no strident divergence among them. This is supported by the observed positive correlation between serum leptin and estradiol in control ( $r = 0.77$ ,  $P < 0.001$ ), fig (1C) and diabetic ( $r = 0.66$ ,  $P < 0.001$ ), fig (2C) girls. In comparison to controls, serum estradiol levels in diabetic girls were non significant during P<sub>1</sub> stage but significantly lower during P<sub>2</sub> and P<sub>3</sub> (table 4). In control boys the significant highest levels of serum leptin were observed at age group 8-10yr and in prepubertal stage. Then a negative inflexion in leptin levels occurred at P<sub>2</sub> stage after the testosterone rise, where negative correlation was observed ( $r = -0.62$ ,  $P < 0.001$ ), fig (3C) suggesting a direct inhibitory action of this steroid hormone on leptin production at the adipose tissue. In diabetic boys serum leptin levels were non correlated with serum testosterone hormone, fig (4C). In comparison to controls, serum testosterone levels at P<sub>2</sub> and P<sub>3</sub> stage in diabetic boys were significantly lower but at P<sub>1</sub> stage were non significantly varied (table 5). Our results are nearly similar to those reported by Garcia-Mayor et al.<sup>(20)</sup> who reported that in normal girls the two hormones leptin and estradiol rose progressively from P<sub>1</sub> to P<sub>3</sub> while in boys leptin decreased from P<sub>1</sub> to P<sub>3</sub> whereas testosterone rose. In a longitudinal assessment of hormonal

and physical alterations during puberty in normal boys reported by Christos et al.<sup>(35)</sup> leptin levels rose by approximately 50% just before the onset of puberty and decreased to the baseline values after the initiation of puberty and testosterone hormone rising and was stable for more than 2 yr. In agreement with our results, luna et al.<sup>(14)</sup> reported that in prepubertal stage estradiol and testosterone levels were non significantly varied in diabetic girls and boys than controls while estradiol levels in diabetic girls during overt puberty were significantly lower than controls, but they added that, the decreased leptin concentration associated with the increase in testosterone levels observed in healthy boys was absent in diabetic boys who showed significantly higher levels of both leptin and testosterone than their matched controls. Also, they reported that a simple linear regression analysis between leptin values and sex steroid values revealed a significant correlation between leptin and estradiol in diabetic girls, while not in healthy girls. In control boys, there was a significant negative correlation with testosterone, whereas in diabetic boys, no correlation was found.

Previous studies had demonstrated the presence of leptin receptors in the ventromedial and arcuate nuclei of the hypothalamic regions which are anatomically associated with the control of appetite and reproductive function. Leptin may be the link which conveys information on the state of adipose tissues to the hypothalamus to initiate pubertal development<sup>(3)</sup>. In experimental animals, administration of leptin

completely restored gonadotropin secretion, secondary sex organ weight, function and fertility in Ob/Ob mice<sup>(55)</sup>. Leptin had also been shown to advance the onset of puberty by an average of 11 days in normal mice<sup>(56)</sup>.

A link between leptin levels and onset of puberty in human had been suggested. Leptin receptor mRNA had been localized to human ovaries and testes<sup>(57)</sup>. It is secreted in a pulsatile manner and its circulating levels display distinct minute to minute variations associated with serum LH and estradiol levels in normal women indicating that leptin may contribute to physiologic levels and rhythmicity of reproductive hormones<sup>(58)</sup>. This may be consistent with our findings that a positive correlation exist between serum leptin levels and estradiol in normal and diabetic girls. Some studies reported that, subjects with inactivating mutations of leptin receptor remain prepubertal and have hypogonadotropic hypogonadism similar to that of the Ob/Ob mouse model obesity<sup>(59)</sup>. Thus it appears that leptin has a role in regulating reproduction. Some evidence come from studies on neuropeptid Y (NPY). NPY is a potent stimulator of food intake but also inhibits gonadotropin secretion. Leptin administration decreases the expression of NPY in the arcuate nucleus and consequently removes the inhibitory actions of NPY on pulsatile GnRH release, also inhibit food intake with reduction of adipose tissue mass<sup>(60)</sup>.

Thus, it has been proposed that leptin may serve as a maker of the critical amount of fat stores necessary for initiation of puberty and maintenance of reproductive ability.

In support of this view came our finding showing that the high levels of leptin as well as obesity observed in diabetic children may reflect leptin resistance and insensitivity which in turn affect the gonadal functions, manifested in diabetic children as a delay in pubertal development where the age of diabetic pubertal children was significantly older and the levels of sex-hormones estradiol and testosterone were significantly lower than healthy children.

**In conclusion**, in healthy control girls studied, serum leptin levels rose progressively in an age related pattern paralleling increased body weight and coincided with pubertal rise of estrogen. In boys initial increase of the levels with age was observed, but after the age of 10yr an inflection at the time of testosterone increase occurred. This suggested that leptin may be a permissive factor that signals the CNS that the metabolic conditions are adequate for initiation of pubertal event. Since diabetic children develop obesity despite having elevated serum leptin concentration than healthy, this would reflect the presence of leptin resistance and insensitivity with subsequent weight gain and pubertal delay.

**Recommendation:** Based on this study, the administration of exogenous leptin (smaller leptin analogue) which is able to cross the blood-brain barrier would be preferable to the obese diabetic children who have leptin insensitivity to the higher endogenous serum leptin concentrations. This finding should be interpreted with caution until further data are available and the optimal



method for leptin administration is defined.

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## مستوى اللبتين في المصل في أطفال ومراهقي مرض السكري من النوع الأول مقارنة بالأصحاء: علاقته مع العمر، الجنس، مؤشر كتلة الجسم، الهرمونات التناسلية ومراحل البلوغ

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### المقدمة:

يعتبر اللبتين هو الناتج من جين السمنة فهو يصنع بواسطة الأنسجة الدهنية ولذلك فهو يلعب دوراً في تنظيم كتلة الدهون بالجسم أيضاً فهو يمثل جزء حيوي لمختلف حلقة جهاز الهيبوثلامس- الغدة النخامية والغدد الصماء. وقد لوحظ أن أطفال مرض السكري يعانون من تأخر النمو والبلوغ وهم أيضاً يصابون بالسمنة وزيادة الوزن.

### هدف البحث

لأن الطفولة والبلوغ هي فترة تتميز بتغيرات في النشاط الأيضي و الهرمونات فقد صممت هذه الدراسة لتهدف إلى:

1. تقييم التغيرات في مستوى اللبتين في المصل في أطفال ومراهقي مرض السكري من النوع الأول ومقارنتهم بالأطفال الأصحاء بالنسبة للعمر الزمني ومراحل البلوغ.
2. تقييم ما إذا كانت هناك علاقة بين مستوى اللبتين والسمنة الملحوظة في أطفال ومراهقي مرض السكري من النوع الأول.

**الأشخاص وطريقه العمل :-**

شملت هذه الدراسة 60 طفلاً ( 32 بنتاً و 28 ولداً) تتراوح أعمارهم بين 6-16 عاماً وقد تم تشخيصهم بالإصابة بمرض السكري من النوع الأول. أيضاً شملت الدراسة مجموعة من الأطفال الأصحاء وعددهم 48 (24 بنتاً و 24 ولداً) وقد تم تقسيم الأطفال الأصحاء والمرضى تبعاً لأعمارهم إلى 4 مجموعات (6-7، 8-10، 11-13، 14-16 عاماً) وتبعاً لمرحل البلوغ إلى 3 مجموعات (مرحلة ما قبل البلوغ، مرحلة البلوغ المبكر ومرحلة البلوغ الواضح) تم حساب مؤشر كتلة الجسم وقياس مستوى الليبتن لجميع الأطفال ومستوى هرمون التستوستيرون للبينين ومستوى هرمون الإستروجين في البنات بطريقة الاليزا.

**النتائج :**

أظهرت الدراسة أن مستوى الليبتن في المصل في مجموع البنات ( المجموعة الضابطة والمرضى ) ارتفع ارتفاعاً ذا دلالة إحصائية مع العمر ومع مراحل البلوغ حيث وصل أعلى مستوى عند عمر 14-16 عاماً ومرحلة البلوغ الواضح وكان مستوى الليبتن في مرضى البنات مرتفعاً ارتفاعاً ذا دلالة إحصائية مقارنة بالمجموعات الضابطة. وقد وجد أنه في مجموع البنين الضابطة إرتفع مستوى الليبتن ارتفاعاً ذا دلالة إحصائية عند عمر 8-10 سنوات وفي مرحلة ما قبل البلوغ ثم إنخفض بعدها انخفاضاً ذا دلالة إحصائية أما في مرضى البنين فقد حدث ارتفاعاً ذا دلالة إحصائية في جميع مراحل العمر والبلوغ وكان مستوى الليبتن في مرضى البنين أعلى من المجموعة الضابطة في جميع مراحل العمر والبلوغ. وعند مقارنة مستوى الليبتن في البنات والبنين كان مستوى الليبتن في مجموع البنات (الضابطة والمرضى) مرتفعاً ارتفاعاً ذا دلالة إحصائية مقارنة بمجموعه البنين (الضابطة والمرضى) في جميع مراحل العمر والبلوغ. وكان متوسط أعمار الأطفال المرضى (بنات وبنين) أعلى من المجموعات الضابطة في جميع مراحل العمر والبلوغ. أيضاً كان مؤشر كتلة الجسم في الأطفال المرضى (بنات وبنين) مرتفعاً ارتفاعاً ذا دلالة إحصائية في جميع مراحل البلوغ مقارنة بالمجموعات الضابطة أما مستوى الإستروجين في مرضى البنات ومستوى التستوستيرون في مرضى البنين فقد إنخفض إنخفاضاً ذا دلالة إحصائية في مرحلتي البلوغ مقارنة بالمجموعات الضابطة.

وقد وجدت علاقة ايجابية ذات دلالة إحصائية بين مستوى الليبتن وكلا من العمر، مؤشر كتلة الجسم وهرمون الاستروجين في البنات ( المجموعة الضابطة ومرضى السكري). وفي مجموعة البنين الضابطة كانت هناك علاقة سلبية ذات دلالة إحصائية بين مستوى الليبتن وكلا من العمر وهرمون التستوستيرون بينما كانت العلاقة مع مؤشر الكتلة ليست ذات دلالة إحصائية. وفي مجموعة اللبينين المصابين بمرض السكري كانت هناك علاقة ايجابية ذات دلالة إحصائية بين مستوى الليبتن وكلا من العمر ومؤشر كتلة الجسم بينما كانت العلاقة مع هرمون التستوستيرون ليست ذات دلالة إحصائية

**الاستنتاج**

وقد خلصنا من هذه الدراسة ان هرمون الليبتن يشارك الهرمونات في مختلف العمليات الفسيولوجية ومن أهمها السمنة وتأخر البلوغ المصاحب لمرض السكري. لذلك فله دور في تنظيم وزن الجسم وبدء حدوث البلوغ وحفظ وظيفة التناسل فيما بعد.