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Association of Sex Hormone Binding Globulin in Obese male Adolescent as One of Cardiovascular Risk Factors

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ABSTRACT

Obesity in adolescents and children has raised to significant levels globally with serious public health consequences. In addition to cardiovascular diseases, childhood obesity predisposes to insulin resistance, type 2 diabetes, hypertension, hyperlipidemia, liver diseases, renal diseases, and reproductive dysfunction. This condition also increases the risk of adult-onset obesity and cardiovascular disease. The aim of this study is to assess some cardiovascular risk factor among Egyptian obese male adolescent subjects and to determine its relation with sex hormone binding globulin (SHBG). The participants of this study will be an Egyptian male adolescent volunteers aged between (12-19) years old and will be were classified according to their Body Mass index (BMI) as control group: apparently healthy non obese, age matched, their BMI is more than 5th percentile to less than the 85th percentile and Group 2: Obese group, their BMI more than 95 percentile. Our result showed that there is negative correlation between Sex Hormone Binding Globulin (SHBG) and Body Mass Index (BMI) which consider a biomarker for metabolic syndrome.

1. Introduction

Obesity is the most public dietary problem today, and it is one of the most serious public health issues in developing countries [1]. The prevalence and severity of childhood obesity are rising [1]. Childhood obesity is a multi-factorial condition caused by a combination of genetics and environment. However, a variety of factors appear to play a role in overweight and other obesity-related chronic diseases. Obesity is primarily caused by insufficient insufficient dietary consumption. Simplistically, obesity caused by an imbalance of caloric consumption and energy expenditure. Therefore obesity in childhood and adolescents depeneds on multiple factors [2].

Childhood obesity is the result of a complex interaction between a number of environmental, genetic, and social factors, including those in the family, community and school [3]. In addition to that, with one in five children and adolescents being overweight or obese, the pediatric obesity epidemic has affected a startling 124 million people.

The body mass index (BMI) of children and adolescents has been steadily rising over the past 40 years, according to a global trend in juvenile obesity. As a result, this issue has recently started affecting the entire planet [4]. Puberty marks the beginning of the developmental stage known as adolescence, which lasts until maturity.

The World Health Organization (WHO) has a clear definition of adolescence as the time between 10 and 19 years old [5]. Age and sex-specific BMI percentages, as defined in growth charts recognized by the Centers for Disease Control and Prevention using historical data from national studies, are typically used to d etermine the status of adolescent overweight and obesity [6]. Comorbidities associated with childhood obesity include but are not limited to those affecting the respiratory, gastrointestinal, musculoskeletal, endocrine, and cardiovascular systems. Many of the comorbidities associated with obesity in young people, including type 2 diabetes (T2D), dyslipidemia, obstructive sleep apnea (OSA), and steatohepatitis, were formerly thought of as "adult" disorders. The seriousness of these comorbidities usually rises with the seriousness of obesity. The danger in this case lies in the association of obesity with affecting many vital function and systems in the body [3].

Obesity in children is a risk indicator for many diseases in the future. Children whose obesity continues into adulthood have a considerably amplified risk of T2D, hypertension, dyslipidemia, and carotid-artery atherosclerosis than do adults who were never suffering with obesity [7]. Although this may be partially mediated by the association between childhood obesity and adult obesity, higher BMI for the duration of childhood has also been linked with an increased risk of deadly and nonlethal cardiovascular events for the duration of adulthood in both men and women [3]. In addition to high blood pressure, low levels of high denisty lipoprotein (HDL) cholesterol, and increased triglycerides, children with obesity are also more likely to have other cardiometabolic risk factors [3].

Insulin resistance and type 2 diabetes mellitus (T2D) like cardiovascular disease, are obesity-related disorders that were formerly assumed to occur in adulthood but are increasingly becoming more common in younger populations. Other than in children and adults, obesity is also substantially linked to reduced levels of adiponectins in teenagers[8], adiponectins are bioactive mediators that facilate the communication between adipose tissues and other biological systems [9]. Lower levels of adiponectin are linked to higher levels of insulin resistance in obese adolescents, implying that the majority of youth with insulin resistance are overweight or obese [8].

In addition to obesity and insulin resistance, low adiponectin levels in young people may also be associated with hypertension and dyslipidemia, which may help to anticipate the clustering of these metabolic syndrome symptoms. Therefore there is a strong relation between obesity and insulin resistance and its complications[6] .

Type 2 diabetes caused by obesity and CVD have both been linked to pro-inflammatory adipokines like leptin [6]. Positional cloning established leptin as a critical molecule in the regulation of human weight and energy balance. Leptin gene is an adipocyte-derived hormone that has long been recognised as a critical regulator of a wide range of biological processes such as energy balance, neuroendocrine function, angiogenesis, bone formation, and reproduction. A growing body of evidence suggests that leptin functions as a pro-inflammatory cytokine during immunological responses. Although pro-inflammatory substances are important mediators of host defensive mechanisms, they have been linked to the development of autoimmune disorders[10] .

A biomarker for metabolic syndrome and a predictor of the risk T2D and CVD is low serum SHBG concentrations in overweight persons. SHBG is the major sex hormone carrier protein in serum, it's primary function is to transport sex steroid hormones to target tissues and moderates the level of free sex hormones that can enter target cells [11] which is expressed under the direction of hormones and dietary factors. BMI has been shown to have a considerable influence on circulating SHBG concentrations, and a sustained negative relationship between BMI and SHBG plasma levels has been identified. Low SHBG levels are thought to be valid biomarkers for a number of potentially significant problems [12].

2. Material and methods

2.1. Sample collection

A total of 40 adolescents, 10 healthy non obese their BMI is more than 5th percentile to less than the 85th percentile as a control group and 30 total patients group their BMI more than 95 percentile divided into two sub groups, 15 over-weight group their BMI 25-29.9 kg/m^2 and 15 obese group their BMI ≥ 30 kg/m^2, age ranged from 12 to 19 years, were enrolled in this study.

Obese adolescents were visitors of National Institute of Diabetes and Endocrinology, Cairo, Egypt. Adolescents with acute or chronic liver and kidney diseases, acute or chronic inflammatory conditions, diabetes mellitus or endocrine diseases that may induce obesity or insulin resistance such as hypothyroidism, hypercotisolism or have drug history that is known to induce obesity, insulin resistance or effect on the level of SHBG were excluded. The study was approved by the Ethics Committee of the National Organization for Teaching Hospitals and Institutes, Cairo, Egypt; the approval is registered under No. I D E 00248.

All Subjects under study were subjected to full history to confirm the diagnosis of simple obesity and to exclude subjects with the exclusion criteria and anthropometric measurements including body weight, height, waist circumference, hip circumference followed by calculation of BMI = kg/m2 where kg is a person's weight in kilograms and m2 is their height in meters squared as well as waist/ hip ratio, Clinical examination and routine examinations including: complete blood picture, liver and kidney function test (to meet exclusion criteria), lipid profile (total cholesterol, triglycerides, high-density lipoprotein (HDL), low density lipoprotein (LDL), hemoglobin A1c (HbA1C), fasting plasma glucose and fasting plasma insulin was determined.

Assessment of the Insulin Resistance Index (IR) using the homeostatic assessment model (HOMA-IR) as follows: HOMA-IR- = fasting insulin (µ/ml) x fasting glucose (mg/dL)/405. Inflammatory markers such as HsCRP were identified. Testosterone, adipokines (leptin and resistin) and sex hormone-binding globulin (SHBG).

2.2 Preparation of samples and biochemical analysis:

Blood samples were drawn by venipuncture in the morning after an overnight fast. For each subject two peripheral blood samples of 2 ml of venous blood were collected on vacutainers tubes containing EDTA, two peripheral blood samples of 4 ml of venous blood were collected on vacutainers tubes with no additive (plain red), one peripheral blood samples of 2 ml of venous blood were collected on vacutainers tubes containing sodium fluoride (glycolysis inhibitor) were collected and serum was separated by centrifugation at 1000-1500 x g for 10 minutes. Complete blood picture (CBC) was determined according to the method described by Sache and Henkel (1996) [13].

C - reactive protein in serum was detected by the principle of agglutination the test specimen (serum) is mixed with Vitro CRP latex reagent and allowed to react, according to Andersen H.C. [14] described by using commercial kits (VITRO SCIENT). Determination of aspartate aminotransferase (AST) and alanine transaminase (ALT) were carried out according to the method of Murray using AST and ALT kits from (SPINREACT), parameters of liver function were expressed by U/L.

Creatinine in serum was determined by Kinetic colorimetric method (fixed rate) Jaffe reaction described by Jaffe [15] using available commercial kit (VITRO SCIENT.) and Urea/BUN in serum was determined by enzymatic colorimetric method (urease) modified Berthelot reaction described by Fawcett & Scott [16] using available commercial kits (VITRO SCIENT.). Parameters of kidney function were expressed by mg/dl.

Serum total cholesterol (TC) and Triglycerides (TG) levels were determined calorimetrically according to the method of by Naito [17] *and* Kaplan [18] respectively, using using a commercial assay kit (SPINREACT). Then high density lipoprotein cholesterol (HDL-c) levels were determined by precipitation method described by Lopes-Virella [19] using available commercial kit (SPECTRUM). Low density lipoprotein cholesterol (LDL- c) concentration was Calculated using formula by Friedewald [20] equation : LDLc= TC – (HDL- c 1/5×TG). Parameters of lipid profile were expressed as mg/dl. HbA1c was determined by ion exchange resin method described by Nathan [21] using available commercial kit (VITRO SCIENT.). Serum fasting blood glucose was determined by enzymatic, colorimetric method (GOD/PAP) with glucose oxidase, and 4-aminoantipyrine, described by Trinder [22] using available commercial kits (VITRO SCIENT.), expressed by mg/dl. Insulin was determined by an enzyme-linked immunosorbent assay (ELISA) technique using commercial kit, purchased from Bio Source International, Inc. Europe S.A **(Flier et al., 1979).**

The function of insulin resistance (HOMAIR) was evaluated by the homeostasis model assessment (HOMA) according to equation by Matthews [23]*:* HOMA-IR = fasting insulin (µIU/mL)× fasting glucose (mmol/L)/22.5. Serum total testosterone was determined by the electrochemiluminescence immunoassay (ECLIA) using Elecsys and cobase analyzers (Roche diagnostics GmbH, Germany).

2.3 RNA isolation and quantitative real time-PCR analysis (qRT-PCR) analysis:

RNA was extracted by TRI Reagent from whole blood samples collected in EDTA tube described by Mackey and Chomczynski with available commercial kits Direct- zol RNA purification product from Zymo Research. Catalog NO. R2050-1.The extracted RNA was used immediately or stored frozen at ≤ - 70º C. The extracted RNA was reverse transcribed into cDNA using the available commercial kit SensiFAST cDNA Synthesis Kit (Bioline reagents Ltd.). Diluted c DNA was stored at 4 °C for 1 week or -20°C for long term storage.To monitor gene expression of leptin, resistin and SHBG, we used quantitative real time-PCR analysis (RT-PCR).

Expression levels of leptin, resistin and SHBG were quantified by q RT-PCR using 5 x HOT FIREPol® Eva Green q PCR Mix Plus (ROX) (Solis BioDyne). A mixture of 4 µl of 5 x HOT FIREPol® EvaGreen q PCR Mix Plus, 0.5 µl primer forward, 0.5 µl primer reverse and 2 µl template c DNA was used, the 20 µl was completed by H_2O PCR grade.

The following primers sequences were used Leptin Forward: 5´-GTG CGG ATT CTT GTG GCT TT-3´ and reverse: 5´-GGA ATG AAG TCC AAA CCG GTG -3´[24], Resistin forward: 5´-GTC TCC TCC TCC TCC CTG TC-3´ and reverse: 5´-CGA CCT CCT GGA TCC TCT-3´ [25] and SHBG forward: 5´-GCC CAG GAC AAG AGC CTA TC-3´ and reverse: 5´-CCT TAG GGT TGG TAT CCC CAT AA -3´ [11]. PCR was carried out for 41 cycles: initial activation at 95ºC for 15 minutes for one cycle then denaturationat 95ºC for 15 seconds, annealing at 58ºC for 20 seconds, and elongation at 72ºC for 20 seconds. Results were normalized against expression of Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) reference gene.

3. Statistical analysis:

Clinical data are expressed as means ± standard deviation (SD). Differences between groups were compared with use of the unpaired Student's t-test. All reported P values are from two-tailed tests, and P values of less than 0.05 were considered to indicate statistical significance.

4. Results

Data in Table 1 showed that the difference in the mean of Age between control and studied groups was not statistically significant.

However, it showed that the studied groups showed significantly higher BMI values compared to the control group. There was no significant difference in the RBCs, Hb, platelets count and CRP between the compared groups. However significant elevation in white blood count was also detected in obese group only as compared to control value (Table 2).

Table 3 showed Significant elevations in serum TC, TG and LDL-c levels along with significant reduction in HDL-c levels were also detected in total patients group and obese group as compared to control values. However there no statistical significant in TG and HDL-c between obese group and control. Furthermore, Table 4 showed that there was no statistical significant in serum creatinine, blood urea nitrogen (BUN) and urea between the compared groups.

Otherwise there was significant elevation in GPT, GOT and total bilirubin in the tested group as compared to control one. There no statistical significant in GPT and GOT between over-weight group and control one. Although there was significant elevation was detected in GOT, GPT and total bilirubin in obese group as compared to control group. However it showed highly statistical significant in Total bilirubin level and GOT in obese group as compared to over-weight group and control group (Table 5).

Data in Table 6 showed that there was significant decrease in fasting blood glucose (FBG) level in studied groups as compared to control one. Otherwise significant elevation was detected in HbA1c, fasting insulin and HOMA-IR in studied groups as compared to control group.

Data in Table 7 clarify that there was significant decrease in total testosterone hormone in studied groups as compared to control one. The data of leptin, resitin and SHBG express CT value which clarify a significant decrease in leptin which indicate higher gene expression in total patients group only as compared to control values. However there was significant decrease in resitin which indicate higher gene expression in studied group as compared to control one. Otherwise significant elevation was detected in SHBG which indicate lower gene expression in total patients group compared to control one.

Table 1. The age and Body Mass Index (BMI) for studied subjects.

Parameters	Control group	Total Patients	
		Over-weight	Obese
Age (years)	15.25 ± 2.27	14.00 ± 2.48 13.83 ± 1.95	14.20 ± 2.99
BMI (Kg/m^2)	17.85 ± 0.90	29.72 ± 4.49* 26.25 ± 1.67*	33.90± 3.02*†

- Data are presented as Mean ± SD.
* Significant difference compared to the control group, p≤ 0.05.
† Significant difference compared to the over-weight group.

Table 2. Complete blood picture and C - reactive protein (CRP) for control and studied subjects.

Parameters	Control group	Total Patient	
		Over-weight	Obese
Red cell count (RBCs) (x10^6/cmm)	4.66 ± 0.21	5.14 ± 0.76 4.71 ± 0.55	5.66 ± 0.63*†
Hemoglobin (Hb) (g/dl)	12.85 ± 0.35	13.64 ± 2.21 12.61 ± 2.12	14.88 ± 1.60
Platelets count (x10^3/cmm)	252.00 ± 56.24	332.09 ± 70.00 337.83 ± 43.67	325.02 ± 91.54
White cell count (WBCs) (10^3/cmm)	4.80 ± 0.45	6.14 ± 1.69 5.25 ± 1.12	7.20 ± 1.65*
C-Reactive protein (CRP) (mg/l)	3.40 ± 0.45	4.28 ± 0.94 4.20 ± 0.96	4.40 ± 0.90

- Data are presented as Mean ± SD.
* Significant difference compared to the control group, p≤ 0.05.
† Significant difference compared to the over-weight group.

Table 3. Lipid profile in control and studied subjects.

Parameters	Control group	Total Patients	
		Over-weight	Obese
Total Cholesterol (TC) (mg/dl)	126.42 ± 10.72	181.38 ± 35.73* 164.2 ± 20.9*	202 ± 38.8*
Triglycerides (TG) (mg/dl)	77.00 ± 3.40	122.71 ± 53.03* 94.8 ± 30	156 ± 55*
High density lipoprotein cholesterol (HDL-c) (mg/dl)	56.57 ± 2.30	38.44 ± 14.56* 42.40 ± 18.18	33.70 ± 5.36 *
Low density lipoprotein cholesterol (LDL-c) (mg/dl)	54 .45 ± 9.54	118.40 ± 28.96* 102.85 ± 17.62*	137.06 ± 28.88*

- Data are presented as Mean ± SD.
* Significant difference compared to the control group, p≤ 0.05.
† Significant difference compared to the over-weight group.

Table 4. kidney profile in healthy control and studied subjects.

Parameters	Control group	Total Patients	
		Over-weight	Obese
S. Creatinine (mg/dl)	0.73 ± 0.16	0.75 ± 0.18 0.80 ± 0.14	0.68 ± 0.20
BUN (mg/dl)	22.42 ± 5.68	19.16 ± 7.00 22.06 ± 7.80	15.55 ± 3.28
Urea (mg/dl)	47.09 ± 10.67	40.25 ± 13.96 46.32 ± 14.96	32.65 ± 6.17

- Data are presented as Mean ± SD.

* Significant difference compared to the control group, p≤ 0.05.

† Significant difference compared to the over-weight group.

Table 5. Liver profile in control and studied subjects

Parameters	Control group	Total Patients	
		Over-weight	Obese
GPT (U/L)	10.57 ± 1.59	35.79 ± 25.99* 20.86 ± 9.41	53.70 ± 28.14*
GOT (U/L)	18.90 ± 3.67	30.10 ± 9.20* 23.61 ± 3.52	37.90 ± 7.77*†
T.Bilirubin (mg/dl)	1.053 ± 0.12	1.40 ± 1.00* 0.55 ± 0.22*	2.53 ± 0.20*†
D.Bilirubin (mg/dl)	0.28 ± 0.06	0.21 ± 0.03 0.22 ± 0.03	0.21 ± 0.03
ALP (U/L)	269.00 ± 12.35	230.00 ± 67.69 220.80 ± 59.66	253.00 ± 80.00

- Data are presented as Mean ± SD.

* Significant difference compared to the control group, p≤ 0.05.

† Significant difference compared to the Sub 1 group.

Table 6. Fasting glucose, glycated hemoglobin, insulin and homeostasis model assessmentof insulin resistance (HOMA- IR) in control and studied subjects.

Parameters	Control	Total Patients	
		Over-weight	Obese
Fasting blood glucose (FBG) (mg/dl)	95.22 ± 3.29	87.86 ± 6.36* 87.91 ± 4.81*	87.80 ± 7.83
Glycated hemoglobin (HBA1C) (%)	5.22 ± 0.14	5.59 ± 0.20* 5.58 ± 0.21*	5.60 ± 0.20*
Insulin Fasting (µIU/ml)	4.50 ± 0.90	14.39 ± 5.21* 14.16 ± 5.92*	14.66 ± 4.18*
HOMA IR	0.99 ± 0.21	3.02 ± 1.24* 3.08 ± 1.36*	2.94± 1.03*

- Data are presented as Mean ± SD.

* Significant difference compared to the control group, p≤ 0.05.

† Significant difference compared to the Over- weight group.

Table 7. Total testosterone, resistin, leptin, SHBG and GAPDH in control and studied subjects.

Parameters	Control	Total Patients	
		Obese	Over-weight
T .Testosterone	3.11 ± 0.68	1.22 ± 1.61* 0.31 ± 0.20*	0.80 ± 0.81*
Resistin	35.31 ± 0.68	30.15 ± 3.03* 30.68 ± 2.79*	29.35 ± 3.19*
Leptin	29.04 ± 0.22	27.01 ± 2.05* 27.74 ± 1.61	26.13 ± 2.17
SHBG	28.5 ± 0.5	34.2 ± 2.8* 33.74 ± 2.6*	35.88 ± 3.0

- Data are presented as Mean ± SD.
* Significant difference compared to the control group, p≤ 0.05.
† Significant difference compared to the over-weight group.

5. Discussion

Obesity in childhood and adolescence is observably a risk factor for first CVD morbidity and mortality, according to evidence from multiple reputable longitudinal researches [7] [26]. The results of our study showed that serum TC, TG and HDL-C levels were highly significant in total patients and obese groups than non-obese adolescents. These results are in accordance with another study observed the 30-year CVD event risk among adolescents with severe obesity treated with and without metabolic and bariatric surgery (MBS), related to adolescences with moderate obesity, overweight, or normal weight [27]. Also in our study we found that non obese adolescents had significantly higher mean values of free testosterone level than obese adolescent males.

Obesity is an insulin-resistant state with an interfering with insulin signal transduction and neurons [28] increases insulin levels, which are assumed to be the reason for decreased SHBG levels and subsequently decreased testosterone levels. Accordingly obesity has an inverse correlation with SHBG.

There was also a significant inverse correlation between testosterone level and BMI in obese adolescent males. These results of our study are similar to that done by Mogri et al. [29] A cross-sectional observational study conducted on 25 obese people in New York found that the testosterone levels in obese men were on average 50% lower than those in lean men.

SHBG has been established to affect glycemic control and to expect both T2D and metabolic syndrome [30]. Our findings are agreement with the studies that demonstrated a negative correlation between SHBG and BMI [12] Taneli et al [31] and [29] also found lower SHBG concentrations in obese boys. Since around half of the total testosterone is bound to SHBG, it is to be expected that lower SHBG concentrations can, at least partially, account for the lower TT concentrations in obese Boys. Taneli also found lower free testosterone concentrations in the obese boys at Tanner stage 2 but not at Tanner stage 4 [31].

Results of our study showed that serum leptin expression level significaly increased in total patients group compared with control group which is agreement with Dencker et al.[32] Who stated that the amount of adipose storage increases along with leptin expression and blood levels. Leptin levels and fat mass have a curved rather than linear connection, suggesting that leptin secretion rises as fat mass rises. But not agreement with Sinha et al. [33] and Ostlund et al. [34] showed that serum leptin is strongly correlated with BMI in healthy and diabetic children.

Christos et al. [35] The increase in leptin concentrations prior to and at the onset of puberty, as well as the subsequent drop to baseline in the time following puberty, were reported to occur in healthy boys despite continuously rising BMI.

As a result, leptin concentration does not increase as BMI rises or as adipose tissue storage grow. Ahmed et al.[36] Reported that when diabetic female patients were grouped according to pubertal status, there was an advanced increase in both BMI and serum leptin levels with development of puberty, conversely, despite the fact those male patients' BMI values increased during adolescence and early adulthood, leptin levels did not increase between pubertal and post pubertal phases.

The most acceptable clarification for this disparity is that men's increased BMI is mostly associated with increased muscle mass, whereas girls' increasing BMI during and after puberty is mainly associated with increased adipose tissues. In addition to this sex-specific differential in body composition, rising androgen levels have direct consequences that may themselves impede peripheral leptin secretion. Accordingly serum leptin concentrations in boys associated with stage of puberty regardless of continually increasing of BMI [37].

Our recent study showed significaly increased in resitin expression in studied groups as compared to the control one in contrast to studies that indicate the relationship between other adipocytokines such as adiponectin and leptin, resistin levels of children and adolescents with pubertal stage and age [38]. According to pubertal stage, resistin levels increased in lean males. A stepwise forward multiple regression model that takes into account variables including age, Tanner stage, estradiol, testosterone, waist and hip circumference, BMI, weight, and height also supports the idea that resistin levels increase throughout pubertal maturation. The only significant independent predictor for resistin was Tanner stage [38]. But our results were confirmed by others which revealed a significant elevation level of resistin in the studied groups as compared to controls [39].

There was a correlation between HOMA-IR and insulin with anthropometric variables and triglycerides levels. Similar data were also found by other studies with children and adolescents [40], which described a relevant positive association between BMI and insulin, HOMA-IR and BMI, HOMA-IR and triglycerides, confirming the findings of our study and showing that insulin resistance is associated with changes in risk for metabolic disorders at maturity [41].

Liver disorders throughout the clinical range of nonalcoholic fatty liver disease (NAFLD) are linked to the rising frequency of obesity, metabolic syndrome, and insulin resistance worldwide [41]. Our study showed an increase of liver enzymes GPT and GOT in obese and overweight groups compared with non-obese groups with agreement with the study demonstrate that increased levels of hepatic enzymes are the hallmarks of the whole spectrum of NAFLD. They are common in obesity, and their prevalence increases progressively with increasing BMI [42].

Glycated hemoglobin (HbA1c) is a regularly used marker for long-term glycemic control. HbA1c predicts the likelihood of an increase in diabetic complications in diabetic patients, in line with its role as a measure of mean blood glucose levels. It may be suggested as a diagnostic principle that identifies more cases of diabetes and pre-diabetes than fasting glucose or oral glucose tolerance tests. HbA1c is a good biomarker for diabetic complications and prediction of diabetic [43].

In this study on statistical analysis a positive correlation found between BMI and HbA1c, which is significant. Sisodia were found similar to our study that is, a significant positive correlation between BMI and HbA1c [43]. Walid Gaafar [44] and Babikr [44] also found a positive correlation between BMI and HbA1c. In many situations, lifestyle changes such as stress reduction, increased physical activity, and dietary changes are sufficient to improve insulin sensitivity and help prevent or treat T2D without the use of exogenous insulin.It can be suggested from this study that weight control as a child, adolescent, or young adult by balanced diet rich in vitamins and minerals, whole grains, milk and dairy products, fruits and vegetables which not only protects growth but also manages childhood obesity may reduce the risk of cardio metabolic disease later in life., according to these findings [6].

Besides we concluded that childhood obesity can be considered as a risk factor for selected adult CVD. In addition to low serum SHBG concentrations in obese individuals can be considered as a biomarker for metabolic syndrome.

6. Reference

1. **S. Karnik and A. Kanekar. (2012).** Childhood obesity: A global public health crisis, Int. J. Prev. Med., vol. **3**, no. **1**: pp. **1–7**, doi: 10.1201/b18227-3.
2. **H. H. El, S. M. Saleh, S. A. Khairy, A. S. Marei, K. Elkelany, and M. F. Al. (2019)** Relationship between dietary intake and obesity among a group of primary school-aged children in Cairo Governorate, pp. **42–53**, doi: 10.4103/JMISR.JMISR.
3. **S. Kumar and A. S. Kelly. (2017).** Review of Childhood Obesity: From Epidemiology, Etiology, and Comorbidities to Clinical Assessment and Treatment, Mayo Clin. Proc., vol. **92**, no. **2**, pp. **251–265**, 2017, doi: 10.1016/j.mayocp.2016.09.017.
4. **J. Kim and H. Lim. (2019).** Nutritional Management in Childhood Obesity, J. Obes. Metab. Syndr., vol. **28**, no. **4**, pp. **225–235**, doi: 10.7570/jomes.2019.28.4.225.
5. **J. K. Das et al. (2017).** Nutrition in adolescents: physiology, metabolism, and nutritional needs, Ann. N. Y. Acad. Sci., vol. **1393,** no. **1**, pp. **21–33**, , doi: 10.1111/nyas.13330.
6. **L. D. Ruiz, M. L. Zuelch, S. M. Dimitratos, and R. E. Scherr. (2020).** Adolescent obesity: Diet quality, psychosocial health, and cardiometabolic risk factors, Nutrients, vol. **12**, no. **1**, pp. **1–22**, doi: 10.3390/nu12010043.
7. **C. Sun, D. Ph, M. Cheung, and D. Ph. (2011).** Childhood Adiposity, Adult Adiposity, and Cardiovascular Risk Factors.
8. **R. Weiss et al. (2014).** Low Adiponectin Levels in Adolescent Obesity: A Marker of Increased Intramyocellular Lipid Accumulation The putative modulatory effects of adiponectin on insulin sen-sitivity may, in part, be mediated via its effects on IMCL lipid content, J Clin Endocrinol Metab, vol. **88**, doi: 10.1210/jc.2002-021711.
9. **C. Antoniades, A. S. Antonopoulos, D. Tousoulis, and C. Stefanadis. (2009).** Adiponectin: from obesity to cardiovascular disease, doi: 10.1111/j.1467-789X.2009.00571.x.
10. **A. M. Abd El-Maksoud et al. (2009).** Early predictors of microvascular complications in type 1 diabetic patients, Clin. Biochem., vol. **42**, no. **13–14**, pp. **1401–1406**, doi: 10.1016/j.clinbiochem.2009.06.008.
11. **H. Li et al. (2016).** Sex Hormone Binding Globulin Modifies Testosterone Action and Metabolism in Prostate Cancer Cells, Int. J. Endocrinol., vol., **2016**, doi: 10.1155/2016/6437585.
12. **R. Simó, C. Sáez-López, A. Barbosa-Desongles, C. Hernández, and D. M. Selva. (2015).** Novel insights in SHBG regulation and clinical implications, Trends Endocrinol. Metab., vol. **26**, no. **7**, pp. **376–383**, doi: 10.1016/j.tem.2015.05.001.
13. **C. Sachse and E. Henkel. (1996).** An evaluation of the CELL-DYN 17000® haematology analyser: Automated cell counting and three-part leucocyte differentiation, Clin. Lab. Haematol., vol. **18**, no. **3**, pp. **171–180**, doi: 10.1046/j.1365-2257.1996.00174.x.
14. **H. C. Anderson and M. McCarty. (1950)** Determination of C-reactive protein in the blood as a measure of the activity of the disease process in acute rheumatic fever, Am. J. Med., vol. **8**, no. **4**, pp. **445–455**, , doi: 10.1016/0002-9343(50)90226-9.
15. **P. Datta, G. A. Graham, and I. Schoen.** Interference by IgG Paraproteins in the Jaffe Method for Creatinine Determination. [Online]. Available: http://ajcp.oxfordjournals.org/.
16. **J. K. Fawcett and J. E. Scott. (1960).** A RAPID AND PRECISE METHOD FOR THE DETERMINATION OF UREA, J. clin. Path, p. **156**, doi: 10.1136/jcp.13.2.156.
17. **C. Liquid.** Cholesterol-LQ Colesterol-LQ, pp. **8–11**.
18. **G. Liquid.** Triglycerides-LQ Triglicéridos-LQ, pp. **8– 11**.
19. **M. F. Lopes-Virella, P. Stone, S. Ellis, and J. A. Coiweil. (1977).** Cholesterol Determination in High- Density Lipoproteins Separated by Three Different Methods, [Online]. Available:https://academic.oup.com/clinchem/article- abstract/23/5/882/5663846.

20. **W. T. Friedewald, R. I. Levy, and D. S. Fredrickson. (1972).** Estimationof the Concentration of Low-Density LipoproteinCholesterol in Plasma,Without Useof the PreparativeUltracentrifuge,. [Online]. Available: https://academic.oup.com/clinchem/article-abstract/18/6/499/5676160.
21. nejm198402093100602**.**
22. **P. Trinder. (1969).** Determination of Glucose in Blood Using Glucose Oxidase with an Alternative Oxygen Acceptor," Ann. Clin. Biochem. Int. J. Lab. Med., vol. **6**, no. **1**, pp. **24–27**, Jan., doi: 10.1177/000456326900600108.
23. **D. R. Matthews, J. R. Hosker, A. S. Rudenski, B. A. Naylor, D. F. Treacher, and R. C. Turner. (1985).** Homeostasis model assessment: insulin resistance and fl-cell function from fasting plasma glucose and insulin concentrations in man.
24. **J. Do¨tsch et al. (1999).** Leptin and Neuropeptide Y Gene Expression in Human Placenta: Ontogeny and Evidence for Similarities to Hypothalamic Regulation.
25. **Y. K. Oh et al. (2017).** Increased expression of resistin in ectopic endometrial tissue of women with endometriosis, Am. J. Reprod. Immunol., vol. **78**, no. **5**, doi: 10.1111/aji.12726.
26. **(1992).** The New England Journal of Medicine Downloaded from nejm.org on June 14, 2022. For personal use only. No other uses without permission. Copyright © Massachusetts Medical Society. All rights reserved 1992.
27. **P. Xu et al. (2020).** Thirty-Year Risk of Cardiovascular Disease Events in Adolescents with Severe Obesity, vol. **28**, no. **3**, pp. **616– 623**, doi: 10.1002/oby.22725.
28. **R. Salvi et al. (2006).** Gonadotropin-releasing hormone-expressing neurons immortalized conditionally are activated by insulin: Implication of the mitogen-activated protein kinase pathway, Endocrinology, vol. **147**, no. **2**, pp. **816–826**, Feb., doi: 10.1210/EN.2005- 0728.
29. **M. Mogri, S. Dhindsa, T. Quattrin, H. Ghanim, and P. Dandona. (2013).** Testosterone concentrations in young pubertal and post-pubertal obese males, Clin. Endocrinol., (Oxf), vol. **78**, no. **4**, pp. **593–599**, doi: 10.1111/cen.12018.
30. **C. K. Roberts, D. M. Croymans, N. Aziz, A. W. Butch, and C. C. Lee. (2013).** Resistance training increases SHBG in overweight/obese, young men, Metabolism, vol. **62**, no. **5**, pp. **725–733**, doi: 10.1016/j.metabol.2012.12.004.
31. **F. Taneli et al. (2010).** The effect of obesity on testicular function by insulin-like factor 3, inhibin B, and leptin concentrations in obese adolescents according to pubertal stages, Clin. Biochem., vol. **43**, no. **15**, pp. **1236–1240**, doi: 10.1016/j.clinbiochem.2010.07.026**.**
32. **M. Dencker, O. L. A. Thorsson, and M. K. Karlsson. (2006).** Leptin is closely related to body fat in prepubertal children aged 8 11 years, no. November 2005, doi: 10.1080/08035250600570561.
33. **M. K. Sinha et al. (1996).** Nocturnal Rise of Leptin in Lean, Obese, and Non – Insulin-dependent Diabetes, vol. **97**, no. **5**, pp. **1344–1347**.
34. **E. Ostlund and W. Yang. (1996**). Richard ronald e. ostlund, gingerich, no. 12.
35. **C. S. Mantzoros, J. S. Flier, and A. D. Rogol. (1997).** A Longitudinal Assessment of Hormonal and Physical Alterations during Normal Puberty in Boys. V. Rising Leptin Levels May Signal the Onset of Puberty*, [Online]. Available: https://academic.oup.com/jcem/article/82/4/1066/2866193.
36. **M. L. Ahmed. (2001).** Elevated Leptin Levels Are Associated with Excess Gains in Fat Mass in Girls, But Not Boys, with Type 1 Diabetes: Longitudinal Study during Adolescence, J. Clin. Endocrinol. Metab., vol. **86**, no. **3**, pp. **1188–1193**, doi: 10.1210/jc.86.3.1188.
37. **E. M. Hammad and A. F. Hassan. (2008).** 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, vol. **28**, no. **1**, pp. **265–286**.

38. **E. Schuster, J. Thiery, W. Kiess, and J. Kratzsch. (2005).** Serum Resistin Levels of Obese and Lean Children and Adolescents : Biochemical Analysis and Clinical Relevance, vol. **90**, no. **8**, pp. **4503–4509**, doi: 10.1210/jc.2005-0437.
39. **M. T. A.-E. P. D. AYMAN S. SOLIMAN, M.D., W. I. R. M. D. AMIRA M. JAWAD, M.D., and N. T. ABD-ELREHEEM, M.D. (2019).** The Association between Resistin, Leptin and Adiponectin with Obesity and Type 2 Diabetes Mellitus, Med. J. Cairo Univ., vol. **87**, no. **12**, pp. **4227–4237**, doi: 10.21608/mjcu.2019.77411.
40. **M. C. D. S. Romualdo, F. J. De Nóbrega, and M. A. M. S. Escrivão. (2014).** Insulin resistance in obese children and adolescents, J. Pediatr. (Rio. J)., vol. **90**, no. **6**, pp. **600–607**, Nov., doi: 10.1016/j.jped.2014.03.005.
41. **J. D. Lin, P. Y. Lin, L. M. Chen, W. H. Fang, L. P. Lin, and C. H. Loh. (2010).** Serum glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) levels in children and adolescents with intellectual disabilities, Res. Dev. Disabil., vol. **31**, no. **1**, pp. **172–177**, Jan., doi: 10.1016/J.RIDD.2009.08.005.
42. **G. Marchesini, S. Moscatiello, S. Di Domizio, and G. Forlani. (2008).** Obesity-Associated Liver Disease, doi: 10.1210/jc.2008-1399.
43. **R. K. Sisodia and M. Chouhan. (2019).** The study of correlation between Body Mass Index and glycemic control-HbA1c in diabetes type 2 patients, Int. J. Adv. Med., vol. **6**, no. **6**, p. **1788**, Nov., doi: 10.18203/2349-3933.ijam20195228.
44. **W. Gaafar Babikr et al.** The correlation of HbA1c with body mass index and HDL-cholesterol in type 2 diabetic patients. [Online]. Available: www.biomedres.info.