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The role of obesity diagnostic body physical and biochemical parameters as alarming signals against complications

Wafaa A. Kandeel[1], Heba A. Elmalt[2], Safinaz E. El-Toukhy[2], Nadia A. Mohammed[2], Eman R. Youess[2], Ola M. Abdelsamie[3] and Elham M. Youssef El abd[4]

[1]Departments of Biological Anthropology, National Research Centre, Cairo, Egypt
[2]Medical Biochemistry Department, National Research Centre, Cairo, Egypt
[3]Child Health Department, National Research Centre, Cairo, Egypt
[4]Biochemistry Department, National Research Centre, Cairo, Egypt

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ABSTRACT

Adolescent's obesity is a worldwide problem which involves chronic low grade inflammation, oxidative stress and insulin resistance. This study aims to evaluate the importance of the different physical and biochemical body parameters in diagnosing obesity as well as displaying the role of inflammatory markers in the pathogenesis of obesity and its related consequences. That may helps in early predicting and management of the expected complications. This cross-sectional study was conducted on 30 obese adolescents and 30 non-obese healthy controls matching in age and sex. Anthropometric measurements were taken. Oxidative stress and inflammatory markers were estimated in blood samples. Moreover, body biochemical parameters, as fasting glucose, insulin, lipid profile, and liver enzymes were also measured. All anthropometric measurements were significantly higher in the obese group as compared to control as well as a statistical significant increase in markers of inflammation. However, PON-1 level was significantly lower in that group. Body biochemical parameters were significantly higher in the obese group. Evaluation of the body physical and biochemical parameters is a crucial step towards management of obesity. This definitely helps in predicting and accordingly early management of the expected complications.

Keywords: Obesity, TNF-α, PON-1, CRP

INTRODUCTION

Obesity in young age is really a global crucial problem whether in developing or developed countries. According to the WHO definition, obesity is considered to be there when the body mass index (BMI) is more than or even equal to 30 [1]. It is a world-wide epidemic considered to be the fifth leading risk for global deaths. Obesity and its associated conditions such as insulin resistance, type 2 diabetes, Dyslipidemia and steatosishepatis, termed as a metabolic syndrome, represent major challenges for basic science and clinical research[1]. Obesity is defined as abnormal or excessive fat accumulation that may impair health. Several studies have documented that peripheral adiposity [especially leg fat] may protect against cardiovascular risk [2]. The link between obesity and inflammation has been derived from the finding that proinflammatory cytokines are overexpressed in obesity .With obesity and progressive adipocyte enlargement, the blood supply to adipocytes may be reduced with consequent hypoxia [3]. Hypoxia has been proposed to be an inciting etiology of necrosis and macrophage infiltration into

adipose tissue that leads to a overproduction of proinflammatory factors like inflammatory chemokines [4]. This results in a localized inflammation in adipose tissue that propagates an overall systemic inflammation associated with the development of obesity-related comorbidities [5].

In obesity there is a chronic low grade inflammation where proinflammatory cytokines like Tumor Necrosis Factor- α (TNF α), interleukins such as IL-6 and others, are released from the hypertrophied white adipose tissue and the macrophages which infiltrate it. [1-5].

Actually in obesity cases, liver suffers from 2 hits, insulin resistance and oxidative stress, both of which lead to each other in a viscous circle [6-8].

TNF-α as a proinflammatory cytokine , exerts numerous effects in adipose tissue including lipid metabolism and insulin signaling whose circulating levels are increased with obesity and decreased with weight loss [9]. An increase in TNF-α promotes the secretion of other pro-inflammatory cytokines IL-6 and TNF-α, and reduces anti-inflammatory cytokines like adiponectin. Evidence suggests that TNF-α induces adipocytes apoptosis and promotes insulin resistance by the inhibition of the insulin receptor substrate 1 signaling pathway [10].

Paraoxonase-1 enzyme (PON-1) is an important antioxidant synthesized in the liver. Its decreased activity in obesity plays an important role in causing oxidative stress [11].

Usually there is an obvious increase in the serum level of the harmful LDL and a decrease in that of the beneficial HDL, Hence, any sort of fatty infiltration of the liver can be blamed for the elevated ALT and AST liver enzymes in obesity, [11, 12].

This study aims to evaluate the importance of the different physical and biochemical body parameters in diagnosing obesity as well as displaying the role of inflammatory markers in the pathogenesis of obesity and its related consequences. That may helps in early predicting and management of the expected complications.

Subjects and Methods:
The study group included 30 obese adolescents from both sexes and their ages ranged from 15-18 years. The control group comprised 30 non-obese adolescents who were age and sex matching with the study group. Adolescents were recruited from ultrasonography clinic of the National Research Center-Dokki- Egypt in the period from September 2013 to May 2014.

Participants were subjected to detailed history for the collection of the demographic data and recording of relevant medical history and medications. Thorough clinical examination; height and weight measurements were recorded for the calculation of body mass index (BMI) as well as the other anthropometric measurements.

The ethical approval was obtained by a signed informed consent from the patients parents and the study was approved by the ethical committee of National Research Centre and all subjects gave their informed consent prior to entering this study.

Morning 10 ml venous blood samples were withdrawn after 12 h over night fasting and divided into two parts; one part in plain polypropylene tubes and left to clot/ The other part was put into polypropylene tubes containing an anticoagulant ant stored -20 until assayed. The serum was separated by centrifugation for 10 minutes at 5000 rpm, and stored at -20 until assayed.

The ethical approval was obtained by a signed informed consent from the patients parents and the study was approved by the ethical committee.

Biochemical Analysis:
Triglycerides (TG) and total cholesterol (TC) levels were assayed by enzymatic colorimetric methods [13, 14]. High density lipoprotein cholesterol (HDL-C) concentrations were measured by enzymatic assay after phosphotungstic acid

and magnesium precipitation [15]. Low density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation when the triglyceride concentrations did not exceed 408 nmol/1 [16].

LDL-C = {Total cholesterol - HDL-C- (Triglyceride/5)}

- **Serum glucose level:** was also determined by the glucose oxidize method [17, 18].
- **Serum Insulin** was measured by using the quantitative Enzyme-Linked Immune-Sorbent Assay (ELISA) using a commercial kit provided by DIA source, Belgium. [19].

The Homeostasis Model Assessment (HOMA-IR) method was used for the calculation of insulin resistance. This method has been validated as a reliable measure of insulin resistance in vivo in humans. HOMA-IR method closely mirrors the glucose clamp technique in the assessment of insulin sensitivity [20]. Higher HOMA-IR scores denoted lower insulin sensitivity and greater insulin resistance.

HOMAIR Fasting Serum insulin (mmol/L) X Fasting Plasma Glucose (mmol/L)/405

- **Serum C-Reactive**: Protein was measured by the using quantitative Enzyme-Linked Immune-Sorbent Assay (ELISA) kit provided by SSF source, USA. [21].

- **Liver enzymes**

Serum alanine aminotransferase (ALT) or GPT and serum aspartate aminotransferase (AST) or GOT were measured by a calorimetric procedure for assaying GOT and GPT activities. End products of the transaminations (oxaloacetate or pyruvate) react with dinitrophenylhydrazine to form a hydrazine complex. This product yields a colored complex when an alkaline diluent is added. The color intensity at 505 nm can be related to enzyme activity by reference to a standard curve. Kit provided by Montgat, Barcelona, SPAIN [22].

- **Serum TNF-*α*:**

Serum TNF-α was quantified using sandwich ELISA kit from bioscience, Bender Med Systems, Austria, which has an inter-assay coefficient of variation of 7.5-10.4 per cent and a lower limit of detection of 0.5 pg/ml. [23] .

- **Serum paraoxonase:**

Serum PON-1 paraoxonase activity was determined as the rate of hydrolysis of paraoxon at 410 nm and 37 °C in a 0.05 mm glycine buffer, pH 10.5 with 1 ml CaCl2 . Activities were expressed as U/L (1 U = 1 μmol of paraoxonase hydrolyzed per minute). Serum PON-1 concentrations were determined by in-house ELISA with rabbit polyclonal antibodies generated against the synthetic peptide CRNHQSSYQTRLNALREVQ which is a sequence specific for mature PON-1. PON-1 specific activities were calculated as the ratios between the activity and the corresponding concentration, and were expressed as U/mg [24].

Anthropometric measurements:

The height (cm), waist and hip circumference (cm) were measured using a measuring tape (to the nearest 0.1 cm). Waist Circumference was measured at the mid point between the lower border of rib cage and the iliac crest. The hip circumference was measured at the level of trochanter (the widest part of the hip region). The weight (kg) was measured to the nearest 0.1 kg using a weighing balance. Obesity was defined as BMI> 30 kg/m2.

Statistical analysis:

Data are presented as mean ± SD the compiled data were computerized and analyzed by SPSS-PC+ version 16 Independent sample's t-test was used between means to analyze the mean difference. A value of $p<0.01$was considered significant and $p>0.01$ was considered insignificant. Relationships between continuous variables were assessed using Pearson's correlation coefficient.

RESULTS

The biochemical parameters of our subjects are presented in Table 1: The results showed that patients had significantly higher values of serum insulin, HOMA-IR, ALT, triglycerides, cholesterol than the control group where $P <0.01$. The HDL and value was lower in the patients group.

Concerning the anthropometric parameters, Table 2 shows that patients had significantly higher measurements than the control ($p<0.01$).

As regards the CRP (inflammatory marker), TNF-α (proinflammatory cytokine) and Paraoxonase-1 (antioxidant enzyme), table 3 shows that patients had significantly higher values of serum CRP, TNF-α, but significantly lower paraoxonase that control subjects where $p<0.01$.

Correlations:
As regards the different correlations in the obese adolescents group, there were significant live correlations between each of the CRP, TNF-α and insulin levels and the others. However, the correlations between PON-1 at one side and each of the VRP, TNF-α and insulin levels on the other side, were all significantly negative correlations (Table 4).

Discussion:
Juvenile obesity in adolescents and even in children who are under 5 years of age is considered a world wide problem in both developing and developed countries [1, 8, 25& 26].

In Obesity there is release of proinflammatory cytokines like TNF-α, IL-6 and others. Proinflammatory cytokines such as IL-6, IL-1, and IL-17 induce the production of CRP which is an acute phase protein [1, 2].

In our study, the CRP level was significantly higher in the obese adolescents group compared to the non-obese control group. This indicated that the obese group suffered from a low grade inflammation.

Matching with this finding, several other very recent studies similarly reported that the CRP levels were higher in their obese adolescents compared to their control ones [2, 27, 28].

In addition other researchers declared that the CRP levels were higher in the obesity cases and correlated significantly with the increased risk of T2DM [2]. Others reported that the high CRP levels in the obese adolescents group significantly correlated with the increased BMI and waist to hip ratio [29, 30].

It is worth mentioning that the high CRP levels, in the obese adolescents of our work study, inversely correlated with the PON-1 level. This is explained by the fact that CRP and inflammatory cytokines directly reduce the number of PON-1transcripts in the liver as noted by similar studies [11].

Finally, there was a significant positive correlation between CRP and the insulin level & also between CRP & the TNF-α.

Concerning the TNF-α, it is a proinflammatory cytokine secreted by specific proinflammatory cells such as the macrophages. It promotes insulin resistance (I.R) through inhibition of the insulin receptor substrate-1 signaling pathway and accordingly it plays an important role in the relationship between obesity and inflammatory diseases such as Diabetes Mellitus D.M. and metabolic syndrome (M.S.). [1-3, 5, 9, 10].

In our obese study group, the TNF-α level was significantly higher than its level in the non obese control group and this was very rational. There was a significant positive correlation between TNF-α and the insulin level. However, the relation between the TNF-α and the PON-1 was a significantly negative one.

Similarly many other recent work studies reported that the TNF-α levels were significantly higher in their obese groups compared to the control groups [28].

Forouzan Z. et al, 2014**,** reported that there was a close relationship between TNF-α (and other inflammatory cytokines) and the size of the visceral adipocyte in obese individuals [10] .

However and contradictory to our results a very recent study found out that there were no significant differences between the overweight adolescents and the control group as regards the TNF-α levels [27].

Similarly and still contradictory to our results, other researchers reported that there were no significant differences between obese and normal weight individuals neither in the TNF-α levels (inflammatory cytokine) nor in the anti-inflammatory cytokines levels [10, 31].

In obesity the liver suffers from two hits. The first hit is "Insulin Resistance" (I.R.), and the second hit is "Oxidative stress" (O.S.) Several research work studies showed that there is a positive correlation between O.S. and insulin resistance I.R. in obese adolescents. Oxidative stress O.S causes a low grade inflammation and causes impaired insulin sensitivity or I.R. Meanwhile, the other way round, insulin resistance I.R causes oxidative stress O.S. as will be explained later. Accordingly we can say in our own words that both oxidative stress O.S. and insulin resistance I.R. are moving in a vicious circle [6-8].

Actually when speaking of mentioning oxidative stress O.S. we should go through two important issues, the PON-1 enzyme and the insulin resistance I.R.

Concerning PON-1, it is a HDL- attached extracellular esterase enzyme mainly synthesized in the liver. [12]. It is an important antioxidant which decreases HDL susceptibility to peroxidation, degrades lipid peroxides and increases cholesterol efflux from macrophages [11]. It attenuates oxidative stress O.S. in serum lipoproteins and macrophages and decreases macrophages uptake of oxidize LDL-C [12].

Insulin resistance I.R. in obese adolescents leads to oxidative stress O.S. because in insulin resistance I.R. there are long periods of hyperglycemia and hyperinsulinemia. The high glucose levels together with cholesterol and triglycerides are major PON-1 in activators [4, 32].

Hyperinsulinemia causes reversal of PON-1enzyme activity [12]. In our study and according to what has out mentioned before, it was very rational to find out that the PON-1 level was much lower in the obese group compared to the control group. There was a negative correlation between insulin level and PON-1. Similarly other studies reported a decrease in the PON-1 activity in obese juveniles [11, 26, 33].

In addition, the association between children obesity and increased O.S. has been reported by many researchers [26, 34, 35]. An interesting research work showed a negative correlation between PON-1 and Epicedial Adipose tissue thickness (EATT). Since the EATT is a component of the visceral adipose tissue, therefore we conclude that similarly there is a negative correlation between PON-1 and visceral adiposity (obesity) [6]. Furthermore, some researchers found out that obese adolescents have an increased oxidative stress Index (OSI) and that it positively correlated with I.R. [8].

In some individuals who suffered from I.R. [in Metabolic syndrome (M.S.) which is rationally a consequence of obesity], there was a low activity of PON-1 and aryl esterase (ARE) [6, 36]. However, one study reported that the PON-1 activity was not low in patients with Metabolic syndrome M.S. who were non-diabetic [6, 37].

Concerning insulin resistance I.R., our study revealed that according to the HOMA-IR test, the obese adolescents group showed a significantly much higher level of I.R. compared to the control group.

Matching with our results, two different studies reported that their study group obese adolescents suffered from I.R. according to the HOMA-IR test [38, 39].

Similarly, Mitrou P et al, 2013 & Mitrou P et al, 2011 reported insulin resistance I.R. or impaired insulin sensitivity (I.S.) in a group of morbidly obese subjects. Both suggested that the IL-6 released from the subcutaneous adipose tissue may have acted as an endocrine mediator of insulin resistance I.R. [40, 41].

In addition, some work studies showed that there was a positive correlation between the increased oxidative stress O.S. values (OSI) and I.R. in obese adolescents [6, 8].

Emanuela F. et al, 2012, reported that evidence suggests that TNF-α promoted I.R. through inhibition of the insulin receptor substrate-1 signaling pathway [5].

One research work found out that there was insulin resistance I.R. in about 38% of a study group of obese adolescents and that there was a strong correlation between insulin resistance I.R. and the impaired fasting glucose level (P=0.04) [42].

Again another one proved alleviation of insulin resistance I.R. with physical exercise which improved glucose uptake and enhanced I.S. [39, 43]. Two different studies proved that there was an association between the I.R. and the low HDL-cholesterol level [44, 45].

Several studies declared that their study group obese adolescents suffered from insulin resistance I.R (HOMA-IR) and that there were significant correlations between insulin resistance I.R and body composition indicators as BMI and waist circumference [44-47]. Other researchers proved a significant correlation between I.R. and the adipose tissue thickness (and even the epicardial adipose tissue thickness (EATT) [6, 48, 49]. Finally, one study reported that the insulin resistance I.R. which existed in obese adolescents improved with the decrease in the total and visceral adiposities (Insulin sensitivity improvement) [39, 50].

Concerning the blood levels of the different body chemical constituents, glucose levels were higher in our obese adolescents study group compared to the control group. This is matching with many very recent studies which reported that in morbid obesity cases, there was an increased glucose production by the liver and an impairment of glucose metabolism sensitivity to insulin in both the adipose tissues and the muscles. Accordingly inactivation of the PON-1 enzyme, I.R and the very possible development of diabetes mellitus all result. [25, 27, 40, 42].

As regards the blood insulin levels, they were significantly higher in the obese group than in the control group.

Similarly, other work studies, reported the same findings. (Chandrasekhar, T. 2014) declared that there was a significant positive correlation between the fasting insulin level and the BMI (Eren, E 2013) found out that the hyperinsulinemia caused reversal of the PON-1 effect oxidative stress (O.S.) [12].

In our study, the HDL level was lower in the obese group compared to the control one. However, the LDL level was higher. Similarly, the cholesterol and the triglycerides levels were significantly much higher in the obese compared to the control group. These findings are at least partially due to the low or even reversed PON-1 activity. This was similarly reported by 2 researchers. [11, 12].

In our research, both liver enzymes ALT and AST were higher in the patients group compared to the control group with the ALT level particularly significantly higher. Similarly, several studies reported the same findings.

An important recent study declared that both liver enzymes are at least mildly increased in liver fat infiltration with the ALT level usually higher than the AST level [51].

Another one confirmed that the association of obesity with elevated serum ALT is determined by central adiposity and hyperinsulinemia. The ALT level was significantly correlated to the liver size in children and adults. It was also declared that the sensitivity of ALT was higher than that of AST [52].

Rodriguez, G et al, 2010 reported that since the ALT enzyme is not commonly found outside the liver, therefore its high serum levels accompany increased liver fat fraction, and increased intra-abdominal visceral adipose tissue. Actually those patients will be more likely to have insulin resistance I.R. [53].

CONCLUSION

In order to protect obese adolescent population from development of diabetes mellitus and cardiovascular diseases later on, we have to go through two ways. First, routine exercise and diet to be considered as a usual habit. Second, monitoring blood glucose and insulin levels as well as the measurement of their insulin resistance status. The inflammatory markers together with the PON-1 are important in determining their state of oxidative stress. Actually all these are the alarming signals to guard against development of life threatening hazards.

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