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ORIGINAL ARTICLES

What is common between overweight and obese adolescent girls regarding oxidative stress and inflammation markers?

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ABSTRACT

Background : - Overweight and obesity are major causes of co-morbidities, including (type 2 diabetes mellitus(T2DM), cardiovascular diseases, various cancers and other health problems. The antioxidant superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzymes are key elements of the internal antioxidant defense system which is crucial in countering oxidative stress produced free radicals .We aimed at a comparative study between overweight and obese adolescent girls regarding their oxidative stress, and inflammation markers. **Results:** Serum levels of 3 nitrotyrosine, nitric oxide markers of oxidative stress, C-reactive protein(CRP) and leptin as markers of inflammation have shown a significantly higher levels among obese girls than their counterparts, whereas low levels of both antioxidant superoxide dismutase (SOD) and glutathione peroxidase (GPx) have been demonstrated lower levels in both studied groups with no statistical difference between them. **Conclusion:** Despite the more significant hazard for inflammatory reaction in obese subjects than their overweight counterparts, yet the later group is in no more safe situation as they express the same low antioxidant level as the former.

Key words: overweight, obese, oxidative stress, inflammation , antioxidant enzymes.

Introduction

Obesity is associated with increased oxidative stress (Sonia *et al*., 2013) and inflammation(Faloia *et al*.; 2011). The world health organization (WHO) 2013, defines obesity as a body mass index (BMI) > 30 kg/m² and overweight as with BMI > of 25 kg/m² (Sikaris 2004). In children and adolescents, the prevalence of overweight has tripled from 5% to 15%.National center for health statistics. Previous literature showed that overweight and obesity are major causes of co- morbidities, including T2DM, cardiovascular diseases, various cancers and other health problems, which can lead to further morbidity and mortality (Chan and Woo 2010). Reactive oxygen species and reactive nitrogen species are produced as by products of normal metabolic processes in all aerobic organisms , the short lived molecules play an important role in normal physiological conditions like signal transduction and gene expression(Lassègue and Griendling 2010). A study carried by (Schopfer *et al*., 2003), revealed that they are the most free radicals in the human body causing increased oxidative/ nitrosative stress and tissue injury under pathological conditions, and responsible for a variety of degenerative process in some human disease (Montero *et al*., 2012).

Peroxynitrite ($ONOO^-$) and other reactive nitrogen species are generated by the reaction of superoxide ($O2^-$) and nitric oxide (NO) (Ignarro 2002). The reaction of RNS with protein- bound tyrosine residues causes formation of 3- nitrotyrosine (Sucu *et al* ., 2003) a marker associated with inflammation (Kulwant *et al*., 2001). Murata and Kawashini 2004 observed that elevation of 3NT has been found to cause DNA damage. NO is considered as a factor with a role in the modulation of food intake and obesity related diseases (Jang *et al*., 2007). The dependent NO inflammatory reactions can be assessed by measurement of 3 nitrotyrosine, a reaction product of tyrosine and NO – derived oxidants (Lassègue and Griendling 2010), but the antioxidant defense systems in the body protect the cells and tissue against these species (Halliwell and Gutteridge 1999), like Glutathione peroxidase (Gpx) and superoxide which is an important indicator of the levels of oxidative stress (Rajeev *et al*., 2011). Increased oxidative stress associated with increased production of ROS is augmented by decreased expression of antioxidant enzymes such as superoxide dismutases (SOD) (Roberts *et al*., 2006), when free radical formation is greatly increased or protective antioxidant mechanisms are compromised oxidative stress occur (Sonia *et al*., 2013). Leptin is an adipocyte derived polypeptide hormone (Bradly *et al*., 2001), that controls body weight through central regulation of food intake and energy expenditure (Ahima and

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Flier 2000). Obese individuals typically have relatively elevated serum protein and is produced by the liver in response to stress (Farah and Mohd 2010) and it has been observed by (Das and Fams 2001) an elevated levels of CRP in obese and overweight children. Despite the more significant hazard for inflammatory reaction in obese subjects than their overweight counterparts, yet the later group is in no more safe situation as they express the same low antioxidant level as the former.

Methods:

Subjects and anthropometric measurements:

The current study was carried out on one hundred and twenty (120) overweight and obese adolescent girls with age range from 13 to 18 years old , through a project conducted in the National Research Centre, Egypt. It was a cross-sectional survey. Two local public schools in Cairo were enrolled in this study regarding adolescents (one preparatory school and one secondary school). Permission to perform the study was granted by the Ministry of Education, and the directors of the school included in the research.

The protocol was approved by the "Ethical Committee" of the "National Research Centre". In accordance with the code of ethics of the world medical association (Declaration of Helsinki) Of the total sample, one hundred and twenty adolescent girls with the complaint of overweight and obesity were included in the current research after obtaining written informed consent from their parents. Student assent was also obtained. The adolescents were required to meet the following inclusion criteria: age, 13–18 years and BMI>95 percentile for age and gender, students were excluded if they had a prior major illness, including type I or II diabetes, took medications or had a condition known to influence body composition, and insulin secretion (eg. Glucocorticoids therapy, hypothyroidism, Cushing's disease). Each adolescent underwent a complete physical examination, including anthropometric measures. The height and the weight were recorded. The height was measured to the nearest 0.5 cm on a Holtain portable anthropometer, and the weight was determined to the nearest 0.1 kg on a Seca scale Balance with the subject dressed minimum clothes and no shoes. Body mass index (BMI) was calculated as Weight (kg) / Height (m^2).

The participating adolescent girls were divided into two groups according to BMI and fat percentage. overweight Group I (sixty subjects) with BMI >25 and Group II(sixty subjects) with BMI >30

Sample collections:

Blood was drawn from the antecubital vein of the students, and sera were separated by centrifugation and kept frozen at -80^{0}C until analysis.

Research methods and procedures:

Biochemical assays:

All samples were assayed in duplicate.

Measurement of Oxidative stress markers:

1-Nitrotyrosine was chosen as a marker of oxidative stress. Fasting plasma concentrations of nitrotyrosine were measured by using a commercial enzyme linked immunosorbent assay kit (OXiSelect Nitrotyrosine ELISA kit Catalog Number STA-305 Cell Biolabs, INC. 10225 Barnes Canyon Road, Suite A103, San Diego, CA 92121)

2-Serum levels of NO were measured using colorimetric Non –enzymatic assay for nitric oxide product no. NB88 (Oxford Biomedical Research Superior Science Reliable Results) the kit can be used to accurately measure as little as 1pmol/ml following the method described by Schmidt *et al*.; 1995.

3-Human Cu/Zn SOD activity was estimated in serum by using Enzyme – linked immuno- sorbent assay ELISA kit produced by Bender Med system GmbH, Austria, Europe, the limit of detection(sensitivity) was determined to be 0.04 mg/ ml.

4-Glutathione peroxidase activity was estimated in erythrocyte lysate by using ELISA kit produced by Bender Med system GmbH, Austria, Europe, the limit of detection (sensitivity) was determined to be 0.04 mg/ ml.

Inflammatory markers:

Serum concentrations of leptin, and hs- CRP were measured by using a sandwich enzyme-linked immunosorbent assay.

Leptin levels were determined with an (ELIZA) procedure using commercial kits (Diagnostics Biochem Canada Inc 1020 Hargrieve Road) and the sensitivity of detection 0.5ng.ml.

Serum CRP levels were determined with an enzyme- linked immunosorbant assay (ELIZA) technique according to the method of Roberts *et al* using commercial kits (BioCheck, Inc 323 Vintage Park Drive Foster City, CA 94404) and the sensitivity of detection level was 0.1 mg\L.

Statistical analysis:

All values are expressed as mean ±SE and the differences between the two groups were calculated by student´s t test. The correlation was done between different parameters using Pearson correlation. All analyses were carried out using SPSS version 16 (IBM , Chicago IL, USA. Statistical software) the statistical significance was set at $p<0.05$

Results:

Subject characteristics:

Main features of obese and overweight adolescent girls are shown in Table (1):

Table 1: Anthropometric measurements of overweight and obese girls.

Parameters	Mean±SE overweight (GroupI)	Mean±SE Obese (Group II)	Significance P value
BMI kg/m	27.65± 0.238	34.56±1.48	0.001
Fat%	38.02±0.8	42.4±1.52	0.013

p<0.0001= very highly significant difference .

Table 2: Comparison between overweight and obese subjects regarding Oxidative stress markers.

Parameters	Mean±SE Overweight (GroupI)	Mean±SE Obese (Group II)	Significance P value
Nitrotyrosine nM	6.18±0.46	30.65±7.2	0.001
Nitric oxide (NO) pmol/ml	21.7±1.34	32.1±1.80	0.005
Superoxide dismutase (SOD) ng/ml	81.64±6.81	69.82±7.03	0.232 NS
Glutathione peroxidase (GPx) nmol/ml	40.58±5.17	36.61±22.1	0.548 NS

p<0.0001= very highly significant difference

Obese subjects had higher plasma nitrotyrosine, and nitric oxide concentrations than did overweight girls. We compared the mean nitrotyrosine of overweight and obese subjects. First , we see the descriptive statistics for the two group the mean of nitrotyrosine, and nitric oxide for overweight is lesser than that of obese (group). That is, obese have an average, higher nitrotyrosine and nitric oxide levels than those of overweight, in addition superoxide dismutase and glutathione peroxidase has shown higher level in overweight than obese group but this difference wasn't statistically significant.

Table 3: Comparison between overweight and obese subjects regarding Inflammatory markers

Parameters	Mean±SE Overweight (Group I)	Mean±SE Obese (Group II)	Significance P value
Leptin ng/ml	7.45±0.968	11.45±1.07	0.008
CRPµg/dl	2.19±0.23	3.98±0.52	0.003

P<0.05 highly significant ,P<0.001 very highly significant

There was a significant increase of leptin levels, and CRP in obese subjects in comparison to the overweight adolescent girls at P< 0.008, P< 0.003., respectively .

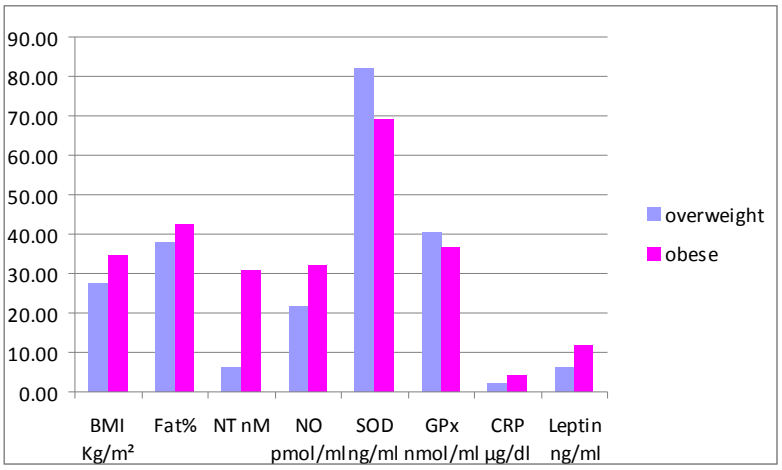


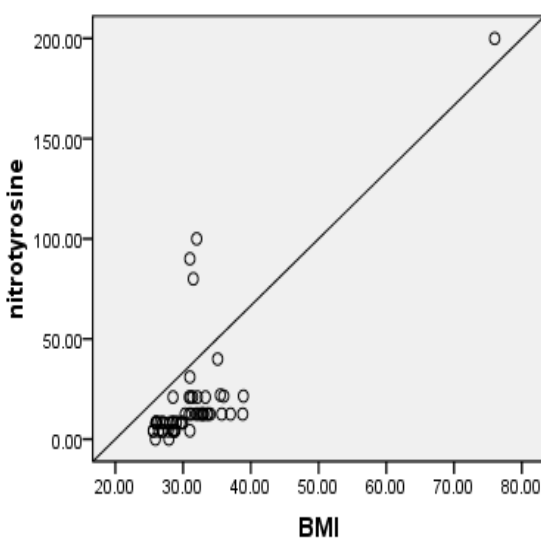
Fig. 1: A histogram showing the mean difference in overweight and obese adolescent girls in the different parameters of the study.

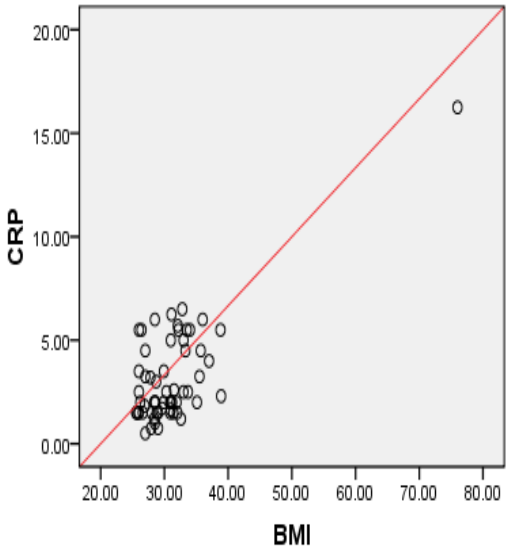
Table 4: Correlation between different parameters of the study.

parameters	Nitric oxide Pmol/ml	Superoxide dismutase ng/ml	Glutathione peroxidase nmol/ml	BMI Kg/m2	Fat percentage	CRPµg/dl	Leptin ng/ml
Nitrotyrosine nM	r=0.262 P<0.043	r=-0.187 P<0.153 NS	r=-0.104 P<0.430 NS	r =0.777 P<0.000	r=0.114 P<0.385 NS	r =0.565 P<0.0001	r=0.243 P<0.06 NS
Nitric oxide ng/ml		r=-0.331 P<0.010	r=-0.134 P<0.306 NS	r=0.438 P<0.000	r=0.077 P<0.559 NS	r=0.266 0.040	r=0.280 p<0.030
Superoxide dismutase ng/ml	r=-0.331 P<0.010		r=0.145 P<0.268 NS	r=-0.254 P<0.05	r=-0.009 P<0.947 NS	r=-0.101 P<0.440 NS	r=0.184 P<0.66 NS
Glutathione peroxidase nmol/m		r=-0.134 P<0.306 NS		r= -0.090 P<0.493 NS	r=-0.096 P<0.465 NS	r=0.125 P<0.340 NS	r=0.095 P<0.472 NS
BMI Kg/m2	r=0.438 P<0.0001	r=-0.254 P<0.05	r =-0.090 P<0.493 NS		r=-0.059 P<0.655 NS	r=0.745 P<0.0001	r=0.417 P<0.001
Fat percentage	r=0.0777 P<0.559 NS	r=-0.009 P<0.947 NS	r=-0.096 P<0.465 NS	r=-0.059 P<0.655 NS		r=-0.164 P<0.211 NS	r=-0.072 P<0.585 NS
CRP µg/dl	r= 0.266 P<0.040	r=-0.101 P<0.440 NS	r= 0.125 P<0.340 NS	r=0.745 P<0.0001	r=-0.164 P<0.211 NS		r=-0.430 P<0.001
Leptin ng/ml	r=-0.280 P<0.030	r=0.184 P<0.160 NS	r=-0.095 P<0.472 NS	r=-0.417 P<0.001	r=-0.072 P<0.585 NS	r=0.430 P<0.001	

* Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.

Nitrotyrosine was positively correlated with BMI, nitric oxide, CRP at P<0.001, r=0.777; P<0.043, r=0.262; P< 0.001, r=0.565 respectively., In addition NO has shown a negative correlation with SOD and glutathione peroxidase at P< 0.01, r=-0.331; p<0.027, r=-0.286 respectively and a positive one with BMI, CRP, Leptin at P< 0.0001, r=0.438; P< 0.04, r=0.266; P< 0.03, r=0.280 respectively, SOD has shown a negative correlation with the BMI at P< 0.05, r=-0.254; but fat % doesn't shown any correlation with any parameter. BMI has shown a positive correlation with CRP, and Leptin at P< 0.001, r=0.747; P< 0.001, r=0.417 respectively, CRP has shown a positive correlation with leptin at P< 0.001, r=0.430.





Discussion:

According to the (WHO 2013) overweight and obesity were defined as an abnormal or excessive fat accumulation that may impair health . Esposito *et al*., 2006 and Sonia *et al*., 2013 revealed that induction of oxidative stress and inflammation (Faloia *et al*., 2011) are the key factors in obesity induced health impairment. The current study aimed at studying both key factors in obesity and the earlier stage of overweight, in a population of adolescent girls. In our study we found a significant increase in both the oxidative stress markers nitrotyrosine and nitric oxide and the inflammatory markers CRP and leptin in obese than overweight adolescent girls. Reactive oxygen species (ROS) are highly reactive derivatives of oxygen metabolism. In health , ROS are maintained at an optimal level due a balance between their production and elimination by enzymatic reaction of superoxide dismutase and glutathione peroxidase stress(Lassègue and Griendling 2010) on the other side, in pathological states such as the metabolic syndrome, an increased oxidant capacity coupled with decreased antioxidant capacity creates an umbalanced environment that results in oxidative stress (Sonia *et al* 2013). The increased levels of nitric oxide and nitrotyrosine and their significant positive correlation with each other demonstrated in this cross study could be attributed to the fact that nitric oxide (NO) and superoxide($O2^{-}$), are required for the nitration reaction, as the generation of 3NT involves both the reactive oxygen species (ROS) such as superoxide and hydrogen peroxide and the reactive nitrogen species (RNS) in nitric oxide, (Sun et al., 2007) therefore, 3NT is being considered as a marker of oxidative and nitrosative stress and also a marker of inflammation since the production of both ROS and RNS usually takes place at the inflammatory site (Villeneuve *et al*., 2003) .In addition the increased production of reactive oxygen species may also enhance the inflammatory response by activating redox-sensitive nuclear transcription factors such as AP-1 and NF-κB. These transcription factors are essential for the inducible expression of genes associated with immune and inflammatory responses, including cytokines, cell adhesion molecules, and inducible NO synthase (Lavrovsky *et al*., 2000).

The current study, revealed a significant increase in NO levels in obese girls than in their overweight counterparts and this was consistent with(Magdalena 2004) and was explained by her that this increase may be the result of increased synthesis of NO in adipose tissue, the activation of the inducible isoform of (iNOS) through inflammatory stimulants produces NO(Beck *et al*., 1999). NO can be transformed in reaction with another radical superoxide ($O2^{-}$) to form peroxynitrate ($ONOO^{-}$) This reaction between NO and $O2^{-}$ is extremely strong and three times faster than the rate at which superoxide dismutases scavenges $O2^{-}$ which explains the inverse correlation found between NO and SOD found in our results. The first evidence of peroxynitrate formation came from SOD as a catalyst of tyrosine nitration to detect peroxynitrite (Ischiropoulos *et al*., 1992), SOD catalyses tyrosine nitration by peroxynitrite, but would generally expected to reduce peroxynitrite formation by scavenging superoxide $O2^{-}$(Sampson *et al*., 1996). When obesity persists for a long time antioxidant sources can be depleted, decreasing the activity of enzymes such as superoxide dismutase (SOD) (Amirkhizi *et al*., 2007), which explains the inverse correlation between nitrotyrosine and SOD.

Previous studies done by (Alba *et al*., 2011 and Ozata *et al*., 2002) established an inverse relationship between adipose tissue and the activity of antioxidant enzymes such as superoxide dismutase and glutathione

peroxidase (GPx). It is worthnoting that these studies compared obese subjects to controls with normal BMI where the literature is scarce in studies comparing obese to overweight subjects. In our study we didn't find any statistically significant difference in the levels of the antioxidant enzymes (SOD and GPx) between obese and overweight subjects . This finding encourages us to speculate a similar susceptibility to oxidative stress between overweight and obese subjects despite our finding of increased levels of oxidative stress markers in the later group than the former . In addition we found a positive correlation between CRP, nitrotyrosine and nitric oxide this was consistent with (Abramson *et al*., 2005 and Cottone *et al*., 2006) who found that CRP was increased by increased oxidative stress. The sensitivity of CRP and other biomarkers of oxidative damage are higher in individuals with obesity and correlates directly with BMI(Phil *et al*., 2006) in contrast antioxidant defense markers are lower according to body fat and obesity (Chrysohoou *et al*., 2007and Harwich *et al*., 2007). In addition our results showed an increase in leptin levels in obese subjects and a positive correlation between leptin and CRP. Elevated leptin levels underlie the low grade proinflammatory state associated with human obesity because in inflammation , leptin acts directly on macrophages to increase phagocytic activity , and proinflammatory cytokine production also excerts an effect on T- cells, monocytes , neutrophil and endothelial cells(Loffreda *et al*., 1998. Fonseca, *et al*., 2007) found that when leptin is administred ,an increased levels of CRP are produced thus providing its inflammatory effect which let (Shamsuzzaman *et al*., 2004) demonstrated that leptin could induce the production of CRP. In addition (Buettner *et al*., 2002) reported a correlation between serum leptin concentrations and CRP that have been shown to be positively associated with BMI (Bastard *et al*., 2000).

Conclusions:

This study highlights an important observation which is that overweight girls are susceptible to the oxidative damage as obese girls, and There is a need to loose weight to approve the potential of antioxidant enzymes and to decrease the oxidative stress and inflammatory markers in obese and overweight adolescent girls.

We concluded from this study , that overweight girls may be as much susceptible to oxidative stress as obese girls , where we suggest future studies to reveal the underlying mechanisms of antioxidative machine exhaustion and the BMI cut-off level above which this machine loses function.

Conflict of interest.

There is no conflict of interest.

Submission declaration and verification.

This work described has not been published previously, and the publication was approved by all authors and if accepted it will not be published elsewhere in English or any other language.

Abbreviations

T2DM: typeII diabetes mellitus, SOD:superoxide dismutase, GPx: glutathione peroxidase, CRP: C- reactive protein, WHO:World health organization, BMI: body mass index, $ONOO^-$: Peroxynitrite,$O2^-$: Superoxide, NO: nitric oxide, RNS: reactive nitrogen species, ROS: reactive oxygen species, 3NT: 3 Nitrotyrosine.

Competing interests

The authors declare that they have no competing interests

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