Some Potential Biological Predictors of Hypertension in Obese Male Rats

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Abstract: Obesity is a leading preventable cause of death worldwide. Patients with obesity are susceptible to hypertension which is a risk factor for all clinical manifestations of atherosclerosis, heart failure, coronary artery disease, stroke and renal disease. The aim of this thesis was to study some potential biological predictors of hypertension in obese male rats. Material and methods: 60 male albino rats were included in this study and classified into 2 main groups. Group 1(control group, n=30) and group 2 (obese group, n=30). Induction of experimental obesity was by feeding rats with high fat diet till reaching Lee index > 0.3. Lee index (cubic root of body weight (g) X 10 / naso-anal length (mm). All the following were measured at 3 months-interval (at the beginning of the experiment, after 3 months, after 6 months): systolic blood pressure (mmHg), serum adiponectin (µg/ml), serum uric acid (SUA) (mg/dl), HsC-reactive proteins (HsCRP) (µg/ml) and triglyceride (TG) (mg/dl). Results: Obesity resulted in significant increase of systolic blood pressure and significant decrease of adiponectin. It also induced a significant increase of SUA, HsCRP, and TG. There is significant negative correlation between adiponectin and body weight ($r=-0.06$ & $P < 0.05$) in obese group. Conclusion: Serum adiponectin, SUA, HsCRP, and TG are biological predictors of hypertension in obese male rats.

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1. Introduction

Obesity is considered as a major obstacle in the maintenance of the human health system (1). Multivariate analysis identified obesity and current smoking as independently associated with uncontrolled blood pressure, both in patients with or without cardiovascular disease (2).

Many hormonal mechanisms have been participated in the regulation of appetite and food intake, storage patterns of adipose tissue, and development of insulin resistance as leptin's , ghrelin, insulin, orexin, cholecystokinin, as well as adiponectin (3). The adiponectin is a protein hormone mediator produced by adipose tissue and modulates a number of metabolic processes, including glucose regulation, fatty acid catabolism (4) and triglyceride clearance (5).

Adiponectin action is thought to modify many obesity-related diseases such as hypertension, type 2 diabetes, and coronary artery disease (6). It correlates negatively with adiposity, and bears an inverse relationship with insulin resistance, atherogenic dyslipidemia, and inflammatory markers (7).

The association of hyperuricemia with the presence of classical coronary risk factors and coronary artery disease (CAD) or myocardial infarction (MI) has been analyzed in many epidemiological studies. Numerous studies have revealed that hypertension, high body mass index (BMI), lipid disorders (especially raised triglycerides--TG level and low high dense lipoprotein cholesterol (HDL-C level), increased creatinine or insulin levels have caused hyperuricemia (8).

However, whether uric acid is an independent risk factor for cardiovascular mortality is still disputed there is still no well-established Pathophysiological link between hyperuricemia and the development of cardiovascular complications (9).

Acute ischemic stroke may trigger an inflammatory response that leads to increased levels of C-reactive protein (CRP). High levels of CRP may be associated with poor outcome because they reflect either an inflammatory reaction or tissue damage (10). Elevated preclinical atherosclerosis in young adults, partly mediated by inflammation (elevated C-reactive protein), high systolic blood pressure, and triglycerides. This association is most marked for those also born preterm (11).

The present work aimed to determine whether serum adiponectin, serum uric acid (SUA), high sensitive C-reactive protein (HsCRP) & serum triglycerides (TG) are potential predictors of hypertension in obese male rats. Also, to find out any association or correlation between those parameters and systolic arterial blood pressure.

2. Material and Methods:

Experimental animals:

The study was carried on 60 male albino rats. The rats were left for a week as a period of acclimatization during which all rats were fed a standard control diet prepared in the laboratory. The

body weight of each rat was recorded every 15 days. Rats were randomly distributed into 2 groups: a) control group receiving control diet (n= 30) b) High fat diet (HFD) – induced obesity group receiving high fat diet (n = 30).

Lee index (cubic root of body weight(g) X 10 / naso-anal length (mm) was assessed every 15 days till reaching Lee index significantly higher than 0.3 where rats were classified as obese (12).

Induction of experimental obesity by high fat diet was introduced by feeding rats with high fat diet. Diet were designed in our laboratory: the composition of control diet is (corn starch 480 g/kg b. wt, sucrose 100 g/kg b. wt, soyabean oil 50 g/kg b. wt, lard 120 g/kg b. wt, casein 190 g/kg b. wt). The composition of high fat diet (HFD) is corn starch 100 g/kg b. wt , sucrose 100 g/kg b. wt, soyabean oil 50 g/kg b. wt, lard 500 kg body wt, casein 190 g/kg b. wt) (13). High fat diets were weekly prepared and stored. The food was renewed every day for 24-week diet course (14).

Measurements:

All the following were measured at 3-months interval (the beginning, 3 and 6 months of the 24-week experimental period):

A) Anthropometric parameters: the body weight (BW) in gm and body length (nasoanal length) in mm were measured and used to determine: Lee index: Body mass index [BMI] Which equal cubic root of body weight (gm) X 10 / naso-anal length (mm)

B) Systolic blood pressure (SBP): was measured by Harvard 50-9331 Rectilinear Recording System, which is a rat tail Monitor.

C) Predictors of hypertension: Retro-orbital blood samples were taken from each rat. The serum was separated and stored at -20^{0}C for subsequent measurement of: adiponectin , SUA, HsCRP & TG.

Adiponectin was detected by Elisa kit from (B-Bridge International, Inc .USA) according to manufacturers instruction (15) & HsCRP was measured by Elisa kit supplied by (Immuno-Biological Laboratories, Inc. Minneapolis, USA) according to manufacturers instruction (16). SUA was measured according to **Fossati *et al.*** (17). TG was estimated by commercial available kit (18).

Statistical Analysis:

The data was encoded and entered using the statistical package SPSS Version 15. The data was expressed as means $\pm$ SD in each group. All data were analyzed by unpaired & paired student's t-test. Correlation between the continuous variables was assessed using Pearson's correlation coefficient.. The level of statistical significance was assumed to be P < 0.05.

3. Results

The results are shown in Tables (1- 3) and Figures (1-6).

Table 1 shows the significant increase in Lee index and SBP of the obese group, but Table 2 shows the significant reduction of serum adiponectin in control and obese groups after 6 months.

Table 2 shows also the significant increase in SUA and HsCRP in obese group at end of 6 months comparing to control and to the beginning. But, there is only significant increase in TG of the obese group comparing to control.

The % of reduction of adiponectin and the % increase of SUA, HsCRP & TG in obese group are illustrated in Table 3.

There is significant positive correlation between adiponectin & HsCRP (r=.+0.6 & P < 0.05) (Figure 5) in control group, but negative correlation between adiponectin and body weight (r= - 0.6 & P < 0.05) (Figure 6) in obese group. There were no correlations between the SBP and adiponectin, SUA or HsCRP in both studied groups.

Table 1: Lee index (BMI)(g/mm) And Systolic blood pressure (SBP) (mmHG) in control & obese groups

	At beginning		At 3 months interval		At 6 months interval	
	Control	Obese	Control	Obese	Control	Obese
Lee index	0.266+0.0097	0.273+0.001*	0.279+0.0012	0.288+0.006*#	0.282+0.001#	0.313+0.0015*#@
SBP	109.4+19	114.3+14.8	100.9+15	132.9+19*#	118.1+5.5 @	227.4+7.7*#@

Results are expressed as mean $^+$ SD
*Significant compared to corresponding value of control rats.
Significant compared to beginning value
@ Significant compared to 3 months value
P value < 0.05 is significant
P value > 0.05 is insignificant

Table 2: Predictors of hypertension: Adiponectin µg/ml, Uric acid mg/dl, High sensitive C-reactive proteins (HsCRP) (µg/ml), triglycerides (mg/dl) in control & obese groups

	At beginning		At 3 months interval		At 6 months interval	
	Control	Obese	Control	Obese	Control	Obese
Adiponectin	3+0.6	3.79+0.57*	3.97+1.01#	2.7+0.97*#	3.7+0.6#	2.4+0.5*#
Uric acid	2.7+0.3	1.6+0.6*	1.9+0.5#	2.5+0.6*#	1.8 +0.3#@	2.7+0.7*#
HsCRP	16.8+1.05	13.8+1.97*	13.5+1.3#	16.9+3.1*#	11.3+1.1#@	17.27+3.7*#@
Triglycerides	99.8+7	101.9+10.6	97.5+11.1	106.96+14.1	95.8+7.3	109.23+9.9*

Results are expressed as mean + SD
*Significant compared to corresponding value of control rats.
Significant compared to beginning value
@ Significant compared to 3 months value
P value < 0.05 is significant
P value > 0.05 is insignificant

Table 3: The percentage change in predictors of hypertension in obese group

Predictors of hypertension	Beginning	3 months	6 months
Adiponectin		- 29 %	- 37 %
Uric acid		+ 56 %	+ 69 %
HsCRP		+ 22%	+ 25.4
Triglyceride		+ 5%	+ 7.2%

HsCRP high sensitive C reactive proteins

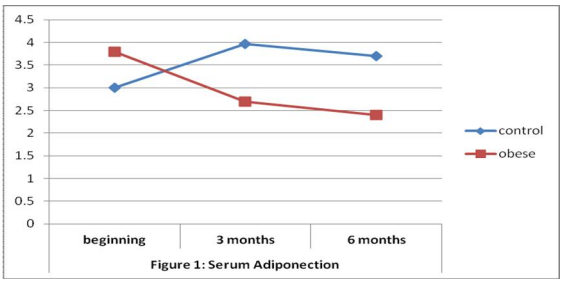

Figure (1): Serum adiponectin levels (µg/ml) in control and obese groups at the beginning of the study and after 3 and 6 months.

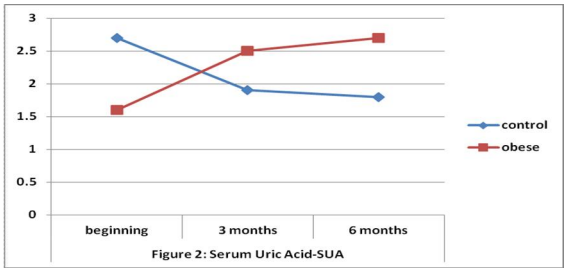

Figure (2): Serum uric acid levels (mg/dl) in control and obese groups at the beginning of the study and after 3 and 6 months.

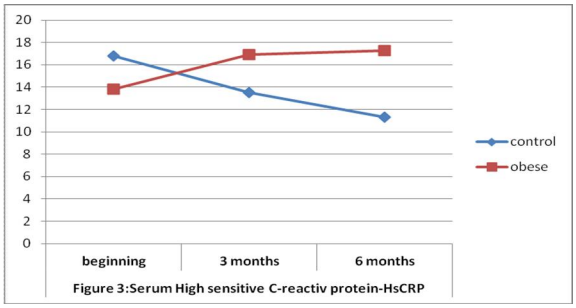

Figure (3): Serum high sensitive C-reactive protein levels (µg/ml) in control and obese groups at the beginning of the study and after 3 and 6 months.

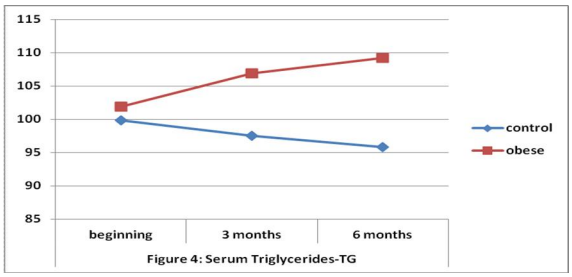

Figure (4): Serum triglyceride levels (mg/dl) in control and obese groups at the beginning of the study and after 3 and 6 months.

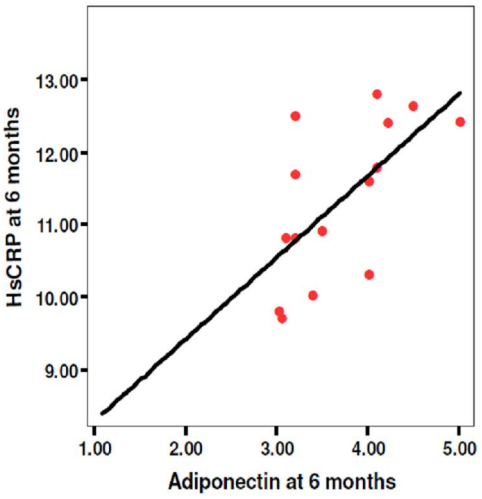

Figure 5: The correlation between HsCRP and adiponectin in control group at end of 6 months.

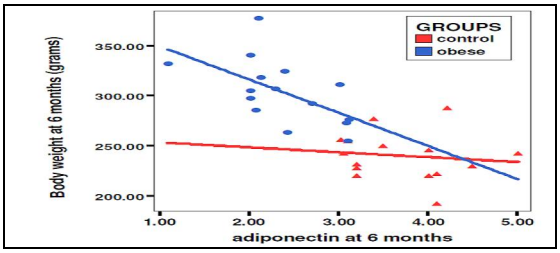

Figure (6): The correlation between adiponectin and body weight in control and obese groups at end of 6 months. (Significant correlation at $P < 0.05$)

4. Discussion

The present study shows that serum adiponectin, SUA, Hs-CRP and TG may be potential predictors of hypertension in obese male rats. The evidence is that, there is statistical significant decrease of adiponectin level in the obese group compared to the corresponding values in the control group after 3 and 6 months periods. Also, there are statistical significant increase of SBP, SUA and Hs-CRP after 3 and 6 months periods. The serum TG increases significantly after 6 months period.

Wang and Schere (19) related the association of low level of adiponectin with high blood pressure to 3 mechanisms: endothelial dysfunctions, increases renin angiotensin system (RAS) activity, and sympathetic nervous system (SNS).

The authors proved that adiponectin regulates endothelial nitric oxide synthase (eNOS) enzymatic activity and nitric oxide (NO) production by several mechanisms. Adiponectin exerts its effects through membrane-bound receptors and adaptor molecules (APPL1) in endothelial cells (19, 20). Adiponectin increases the stability and half- life of eNOS mRNA (21) and stimulates the phosphorylation of eNOS,

which together lead to increase NO production. These results suggest that hypoadiponectinemia can cause decreased endothelium-derived NO production and subsequent endothelial dysfunction, ultimately contributing to the development of hypertension. Adiponectin stimulates phosphorylation of eNOS at Ser-1177 in human endothelial cells through its ability to activate AMP-activated protein kinase signaling (22).

In addition to the above-mentioned signaling pathway, adiponectin promotes endothelial cell function through another mechanism. It promotes revascularization in response to tissue ischemia through endothelial cyclooxygenese-2 (COX-2)-dependent mechanism (23). Deletion of COX-2 in an endothelial specific manner results in suppression of adiponectin-induced revascularization response in ischemic muscle. In cultured endothelial cells, recombinant adiponectin treatment significantly increases COX-2 expression and promotes endothelial cell function (24).

Clinical studies shows that inhibition of RAS with angiotensin converting enzyme inhibitors and angiotensin II receptor blockers can decrease blood pressure and increase circulating adiponectin level in hypertensive patients (25). Several mechanisms have been proposed to explain the stimulatory effect of angiotensin II receptor blocking on circulating adiponectin levels. Angiotensin II inhibits adiponectin production through angiotensin II receptor subtype 1, and angiotensin II receptor blockers may elicit their effect by inhibiting angiotensin II receptor subtype 1 signaling (19).

Angiotensin II infusion is associated with increased generation of reactive oxygen species (26) which may be one of the underlying reasons for the suppression of adiponectin production, because hydrogen peroxide has been shown to inhibit adiponectin expression (27).

Angiotensin II receptor blockers may increase adiponectin production directly by activating the nuclear receptor PPARγ. In 2004, 2 pioneering articles demonstrated that some angiotensin II receptor blockers act as partial peroxisome proliferators activated receptor-γ (PPARγ) agonists *in vitro* and *in vivo* (28 & 29).

SNS overdrive has been shown to suppress adiponectin expression. The treatment with norepinephrine synthesis inhibitors attenuated the suppression. β-Adrenergic receptor antagonist treatment releases this suppression, suggesting a local effect of sympathetic neurotransmitter signaling in adipose tissue (30).

Because adiponectin has been found in cerebrospinal fluid and administration of adiponectin centrally affects energy homeostasis, it is tempting to speculate that adiponectin may be involved in the regulation of SNS activity from the brain (31). Adiponectin affects SNS activity via central regulation (32).

Analysis of mutations in human adiponectin gene provides further information about the link between adiponectin and hypertension. Among several single-nucleotide polymorphisms of adiponectin gene, single-nucleotide polymorphism at position 164 has been associated with hypoadiponectinemia and high blood pressure in Japanese population (33).

The potential mechanisms involved with the association of hyperuricemia and hypertension include the following: 1.Decrease renal blood flow (decreased GFR) stimulating urate reabsorbtion, 2. Microvascular (capillary) disease that results in local tissue ischemia. 3.Ischemia with associated increased lactate production that blocks urate secretion in the proximal tubules and increased uric acid synthesis due to increased RNA-DNA breakdown and increased purine (adenine and guanine) metabolism,. 4. Ischemia induces increased xanthine oxidase (XO) production and increased SUA and ROS. These associations with ischemia and XO induction may help to understand why hyperuricemia is associated with preeclampsia and congestive heart failure (34).

The results of Lago *et al.* (35) coincide with our results. The authors suggested that visceral fat accumulation play an essential role in the development of the coexistent disorders in the metabolic syndrome (hyperlipidemia, diabetes, hypertension).

It was reported that, CRP is associated with components of metabolic syndrome, including abdominal obesity. Cytokine production by adipocytes might mediate the elevation of CRP levels. Adipose tissue secretes a number of cytokines, among which is interleukin 6 (IL-6). IL-6 regulates hepatic production of CRP (36).

Our results are in harmony with that of Kawamoto *et al.* (37) who proved that adiponectin was significantly lower in subjects with prehypertension and hypertension than those with normotension. Lower serum adiponectin were positively associated with prehypertension and hypertension. Serum adiponectin concentrations were inversely associated with blood pressure (BP) in the general male population.

Ohashi *et al.* (20) & Cohen *et al.* (38) proved that obesity, in particular, visceral fat accumulation, is implicated in the deregulated secretion of adipocytokines, which can contribute to the development of metabolic syndrome and cardiovascular diseases. Adiponectin is an adipocytokine that is exclusively secreted from

adipose tissue, but its plasma levels are reduced in obese subjects, especially those with visceral fat accumulation. Adiponectin has a variety of protective properties against obesity-linked complications, such as hypertension, metabolic dysfunction, atherosclerosis, and ischemic heart disease. Adiponectin exerts the beneficial effects on vascular disorders by directly affecting components of vascular tissue.

Our results also coincide with that of Tanimura *et al.* (39) who documented that Hypoadiponectinemia was positively associated with systolic blood pressure and the prevalence of hypertension

The results coincide with that of Waring & Esmail (40) who concluded that Dietary supplementation of 2 % oxonic acid causes a significant increase in blood pressure. Barbosa *et al.* (41) proved that, close correlation was found between the resultant increase in SUA concentration and rise in blood pressure. Tamakoshi et al (42) have shown a statistically significant positive correlation between CRP and body mass index, total cholesterol, triglycerides, LDL, fasting glucose, fasting insulin, uric acid , systolic blood pressure and diastolic blood pressure and a significant negative correlation of CRP with HDL-C.

Hs-CRP proved to be the strongest and most significant predictor of the risk of future cardiovascular events regardless of the LDL cholesterol level; the data indicate that use of pravastatin resulted in decreased levels of Hs-CRP in a manner largely independent of LDL cholesterol. The data raise the possibility that the addition of Hs-CRP to standard lipid screening will generate an improved method for identifying persons at high risk for future cardiovascular events, who would thus be candidates for primary-prevention interventions such as the use of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (43).

In conclusion, in this prospective evaluation of some biological predictors of hypertension in obese male rats, serum adiponectin, SUA, Hs-CRP and TG are proved to be significant predictor of the risk of future hypertension. Thus, these data raise the possibility that the addition of adiponectin, SUA and Hs-CRP to standard lipid screening will generate an improved method for identifying obese persons at high risk for future hypertension, who would thus be candidates for primary-prevention interventions.

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