Reciprocal Relationship between Obesity and Angiogenesis and Accentuated Pro-Inflammatory Response

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Abstract: Objectives: To determine serum levels of vascular endothelial growth factor (VEGF), interleukin-13 (IL-13) and C-reactive protein (CRP) after induction of obesity and diabetes in rats as a trial to elucidate their role in pathogenesis of obesity.Materials & Methods: The study included 45 male albino rats divided into Control group received no medications, Obese and Diabetic groups maintained on high-fat diet (HFD) for 2 weeks and then 15 rats were injected intraperitoneally with a single dose of streptozotocin (STZ) and diabetes was confirmed on the 3rd day. Venous blood samples were obtained at two weeks after induction of obesity and assurance of development of diabetes for ELISA estimation of serum VEGF, IL-13 and CRP .Results: Pre-induction blood glucose, serum insulin, VEGF, IL-13 and CRP levels were non-significantly different among studied groups. Post-induction blood glucose and serum insulin levels were significantly higher compared to control and pre-induction levels with significantly higher levels in diabetic versus obese groups. Post-induction serum VEGF, IL-13 and CRP levels in obese and diabetic groups were significantly higher compared to both control group and pre-induction levels with significantly higher post-induction serum IL-13 and CRP level. However, post-induction serum VEGF levels were non-significantly higher in diabetic compared to obese group. Conclusion: The obtained indicated that obesity and obesity-associated type-2 diabetes mellitus represent double-crossing vicious circles of obesity, disturbed immune system and promoted angiogenesis.

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1. Introduction

Adipose tissue is one of the most highly vascularized tissues in the body, and a close functional relationship exists between fat tissue and its vasculature. Adipose tissue is well-known for its angiogenic capacity, and has been used clinically to promote wound healing and revascularization. It remains unknown if new vessel formation in fat requires blood circulating progenitors, such as bone marrow-derived cells (BMDCs, a process known as vasculogenesis). Vascular endothelial growth factor-A (VEGF-A or VEGF) is believed to be responsible for most of adipose tissue's angiogenic capacity, and adipogenesis is dependent on VEGF-mediated formation of new blood vessels, (Hausman et al., 2004; Cao, 2007; Nishimura et al., 2007).

VEGF is a master regulator of both physiologic and pathologic angiogenesis. VEGF binds to two tyrosine kinase receptors-VEGFR1 and VEGFR2. VEGFR2 activation promotes endothelial cell growth, survival, and migration, and increases vascular permeability. VEGFR1 was involved in pathologic angiogenesis and the recruitment of BMDCs, including macrophages and endothelial precursor cells. Whereas VEGFR2 is primarily expressed by endothelial cells, VEGFR1 is expressed by multiple cell types, including cells of the myeloid lineage, e.g., macrophages. These have recently been recognized as significant contributors to adipose tissue composition and function, (Hattori et al., 2002, Ferrara, 2004, Shibuya 2006).

The rapid rise in the incidence of obesity has emerged as one of the most pressing global public health issues in recent years. Obesity behaves like an epidemic with escalating progress up to a fact that the number of overweight and obese people in the world overtook the number of malnourished, (Frossard et al., 2009, Demerath et al., 2009).

Obesity, and especially visceral adipose tissue accumulation, increases the risk of developing type 2 diabetes (Jensen 2008). The greater risk of type 2 diabetes in the obese can, at least partly, be explained by changes in adipose tissue function (Bastard et al. 2006; Hajer et al. 2008). The classical perception of adipocytes merely as a storage site for excess lipid has changed dramatically over the last decade. This is attributed to the discovery that adipose tissue can function as an active endocrine organ, co-regulating whole-body metabolism, (Rasouli & Kern 2008).

The obesity epidemic has been attributed to the ready availability, abundance, and over-consumption of high-energy content food. However, data from the United States Department of

Agriculture Economic Research Services as well as the obesity prevalence data from the Behavioral Risk Factor Surveillance System and the National Health and Nutrition Examination Survey at the Centers for Disease Control and Prevention showed that total calorie intake and consumption of high fructose corn syrup and intake of other major food types, including chicken, dairy fats, salad and cooking oils, and cheese did not correlate with obesity trends, (Shao and Shin, 2011).

The data indicated that underlying etiological causes of obesity, whether behavioral, environmental, genetic, or a combination of several of them have an impact on its pathogenesis with subsequent associated morbidities up, thus the present study aimed to determine serum levels of multiple parameters after induction of obesity and subsequently diabetes in rats as a trial to elucidate their role in pathogenesis of obesity.

2. Materials and Methods

Animals

The present study comprised 45 male albino rats with weight range of 250-300 grams. Rats were grouped and kept in separate animal cages, under the prevailing atmospheric conditions and maintained on a balanced diet (bread, barely, carrots, lettuce, milk) and fresh-water supply.

Grouping:

- Control group included 15 animals received no medications and kept under the same conditions as prior to start of the study.
- Obese group included 15 rats had induced obesity
- Diabetic group included 15 rats had induced NIDDM.

Induction of obesity:

Obesity was induced by feeding rats with high-fat diet (HFD) consisting of 22% fat, 48% carbohydrate and 20% protein for two weeks.

Induction of diabetes:

Type 2 diabetes mellitus (NIDDM group) was induced by feeding rats with high-fat diet (HFD) consisting of 22% fat, 48% carbohydrate and 20% protein. After two weeks, rats were injected intraperitoneally with a single dose of streptozotocin (STZ) (Sigma) in a dose of 50 mg/kg body weight dissolved in 0.2 ml of citrate buffer (pH 4.5), (Islam & Choi, 2007). On the third day of injection, the animals were checked for the presence of glucose in the urine using enzymatic test strips as STZ induces diabetes within 3 days by destroying the beta cells, (Karunanayake et al., 1975). Confirmation was done by measuring fasting blood glucose levels by taking a drop of blood from the rat-tail using a glucose-measuring device (Glucocheck). Rats had blood glucose levels of ≥200 mg/dl were considered diabetic, (Islam and Choi, 2007).

Biochemical Evaluation

Venous blood samples, withdrawn from the tail vein, were obtained at two weeks after animal collection in Control group, after induction of obesity by reaching doubling of the initial weight in obese group and after assurance of development of diabetes in diabetic group. Blood samples were divided into 2 parts:

A) The first was put in a tube containing sodium fluoride (2 mg sodium fluoride/ ml blood) to prevent glycolysis. Plasma was separated by centrifugation and used for estimation of glucose by glucose oxidase method, (Tinder, 1969).

B) The second part was allowed to clot then serum was separated by centrifugation at 3000 rpm for 10 min. Serum was removed, divided into 2 parts:
   1. The first part was used for RIA determination of serum level of insulin, (Gordon et al., 1985).
   2. The second part was placed in pyrogen-free Eppendorf tubes and stored at -80°C until ELISA assayed (within one month) for estimation of serum levels of VEGF, (Ferrara and Alitalo, 1999), IL-13, (Zurawski G and de Vries, 1994) and CRP, (Thompson et al., 1992) using Quantikine ELISA kits from R & D Systems, Inc., (Minneapolis, MN).

3. Results

Pre-induction blood glucose levels were non-significantly different in diabetic and obese groups compared to control group. However, post-induction blood glucose levels in diabetic group were significantly higher compared to its pre-induction levels to levels estimated in both other groups. Moreover, blood glucose levels estimated after induction of obesity were significantly higher compared to their pre-induction levels and to control group despite the maximal levels did not approach the minimum for the diabetic group, (Fig. 1).

Similarly, pre-induction serum insulin levels showed non-significant difference among studied groups. However, post-induction serum insulin levels were significantly higher in both obese and diabetic groups compared to their pre-induction levels and to control levels with significantly higher levels in diabetic compared to obese groups, (Fig. 2).

Pre-induction serum VEGF levels were non-significantly different in diabetic and obese groups compared to control group. However, post-induction serum VEGF levels in obese and diabetic groups were significantly higher compared to both control group and their pre-induction levels with non-significantly higher post-induction levels in diabetic compared to obese group, (Table 2, Fig. 3).

Also, pre-induction serum IL-13 and CRP levels were non-significantly different among studied groups. However, post-induction serum IL-13 and CRP levels in obese and diabetic groups were significantly higher compared to both control group and their pre-induction levels. Moreover, post-induction serum IL-13 and CRP levels in diabetic group were significantly higher compared to obese group, (Table 2, Fig. 4, 5).

Table (1): Mean (±SD) levels of blood glucose and serum insulin estimated in studied animals pre- and post-induction compared to control levels

Group	Fasting blood glucose (mg/dl)		Fasting serum insulin (µIU/ml)	
	Pre-induction	Post- induction	Pre- induction	Post- induction
Control	87±6.3 (77-100)		0.91±0.14 (0.65-1.12)	
Obese	85.8±6.7 (75-95)	96.2±11.4 (80-115)*†	0.93±0.11 (0.72-1.07)	2.43±0.96 (1.23-4.25)*†
Diabetic	85.1±8.9 (70-104)	230.3±25.6 (200-265)*†‡	0.94±0.11 (0.77-1.08)	3.6±1.67 (2.12-8.65)*†‡

Data are presented as mean±SD Pre: prior to initiation of therapy Post: at end of 6-wks therapy
*: significant difference versus control group †: significant difference versus pre levels
‡: significant difference versus obese group

Table (2): Mean (±SD) serum levels of VEGF, IL-13 and CRP estimated in studied animals pre- and post-induction compared to control levels

		Control	Obese	Diabetic
VEGS	Pre-induction	203.3±46.9 (130-270)	191±52.3 (120-280)	198.5±47.5 (125-260)
			Z=0.369, p_1>0.05	Z=0.711, p_1>0.05
	Post-induction		360.3±106.4 (200-540)	403.7±124 (230-625)
			Z=3.181, p_1=0.001	Z=3.352, p_1=0.001
			Z=3.297, p_2=0.001	Z=3.409, p_2=0.001
				Z=0.700, p_3>0.05
IL-13 (pg/ml)	Pre-induction	1.91±0.52 (1.2-2.8)	1.99±0.48 (1.25-2.72)	2.03±0.47 (1.25-2.64)
			Z=0.369, p_1>0.05	Z=0.477, p_1>0.05
	Post-induction		2.77±0.82 (1.67-4.17)	3.47±1.07 (2.15-5.12)
			Z=2.987, p_1=0.004	Z=3.124, p_1=0.002
			Z=2.102, p_2=0.036	Z=2.613, p_2=0.009
				Z=1.988, p_3=0.047
CRP	Pre-induction	0.508±0.22 (0.2-1.03)	0.52±0.19 (0.26-0.94)	0.53±0.21 (0.33-0.98)
			Z=0.798, p_1>0.05	Z=0.568, p_1>0.05
	Post-induction		3.14±1.19 (1.67-4.17)	4.13±1.04 (2.15-5.12)
			Z=3.455, p_1=0.001	Z=3.782, p_1=0.001
			Z=3.408, p_2=0.001	Z=3.421, p_2=0.001
				Z=1.988, p_3=0.047

Data are presented as mean±SD Pre: prior to initiation of therapy Post: at end of 6-wks therapy
p_1: significant difference versus control group p_2: significant difference versus pre levels
p_3: significant difference versus obese group

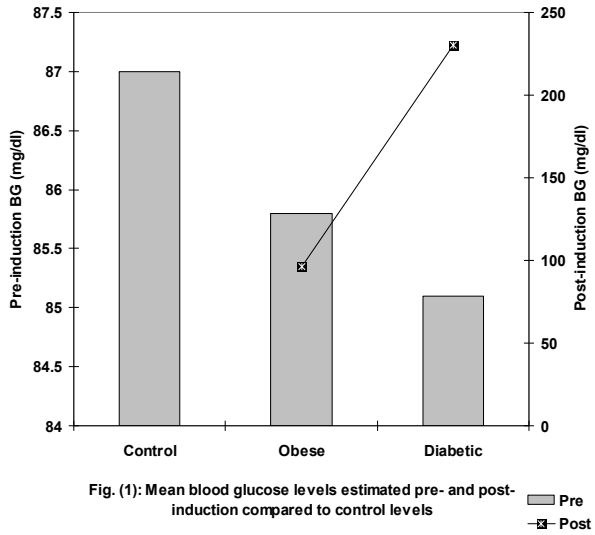

Fig. (1): Mean blood glucose levels estimated pre- and post-induction compared to control levels

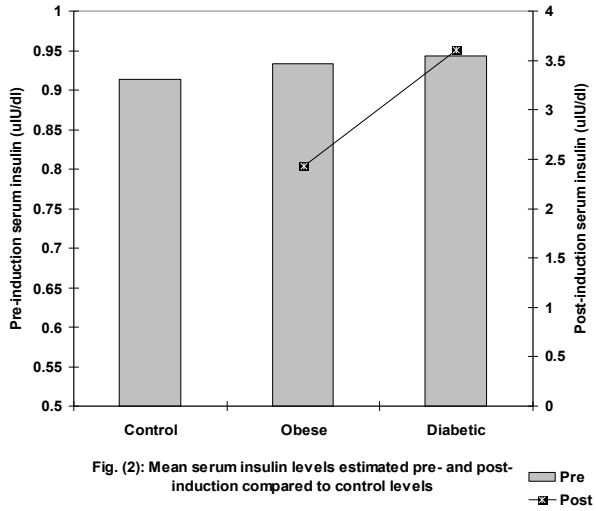

Fig. (2): Mean serum insulin levels estimated pre- and post-induction compared to control levels

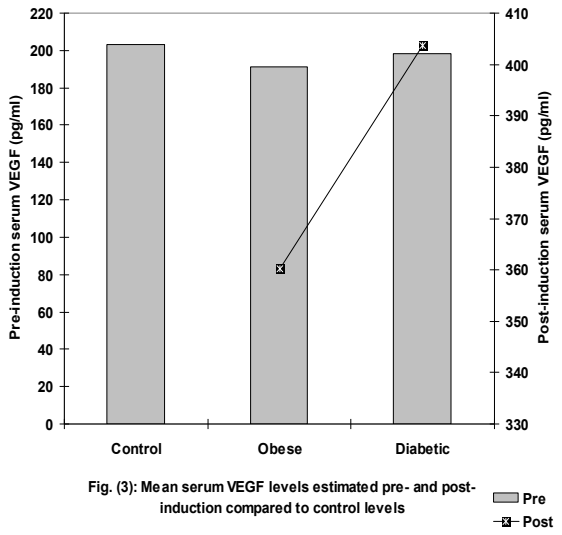

Fig. (3): Mean serum VEGF levels estimated pre- and post-induction compared to control levels

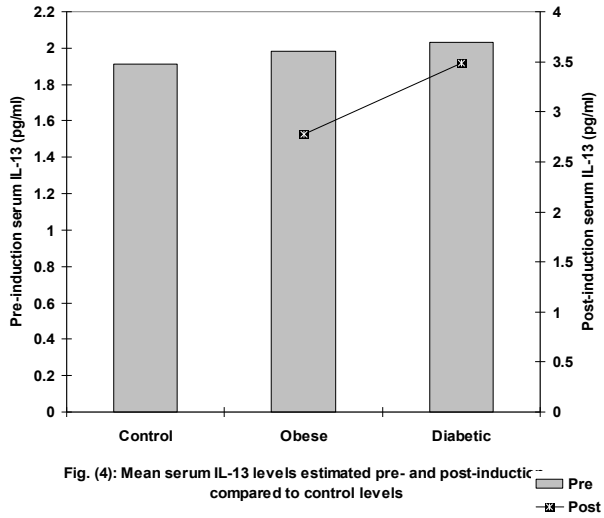

Fig. (4): Mean serum IL-13 levels estimated pre- and post-induction compared to control levels

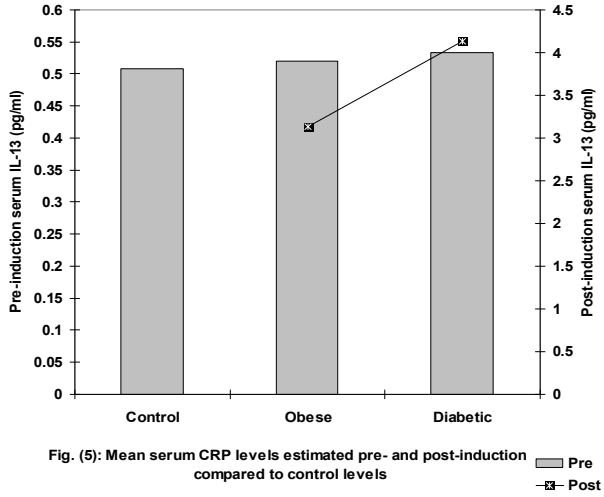

Fig. (5): Mean serum CRP levels estimated pre- and post-induction compared to control levels

4. Discussion

Induction of obesity and subsequently diabetes in studied animals induced disturbed biochemical milieu in both obese and diabetic rats. These data are represented as the non-significant difference of all studied parameters among studied groups prior to induction of obesity or diabetes. Serum VEGF levels were significantly higher in obese and diabetic groups after induction compared both to control group and to their pre-induction levels. These findings point to a fact that there was reciprocal relation between obesity and angiogenesis but it is evident that obesity initiates angiogenesis to cope with the need of adipose tissue for maintenance of vascularity of hypertrophied adipocytes and in turn neovascularization allows for initiation of hyperplasia to synthesis more adipocytes and so on.

In support of these data, Gómez-Ambrosi et al., (2010) reported that in murine model of obesity, serum VEGF-A was significantly increased after 12

weeks on a high-fat diet or in ob/ob mice and concluded that these data indicate the involvement of VEGF in the expansion of adipose tissue that takes place in obesity in relation to the need for increased vascularization, suggesting that manipulation of the VEGF system may represent a potential target for the pharmacological treatment of obesity. Loebig et al., (2010), under euglycemic conditions, found VEGF concentrations differed significantly between BMI-groups with higher concentrations in obese subjects as compared to normal weight and low weight subjects with a positive correlation between concentrations of circulating VEGF levels and BMI and concluded that this relationship may be part of some pathogenetic mechanisms underlying obesity and type 2 diabetes.

Belo et al., (2011), documented that expansion of adipose tissue in obesity is associated with angiogenesis and adipose tissue mass depends on neovascularization with VEGF is the main angiogenic factor in the adipose tissue, and its expression is tightly regulated at both transcriptional and translational levels. Moreover, Belo et al., (2011) suggested that VEGF CAG haplotypes affect susceptibility to obesity in children and adolescents

Estimated serum levels of IL-13 and CRP were significantly higher in obese and diabetic animals compared to their pre-induction levels and to control animals; these data indicating a possible relation between obesity and inflammation either as pathogenic factors or as a consequence. However, the reported significantly higher serum levels of CRP and IL-13 in diabetic compared to obese animals illustrates the impact imposed by diabetes on the immune system and goes in hand with Mohanty, (2000), who reported that glucose is proinflammatory and even a 75-g glucose load given orally to normal subjects results in profound oxidative stress and inflammatory changes at the cellular and molecular level without increased plasma glucose concentrations to the pathological range and in spite of endogenous insulin secretion.

In parallel with the obtained results Thompson et al., (2011), found CRP levels were significantly higher among individuals with a higher BMI and waist circumference and each standard deviation increase in waist circumference was associated with about 46% higher risk of elevated CRP. Ndumele et al., (2011), reported that hepatic steatosis, obesity and the metabolic syndrome were all independently associated with high high-sensitivity CRP levels.

Also, Surendar et al., (2011), reported that both Th1 and Th2 cytokines showed up-regulation in metabolic syndrome with significantly higher serum levels of IL-12, IL-4, IL-5 and IL-13 compared with obese without manifestations of metabolic syndrome and both Th1 and Th2 cytokines showed a positive significant association with fasting plasma glucose level, insulin resistance and high-sensitivity C-reactive protein, but negative association with adiponectin.

In support of the obtained results, Doupis et al., (2011), reported that VEGF was significantly higher in the obese non-diabetic subjects compared to lean non-diabetics, tumor necrosis factor-α was higher in the obese diabetic patients and C-reactive protein was higher in both the obese non-diabetic and diabetic subjects and concluded that diabetes and obesity affect equally the endothelial cell function but the smooth muscle cell function is affected only by diabetes, and these findings may be related to differences that were observed in the growth factors and inflammatory cytokines.

In trial to explore obesity-associated disturbed immune milieu, Meijer et al., (2011), concluded that human adipocytes express many cytokines/chemokines that are biologically functional and are able to induce inflammation and activate CD4+ cells independent of macrophages; this suggests that the primary event in the sequence leading to chronic inflammation in adipose tissue is metabolic dysfunction in adipocytes, followed by production of immunological mediators by these adipocytes, which is then exacerbated by activated adipose tissue macrophage, activation and recruitment of immune cells.

The obtained results and review of literature indicated that obesity and obesity-associated type-2 diabetes mellitus represent double-crossing vicious circles of obesity, disturbed immune system and promoted angiogenesis. Further studies were advocated for evaluation of the impact of weight reduction programs on such vicious circles.

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