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EXPERIMENTAL STUDY

Changes of serum angiogenic biomarkers and their correlations with serum leptin concentration

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Abstract: *Aim:* Obesity is considered as a major health problem. Angiogenic vessels by providing oxygen, nutrients and growth factors trigger growth and survival signals in adipocytes. We aimed to investigate the effect of high-fat diet (HFD) on serum angiogenic biomarkers including vascular endothelial growth factor (VEGF), soluble VEGF receptor 1 (sVEGFR1), nitric oxide (NO) concentrations and their correlations with serum leptin level in obese and control groups.
Methods: Twenty male C57BL/6 mice were randomly assigned into the control and obese groups. Obese group received HFD for 15 weeks. At the end of experiment, blood samples were collected for blood glucose, serum insulin, VEGF, sVEGFR1, NO and leptin level measurements and correlation between serum angiogenic factors and leptin levels were analyzed.
Results: HFD induced higher serum NO and leptin levels compared to the control group, while, it did not affect serum VEGF and sVEGFR1 concentrations. There was a strong positive correlation between serum leptin and NO levels (r=0.78), however, a weak correlation was found between serum leptin and VEGF and VEGFR-1 concentrations.
Conclusion: It seems that the angiogenic activities in obese mice are through the mechanisms that were not regulated by VEGF or VEGF receptors rather; other factors such as leptin and NO are involved *(Tab. 1, Fig. 4, Ref. 32)*. Text in PDF *www.elis.sk.*
Key words: obesity, angiogenesis, leptin, angiogenic factors.

Obesity, nowadays with rapid and alarming increase worldwide, is considered as a major health problem. It is a multifactorial disease where interaction of genetic predisposition and environmental factors is involved (Li et al, 2011). Obesity and overweight increase the risk of most common and severe human diseases including hypertension, cardiovascular disease, type 2 diabetes, certain types of cancer, gallstones and osteoarthritis (Shamseddeen et al, 2011). Based on statistics, the number of overweight people worldwide from 937 million in 2005 is estimated to increase to 1.35 billion in 2030 and obese individuals from 396 million to 573 million (Kelly et al, 2008).

Angiogenesis, a complex process of new blood vessel formation, is controlled by a precise balance between multiple pro and anti-angiogenic molecules (Distler et al, 2003). Angiogenesis consists of the three stages: the first, selection of some endothelial cells namely "tip cells" inside the capillary to begin angiogenic expansion. "Tip cells" have a master role while new vessels grows. These cells react to the angiogenic factor VEGF (vascular endothelial growth factor). Thus, VEGF empowers "tip cells" for invasion and migration. The third stage: maturation of newly formed vessels that in this stage consists of inhibition of endothelium proliferation and migration of new capillaries, stability of already existing new vascular tubes (fusion of the newly formed vessels with others) (Karamysheva, 2008).

Adipose tissue as a highly vascularized tissue is characterized with an unique plasticity feature for its ability in dynamic expansion or shrinkage in exposure with excess or demand of energy, thus, maintenance of systemic energy homeostasis (Rosen and MacDougald, 2006; Sethi and Vidal-Puig, 2007). During embryogenesis, adipose tissue development spatially and temporally is associated with microvessel growth (Cao, 2007). Angiogenic vessels by several mechanisms including supplying oxygen, nutrients and removing of waste products (Kamba et al, 2006), providing of growth factors and cytokines (Sun et al, 2012) and finally supplying circulating stem cells, trigger growth and survival signals in adipocytes and adipose tissue (Cao, 2010). Thus, angiogenesis is critical and rate limiting step for adipose tissue expansion (Sun et al, 2011). Interaction between endothelial cells and adipocytes cause that a dysfunction of each compartment would have a major effect on the other system (Jansson, 2007). In rapid expansion of adipose tissue, hypoxia is an important factor for the induction of angiogenesis (Bouloumie et al, 2002). Thus, adipose tissue produces HIF-1α (hypoxia inducible factor 1α) that in turn induces angiogenic factors (Trayhurn et al, 2008; Hosogai et al, 2007).

Leptin is an adipocyte derived hormone that substantially regulates food intake and energy homeostasis and also has a direct angiogenic activity (Suganami et al, 2004). The objective of present study was to evaluate the effect of high-fat diet (HFD) on some biomarkers of angiogenesis including VEGF, sVEGFR1

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(soluble VEGF receptor 1) and NO (nitric oxide) in normal and diet – induced obese mice.

Materials and methods

Animals

Twenty male mice (C57BL/6, 20–30 g, 5 weeks old) were purchased from the Pasteur Institute of Iran. All animals were housed in polypropylene cages on a 12 h light-dark cycle at 25 °C room temperature. All animals had 7 days to acclimatize to the laboratory conditions and were fed with the standard or HFD chow ad libitum during this time, and had free access to water throughout the study. The ethical committee of the Isfahan University of Medical Sciences approved all study protocol. After one week, the animals were randomly divided into the two groups: obese and control (n = 10 each).

Animal diets

For induction of diet-induced obesity, the obese group consumed HFD (labratories BioServ, Cat #F3282, USA) included 59 % fat, 27 % carbohydrate, 14 % protein) (Guo et al, 2009) for 15 weeks. The control group received the standard diet (Pasteur Institute, Iran). Body weights of the animals were monitored weekly. At the end of the nutritional period, the animals were anaesthetized. Blood samples were collected for blood glucose and serum insulin, VEGF, sVEGFR1, NO and leptin measurements.

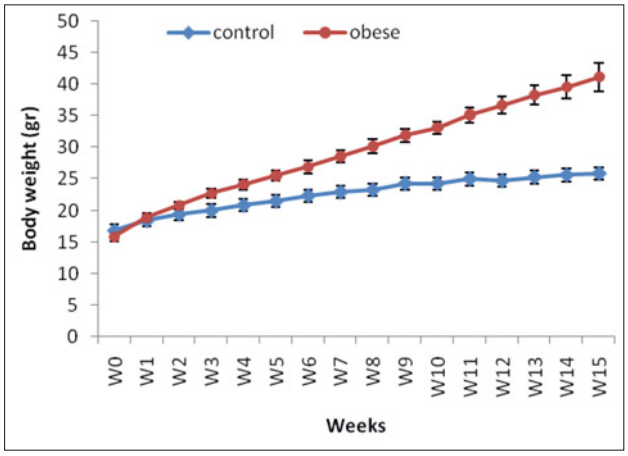

Fig. 1. Changes of body weight after 15 weeks high-fat or standard diets. * p < 0.05 compared to control.

Biochemical analysis

Blood samples were centrifuged for 30 minutes. The serums were removed and stored at –20 °C for subsequent analysis. The serum levels were measured in each case by sandwich enzyme immunoassay using specifically available kits. Mouse VEGF and sVEGFR1 ELISA kits (R&D systems, Minneapolis, MN, USA), mouse Leptin kit (Invitrogen, Camarillo, CA 93012), mouse Insulin kit (Mercodia, Uppsala, Sweden) and serum nitrite (Promega Corp, USA) were measured according to the manufacturer's instructions. Then, samples were read within the linear range of the assay and the accuracy of the analysis was confirmed by the controls provided in each assay kit.

Statistical analysis

All values are expressed as the mean ± S.E.M. The statistical software SPSS version 16 was used for data analysis. The significance of differences between groups was assessed with the Student's t–test. Correlation analysis was examined using Pearson's correlation coefficient. p value less than 0.05 was considered statistically significant.

Results

Body weight

We monitored body weight of the animals weekly and found that the animals consuming HFD had significantly increased body weight than that of the control group (Fig. 1).

Blood glucose and serum insulin

We observed that a consumption of the HFD increased blood glucose and serum insulin concentration compared to the standard diet (Tab. 1).

Serum nitrite, VEGF and sVEGR1 measurements

As shown in the figure 2A, serum nitrite levels in obese mice were significantly higher than in the control group ($p < 0.05$), while, serum VEGF and sVEGFR1 concentrations were not significant different between obese and control groups ($p > 0.05$) (Fig. 2B, C).

Serum leptin measurement

We found that serum leptin level in obese animals was significantly higher than in the control group ($p < 0.05$) (Fig. 3).

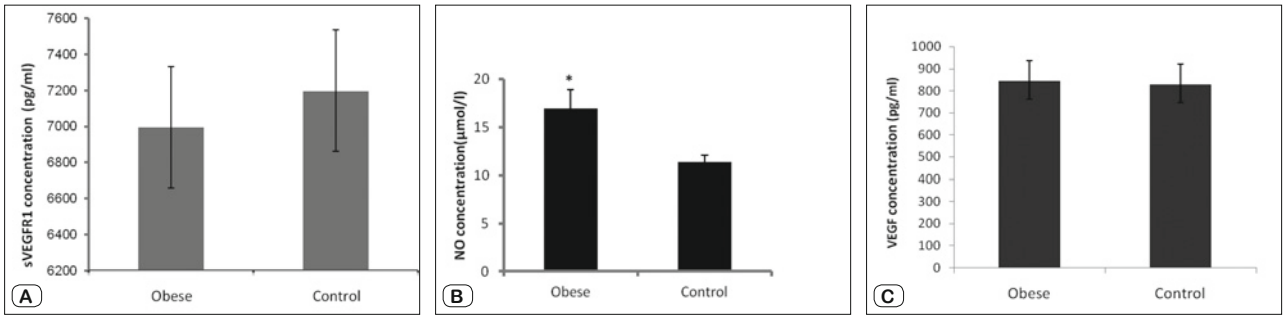

Fig. 2. Effect of high-fat or standard diet on serum NO (A), VEGF (B) and sVEGFR1 (C) concentrations. Data are expressed as the mean ± S.E.M. * p < 0.05 vs control group,

Tab. 1. Effect of high fat diet on blood glucose and serum insulin level after nutritional period of 15 weeks. Data are presented as mean ± S.E.M. *P<0.05 compare to control.

	Control	High-fat diet
Blood Glucose (mg/dl)	153.43±5.84	200.14±5.75*
Serum Insulin (µg/l)	0.1772±0.05	2.48±0.43*

Correlation analysis

To examine the correlation between the serum leptin level and serum angiogenic factors, we performed a correlation analysis and we found a strong positive correlation between serum leptin and nitrite levels ($r = 0.78$; $p < 0.05$) (Fig. 4A), while there was a weak correlation between serum VEGF and leptin concentrations ($r = –0.053$; $p > 0.05$) (Fig. 4B) and between serum sVEGFR1 and leptin concentrations ($r = 0.04$; $p > 0.05$) (Fig. 4C).

Discussion

In this study, the effect of HFD on serum angiogenic factors (VEGF, sVEGFR1 and NO) and their correlation with serum leptin was examined. Our results showed that HFD increased serum concentration of NO and leptin in obese mice compared to the normal diet group whereas it had no significant effect on serum concentration of VEGFA and sVEGFR1 in the obese and control groups.

As during embryogenesis, adipose tissue development is associated with microvessel growth, adipose tissue expansion requires a functional vascular system (Cao, 2010). Adipose vasculature provides oxygen, nutrients, growth factors and cytokines for adipocytes (Sun et al, 2012). Hypoxia in rapidly expanding adipose tissue leads to production of angiogenic factors (Trayhurn et al, 2008). VEGF is a major angiogenic factor that stimulates mostly angiogenesis through binding with VEGFR2 receptor (Christiaens and Lijnen, 2010). In our study, we demonstrated that HFD did not affect serum concentration of VEGF compared to the standard diet. A recent study on C57BL/6 mice showed that HFD did not affect plasma concentration of VEGF compared to AIN93G diet (Yan et al, 2012). Thus, possibly the angiogenic activities in obese mice are through the mechanisms that were not regulated by VEGF or VEGF receptors rather, other factors such as leptin and NO can be involved. However, other study indicated that serum VEGF level in obese mice and humans was significantly higher than that of the control group (Gomez-Ambrosi et al, 2010).

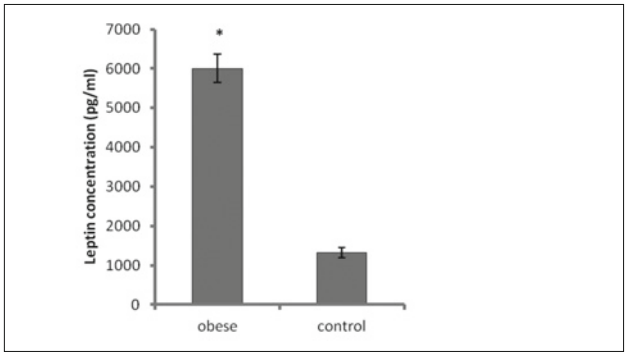

Fig. 3. Serum leptin concentration in obese and control mice. Data are expressed as the mean ± S.E.M. * p < 0.05 vs control group.

VEGF as the main regulator of angiogenesis binds to two tyrosine kinase receptors of VEGFR1 and VEGFR2. VEGFR2 is predominant effector of proangiogenic signalling, while, VEGFR1 leads to anti or proangiogenic signalling (Tam et al, 2009; Verhoef et al, 2006). sVEGFR1 is a truncated version of the cell membrane spanning VEGFR1 that inhibits angiogenic signalling through sequestration of VEGF ligands and involved in pathological angiogenesis (Wu et al, 2010). In the present study, HFD did not change serum concentration of sVEGFR1 and there was no correlation between serum leptin and sVEGFR1. However, in other study in transgenic rats for the human renin gene (hREN) as an established obesity/ metabolic syndrome, an inverse correlation between sVEGFR1 level and body weight was demonstrated (Herse et al, 2011).

In the present study, HFD increased serum nitrite level as a marker of NO production compared to the control group. Besides, the role of NO in food intake (Jahng et al, 2005) is involved in angiogenesis process and has various antiatherosclerotic actions via inhibition of leukocyte adhesion and prevention of smooth muscle proliferation and protection of vasculature (Wohlfart et al, 2008). Abnormalities in the NO cyclic GMP pathway located at the subendothelial space could be involved in an impaired vascular response and endothelial dysfunction in diabetic subjects (Maejima et al, 2001). It was demonstrated that adipose tissue surrounding blood vessels is a source of NO overproduction in HFD mice (Gil-Ortega et al, 2010). Benkhoff et al showed that HFD increased cerebral nNOS expression and cerebral and plasma nitrite levels in humans and mice (Benkhoff et al, 2012). Other study demonstrated that 19 weeks of HFD significantly increased hypothalamic NO production in mice C57BL/6 that was probably caused by nNOS expression and excessive production of NO in the hypothalamus may be involved in an insensitivity to leptin through downregulation of long form leptin receptor (LEPR–b) expression that is in line with our study (Jang et al, 2007).

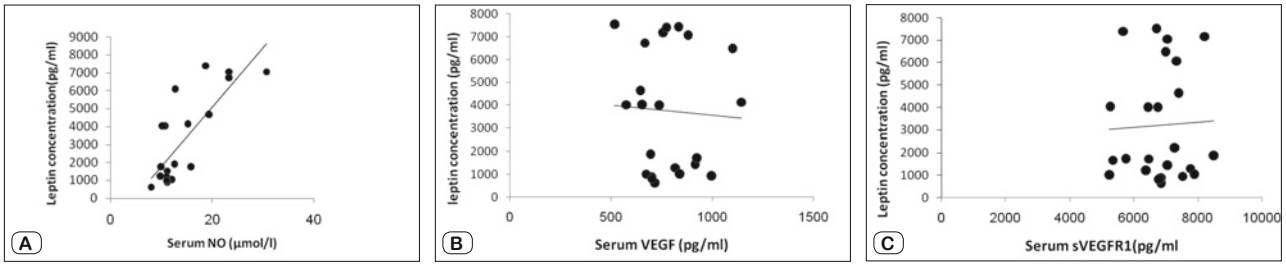

Fig. 4. Correlations between serum leptin concentrations and serum NO (r = 0.78) (A), VEGF (r = –0.053 (B) and sVEGFR1 (r = 0.04 (C) in control and obese mice.

In the present study, we also found a strong correlation between serum nitrite and leptin levels. Leptin is considered as an adipose tissue derived hormone and its level is correlated with the adipose mass, substantially regulates food intake and energy homeostasis and possess direct proangiogenic activity (Anagnostoulis et al, 2008). In this study, HFD significantly increased serum leptin levels and it seems that the angiogenic activities in obese mice are through the mechanisms that were not regulated by VEGF or VEGF receptors, rather other factors such as leptin and NO can be involved.

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