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Original Research

Ghrelin did not change coronary angiogenesis in diet-induced obese mice

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Abstract: Ghrelin is a 28 amino acids peptide that initially was recognized as an endogenous ligand for growth hormone secretagogue receptor (GHSR). Recently, a number of studies demonstrated that ghrelin is a cardiovascular hormone with a series cardiovascular effect. The main objective of this study was to investigate the effect of systemic ghrelin administration on angiogenesis in the heart and its correlation with serum leptin levels in normal and diet-induced obese mice. 24 male C57BL/6 mice were randomly divided into four groups: normal diet (ND) or control, ND+ghrelin, high-fat-diet (HFD) or obese and HFD+ghrelin (n=6/group). Obese and control groups received HFD or ND, respectively, for 14 weeks. Then, the ghrelin was injected subcutaneously 100µg/kg twice daily. After 10 days, the animals were sacrificed, blood samples were taken and the hearts were removed. The angiogenic response in the heart was assessed by immunohisochemical staining. HFD significantly increased angiogenesis in the heart expressed as the number of CD31 positive cells than standard diet. Ghrelin did not alter angiogenesis in the heart in both obese and control groups, however, it reduced serum nitric oxide (NO) and leptin levels in obese mice. There was a strong positive correlation between the number of CD31 positive cells and serum leptin concentration (r=0.74). Leptin as an angiogenic factor has a positive correlation with angiogenesis in the heart. Although systemic administration of ghrelin reduced serum leptin and NO levels in obese mice, however, it could not alter coronary angiogenesis.

Key words: Obesity; Angiogenesis; Ghrelin; Coronary.

Introduction

Nowadays, obesity is considered as an epidemic health problem (1). Obesity as a multifactorial disease is created following interaction of genetic and environmental factors. Clinical studies have demonstrated a complex and even paradoxical relationship between obesity in particular visceral adiposity and cardiovascular diseases such as heart failure, coronary heart disease and myocardial infarction (2).

Angiogenesis that is defined the formation of new blood vessels from preexisting ones, participates substantially in a series of physiological and pathological process such as wound healing, chronic inflammation and revascularization of the myocardium following myocardial infarction (3, 4). Obesity is characterized as a chronic, low grade inflammation status and recruitment of adipose tissue macrophages as an important source of pro-inflammatory cytokines plays a critical role in adipose tissue neovascularization (5). Also growing adipocytes in obesity produce numerous angiogenic factors including VEGF (vascular endothelial growth factor), FGF2 (fibroblast growth factor), resistin, leptin and etc (6). In recent years, epicardial adipose tissue located along the large coronary arteries (perivascular) and the surface of the ventricles through vascular remodeling, endothelial dysfunction and inflammation mechanisms besides abdominal adipose tissue has been addressed in cardiometabolic complications including atherosclerosis in obesity (7-9).

Ghrelin an endogenous ligand of the GHSR (growth hormone secretagogue receptor) mainly is released from the stomach (10). Although, ghrelin participate in food intake and energy homeostasis, recently, a number of studies demonstrated that it is a cardiovascular hormone (11) with series cardiovascular effects consist of promoting of vascular endothelial function through inhibition of proinflammatory interleukins (12), counteract against atherosclerosis lesions (13) and increase in LV (left ventricular) ejection fraction in CHF (chronic heart failure) patients (14).

The main objective of this study was to investigate the effect of systemic ghrelin administration on the angiogenic response in the heart and its correlation with serum leptin levels in normal and diet-induced obese mice.

Materials and Methods

Animals and experimental groups

Twenty four male mice (C57BL/6, 20-30g, 5 weeks old) were purchased from Pasteur Institute of Iran. All animals were maintained on a regular light-dark cycle (12 h light, 12 h dark) at 25˚C room temperature. The animals had seven days to acclimatize to the laboratory conditions and kept on standard or high-fat diet (HFD) chow ad libitum during this time and had free access to water throughout the study. After one week, the animals were randomly divided into four groups: normal diet (ND) or control, ND+ghrelin, HFD or obese and

HFD+ghrelin (n=6 each). The ethical committee of the Isfahan University of Medical Sciences approved all study protocol.

Animal diets and ghrelin administration

To obtain the diet-induced obesity model, the obese groups consumed HFD (labratories BioServ, Cat #F3282, USA) included (59% fat, 27% carbohydrate, 14% protein) for 14 weeks (15). Control groups received standard diet (Pasteur Institute, Iran). Body weights of the animals were monitored weekly. After 14 weeks, the mice were randomized to treatment with ghrelin (S.C; twice daily, at the dose of 100 µg/kg, Tocris Co. Bristol, UK) for 10 days (16) or did not treat.

Immunohistochemistry

After 10 days, the animals were scarified. The hearts were excised and fixed in 10% formalin solution and then embedded in molten paraffin. Four micrometer sections were cut and incubated with primary antibody (rabbit anti-mouse CD31; 1:50; Abcam Co.) to identify endothelial cells. The angiogenic response was expressed as the numbers of CD31-positive cells which were counted using an Olympus light microscope at ×40 magnification in five different fields for each heart and reported as a number of CD31 positive cells per field (17).

Biochemical analysis

The serum levels of NO (nitric oxide) and leptin were quantified using ELISA kits (serum nitrite:Promega Corp, USA and mouse Leptin kit: Invitrogen, Camarillo, CA 93012) according to the manufacturer's instructions. Then, the 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 mean ± S.E.M. The statistical software SPSS version 16 was used for data analysis. The significance of differences between groups was assessed with one-way ANOVA. Correlation analysis was examined using pearson's correlation coefficient. A P value less than 0.05 was considered statistically significant.

Results

Body weight

The animals on the HFD had higher body weight than that control group fed with ND ($p<0.05$) (Fig.1).

Angiogenic response in the heart: effect of ghrelin

CD31 is an endothelial surface antigen and indicator of capillary density. Thus, CD31 positive cells stained by immunohistochemistry demonstrate endothelial cells and we can estimate angiogenesis process in tissue (16). Analysis of angiogenesis in the hearts demonstrated that the number of CD31 positive cells in obese mice were significantly higher in comparison with control group ($p<0.05$) (Fig.2E). Ghrelin administration could not alter the number of CD31 positive cells in both obese mice and control groups ($p>0.05$). Samples of immunohistochemical staining were presented in Fig2 A-D.

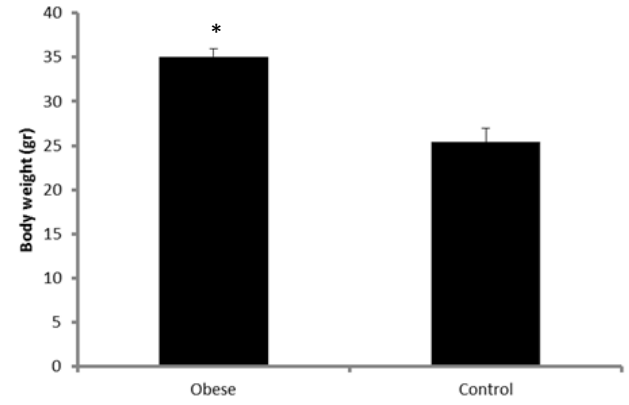

Figure 1. Body weight in obese and control groups after 15 weeks of receiving diets. Values are expressed as Mean± SEM; *:p <0.05 compare to control.

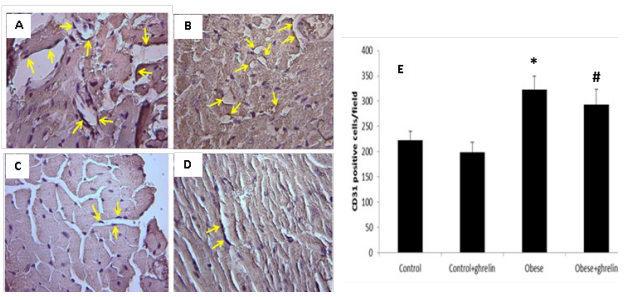

Figure 2. Effect of HFD and ghrelin on the number of CD31 positive cells in the heart. The histological sections were stained with immunohistochemical technique. A: obese+ghrelin; B: obese; C: control+ghrelin; D: control. HFD loading significantly increased the number of CD31 positive cells than normal diet and ghrelin administration did not change the number of CD31 positive cells in obese and control groups; *:$p<0.05$ compared to control and control+ghrelin; #: $p<0.05$ compared to control+ghrelin (E). Arrows indicate the CD31 positive cells. Data are shown as mean ± SEM. (n=6 each group).

Effect of ghrelin on serum biomarkers of angiogenesis

HFD produced higher serum leptin and NO levels in experimental mice compare to control animals (Fig3A,B) ($p<0.05$). Ghrelin administration reduced serum NO and leptin levels in obese mice ($p<0.05$) without significantly changes of those factor in control group (Fig3C).

Correlation analysis

Scatter plots in Figure 4 presents a strong positive correlation between the number of CD31 positive cells in the heart and serum leptin levels ($r=0.74$; $p<0.05$).

Discussion

This study was examined the effect of ghrelin treatment on angiogenic response in the heart and its correlation with serum leptin levels in normal and diet-induced obese mice.

In the present study, we demonstrated that HFD increased the number of CD31 positive cells in the heart compare to ND group. A study on Otsuka Long Evans Tokushima Fatty (OLETF) and control Long Evans Tokushima (LETO) rats demonstrated that left ventricular subendocardial total capillary density in

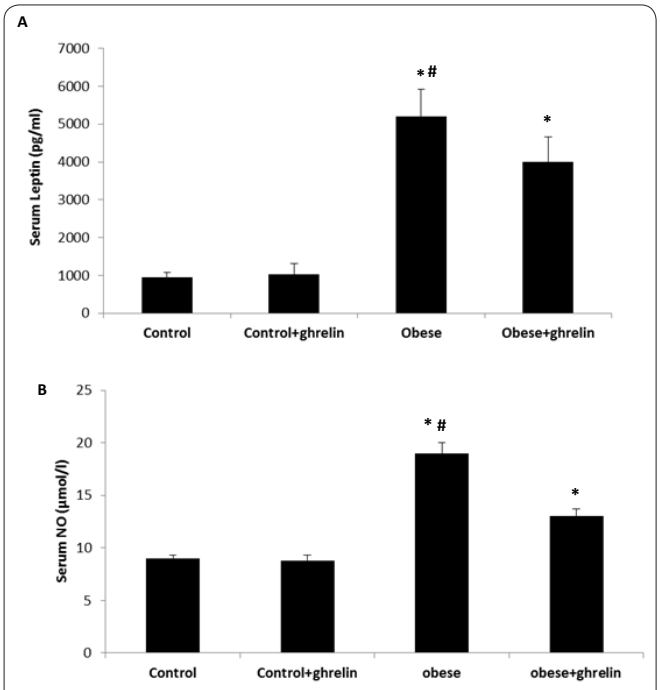

Figure 3. Serum leptin (A) and NO (B) concentrations in obese and control groups. Ghrelin administration reduced serum NO and leptin levels in obese mice. Data are shown as mean ± SEM. *: P<0.05 compare to control groups; #: p<0.05 compare to obese+ghrelin.

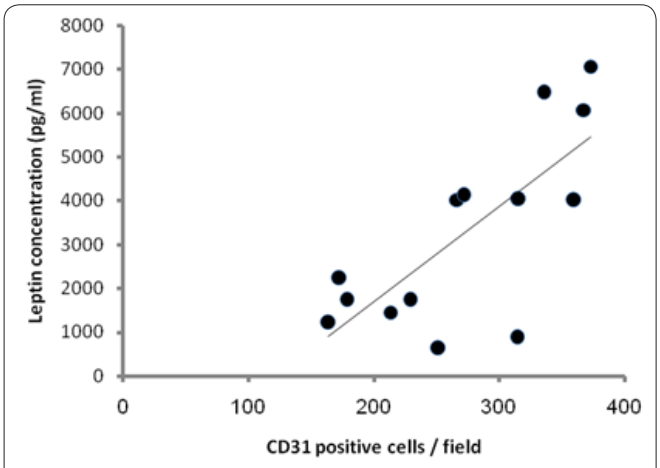

Figure 1. Correlation between the number of CD31 positive cells/field and serum leptin concentration (r=0.74).

OLETF rats was significantly higher than that in LETO at 20 weeks (18). Possibly, a capillary network remodeling for compensatory improvement to efficiently O_2 transport in a setting of HFD and obesity can be involve in angiogenic response. In contrast, in another study on male mice that were fed with HFD or control diet for 14 weeks showed that the circulating number and function of EPC_s (endothelial progenitor cells) as well as heart function were markedly decreased compare to that in normal mice (19) which could involve in angiogenesis process.

We also demonstrated that serum leptin and NO concentrations were significantly increased in obese mice than that control group. Leptin is an adipocyte derived hormone that its serum levels are correlated with body mass index (20). NO is a free radical and one of the important regulators of angiogenesis that is derived from L-arginine by NO synthase (NOS) (21). Studies have shown that HFD leads to hyperleptinemia and leptin resistance (22). In addition, leptin can activate directly NO production (23). Increased NO through vasodilatation effect during obesity state can be a protective mechanism against deleterious outcome of HFD on the vascular system.

We also showed that ghrelin administration could not alter angiogenic response in obese and control groups, while, ghrelin treatment reduced serum NO and leptin levels in obese mice. Ghrelin is an acylated 28 amino acid peptide that is produced mainly in the gastric oxyntic mucosa in the X/A cells (10, 11). It substantially regulates appetite, energy and body weight and newly has been recognized as a cardiovascular hormone (11, 24). A study demonstrated that acute administration of ghrelin in post-MI rats compared with saline controls, substantially decreased LV end diastolic pressure and LV enlargement (25). A few studies have been done on the effect of ghrelin on coronary angiogenesis. Recently Yuan MJ et al. revealed that in myocardial infarction (MI) model in rats, chronic ghrelin administration after MI, increased angiogenesis through increment of VEGF expression and inhibition of apoptosis (26). Other study demonstrated that ghrelin treatment in MI model in rats reduced post MI remodeling through inhibition of the inflammatory response and MMP_s (matrix metalloproteinase)(27). Obesity is associated with a low grade systemic inflammation condition and a number studies have shown that ghrelin in congestive heart failure and MI models exert an anti-inflammatory effect through the decrease in expression and production of inflammatory cytokines (28), whereas, leptin has proinflammatory effects to increase of a number of inflammatory cytokines. Dixit VD et al. exhibited a reciprocal regulatory network between ghrelin and leptin in a setting of inflammation and immune cell activation (29). Thus ghrelin and leptin have mutually antagonistic effects on inflammatory cytokine expression in obesity and could involve in angiogenic response.

In correlation analysis we found a strong positive correlation between the number of CD31 positive cells and serum leptin levels. Leptin involves in cardiovascular complications including hypertension, diabetes, coronary heart disease and stroke (30). However, a community based framingham heart study has reported the cardioprotective effect of leptin on left ventricle remodeling (31). On the other hand, leptin mostly regulates food intake and energy homeostasis and interestingly is considered as a potent angiogenic factor and has the mitogenic effect (32). Thus, it seems that leptin effects on cardiovascular system are intricate and paradoxical. Possibly, the higher serum leptin concentration in obese groups is responsible for increased capillary density in the hearts of obese group, although the functional or non-functional of these capillaries needs more studies.

In conclusion, HFD increased coronary angiogenesis, serum leptin and NO concentrations. Ghrelin although reduced serum leptin and NO levels in obese animals, however, could not change coronary angiogenesis.

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References

1. Shamseddeen H, Getty JZ, Hamdallah IN, Ali MR. Epidemiology and economic impact of obesity and type 2 diabetes. The Surgical clinics of North America 2011;91:1163-72, vii.
2. Gropler RJ. The road connecting obesity and coronary vasomotor function: straight line or U-turn? JACC Cardiovascular imaging 2012;5:816-8.
3. Carmeliet P. Angiogenesis in health and disease. Nature medicine 2003;9:653-60.
4. Wang L, Chen Q, Li G, Ke D. Ghrelin stimulates angiogenesis via GHSR1a-dependent MEK/ERK and PI3K/Akt signal pathways in rat cardiac microvascular endothelial cells. Peptides 2012;33:92-100.
5. Tahergorabi Z , Khazaei M. The relationship between inflammatory markers, angiogenesis, and obesity. ARYA Atheroscler. 2013;9(4):247-53.
6. Voros G, Maquoi E, Demeulemeester D, Clerx N, Collen D, Lijnen HR. Modulation of angiogenesis during adipose tissue development in murine models of obesity. Endocrinology 2005;146:4545-54.
7. Ding J, Hsu FC, Harris TB, Liu Y, Kritchevsky SB, Szklo M, et al. The association of pericardial fat with incident coronary heart disease: the Multi-Ethnic Study of Atherosclerosis (MESA). The American journal of clinical nutrition 2009;90:499-504.
8. Mahabadi AA, Massaro JM, Rosito GA, Levy D, Murabito JM, Wolf PA, et al. Association of pericardial fat, intrathoracic fat, and visceral abdominal fat with cardiovascular disease burden: the Framingham Heart Study. European heart journal 2009;30:850-6.
9. Payne GA, Kohr MC, Tune JD. Epicardial perivascular adipose tissue as a therapeutic target in obesity-related coronary artery disease. British journal of pharmacology 2012;165:659-69.
10. Tahergorabi Z , Rashidi B , Khazaei M . Ghrelin Does not Alter Aortic Intima-Media Thickness and Adipose Tissue Characteristics in Control and Obese Mice. Iran J Basic Med Sci. 2013; 16(8): 942–945.
11. Zhang G, Yin X, Qi Y, Pendyala L, Chen J, Hou D, et al. Ghrelin and cardiovascular diseases. Current cardiology reviews 2010;6:62-70.
12. Cheyuo C, Wu R, Zhou M, Jacob A, Coppa G, Wang P. Ghrelin suppresses inflammation and neuronal nitric oxide synthase in focal cerebral ischemia via the vagus nerve. Shock 2011;35:258-65.
13. Kadoglou NP, Lampropoulos S, Kapelouzou A, Gkontopoulos A, Theofilogiannakos EK, Fotiadis G, Kottas G. Serum levels of apelin and ghrelin in patients with acute coronary syndromes and established coronary artery disease—KOZANI STUDY. Translational Research. 2010 May 31;155(5):238-46.
14. Nagaya N, Moriya J, Yasumura Y, Uematsu M, Ono F, Shimizu W, et al. Effects of ghrelin administration on left ventricular function, exercise capacity, and muscle wasting in patients with chronic heart failure. Circulation 2004;110:3674-9.
15. Peyot ML, Pepin E, Lamontagne J, Latour MG, Zarrouki B, Lussier R, et al. Beta-cell failure in diet-induced obese mice stratified according to body weight gain: secretory dysfunction and altered islet lipid metabolism without steatosis or reduced beta-cell mass. Diabetes 2010;59:2178-87.
16.Tahergorabi Z, Khazaei M, Rashidi B. Systemic administration of ghrelin did not restore angiogenesis in hindlimb ischemia in control and diet-induced obese mice. Bratisl Lek Listy. 2015;116(1):35-40.
17. Peng X, Wang J, Lassance-Soares RM, Najafi AH, Sood S, Aghili N, et al. Gender differences affect blood flow recovery in a mouse model of hindlimb ischemia. American journal of physiology Heart and circulatory physiology 2011;300:H2027-34.
18. Campbell DJ, Somaratne JB, Prior DL, Yii M, Kenny JF, Newcomb AE, Kelly DJ, Black MJ. Obesity is associated with lower coronary microvascular density. PLoS One. 2013;8(11):e81798.
19. Tsai TH, Chai HT, Sun CK, Yen CH, Leu S, Chen YL, et al. Obesity suppresses circulating level and function of endothelial progenitor cells and heart function. Journal of translational medicine 2012;10:137.
20. Hou N, Luo JD. Leptin and cardiovascular diseases. Clinical and experimental pharmacology & physiology 2011;38:905-13.
21. Nematollahi S, Nematbakhsh M, Haghjooyjavanmard S, Khazaei M, Salehi M. Inducible nitric oxide synthase modulates angiogenesis in ischemic hindlimb of rat. Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia 2009;153:125-9.
22. Dyck DJ. Leptin sensitivity in skeletal muscle is modulated by diet and exercise. Exercise and sport sciences reviews 2005;33:189-94.
23. Tahergorabi Z, Khazaei M. Leptin and its cardiovascular effects: Focus on angiogenesis. Adv Biomed Res. 2015 May 6;4:79.
24. Tesauro M, Schinzari F, Caramanti M, Lauro R, Cardillo C. Cardiovascular and metabolic effects of ghrelin. Current diabetes reviews 2010;6:228-35.
25. Soeki T, Kishimoto I, Schwenke DO, Tokudome T, Horio T, Yoshida M, et al. Ghrelin suppresses cardiac sympathetic activity and prevents early left ventricular remodeling in rats with myocardial infarction. American journal of physiology Heart and circulatory physiology 2008;294:H426-32.
26. Yuan MJ, He H, Hu HY, Li Q, Hong J, Huang CX. Myocardial angiogenesis after chronic ghrelin treatment in a rat myocardial infarction model. Regulatory peptides 2012;179:39-42.
27. Huang CX, Yuan MJ, Huang H, Wu G, Liu Y, Yu SB, et al. Ghrelin inhibits post-infarct myocardial remodeling and improves cardiac function through anti-inflammation effect. Peptides 2009;30:2286-91.
28. Li WG, Gavrila D, Liu X, Wang L, Gunnlaugsson S, Stoll LL, et al. Ghrelin inhibits proinflammatory responses and nuclear factor-kappaB activation in human endothelial cells. Circulation 2004;109:2221-6.
29. Dixit VD, Schaffer EM, Pyle RS, Collins GD, Sakthivel SK, Palaniappan R, et al. Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells. The Journal of clinical investigation 2004;114:57-66.
30. Liu J, Butler KR, Buxbaum SG, Sung JH, Campbell BW, Taylor HA. Leptinemia and its association with stroke and coronary heart disease in the Jackson Heart Study. Clinical endocrinology 2010;72:32-7.
31. Lieb W, Sullivan LM, Aragam J, Harris TB, Roubenoff R, Benjamin EJ, et al. Relation of serum leptin with cardiac mass and left atrial dimension in individuals >70 years of age. The American journal of cardiology 2009;104:602-5.
32. Cao Y. Angiogenesis modulates adipogenesis and obesity. The Journal of clinical investigation 2007;117:2362-8.