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Circulating Level of Soluble Leptin Receptor and its Association with Cardiometabolic Risk Factors among Obese Subjects in Kerala, South India

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Authors' contributions

This work was carried out in collaboration among all authors. Authors KTS and KSM made substantial contributions to the conception and design of the study. Authors KSM, KBL and LV managed the data collection and the literature searches. Author KSM executed the experiment. All authors participated in drafting the article. All authors read and approved the final manuscript.

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ABSTRACT

Background and Aims: Leptin, the peptide hormone secreted mainly by adipose tissue is reported to play the central role in the pathogenesis of obesity. Leptin exerts its biological effects through specific receptor molecules present in target tissues. Among the different isoforms of leptin receptor, the Soluble Leptin Receptor (SLR) is the major leptin binding protein seen in circulation which modulates the bioavailability of leptin. Our objectives were to analyse the level of circulating SLR among obese subjects and its association with biomarkers of obesity, serum leptin, insulin and cardiometabolic risk factors in comparison with healthy age and sex matched control subjects.
Methods: About 173 study participants of both genders were selected and grouped as case (n=102) and control (n=71) with a cut off point of BMI 25kg/m^2. Waist to hip ratio (WHR) and body

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fat percentage (BF%) were calculated from anthropometric measurements. Leptin, insulin, soluble leptin receptor were estimated in fasting blood samples by sandwich ELISA method. Fasting plasma glucose and lipid profile were measured by standard enzymatic methods in autoanalyzer. Homeostasis Model Assessment of Insulin resistance (HOMA-IR) was calculated. Comparison between groups was done by independent sample 't' test. *P* values <.05 were considered statistically significant.

Results: The SLR level was found to be increased in obese group in comparison with control group(*P* =.001). A significant increase in serum leptin and insulin level was observed in obese group when compared to control (*P* =.001). Obese group showed more than two fold increase in insulin resistance expressed as HOMA-IR when compared to control subjects (*P* =.001). But no significant difference in the synthesis of insulin expressed as HOMA-beta between the groups. No significant difference in serum lipoprotein levels was observed between the two groups.

Conclusion: Increased level of circulating soluble leptin receptor has been observed in obese subjects in comparison with control subjects and is associated with hyperleptinemia, hypertension and insulin resistance.

Keywords: Soluble leptin receptor; leptin; insulin resistance; obesity; Kerala.

1. INTRODUCTION

Obesity is a major cause accountable for the increased incidence of diabetes, hypertension, dyslipidemia, metabolic syndrome, cardiovascular diseases etc. among the population in Kerala [1,2]. Leptin, the peptide hormone secreted by the adipose tissue in response to body fat has been reported to be elevated in obesity [3,4]. Leptin exerts it's physiological functions by binding with specific receptors in target tissues. Leptin receptor is a single transmembrane domain receptor which displays a structural similarity to class 1 cytokine receptor family [5]. The Leptin receptor is produced in several alternatively spliced forms designated as LEPRa –LEPRf, that share the common extracellular domain with more than 800 amino acids and the transmembrane domain with 34 amino acids, and have a variable intracellular domain characteristic of each of the isoforms [6]. There are three classes of leptin receptor isoforms such as short, long and secreted forms. The 'short forms' of LEPR may have roles in binding (LEPRe), transport (LEPRa), and clearance (LEPRc and LEPRd) of leptin [7] whereas only the 'long form' (LEPRb) encodes all protein motifs capable of activating the janus kinase – signal transduction and activation (JAK-STAT) pathway which in turn stimulates transcription of target genes [8]. The 'secreted forms' are formed either by the alternative splicing of mRNA species or by the proteolytic cleavage of membrane bound forms of leptin receptor. The secreted forms of leptin receptor (Ob-Re) consists entirely of the extracellular ligand-binding domain and it lacks the transmembrane residues and intracellular domain responsible for signal transduction. The extracellular domain binds the circulating leptin, thereby regulating the concentration of free leptin and is known as soluble leptin receptor (SLR) [9]. SLR is the cleaved portion of the extracellular domain of the membrane bound leptin receptor and acts as the main binding protein for leptin in human blood and inhibits leptin transfer thereby modulating the bioavailability of leptin [10].

To exert it's action, leptin must reach the brain through blood brain barrier by binding with specific leptin receptor. The increase in adiposity increases the serum leptin levels which can lead to the development of resistance at the blood brain barrier due to impaired transport of leptin across the blood-brain barrier in obesity [11]. The mechanisms involved in this effect remains unknown. Less amount of leptin reaching the brain may lead to reduced activation of the signalling pathway for body weight regulation. Studies reported that reduced brain access may be the source of leptin resistance in obesity and further increase in body weight [12,13] According to certain studies, the high levels of circulating leptin could activate the mechanisms of desensitization and downregulation, causing the degradation of leptin receptors [14]. Increased levels of SLR point out regulation of leptin signalling, but the exact mechanism of SLR formation is not yet completely elucidated. However it has been proposed that increased leptin levels, cellular stress and inflammation and reduced cellular expression of leptin receptors may in part mediate serum SLR levels. Free Leptin Index (FLI), the ratio of total leptin and SLR concentrations is the biomarker of leptin

resistance and the status of leptin action. Research in rodent models reported that the SLR modulates serum leptin levels by delaying its clearance thereby determines the amount of free as well as bound leptin levels in serum [15]. In this background, the present study was designed to determine the level of serum soluble leptin receptor among obese subjects and to compare with that in age and sex matched control subjects. Adiposity measures, cardiometabolic risk biomarkers and insulin resistance was also analysed. We also measured the serum leptin level in order to analyse role of SLR in the regulation of serum leptin level.

2. MATERIALS AND METHODS

The study participants were selected from the obesity clinic Govt. Medical College, Trivandrum and also from the outpatient units of Sree Gokulam Medical College and Research Foundation, Trivandrum. Obese individuals with a BMI≥25, with no history of any prior medication were selected as cases. Healthy subjects with BMI≤24.9 were selected as controls. Subjects with a history of drug intake affecting the study variables (BMI, blood glucose, insulin, leptin, lipid profile etc.), those with thyroid diseases, polycystic ovarian diseases (PCOD), pregnant and post-partum women and those taking oral contraceptive pills were excluded from the study. Subjects under the age of 20years, pregnant and post-partum women, those with polycystic ovarian diseases (PCOD) and thyroid diseases and those taking oral contraceptive pills were excluded from the study.

A detailed informed consent was obtained from each subject and the data were confidential. Sampling was done after getting ethical approval from both institutions. All participants answered a life style questionnaire. Information about age, gender, race, socioeconomic factors, lifestyle habits and family history of diseases was gathered from questionnaire. Body height was recorded with the subjects wearing light clothes and no shoes. Body height measured to the nearest 0.1cm with a fixed stadiometer. Body weight determined to the nearest 0.1kg in the fasting state using a standard weighing machine. BMI was calculated using the standard formula. Waist and hip circumferences measured with a fibre reinforced plastic tape to the nearest 0.1cm at the level of umbilicus (L4-L5) and the maximum extension of the buttocks respectively at the end of expiration with the subject upright and his/her hands by the side. Body fat (%) was calculated by using anthropometric measurements [16]. Blood pressure was measured with a mercury column sphygmomanometer on the right arm of the subjects in relaxed sitting position after 10minutes rest.

Blood samples were collected by venepuncture into sterile vacutainer tubes with/without EDTA. Blood glucose and lipid profile was done on the same day of sample collection in 'Siemens Dimension' fully autoanalyzer using Flex reagent cartridges. The serum and plasma were separated as aliquots of 1ml and stored in a deep freezer at -80^{0}C for future analysis of leptin (DRG Cat No:191001100), soluble leptin receptor (BioVendor Cat No:RD194002100) and insulin (DRG Cat No:EIA 2935) by ELISA sandwich method according to the respective kit's instructions. Free Leptin Index (FLI) is calculated as the ratio of serum total leptin to SLR. Homeostasis model assessment (HOMA-IR) of insulin resistance derives an estimate of insulin resistance from fasting glucose and insulin concentrations [17]. Homeostasis model assessment (HOMA- β) of insulin secretion by beta cells of pancreas was calculated using the standard formula(19,20). Insulin sensitivity expressed as Quick Insulin sensitivity Check Index (QUICKI) was calculated as previously defined from fasting glucose and insulin values [18].

The data were analysed using Statistical Package for Social Sciences (SPSS) for Windows version 17.0 (SPSS Inc, Chicago, IL, USA). Study variables were expressed as mean ± SEM and the significance of study variables between groups was tested by unpaired Student's t test. The statistical significance was defined by P <0.05.

3. RESULTS

The mean BMI of obese group was 29.2 kg/m^2 while the control group had the mean BMI of 21.45kg/m^2. About 51% of each group were males and 49% females. There was no significant difference in age between the groups (P =.50). Statistically significant increase (P=.001) in adiposity measures such as weight, BMI, waist and hip circumferences and body fat was observed in obese group compared to control (Fig. 1). The mean waist circumference of obese group was 100.3cm and that of control group was 82.4cm. The average height was more in control group and the difference in height

was not statistically significant between the two groups (*P* =.50). The average body fat percentage in obese group was 37.5 while that of control group was 26.6. The increase in both systolic and diastolic blood pressure in obese group was found to be statistically significant in comparison with control group(*P* =.001).

Fasting Plasma Glucose and insulin levels showed a statistically significant increase (*P* =001) in obese group compared to control (Table1). Obese group showed more than two fold increase in HOMA-IR (*P* =.001) along with a significant increase in serum insulin level (*P* =.001) than control. But there was no statistically significant difference in the synthesis of insulin expressed as HOMA-β between the groups. The insulin sensitivity expressed as Quick Insulin Sensitivity Check Index (QUICKI) decreased significantly in obese group (0.29±0.003) in comparison with control (0.33 ± 0.005) (*P* =.001). There was no statistically significant difference in serum lipoprotein levels between the groups.

The mean leptin level of obese group was 60.9ng/ml with a significant difference from control group of 13.2ng/ml. The concentration of serum SLR in obese group (24.26 ± 0.98, mean± SEM) showed a statistically significant increase in comparison with that in control group (18.84 ± 0.46). The mean FLI showed approximately four fold increase in obese group when compared to control group (*P* =.02).

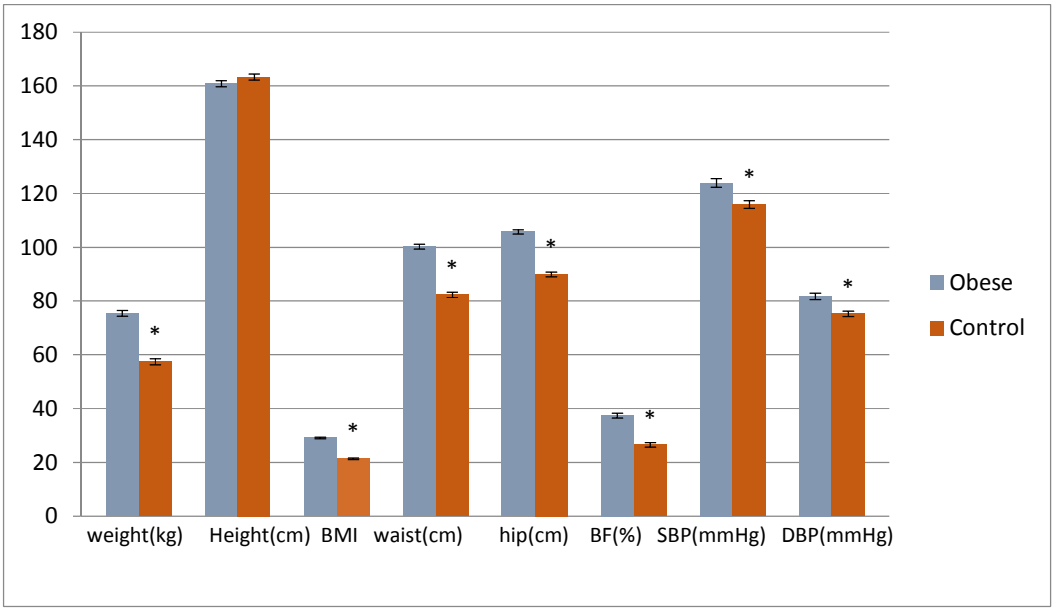

Fig. 1. Anthropometric measurements and blood pressure of study subjects
*BF% : body fat percentage SBP : systolic blood pressure DBP: diastolic blood pressure. * P =.001*

Table 1. Fasting plasma glucose, markers of insulin resistance and lipid profile in study groups

Study variable	Control(n=71)	Obese (n=101)	't' value	*P* value
Glucose (mg/dl)	90.7 ± 2.6	105.6 ± 3.09	3.81	.001
Insulin (µIU/ml)	15.13 ± 1.95	36.76 ± 2.27	7.51	.001
HOMA-IR	3.5 ± 0.4	9.82 ± 0.8	6.23	.001
HOMA- β (%)	348.3 ± 63.9	399.1 ± 29.0	0.21	.50
QUICKI	0.33 ± 0.005	0.29 ± 0.003	4.7	.001
Total cholesterol(mg/dl)	211.6 ± 5.1	209.5 ± 3.7	0.37	.50
Triglycerides (mg/dl)	107.7 ± 7.4	120.6 ± 7.0	0.11	.50
HDL cholesterol (mg/dl)	52.95 ± 1.6	48.6 ± 1.2	0.01	.50
LDL cholesterol (mg/dl)	137.3 ± 4.9	135.4 ± 3.5	0.37	.50

values are expressed as mean ±SEM. P<.05 considered statistically significant

Table 2. Serum leptin, SLR and FLI in the study groups

Study variable	Control n = (71)	Obese (n=101)	't' value	*P* value
Leptin(ng/ml)	13.2 ± 2.07	60.6 ± 4.41	12.0	.001
SLR(ng/ml)	18.84 ± 0.46	24.26 ± 0.98	9.58	.001
FLI	0.64 ± 0.12	2.98 ± 0.28	2.16	.02

values are expressed as mean ±SEM. P<.05 considered statistically significant

4. DISCUSSION

In our study population the level of serum soluble leptin receptor (SLR) was found to be increased in the obese group when compared to age and sex matched control group. This was consistent with previous findings [19,20,21]. Contradictory finding was also accounted reporting the decreased levels of SLR associated with obesity, but this study mentioned that increased SLR levels may heighten the physiological actions of leptin in lean subjects more than in obese subjects [22]. Devos *et al*. reported that complexes of leptin with SLR reflect a molecular ratio of 1:1 [23]. Circulating soluble leptin receptor when present in two fold or greater, suppresses leptin actions *in vitro* and *in vivo* [24]. This study indicated that the increased levels of SLR may be one cause for at least partial leptin resistance.

Many studies consider hyperleptinemia as a key marker of leptin resistance [24,25]. We confirmed these findings in our study population as increased levels of serum leptin in obese group associated with increased body fat, elevated levels of SLR and insulin resistance in comparison with control group. Enriori et al. reported that hyperleptinemia associated with obesity is a sign of resistance to leptin's weight reducing effect [26].

Montez et al. reported that leptin resistance is an important phenomenon as it causes further dysregulation of metabolic and inflammatory pathways, worsening insulin resistance, ultimately leads to progressive organ injury in obese patients with metabolic syndrome [27]. FLI was found to be increased in obese group in our study population in comparison with control subjects indicating leptin resistance. This may be due to hyperleptinemia associated with decreased cellular leptin receptor expression along with increased levels of SLR. Different mechanisms were implicated in leptin resistance such as saturation of leptin transport across the blood brain barrier, inhibition of leptin receptor activation and signal transduction, increased leptin receptor degradation etc. [28,29]. Leptin receptors located mainly in the hypothalamus play a major role in leptin signal transduction and a defect in either leptin or leptin receptor may contribute to the development of obesity [30]. The mutation in leptin receptor gene (*LEPR*) causes splicing abnormality that resulted in truncated receptor, aberrant signal transduction, leptin resistance and obesity.

Lipotoxicity and apoptosis increase the rate of Ob-R cleavage through different mechanisms. According to Michael Schaab *et al.* high leptin levels and ER stress cause decreased SLR levels. While increased SLR levels seem to directly block leptin action, reduced amounts of SLR may reflect decreased membrane expression of Ob-R [31].

Zastrow *et al.* reported that increased level of SLR may suppress the biological action of leptin by inhibiting the binding of leptin to specific membrane bound receptors in vitro while in vivo, increased SLR may delay leptin clearance and increase the available leptin pool in the circulation [19]. Gruzdeva *et al.* reported that in epicardial adipocytes, leptin secretion levels were found to be higher relative to those of adipocytes from subcutaneous adipose tissue [32]. So, it is clear that the number of leptin receptors as well as their localization might contribute to the development of leptin resistance. At the level of pancreatic beta cell, leptin resistance may contribute to dysregulation of the adipo-insular axis and promote the development of hyperinsulinemia [33]. In our study population, serum insulin level was found to be increased among obese group with two fold increase in insulin resistance in comparison with control subjects; while there was no statistically significant difference in the synthesis of insulin expressed as HOMA-β between the groups. At the same time, the insulin sensitivity expressed as QUICKI decreased significantly in obese group in comparison with control.

5. CONCLUSION

Increased levels of circulating soluble leptin receptor have been observed in obese subjects

in comparison with age and sex matched control subjects. The increased level of circulating soluble leptin receptor was found to be associated with hypertension, visceral obesity, hyperleptinemia and insulin resistance. No significant association was observed between serum lipoproteins and soluble leptin receptor.

CONSENT AND ETHICAL APPROVAL

This study was conducted in accordance with Declaration of Helsinki. Ethical approval was obtained from the Human ethics committee of both institutions. The study subjects were explained the entire study in detail and written informed consent was obtained with due negligence.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Anandhasayanam A, Kannan S, Md. Dinsar and Saranya P. Prevalence of Obesity and Its Link between Various Disorders in a Semi Urban District in Kerala. Int J Pharm Sci Res. 2016;7(9):3826-3834.
2. Vatakencherry RMJ, Saraswathy L. Prevalence of Metabolic syndrome among adults in a teaching hospital in Kochi, Central Kerala: A cross-sectional study. J Family Med Prim Care 2019 ;8(6):2079-2083.
3. Mishra S, Harris TB, Hue T, Miljkovic I, Satterfield S, de Rekeneire N, et al. Hyperleptinemia, adiposity, and risk of metabolic syndrome in older adults. J Nutr Metab 2013:327079. DOI:10.1155/2013/327079. PMCID: PMC3888758.
4. Farr OM, Gavrieli A, Mantzoros CS. Leptin applications in 2015: what have we learned about leptin and obesity? Curr Opin Endocrinol Diabetes Obes. 2015;22(5):353-359.
5. Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R et al. Identification and expression cloning of a leptin receptor, OB-R. Cell.1995;83(7):1263-1271.
6. Bornstein SR, Abu-Asab M, Glasow A, Path G, Hauner H, Tsokos M et al. Immunohistochemical and ultrastructural localization of leptin and leptin receptor in human white adipose tissue and differentiating human adipose cells in primary culture. Diabetes. 2000;49(4):532-538.
7. Margetic S, Gazzola C, Pegg GG, Hill RA. Leptin: a review of its peripheral actions and interactions. Int J Obes Relat Metab Disord. 2002;26(11):1407-1433.
8. Lammert A, Kiess W, Bottner A, Glasow A, Kratzsch J. Soluble leptin receptor represents the main leptin binding activity in human blood. Biochem Biophys Res Commun. 2001;283(4):982-988.
9. Wada N, Hirako S, Takenoya F, Kageyama H, Okabe M, Shioda S. Leptin and its receptors. J Chem Neuroanat. 2014 ;61(2):191-199.
10. Banks WA, DiPalma CR, Farrell CL. Impaired transport of leptin across the blood-brain barrier in obesity. Peptides.1999;20(11):1341-1345.
11. Caro JF, Kolaczynski JW, Nyce MR, Ohannesian JP, Opentanova I, Goldman WH et al. Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. Lancet. 1996;348(9021):159-161.
12. Schwartz MW, Peskind E, Raskind M, Boyko EJ, Porte D Jr. Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. Nat Med.1996;2(5):589-593.
13. Maness LM, Banks WA, Kastin AJ. Persistence of blood-to-brain transport of leptin in obese leptin-deficient and leptin

receptor-deficient mice. Brain Res.2000;873(1):165-167.

14. Barr VA, Lane K, Taylor SI. Subcellular localization and internalization of the four human leptin receptor isoforms. J Biol Chem. 1999;274(30):21416-21424.
15. Huang L, Wang Z, Li C. Modulation of circulating leptin levels by its soluble receptor. J Biol Chem. 2001;276(9):6343-6349.
16. Lean ME, Han TS, Deurenberg P. Predicting body composition by densitometry from simple anthropometric measurements. Am J Clin Nutr. 1996;63(1):4-14.
17. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-419.
18. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab. 2000;85(7):2402-2410.
19. Zastrow O, Seidel B, Kiess W, Thiery J, Keller E, Böttner A, Kratzsch J. The soluble leptin receptor is crucial for leptin action: evidence from clinical and experimental data. Int J Obes Relat Metab Disord. 2003;27(12):1472-1478.
20. Lammert A, Kiess W, Bottner A, Glasow A, Kratzsch J. Soluble leptin receptor represents the main leptin binding activity in human blood. Biochem Biophys Res Commun. 2001;283(4):982-988.
21. Medici V, Ali MR, Seo S, Aoki CA, Rossaro L, Kim K et al. Increased soluble leptin receptor levels in morbidly obese patients with insulin resistance and nonalcoholic fatty liver disease. Obesity (Silver Spring). 2010; 18(12):2268-2273.
22. 22.Ogier V, Ziegler O, Méjean L, Nicolas JP, Stricker-Krongrad A. Obesity is associated with decreasing levels of the circulating soluble leptin receptor in humans. Int J Obes Relat Metab Disord. 2002;26(4):496-503.
23. Devos R, Guisez Y, Van der Heyden J, White DW, Kalai M, Fountoulakis M, et al. Ligand-independent dimerization of the extracellular domain of the leptin receptor and determination of the stoichiometry of leptin binding. J Biol Chem. 1997;272(29):18304-18310.
24. Posey KA, Clegg DJ, Printz RL, Byun J, Morton GJ, Vivekanandan-Giri A, et al. Hypothalamic proinflammatory lipid accumulation, inflammation, and insulin resistance in rats fed a high-fat diet. Am J Physiol Endocrinol Metab. 2009;296(5):E1003-1012.
25. Rehman Khan A, Awan FR. Leptin Resistance: A Possible Interface Between Obesity and Pulmonary-Related Disorders. Int J Endocrinol Metab. 2016;14(1): e32586.
26. Enriori PJ, Evans AE, Sinnayah P, Jobst EE, Tonelli-Lemos L, Billes SK, et al. Diet-induced obesity causes severe but reversible leptin resistance in arcuate melanocortin neurons. Cell Metab. 2007;5(3):181-194.
27. Montez JM, Soukas A, Asilmaz E, Fayzikhodjaeva G, Fantuzzi G, Friedman JM. Acute leptin deficiency, leptin resistance, and the physiologic response to leptin withdrawal. Proc Natl Acad Sci USA. 2005;102(7):2537-2542.
28. Zabeau L, Lavens D, Peelman F, Eyckerman S, Vandekerckhove J, Tavernier J. The ins and outs of leptin receptor activation. FEBS Lett. 2003;546(1):45-50.
29. El-Haschimi K, Pierroz DD, Hileman SM, Bjørbaek C, Flier JS. Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. J Clin Invest. 2000;105(12):1827-1832.
30. Shimizu H, Oh-I S, Okada S, Mori M. Leptin resistance and obesity. Endocr J. 2007;54(1):17-26.
31. Schaab M, Kausch H, Klammt J, Nowicki M, Anderegg U, Gebhardt R, et al. Novel regulatory mechanisms for generation of the soluble leptin receptor: implications for leptin action. PLoS One. 2012;7(4): e34787.
32. Gruzdeva O, Uchasova E, Dyleva Y, Borodkina D, Akbasheva O, Belik E, et al. Relationships between epicardial adipose

tissue thickness and adipo-fibrokine indicator profiles post-myocardial infarction. Cardiovasc Diabetol. 2018; 17(1):40.

33. Seufert J. Leptin effects on pancreatic beta-cell gene expression and function. Diabetes. 2004;53 Suppl 1:S152-8.

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