

TITLE:

Impact of significant surgical reduction of adipose tissue on plasma levels and genetic expression of adipokines associated with ischemic heart disease in type I obesity.

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INTRODUCTION:

Overweight and obesity belong to the main public healthcare problems in many developed countries. Causes of obesity are considered to be the combination of genetic, epigenetic and environmental factors. In case of morbid obesity, the human organism suffers from an excess of subcutaneous and visceral adipose tissue.¹ Adipose tissue plays a principal role in overall metabolic homeostasis, and its dysfunction can lead to metabolic disorders, most notably type 2 diabetes and cardiovascular disease.² Mature adipocytes express all components of the renin-angiotensin system (RAS) including angiotensin II (Ang II), and also express the type 1 (AT1) and type 2 (AT 2) angiotensin receptor subtypes.³ For cardiovascular disease, perivascular adipose tissue (PVAT) is a specific adipose tissue and now recognized to be an endocrine organ with important physiological and pathological effects on vascular homeostasis and vascular diseases.⁽⁴⁾ PVAT dysfunction may induce several pathophysiological states such as obesity, atherosclerosis or hypertension.⁴⁻⁶ There are essentially two major types of adipose tissue with different functions: brown adipose tissue (BAT) containing multiple lipid droplets and densely packed mitochondria, and white adipose tissue (WAT) with large lipid droplets and relatively low expression levels of UCP-1(mitochondrial uncoupling protein-1).⁴ Ang II is produced from angiotensin I (Ang I as a result of the catalytic action of angiotensin-converting enzyme I (ACE-1). Ang II plays an important role in the regulation of blood volume, blood pressure, cardiac function, and electrolyte balance.⁷

On the other hand, Leptin is a protein classified within the adipokine family and is currently recognized as a hormone consisting of 167 amino acids with a molecular weight of 16 kD. It is predominantly synthesized in adipocytes, and its production is contingent upon the volume of fat deposits in the body. It is known that leptin inhibits the orexigenic pathway and stimulates the anorexigenic pathway, underscoring its pivotal role in body weight regulation.⁸ The relationship between leptin and the RAAS is bi-directional. Leptin not only stimulates sympathetic nervous system activation and angiotensin-dependent mechanisms, but it also seems to be a major driver in the aldosterone production in obese patients.⁹ Moreover, leptin appears to play a role in heart protection. The increased blood volume in obese patients increases cardiac output and stimulates biomechanical stress and structural remodeling that may result in cardiac hypertrophy. A correlation between plasma leptin levels and cardiac hypertrophy has been clinically demonstrated.¹⁰ There is evidence for both angiotensin and leptin receptors in several brain regions involved in regulation of sympathetic nerve activity. Ang II may modulate the action of leptin and adiponectin in obesity. Blocking of RAS increases

plasma adiponectin suggesting a role of Ang II in decreased levels of adiponectin. Supporting this, Ang II infusion decreased plasma adiponectin and adiponectin mRNA in adipose tissue. Ang II also interacts with adiponectin in their target cells.¹¹

Considering the role of Ang II and Leptin in the regulation of obesity, I hypothesized that significant surgical reduction in adipose tissue mass leads to decrease in its cell population which may pose significantly reduced production of adipokines associated with ischemic heart disease in obese people.

OBJECTIVES:

1. Analysis of adipokine levels (Leptin, Angiotensin-II) in patients with type I obesity (Quazi Experimental).
2. mRNA expression analysis of leptin, leptin receptor and Angiotensin-II (Quazi Experimental).
3. Investigation of genetic variations in the Leptin, Leptin receptors, Angiotensin-I and Angiotensin-II receptors (Case Control).
4. Confirm SNPs of selected sample.
5. Determine the association of genetic variation in Leptin receptor, Angiotensin-I and Angiotensin-II receptors with Leptin and Angiotensin-II levels.
6. Determine effect of genetic variation on the transcriptional regulation.

OPERATIONAL DEFINITION:

Significant Fat Reduction

A removal of 1 liter or more lipoaspirate through liposuction will be considered as significant.

Tumescence

Injection of fluid in the fat removal area containing Ringer Lactate each liter with 40ml 2% xylocaine and 1ml of 1:1000 Epinephrine till the area is blanched and showing fountain sign.

Polymorphism

Defined a discontinuous genetic variation resulting in the occurrence of several different forms or types of individuals among the members of a single species. A discontinuous genetic variation divides the individuals of a population into two or more sharply distinct forms.

HYPOTHESIS:

Significant surgical reduction of adipose tissue reduces the level of adipokines (Leptin and Angiotensin) associated with cardiac risk in plasma and their expression in adipose tissue.

MATERIAL AND METHODS:

Study design Case control study.

Setting The study will be conducted at Advanced Research Centre for Biomedical Sciences (ARCBS), KEMU and Jinnah Burn and Reconstructive Surgery Center / AIMC, Lahore.

Duration The study will be expected to complete within two years after the approval of synopsis.

Sample size Sample size of 212 patients (106 patients in each group) is estimated by using 95% confidence level and 10% absolute precision with expected percentage LEPrs7799039 as in FEN as 19.1% and healthy control as 14.1%.⁽¹²⁾

$$n = \frac{Z_{1-\alpha/2} [P_1(1-P_1) + P_2(1-P_2)]}{d^2}$$

$Z_{1-\alpha/2}$ = confidence level 95 % = 1.96

P_1 = population proportion I = 19.1%

P_2 = population proportion II = 14%

d^2 = absolute precision 10%

1. SNP analysis will be done in all the 212 subjects with 106 taken as cases (Obese type 1) and 106 as controls (Normal BMI).
2. Out of 106 cases, liposuction will be performed in selected subjects.
3. Similarly, sequencing will be done in selected subjects.

Sampling technique Non Probability Consecutive Sampling.

SAMPLE SELECTION:

Inclusion Criteria: Type I Obese adults, both males and pre-menopausal females, Patients requiring staged liposuction, American Society of Anesthesiology (ASA) class 1 and 2 (as determined by consultant anesthesiologist)

Exclusion Criteria: Patients who are with Diabetes mellitus, Metabolic syndrome, Endocrine disorders, on statin drugs, not willing to participate.

DATA COLLECTION PROCEDURE:

Informed consent will be obtained from the study subjects for donation of blood and adipose tissue samples in the proposed study, which will be conducted under a protocol approved by the Institutional Review Board of King Edward Medical University (Appendix I). The information of the study subjects will be kept confidential, and the subjects will be recruited against lab record number or archive number.

Sample Collection: Patients will undergo lipo-aspiration under tumescence by Colman's technique. Each unit of tumescent fluid will include xylocaine 40ml, Hartmann's solution 1000ml and 1ml adrenaline. A multi hole 5mm liposuction cannula attached with suction machine will be inserted through the incisions using controlled back and forth motion. All the patients will be followed fortnightly. Analysis of pre and post procedure blood and adipose tissue samples will be done. For DNA extraction, 5ml blood will be collected.

Quantification of Serum Adipokines levels by ELISA: Serum will be separated after clotting through centrifugation at 3000 rpm and stored at -70°C. ELISA will be performed on samples by standard protocol for the quantification of adipokines (Leptin, Angiotensin II) pre-operatively and three months post operatively

DNA Extraction: 5ml blood will be collected in EDTA-coated tubes. Genomic DNA will be extracted from whole blood by using commercially available kits.

Restriction Fragment Length Polymorphism Analysis of SNPs: The selected SNPs will be amplified by polymerase chain reaction (PCR) by using the primers given in Table 1. Amplicons will be detected on 2.5% agarose gel and stained with DNA gel stain. DNA bands will be detected by gel documentation instrument under UV light. PCR product will be purified by using commercially available kit and digested with restriction enzymes. The restriction product will be analyzed by agarose gel electrophoresis. The details of restriction enzymes and PCR product size is given in Table 1.

Table 1: Primer sequences and restriction enzymes for RFLP of selected SNPs

Gene	SNP	Primer Sequence	PCR Product (bp)	Restriction Enzyme	Restriction Product (bp)
Angiotensin II subtype 1 receptor (AT1R)	Rs5186 (A1166C) ⁽⁹⁾	F:ata atg taa gct cat cca cc R:gag att gca ttt ctg tca gt	350	HpyF31	AA:350 CC:211, 139 AC:350, 211, 139
Angiotensin II subtype 2 receptor (AT2R)	Rs14035430 (A1675G) (13)	F:aga gat ctg gtg cta tta cg R:cac ttg aag act tac tgg ttg	310	HYP188III	AA: 310 GG: 206, 104 GA: 310, 206, 104
Leptin	Rs7799039 (-2548G/A) (14)	F:ttt cct gta att ttc ccg tga g R:aaa gca aaa gac agg cat aaa aa	242	HhaI	GG: 242 GA:242, 181, 61 AA: 181, 61
Leptin Receptor	Rs1137101 (223Q/R) (14)	F:gcc taa tcc ag tatt tta tat ctg R:gcc act ctt aat acc ccc cag tac	416	MspI	AA: 416 Ag: 416, 229, 187 GG:229, 187

Sequencing of SNPs: Sequencing of SNPs will be done using specific primers by Sanger Sequence using ABI 3500, Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific, USA) and the sequences will be analyzed using the BLAST program at National Center for Biotechnology Information (NCBI). Amino acid sequences will be predicted by the standard code and will be aligned using the ExPASy online tool for translation. Sequencing will be done on study subjects and compared with same number of healthy persons for comparison.

RNA Isolation and cDNA Synthesis: RNA will be isolated from subcutaneous adipose tissue by using commercially available kits. 1ug RNA will be reverse transcribed using commercially available Transcription First Strand cDNA kit and oligo (dT) primers.

Real-time PCR Analysis for Gene Expression Analysis: Quantitative real-time PCR analysis will be carried out by using SYBR Green real-time PCR Master Mix and an ARIAMix, real-time PCR system (Agilent Technologies). Cycling conditions for real-time PCR will be 3

min at 95°C, followed by 40 cycles of 30 S at 95°C, 30 S at 60°C and 30 S at 72°C. Results will be analysed using the $\Delta\Delta C_t$ method and will be presented as fold difference in mRNA level relative to GAPDH. The expression profile of transcription factors will be analyzed.

Table 2: Primer sequences for real-time PCR analysis

Sr No	Genes	Primer Sequence (5'-3')
1	LEP ⁽¹⁵⁾	F: ttt cac aca cgc agt cag tct R: cca gga atg aag tcc aaa cc
2	GAPDH ⁽¹⁵⁾	F: ttg cca tgg gtg gaa tca ta R: tcg gag tca acg gat ttg gt
3	Angiotensin Receptor I ⁽¹⁶⁾	F: aac tca ctg atg cca tcc cag R: tcc tct gag ctg tgg agc ct
4	Angiotensin Receptor II ⁽¹⁶⁾	F: tgt tta ggc act aag caa gct gat R: aat gtc agc agc tcc tag ttc tta tg
5	Angiotensin ⁽¹⁷⁾	F: tgg aca gca ccc tgg ctt tca a R: aca ctg agg tgc tgt tgt cca c
6	Angiotensin ⁽¹⁷⁾	F: gtg atg gca gag atg gca c R: aga ggc tga cct tca cat cc

DATA ANALYSIS PRODECURE:

Data will be entered and analyzed using statistical Package for Social Sciences (SPSS-26). Quantitative variables like age, BMI, lipid profile and adipokine levels will be presented as mean $\pm$ SD. Qualitative variables like family history of obesity, family history of heart diseases and gender will be presented frequency and percentages. Paired t-test will be used to compare clinical parameters like serum adipokines levels and adipokines expression levels. Odds ratio and 95% CI will be used for SNP analysis. Whereas p value <0.05 will be taken as statistically significant. Pearson equation will be used to determine association of adipokines levels with clinical parameters and expression levels.

OUTCOME & UTILIZATION:

The results will be utilized to determine impact of surgical reduction on plasma level and expression of the adipokines in minimizing cardiovascular risk factors.

SCHEDULE/PHASING:

Phases (Description)	Duration (24 Months)			
	1-10 Months	11-16 Months	17-22 Months	23-24 Months
Phase I a) Sample collection b) ELISA c) DNA Expression d) PCR analysis of selected SNPs e) PCR gel electrophoresis f) RFLP analysis of selected SNPs g) RFLP gel electrophoresis h) RNA extraction i) cDNA synthesis j) Real time PCR analysis				
Phase II a) PCR Product purification b) Sequence analysis of SNPs				
Phase III Analysis of data, conclusion, article writing and thesis				
Total Duration Final submission				

REFERENCES:

1. Šebunova N, Štšepetova J, Kullisaar T, Suija K, Rätsep A, Junkin I, et al. Changes in adipokine levels and metabolic profiles following bariatric surgery. *BMC Endocr Disord.* 2022;22(1):33.
2. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. *Nature.* 2006;444(1):875-80.
3. Liang X, Sun J, Guan H, Zhu Q, Yao W. Angiotensin II inhibits adipogenic differentiation and promotes mature adipocyte browning through the corepressor CtBPI. *Biomedicines.* 2022;10(12):1-11.
4. Liang X, Qi Y, Dai F, Gu J, Yao W. PVAT: an important guardian of the cardiovascular system. *Histol Histopathol.* 2020;35(8):779-87.
5. Lian X, Gollasch M. A clinical perspective: contribution of dysfunctional perivascular adipose tissue (PVAT) to cardiovascular risk. *Curr Hypertens Rep.* 2016;18(11):82.
6. Nava E, Llorens S. The local regulation of vascular function: from an inside-outside to an outside-inside model. *Front Physiol.* 2019;10(1):1-15.
7. Camargo RL, Bombassaro B, Monfort-Pires M, Mansour E, Palma AC, Ribeiro LC, et al. Plasma angiotensin II is increased in critical coronavirus disease 2019. *Front Cardiovasc Med.* 2022;9(1):1-9.
8. Vilariño-García T, Polonio-González ML, Pérez-Pérez A, Ribalta J, Arrieta F, Aguilar M, et al. Role of leptin in obesity, cardiovascular disease, and type 2 diabetes. *Int J Mol Sci.* 2024;25(4):1-21.
9. Dadarlat-Pop A, Pop D, Procopciuc L, Sitar-Taut A, Zdrengea D, Bodizs G, et al. Leptin, galectin-3 and angiotensin II type I receptor polymorphism in overweight and obese patients with heart failure-Role and functional interplay. *Int J Gen Med.* 2021;14(1):1727-37.
10. Kang KW, Ok M, Lee SK. Leptin as a key between obesity and cardiovascular disease. *J Obes Metab Syndr.* 2020;29(4):248–59.
11. Mosquera-Sulbarán J, Ryder E, Vargas R, Pedreáñez A. Angiotensin II and human obesity. A narrative review of the pathogenesis. *Invest Clin.* 2022;63(4):435-53.
12. Elmeherat A, Kishk TF, Abdullah AF, Taalab AAA. The effects of large volume liposuction on body weight. *Egypt J Plast Reconstr Surg.* 2021;45(1):33-9.
13. Nguyen-Thanh T, Nguyen-Vu PT, Le-Thi QA, Phan-Thi TN, Ha TM. Association between maternal and fetal genetic variants and preeclampsia: Evidence from a meta-analysis. *Mol Biology.* 2024 Aug 1;46(8):8282-300.
14. Uysalol EP, Uysalol M, Serin I, Pehlivan M, Oyaci Y, Pehlivan S, et al. The effect of leptin gene polymorphism (LEP rs779939 and LEPR rs1137101) on febrile neutropenia. *Heliyon.* 2024;10(7):1-8.
15. Hattori N, Takamatsu H, Iida N, Asano N et al. Epigenetic disruption of adipogenic gene enhancers in dedifferentiated liposarcomas and its therapeutic value. *Front Onco.* 2025; 30(15):1419877.
16. Königshoff M, Wilhelm A, Jahn A, Sedding D, Amarie OV, Eul B, et al. The angiotensin II receptor 2 is expressed and mediates angiotensin II signaling in lung fibrosis. *Am J Respir Cell Mol Biol.* 2007;37(6):640-50.
17. Hennige AM, Staiger H, Wicke C, Machicao F, Fritsche A, Häring HU, et al. Fetuin-A induces cytokine expression and suppresses adiponectin production. *PLoS One.* 2008;3(3):1-9.