

## **Relationship Between *Ob* Gene Product and Lipid Profile in The Pathogenesis of Atherosclerosis**

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## **ABSTRACT**

Leptin, *Ob* gene product is overexpressed in atherosclerotic lesions, and leptin signaling is implicated in promoting both thrombosis and atherosclerosis in mice models, suggesting a role for leptin in CVD. Leptin is also known to promote pro-inflammatory signaling through cytokines and growth factors that may contribute to the progression of atherosclerosis. Hence, in the present study, we investigated the role of leptin in the pathogenesis of atherosclerosis by measuring its levels in atherosclerosis subjects and analyzing its correlation to anthropometric variables such as waist to hip ratio, body mass index, and biochemical parameters as lipids and glucose. Leptin levels correlate to serum glucose, triglycerides, waist to hip ratio, body mass index, and blood pressure in atherosclerosis subjects with increased body mass index. Our results show that leptin influences the lipid profile to induce the pathogenesis of atherosclerosis.

**Keywords:** leptin, lipid profile, glucose, atherosclerosis, body mass index

## **INTRODUCTION**

CVDs, including heart disease, vascular disease, and atherosclerosis, are the most critical global health threat, contributing to more than one-third of the global morbidity (Agha M, Agha R. 2017). In most cases, these clinical conditions result from atherosclerosis, which was once identified as a lipid-storage disease and obesity is currently viewed as an underlying risk factor for CVD (Babu et al., 2020). Furthermore, epidemiological observations and metabolic investigations consistently demonstrate that the accumulation of excess visceral fat is related to an increased risk of CVD and several metabolic and inflammatory perturbations (Beamont et al., 1972, Chandrasekaran et al., 2020, Cole et al., 1997.) Key features associated with excess visceral fat/ectopic fat accumulation include insulin resistance, atherogenic dyslipidemia, hypertension, impaired fibrinolysis/increased risk of thrombosis, and inflammation (Kannan et al., 2018). These metabolic features, most commonly found in viscerally obese patients, are often referred to collectively as metabolic syndrome.

Adipose tissue is recognized as an important player in obesity-mediated CVD. Adipocytes produce large numbers of hormones, peptides, and other molecules that affect cardiovascular function, not only in an endocrine manner but also by autocrine and paracrine mechanisms (Nalini et al., 2019). This might lead to cytokine-mediated inflammatory changes in the liver, systemic inflammation and atherosclerosis. One of the key vasoactive substances produced by adipocytes is leptin, an important food intake regulator.

Leptin is a novel and very promising molecule of research that may mediate obesity and cardiovascular diseases. Since its discovery in 1994, major advances are made in understanding neuroendocrine mechanisms regulating appetite, metabolism, inflammation, adiposity, sympathetic tone and blood pressure (Dardeno et al., 2010, Devarajan et al., 2021, Haouari et al., 2019, Elsayed et al., 2008 ). In the present study, we evaluated the relationship between leptin and obesity in the pathogenesis of atherosclerosis. Also, we have suggested prominent remedies to treat obesity and associated comorbidities effectively (Golia et al., 2015, Gomez-Delgado et al., 2021, Hema et al., 2019, Jayaraman et al., 2021).

## **Materials and methods**

### **Choice of CVD and non-CVD subjects**

Control (non-CVD) subjects chosen for the present study were those who had no history of incidence of CVD. They belonged to the age group of 35 – 75 and were devoid of diabetes, and heart disease, thyroid disorders, and arthritis. None of the subjects were on medication known to affect insulin action or plasma lipoprotein – lipid levels and they were not on any

inflammatory drug either before or at the time of the study. Individuals using aspirin as a chronic medication were excluded from the study. Subjects were not under any medication for at least 3 months before the study.

Written consent was obtained from the above subjects to participate in the present study. Trained interviewers administered questionnaires to obtain information on each subject's date of birth, occupation, current cigarette smoking and alcohol use and random blood samples (5 mL) were collected from them. The blood was then allowed to clot and it was retracted and separated by centrifugation at 3500 rpm for 10 minutes. Serum was separated using standard protocols and stored at -80°C.

### **Anthropometric Measurements**

Anthropometric measurements, including height and weight were recorded using standard procedures. Standard weighing machine that had a precision of 0.1Kg was used and the heights of the subjects were recorded using a vertically mobile scale and expressed to the nearest centimeter.

BMI was calculated from height and weight as follows:

$$\text{BMI} = \frac{\text{Weight (Kg)}}{\text{Height(m}^2\text{)}}$$

Waist and hip circumferences were measured in erect position, with abdomen relaxed, arms at the sides and the foot together. The measurement was taken at the level of narrowest part of the torso, as seen from the anterior aspect and was recorded to the nearest 0.1cm. Hip circumference was measured horizontally at the level of the maximum extension of the buttocks posteriorly. Waist to hip ratio (WHR) was then calculated as follows:

$$\text{WHR} = \frac{\text{Waist circumference (inches)}}{\text{Hip circumference (inches)}}$$

Blood pressure was recorded by trained nurses using mercury sphygmomanometer. **Serum Biochemical Analysis.** Serum glucose was estimated using GOD-POD method (Kim et al., 2020), Serum triglyceride was measured using GPO-POD-ESPT method (Ku et al., 2010 ), Serum cholesterol was measured using CHOD-POD method (Lovren et al., 2015 ), Serum HDL-cholesterol was measured using direct method (Shanthi et al., 2016 ); VLDL and LDL were measured by indirect method.

**Statistical analysis.** Data obtained in chapters I, II and III were analyzed using SPSS software version 17.0 and the results were presented as mean±SD. As the data obtained were not

normally distributed, Mann-Whitney U test was used. In addition, the relationship between continuous variables were evaluated using Spearman's rank correlation technique (Kruskal-Wallis analysis of variance by ranks test was performed). A value of  $p < 0.05$  was considered statistically significant.

## Results

Serum leptin values were found to be significantly higher in atherosclerosis subjects than in control (non-CVD) subjects (normal weight subjects -  $23.1 \pm 5.1$  ng/mL versus  $5.7 \pm 3.8$  ng/mL,  $p < 0.0001$ ; overweight subjects -  $30.2 \pm 3.7$  ng/mL versus  $10.5 \pm 4.2$  ng/mL,  $p < 0.0001$ ; obese subjects -  $42.8 \pm 4.5$  ng/mL versus  $29.3 \pm 9.1$  ng/mL,  $p < 0.0001$ ). A positive correlation is observed between leptin and BMI in these subjects ( $r = 0.57$ ,  $p < 0.0001$ ). Serum leptin value was found to be higher in normal weight atherosclerosis when compared to that of overweight control subjects. This in turn is indicative of the possible leptin resistance in them during pathological conditions.

**Table 1. Values Of Serum Triglycerides In Atherosclerosis And Control Subjects**

Bmi class (kg/m <sup>2</sup> )	Subjects	N	Triglycerides (mg/dl)
Normal Weight	Control subjects	21	$124.6 \pm 14.3$
	Atherosclerosis subjects	22	$145.46 \pm 25.1$
	P value <sup>#</sup>		0.23
Over Weight	Control subjects	46	$144.13 \pm 21.3$
	Atherosclerosis subjects	45	$173.9 \pm 18.9$
	P value <sup>#</sup>		0.005**
Obese	Control subjects	33	$167.9 \pm 25.8$
	Atherosclerosis subjects	33	$149.1 \pm 18.8$
	P value <sup>#</sup>		0.644

**Table 2. Values Of Serum Glucose In Atherosclerosis And Control Subjects**

Bmi class (kg/m <sup>2</sup> )	Subjects	N	Glucose (mg/dl)
Normal Weight	Control subjects	21	113.9±15.4
	Atherosclerosis subjects	22	138.9±22.3
	P value <sup>#</sup>		0.09
Over Weight	Control subjects	46	108.9±18.2
	Atherosclerosis subjects	45	148.4±17.6
	P value <sup>#</sup>		0.005**
Obese	Control subjects	33	114.9±11.9
	Atherosclerosis subjects	33	139.3±23.6
	P value <sup>#</sup>		0.1086

Table 1 and table 2 illustrate the values of serum glucose and triglycerides in atherosclerosis and control subjects. The results obtained for atherosclerosis subjects were found to be similar to that observed in AMI subjects . Serum glucose, triglycerides, cholesterol, HDL and LDL were found to be higher in overweight atherosclerosis when compared to control subjects (serum glucose - 148.4±17.6 mg/dL versus 108.9±18.2 mg/dL, p=0.005; serum triglycerides - 173.9±18.9 mg/dL versus 144.13±21.3 mg/dL, p=0.005; serum cholesterol - 233.2±25.1 mg/dL versus 174.4±23.4 mg/dL, p<0.0001; serum HDL - 41.4±9.1 mg/dL versus 35.1±9.8 mg/dL, p=0.008; serum LDL - 148.6±53.1 mg/dL versus 112.02±12.4 mg/dL, p=0.0005). In obese category, both control and atherosclerosis subjects were found to possess a higher level of lipid profile and hence no significant differences were observed. Also, both systolic and diastolic blood pressure were found to be significantly higher in overweight atherosclerosis when compared to control subjects (systolic blood pressure - 134.4±16.6 mm Hg versus 122.8±20.5 mm Hg, p=0.003; diastolic blood pressure - 90±12.6 mm Hg versus 84.3±15.5 mm Hg, p<0.0001).

Waist to hip ratio was also found to possess a positive correlation with triglycerides (r=0.42, p=0.0001) and VLDL (r=0.45, p<0.0001) in atherosclerosis subjects. The results obtained in atherosclerosis subjects are found to be comparable with that of AMI subjects.

## **Discussion**

Triglycerides are reported to exhibit a long-standing association with CVD. The finding of triglyceride-rich lipoproteins in human atheromata provides substantial pathophysiologic evidence for its direct role in atherogenesis [Nalini et al., 2015 ]. Serum triglycerides were found to be higher in both AMI and atherosclerosis subjects when compared to that of control subjects. In addition, serum leptin levels were found to be positively correlated to serum triglycerides in atherosclerosis subjects. Serum triglycerides also positively correlate with BMI, cholesterol, HDL and VLDL in all the subjects under study (viz., atherosclerosis subjects and control subjects).

Moreover, serum cholesterol, HDL, LDL and VLDL were found to be higher in overweight AMI and atherosclerosis subjects than in control subjects. Higher VLDL triglyceride output are reported to activate cholesteryl ester transfer protein, resulting in triglyceride enrichment of LDL and HDL [Nordestgaard BG, Varbo 2015]. Experimental studies suggest that (i) hypertriglyceridemic HDL may be dysfunctional, (ii) small, dense LDL particles may be more susceptible to oxidative modification, and (iii) an increased number of atherogenic particles may adversely influence CVD risk . Thus, the increased values of triglycerides observed during obese conditions might act as an added risk for CVD incidence.

Adipose tissue distribution, rather than total fat, is associated with risk for diabetes, hyperlipidemia, and CAD (Ponnulakshmi et al., 2019, Raskin Erusan et al., 2008). One large cohort study shows a 60% greater relative risk of death with more centralized pattern of fat distribution (Rajagopal et al., 2012). Waist to hip ratio is the most commonly used index of central fat distribution and obesity assessed by waist to hip ratio is a better predictor of CVD and CHD mortality than waist circumference, which is a better predictor than BMI (Rajendran et al., 2012, Redinger 2007).

Waist to hip ratio exhibit a significant positive correlation to triglycerides and VLDL in atherosclerosis subjects but such a correlation was not observed in control (non-CVD) subjects. All these observations suggest that increased visceral obesity associated with elevated serum levels of leptin, triglycerides, and VLDL may act synergistically to increase one's risk of acquiring CVD. Decreasing obesity with anti-oxidant and hypolipidemic agents including phytochemicals, nutraceuticals etc would be a promising approach to prevent the development of CVD and other dreadful diseases as well (Rocha et al., 2009, Trinder 1959, Young 2000).

## **Author contribution**

Mathangi R, Selvaraj J, Reji M conceived of the presented idea Jaideep M<sup>4</sup>, Ponnulakshmi R, Nalini D Samyuktha. P, Pugazharasan encouraged and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

## **Acknowledgment**

**Conflict of interest** .Nil

## **Study significance**

In the present study, we investigated the role of leptin in the pathogenesis of atherosclerosis by measuring its levels in atherosclerosis subjects and analyzing its correlation to anthropometric variables such as waist to hip ratio, body mass index, biochemical parameters such as lipids and glucose. Leptin levels correlate to serum glucose, triglycerides, waist to hip ratio, body mass index, and blood pressure in atherosclerosis subjects with increased body mass index. Our results show that leptin influences the lipid profile to induce the pathogenesis of atherosclerosis.

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