Abstract: The incidence of obesity worldwide has increased drastically during recent decades. Currently, obesity is viewed as a pro-inflammatory state with a rise in inflammatory parameters such as C-reactive protein (CRP), nitric oxide (NO) along with the increase in the secretion of leptin. Evaluation of these parameters in lean and obese subjects might enable us to understand the link between obesity, BMI and inflammation. In this connection, we have estimated serum leptin, CRP, NO, serum triglycerides, cholesterol, and lipid profile in normal, underweight, overweight and obese subjects. Interestingly leptin level is found to be higher in obese and overweight subjects. A profound positive correlation has been observed between obesity, serum leptin and CRP suggesting a possible link between obesity, inflammation and metabolic syndrome. 

Keywords: Inflammation, cardiovascular disease (CVD), nitric oxide (NO), leptin, C-reactive protein (CRP)

Introduction

Obesity is an increasingly prevalent metabolic disorder among developed- as well as developing-countries with its pathogenesis being multifactorial incorporating both genetics and lifestyle (Ren, 2004). Uncontrolled obesity when sustained dramatically enhances the propensity of a cluster of metabolic diseases such as cardiac dysfunction, ventricular hypertrophy, reduced diabetic compliance, increased blood pressure, arteriosclerosis and other complications such as diabetes mellitus, insulin resistance, hyperinsulinemia, hyperlipidemia, hyperurecemia, low plasma high density lipoprotein-cholesterol, collectively known as metabolic syndrome (Sowers, 1998; Unger & Orci, 2001). Although, it is generally accepted that activation of the sympathetic nervous system and impairment of endothelial function are the two phenotypical traits in obesity, the cellular mechanisms responsible for sympathetic hyperactivity and endothelial dysfunction have not been clearly elucidated (Steinberg et al., 1996; Julius et al., 2000; Kuo et al., 2004). Therefore, direct and independent impact as well as the consequence of obesity on cardiovascular function may be largely obscured.

It is not until 1994 that the discovery of the non-glycosylated 16kDa peptide hormone, leptin (ob gene product), brought a complete revolution to the understanding of obesity (Zhang et al., 1994, Caro et al., 1996, Leibel et al., 1997, Freidman & Halas, 1998). Leptin is known to play a key role in regulating energy intake and its expenditure, including the regulation of appetite and metabolism. Its key role is also known in reproduction, haematopoiesis, immune response, Cushing syndrome, thyroid disease and growth hormone deficiency and is also found to act as a novel, independent risk factor for PCOS (Mani Ravishankar Ram et al., 2005). Leptin is synthesized in adipose tissue and interacts with six type of receptors (ob Ra - ob Rf). ob Rb is the only isoform that contains active intracellular signaling domains. It is shown that administration of leptin to genetically obese (ob/ob) mice reduces food intake and body weight whereas humans, in spite of their high serum leptin level tend to remain obese, indicative of leptin resistance. Leptin is also found to be involved in intricate cascade of cardiovascular events and can promote the risk of its incidence through elevated blood pressure, by provoking ADP-induced platelet aggregation, stimulating cytokine release, promoting calcification process (Bridget Canavan et al, 2005) and triggering inflammatory response such as adhesion of leukocyte and monocytes to the endothelium. Hence, a detailed study on the correlation of leptin to obesity and other associated metabolic disorders such as CVD becomes essential in order to exploit therapeutic targets essential for the same.

Obesity is viewed as a proinflammatory state with inflammatory markers crucial for the onset of the disease (Thomas A. Pearson et al., 2003). Mounting evidences suggest a role for inflammation in augmenting atherosclerotic disease process. All the stages of atherosclerosis i.e., initiation, growth and complication of the atherosclerotic plaque might be considered to be an inflammatory response to injury. The major risk factors that promote atherogenesis- cigarette smoking, hypertension, atherogenic lipoproteins and hyperglycemia are well established (Tracy, 1998; Ross, 1999). These risk factors give rise to a variety of noxious stimuli that elicit secretion of both leukocyte soluble adhesion molecules which can facilitate the attachment of monocytes to endothelial cells and chemotactic factors, and encourage the monocyte migration into the subintimal space. The transformation of monocytes into macrophages and the uptake of cholesterol,
lipoproteins are thought to initiate the fatty streak (Libby & Ridker, 1999; Plutzky, 2001). Further, attraction and accumulation of macrophages, mast cells, and activated T-cells within the growing atherosclerotic lesion take place. Oxidized low density lipoproteins may be one of the several factors that contribute to loss of smooth muscle cells through apoptosis in the atherosclerotic plaque cap and secretion of metalloproteinases and other connective tissue enzymes by activated macrophages may breakdown collagen, weakening the cap and making it prone to rupture. The disruption of the atherosclerotic plaque then exposes the atheronecrotic core to arterial blood, which induces thrombosis. Thus, virtually every step in atherogenesis is believed to involve cytokines, cells that are characteristic of inflammation and other bioactive molecules of which leptin might exert a major role. In obese condition, there is an increase in the level of these cytokines due to their secretion from adipocyte and this could potentiate atherosclerosis.

Insights into potential targets of measurements provide means to identify and monitor the relationship between obesity and ongoing inflammatory process. Hence, the present study is intended to evaluate the inflammatory mediators such as leptin, nitric oxide (NO) and acute phase proteins such as CRP in relation to BMI which in turn might provide a better understanding of the link between obesity, inflammation and metabolic syndrome.

**Materials and methods**

**Subjects**

The present study is carried out in 62 normal subjects from Chennai based population. 21 subjects under study are young, healthy, control subjects with normal BMI (18-25) belonging to the age group of 20-34 years. 41 subjects under the study belong to the age group of 35-60 years of which 2 are underweight, 12 are normal, 17 are overweight and 10 are obese. All the subjects considered for the study are healthy with no previous history of incidence of any CVD. They were also devoid of medication for at least 60 days before blood collection. After obtaining a written consent, 5ml fasting blood is drawn from them. The blood is then allowed to clot and it is retracted and centrifuged at 2000g for 15 minutes. The serum is separated carefully and stored at -80°C until analysis.

Anthropometric measurements, including height and weight are taken using standard protocols. Height is measured to the nearest 0.1cm on a scale. Weight is measured to nearest 0.1kg on a portable stadiometer.

**ELISA method for measuring leptin, insulin and NO concentration**

Serum leptin concentration is measured using the Sandwich Leptin Elisa Kit (Diagnostic Biochem Canada Inc.), insulin level is measured using Insulin ELISA kit (United Biotech Inc. California, USA) and nitric oxide is estimated using Griess reagent (Promega, USA). All the analyses are performed according to the manufacturer's instructions.

**Immunoturbidometric analysis of serum CRP concentration**

Serum CRP level is estimated using CRP-ultra kit (Spinreact, Spain) by immunoturbidometry according to the manufacturer's instruction.

**Serum biochemical analysis**

Serum glucose is estimated using glucose oxidase-peroxidase method (Trinder, 1959), cholesterol measured by the cholesterol oxidase-peroxidase method (Beaumont et al., 1972), high density lipoprotein (HDL)- cholesterol is measured by the glycerol-3-phosphate oxidase-peroxidase-N-ethyl-methylanilin propan sulphonate sodic method (Holvey, 1972), triglyceride is measured using GPO-POD-ESPT method (Cole et al., 1997) using autoanalyzer (BAYER RA 50; Bayer company India, Guindy, Chennai, India).

**Statistical analysis**

For each experimental series, data are presented as mean±SEM. Statistical significance (P<0.05) for each variable is estimated using t-test.

**Results**

Anthropometric measurements including BMI and biochemical parameters including serum triglycerides, cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL and inflammatory parameters such as leptin, CRP and other parameters including NO, insulin are analyzed in 62 normal subjects from Chennai based population. The results thus obtained are tabulated (Table 1).

**Correlation between leptin and BMI**

A profound positive correlation is observed between serum leptin and BMI in the subjects considered for the present study. Normal subjects belonging to the age group of 20-35 years are found to possess normal serum leptin level (5.1±0.8ng/ml). There is a linear relationship between leptin and BMI, with obese subjects possessing significantly high serum leptin (33.9±4.1ng/ml) when compared to that of overweight (27.5±4.7ng/ml), control (14.6±3.8ng/ml) and underweight (7.5±3.5ng/ml) subjects. Interestingly, subjects belonging to the higher age group (35-60 years) are found to possess higher level of serum leptin irrespective of their BMI. It can be noted that subjects belonging to the higher age group especially obese subjects...
have higher value of leptin suggesting that this could be a risk factor for the incidence of metabolic disorders.

**Table 1. Anthropometric measurement for obesity incident from Chennai-based population**

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<tr>
<td>Triglycerides (mg/dl)</td>
<td>149.0±11.7</td>
<td>79.0±19.0</td>
<td>160.0±5.9</td>
<td>149.0±11.1</td>
<td>195.6±48.6</td>
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<tr>
<td>Cholesterol (mg/dl)</td>
<td>181.5±6.6</td>
<td>88.5±24.5</td>
<td>195.0±5.8</td>
<td>177.2±5.6</td>
<td>184.9±8.7</td>
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<tr>
<td>HDL (mg/dl)</td>
<td>45.5±0.58</td>
<td>45.0±4.0</td>
<td>52.3±0.8</td>
<td>46.05±1.3</td>
<td>48±1.9</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>108±8.4</td>
<td>28.0±17.0</td>
<td>111.7±3.9</td>
<td>103.17±4.7</td>
<td>93±0.4</td>
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<td>VLDL (mg/dl)</td>
<td>31.7±0.17</td>
<td>15.5±3.5</td>
<td>31.9±1.2</td>
<td>31.3±3.0</td>
<td>28.5±4.0</td>
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<tr>
<td>TotalCHO/HDL ratio</td>
<td>3.9±0.17</td>
<td>1.95±0.35</td>
<td>3.6±0.45</td>
<td>3.9±0.10</td>
<td>3.1±0.4</td>
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<tr>
<td>LDL/HDL ratio</td>
<td>2.4±4.1</td>
<td>0.58±0.32</td>
<td>2.5±0.17</td>
<td>2.3±9.6</td>
<td>3.1±0.5</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>5.6±3.1</td>
<td>1.05±7.07</td>
<td>2.8±1.2</td>
<td>3.7±3.5</td>
<td>6.0±6.4</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>14.6±3.8</td>
<td>7.5±3.5</td>
<td>5.1±0.8</td>
<td>27.5±4.7</td>
<td>33.9±4.1</td>
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<tr>
<td>NO (µM/L)</td>
<td>7.0±1.3</td>
<td>3.95±1.05</td>
<td>9.95±1.05</td>
<td>5.8±0.7</td>
<td>6.4±0.8</td>
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Serum triglycerides level and LDL/HDL ratio is found to be normal in control subjects (149.0±11.7mg/dl and 2.4±4.1 respectively). Obese subjects are found to exhibit high serum triglycerides (195.6±48.6 mg/dl) than overweight (149.0±11.1mg/dl), normal (160.0±5.9mg/dl) and underweight subjects (79.0±19.0mg/dl). LDL/HDL ratio is also found to be high in obese subjects (3.1±0.5). Furthermore, serum HDL level is found to be significantly high in normal subjects (52.3±0.8mg/dl) when compared to that of other subjects under study (underweight subjects- 45.0±4.0mg/dl, control subjects - 45.5±0.58mg/dl, overweight subjects - 46.05±1.3mg/dl, and obese subjects- 48.0±1.9mg/dl). Elevated triglyceride level is one of the symptoms of metabolic syndrome with triglycerides acting as a main form of storage of excess calories in fat. Therefore, increased triglycerides and low HDL observed in obese subjects with an elevated level of leptin suggest their increased risk for the incidence of CVD.

CRP is a signaling product of inflammatory cytokines such as TNF-α, IL-6 and reports suggest that leptin could be involved in synthesis of CRP in obesity. Since there is an excess secretion of this adipokine, CRP is also expected to be elevated and could be a link between obesity and inflammatory disorders. Serum CRP is found to be normal in normal subjects (2.8±1.2mg/L) but, elevated in obese (6.0±6.4 mg/L), overweight (3.7±3.5mg/L) and control subjects (5.6±3.1mg/L). Underweight subjects are found to possess a borderline level of serum CRP level (1.05±7.07mg/L). Recently, CRP is being used as an important parameter for monitoring the severity of the heart diseases. Its increase in obese subjects in turn is suggestive of an increased risk for cardiovascular events. No significant relationship between CRP level and BMI has been established so far and an extensive analysis is necessary to examine the correlation between leptin, CRP and BMI and their relation to inflammation associated CVD.

Multiple risk markers for atherosclerosis and cardiovascular disease act in a synergistic way through inflammatory pathways. Hence, analysis of the inflammatory markers has gained importance in the elucidation of the role of inflammation in obesity, CVD and metabolic disorders. NO, an essential molecule involved in vasodilation is found to be normal in normal subjects (9.95±1.05µM/L), but low in all other subjects as evidenced from our study (obese, overweight, control and underweight subjects possessing a value of 6.4±0.8µM/L, 5.8±0.7µM/L, 7.0±1.3µM/L and 3.95±1.05µM/L respectively).

**Discussion**

The incidence of obesity is increasing dramatically in recent years, making it one of the most pressing public health concerns worldwide. Obesity is associated with co-morbid conditions, most notably diabetes, CAD and hypertension and the co-existence of these diseases has been termed metabolic syndrome (Hotamisligil & Gökhan, 2006).

Also, the identification of the adipokine leptin might provide a molecular link to obesity. Leptin is recognized as a central mediator in endocrine circuit regulating energy homeostasis (Hotamisligil & Gökhan, 2006) and has permitted a more detailed understanding of energy balance. An elevated expression of leptin and inflammatory cytokines (TNF-α, IL-6 and IL-8) in serum and their increased expression in the ovary of thin PCOS subjects were reported suggesting a possible role for leptin in reproduction and inflammation (Mani Ravishankar Ram, 2005). In the present study, obese subjects are found to possess high value of...
serum leptin suggestive of leptin resistance. Nevertheless, a complete understanding of the molecular components of the leptin pathway is necessary to interpret its actual role in obesity and this will aid in the development of effective treatment for obesity and the metabolic syndrome.

CRP, a clinically important prognostic tool to metabolic syndrome is found to be elevated in obese subjects. CRP is also an easily measured inflammatory biomarker that has proven to be a strong, independent predictor of both incident diabetes (Aruna D Pradhan et al., 2001; Freeman et al., 2002) and incident cardiovascular diseases (Ridker et al., 1997; Danesh et al., 2000). The metabolic syndrome describes a high risk population having 3 or more of the following clinical characteristics: upper-body obesity, elevated triglyceride level, low HDL, hypertension and abnormal glucose. All of these attributes, however, are associated with increased level of CRP (Koenig et al., 1999; Ridker et al., 2000; 2001; 2002; Tracy et al., 1997). Also, leptin has been reported to stimulate hepatic CRP synthesis through TNF-α and IL-6 signaling pathways. Hence, increased CRP level observed in obese subjects might be due to increased triglyceride level, low HDL and leptin resistance.

Metabolic syndrome is in turn a combination of medical disorders that increase one’s risk for cardiovascular disease and diabetes. However an in depth study on the analysis of the role of adipokines such as leptin and its relation to other cytokines in promoting atherosclerosis will enable us to explore the mechanistic and signaling pathways that act as a crucial link between obesity, inflammation and CVD.

Acknowledgements

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