Human Biology Review
www.humanbiologyjournal.com

Human Biology Review(ISSN 22774424) 7(2) 2018
Original scientific paper (Kaur et al.pp.205-225)
Revised and Accepted on April 27, 2018

Association of Metabolic and Inflammatory Biomarker Profile with Body Mass Index in Perimenopausal Women

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Citation: Kaur T, Singh B, Gupta SK and Kaur G. 2018. Association of Metabolic and Inflammatory Biomarker Profile with Body Mass Index in Perimenopausal Women. Human Biology Review, 7 (2), 205-225.

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ABSTRACT

Background: *Obesity is known to cause inflammation and alter the levels of cytokines, adipokines and insulin in humans. Our aim was to investigate the relationship of BMI with serum lipid levels, leptin, estradiol, cortisol, insulin and inflammatory cytokines in working perimenopausal women.*

Methods: *62 healthy perimenopausal women working in Guru Nanak Dev University, Amritsar (India) were grouped into three BMI categories: BMI<25 kg/m^2 (normal weight), 25 kg/m^2≤BMI<30 kg/m^2 (overweight) or BMI≥30 kg/m^2 (obese). Basic parameters, i.e. blood pressure, lipid profile and fasting blood glucose were monitored among the three categories. Differences in the circulating levels of leptin, insulin, cortisol, estradiol, TNFα, IL-1β and IL-6 were evaluated. Additionally, Western blot analysis for neurological marker GFAP and inflammatory cytokines was also performed.*

Results: *Blood pressure showed significant increase in the overweight and obese subjects as compared to normal weight women. Serum cholesterol, triglycerides, LDL and fasting blood glucose showed trend to increase with BMI. Leptin and TNFα increased significantly in overweight and obese subjects as compared to normal weight group. Insulin, cortisol and IL-6 showed increase only in obese subjects. Level of estradiol declined significantly with increase in BMI whereasIL-1β did not show any difference among the three categories.*

Conclusions: *The association of overweight and obesity with dyslipidemia, serum leptin, cortisol, insulin and inflammatory cytokines was independent of age and menopausal status and showed direct relationship with BMI values in perimenopausal women.*

KEYWORDS:*Perimenopause; BMI; Inflammation; Estradiol; Leptin*

INTRODUCTION

Obesity is considered as a world health problem as it has reached pandemic proportions in the 21st century. Obesity, also known as overnutrition-induced disease, is defined as a stage of accumulation of fatty acids and increased adipose tissue mass as compared to lean mass (Kaur et al., 2016). The terms 'overweight' and 'obesity' refer to body weight that is greater than what is considered healthy for a certain height (http://www.nhlbi.nih.gov/health/health-topics/topics/obe/#). The global epidemic of overweight and obesity has become so extensive that it has been termed as 'globesity' by the World Health Organization. According to the data collected from 188 countries in 2014, around 2.1 billion people (30% of the world's population) are either overweight or obese(Ng et al., 2014). More than 50% of the 671 million obese (BMI ≥ 30 kg/m^2) individuals in the world reside in ten countries: USA, China, India, Russia, Brazil, Mexico, Egypt, Germany, Pakistan and Indonesia (listed in order of the number of obese individuals) (Ng et al., 2014).In clinical practice, obesity is usually measured by Body Mass Index (BMI), waist circumference or waist-to-hip circumference ratio (Misra et al., 2009).BMI is the most researched and widely used tool to calculate overweight and generalized obesity in children as well as adults. Waist circumference and waist-to-hip circumference ratio are used as a measure for abdominal obesity. BMI has been linked to the incidences of various conditions such as cancer (Renehanet al., 2008) and chronic obstructive pulmonary disease(Celli et al., 2004).The individuals are usually categorized into underweight, normal weight, overweight and obese categories based on BMI cut-off values.

The ectopic accumulation of adipose tissue in obesity leads to adverse effects on functioning of many organs of body such as liver, heart, kidneys, skeletal muscle, pancreas, joints and central nervous system. This collectively contributes to development of various metabolic disorders such as hypertension, type 2 diabetes mellitus, atherosclerosis, hypercholesterolemia, non-alcoholic fatty liver disease and risk for arthritis (Rasouliet al., 2007). Pathophysiology of many obesity related disorders associates with the involvement of inflammation(Gallettiet al., 2012; Akash et al., 2013; Danieleet al., 2014; Paulettoand Rattazzi, 2015).Adipose tissue secretes a number of adipokines, which play a central role in energy and vascular homeostasis as well as immunity(Maury and Brichard, 2010).The dysregulation of adipokine signalling in obesity leads to inflammation marked by the significant upregulation of pro-inflammatory cytokines such as TNFα. IFN-γ, IL-1β, IL-6 and IL-12(Schmidt et al.,

2015).Overweight and obesity are also responsible for hyperlipidemia, which collectively count as the risk factors for early diabetic neuropathy(Smith and Singleton, 2013). Leptin, insulin and cortisol in conjunction with glucose have also been implicated in fat metabolism and development of obesity(Koester-Weber et al., 2014).

Perimenopause is a phase marked by extreme changes in body such as altered levels of reproductive and stress hormones, increased fat storage and altered body fat distribution(Chrousoset al., 1998).Majority of women in perimenopause gain weight because of fat storage, particularly in the abdomen(Kontogianniet al., 2004).The current study was carried out in the perimenopausal working women fromGuru Nanak Dev University, Amritsar, Punjab (India) in the age group of 35-58 years. Punjab is one of the economically advanced states of the country with high per capita income. The state houses a high percentage of affluent and well-to-do families who are exposed to a modern sedentary lifestyle. Moreover, the people are accustomed to consume oily, spicy and processed food. The combined effect of sedentary lifestyle and unhealthy diet makes people prone to obesity. A study conducted in 2005 reported the prevalence of 43.88% obesity in the urban females of Punjab(Sidhu et al., 2005). A 2010 study has reported the prevalence of 70.30% and 75.09% obesity among premenopausal and postmenopausal working women, respectively, in Jalandhar district of Punjab (Khokhar et al., 2010).A survey conducted in 2014-2015 reported the presence of 29.3% and 30.1% overweight subjects among rural and urban females of Punjab, respectively. The prevalence of obesity was 11.4% and 19.9% among rural and urban females, respectively(Tripathy et al., 2016).

Considering higher prevalence of obesity in urban women, we particularly included the working perimenopausal women in our study. The socio-economic status of urban upper middle class population of Punjab is similar to that of developed societies, especially with respect to nutritional intake and living conditions, which has led to the changes in the patterns of food consumption, dietary intake and physical activity levels. This has contributed to the increase in overweight and obesity among the population of Punjab, especially women. So, this study was planned to investigate the differences in metabolic profile among the normal weight, overweight and obese perimenopausal subjects. The cut off values used for the current study were: BMI 17.7-24.9 kg/m^2 (normal weight); BMI 25.0-29.9 kg/m^2 (overweight) and BMI $\geq$ 30.0 kg/m^2 (obese). Besides lipid profile, the markers of stress and inflammation were studied in our selected group. Among the three BMI categories, we examined differences in the levels of leptin,

which is directly linked to the amount of body fat. Further, the differences in the levels of estradiol and cortisol in the perimenopausal women of three groups were also studied. As obesity is known to cause central and peripheral inflammation, so the levels of circulating pro-inflammatory cytokines TNFα, IL-1β and IL-6 were also compared among the subjects indifferent BMI categories using ELISA and Western blotting. Expression level of GFAP, which is a marker for neurological disorders, was also studied among the three groups by Western blotting.

SUBJECTS AND METHODS

Subjects

The women volunteers in the perimenopausal age group of 35-58 yearswere recruited for the study from Guru Nanak Dev University. For this purpose, a two-day medical check-up camp was organized in the Health Centre of the university. After an initial screening on first day, 62 subjects were included in the study. Weight, height and blood pressure (BP) of the subjects were recorded using a weighing scale, stadiometer and sphygmomanometer, respectively. BMI was calculated using formula: weight in kg divided by square of height in meters.Among these subjects, 23women were in normal weight category, 24 in overweight category and 15 in obese category (as defined in WHO 2000 report). No subject was reported to have any disease history. All the subjects provided written informed consent before participating in the study. The study was approved by the Institutional Ethical Review Committee. All the experiments were performed in accordance with the institutional guidelines and ethical standards as laid down in the Declaration of Helsinki. Blood samples were collected from the subjects after 12 hours of fasting on second day, which were allowed to clot for 30 minutes at room temperature, followed by centrifugation at 2000 rpm for 15 minutes. The serum was separated and used for further metabolic analyses.

Analysis of lipid profile and fasting blood glucose (FBG)

The analysis of cholesterol, triglycerides, HDL and FBG for each sample was done on Biochemical Analyzer XL 640 (Erba Mannheim, Germany), a fully automated, random access, clinical chemistry analyzer using XL system packs. The values of LDL and VLDL for each

sample were calculated using lab management software 'PathoGold Express' by Birlamedisoft Pvt. Ltd. (Pune, India).

Estimation of leptin level

The level of leptin in each sample was analysed using sandwich ELISA kit from Sigma Aldrich (St. Louis, MO, USA) based on colorimetric detection according to manufacturer's protocol. The concentration of leptin in each sample was calculated using kit provided standards and Sigma Plot software.

Estimation of insulin, cortisol and estradiol

The insulin, cortisol and estradiol level in serum for each sample was assayed using Access 2 Immunoassay System (Beckman Coulter, CA, USA), an automated dioxetane-based chemiluminescent detection system using calibrators and assay kits for insulin, cortisol and estradiol respectively. HOMA-IR (insulin resistance index) was calculated using formula: [Insulin (μU/mL) * blood glucose (mg/dL)] / 405

Estimation of pro-inflammatory cytokines levels in serum by ELISA

The levels of pro-inflammatory cytokines TNFα, IL-1β and IL-6 were assayed using sandwich ELISA kits from Cayman Chemical Company (Ann Arbor, MI, USA) based on colorimetric detection according to manufacturer's protocol. The concentrations of TNFα, IL-1β and IL-6 in each sample were calculated using kit provided standards and Sigma Plot software.

Expression study of proteins by Western blotting

The protein estimation in serum samples was done by Bradford method. The samples were processed and used for Western blot analysis according to the previously described method(Kaur et al., 2017). Primary antibodies used were mouse anti-TNFα (1:1000), mouse anti-IL-1β (1:1000), mouse anti-IL-6 (1:1000) and mouse anti-GFAP (1:2000) (Sigma Aldrich, St. Louis, MO, USA) and secondary antibody used was goat anti-mouse IgG-HRP (1:2500) (Merck Millipore, USA). Albumin was used as internal control for protein loading.

Statistical Analysis

Values are expressed as Mean±SEM. Sigma Stat for Windows (version 3.5) was used to analyze the results.Significance of means was determined by One-way ANOVA. Multiple comparisons of mean differences among the variables were done using Holm-Sidak post-hoc test. Values with $p \leq 0.05$ were considered as statistically significant.

RESULTS

Overweight and obese individuals showed increased BP and adverse lipid profile

The differences in the body weight, BP, lipid profile and FBG amongst different groups are shown in Table 1.

Table 1: Body weight, BMI, Blood pressure and serum levels of Cholesterol, Triglycerides, HDL, LDL, VLDL and FBG and HOMA-IR in normal weight, overweight and obese subjects.

	Normal Range	Normal Weight (n=23)	Overweight (n=24)	Obese (n=15)
Body Weight (kg)		55.91±0.95	70.92±1.01*	76.00±1.93* #
BMI (kg/m^2)		22.70±0.35	26.91±0.20*	31.55±0.63* #
Age (years)		44.22±1.52	45.53±1.58	47.30±1.62
Systolic BP (mm Hg)	120	121.96±2.22	135.17±2.53*	143.67±4.35*
DiastolicBP (mm Hg)	80	77.30±1.12	84.04±1.12*	88.67±2.56* #
Cholesterol(mg/dL)	<200.0	180.04±3.97	186.83±4.02	188.67±6.05
Triglycerides(mg/dL)	<150.0	147.61±2.11	152.83±2.62	153.27±3.46
HDL(mg/dL)	40.0-60.0	48.91±0.66	47.50±0.72	47.40±0.62
LDL(mg/dL)	<100.0	99.44±3.40	104.85±3.61	110.23±5.83
VLDL(mg/dL)	2.0-30.0	29.70±0.46	30.61±0.53	30.78±0.68
FBG (mg/dL)	70.0-100.0	80.78±1.52	88.08±2.36	87.07±3.28
HOMA-IR		2.73±0.07	2.94±0.08	3.16±0.13*

Values are represented as Mean±SEM. * $p \leq 0.05$ normal weight v/s overweight and obese subjects, # $p \leq 0.05$ overweight v/s obese subjects, Holm-Sidak method after one-way ANOVA.

The overweight and obese subjects exhibited higher BP and higher levels of FBG. Systolic BP showed significant increase by 10.8% and 17.8% in overweight and obese subjects, respectively, as compared to normal weight women. Diastolic BP also showed significant increase by 8.7% and 14.7% in overweight and obese subjects. The level of FBG increased by 9.03% and 7.8% in overweight and obese subjects as compared to normal weight group.

The serum levels of cholesterol, triglycerides and LDL showed slight but consistent increase in overweight (by 3.7%, 3.5% and 5.4% respectively) and obese subjects (by 4.8%,

3.8% and 10.8% respectively) as compared to the normal weight group although the changes were not statistically significant. On the other hand, the level of HDL and VLDL in both overweight and obese women was similar to the normal weight group.Moreover, the levels of triglycerides and LDL werehigher than the normal range in both overweight and obese subjects with obese women showing more pronounced increase.

Hyperleptinemia and hyperinsulinemia in overweight and obese subjects

Serum levels of leptin and insulin are shown in Fig. 1 (a-c).The level of leptin in overweight and obese groups was significantly ($p<0.001$) increased by 40.3% and 70.7% respectively, as compared to normal weight subjects. The level of insulin was not different between normal weight and overweight subjects but it was significantly higher ($p\leq0.05$) in obese women as compared to overweight subjects.Insulin resistance index (HOMA-IR) also showed increase in overweight and obese subjects as compared to normal weight subjects (Table 1).

Obese individuals showed increase in cortisol and decrease in estradiol levels

Serum levels of cortisol and estradiol are shown in Fig. 1 (a, d, e). The overweight subjects did not show any change in the level of cortisol as compared to normal weight group, whereas, it increased by 8.3% in obese subjects. On the other hand, the level of estradiol significantly decreased by 21% and 34.4% in overweight and obese subjects ($p\leq0.05$), respectively, as compared to normal weight subjects.

Obese individuals showed upregulated levels of pro-inflammatory cytokines

The results of ELISA based estimation of TNFα, IL-1β and IL-6 among the three groups are shown in Fig. 2 (a-d). Serum levels of TNFα were approximately 1.4-fold and 1.7-fold higher in overweight and obese category, respectively, compared to normal weight subjects ($p\leq0.02$). The rate of increase in level of TNFα correlated with that of serum leptin, whereas, IL-1β level was not different among the three groups. The level of IL-6 increased by 13.8% in obese subjects as compared to normal weight subjects. However, the normal weight and overweight subjects showed similar level of IL-6.

(a)

	Normal Range	Normal Weight (n=23)	Overweight (n=24)	Obese (n=15)
Leptin (ng/mL)	4.1-25.0	13.40±1.27	18.77±1.26*	22.15±1.58*
Insulin (µU/mL)	2.0-20.0	13.35±0.43	12.96±0.46	15.00±0.66#
Cortisol (µg/dL)	5.0-25.0	16.87±0.74	16.29±0.75	18.27±1.03
Estradiol (pg/mL)	20-443 (depending upon stage of ovarian cycle)	99.91±7.80	78.92±8.98	65.53±9.45*

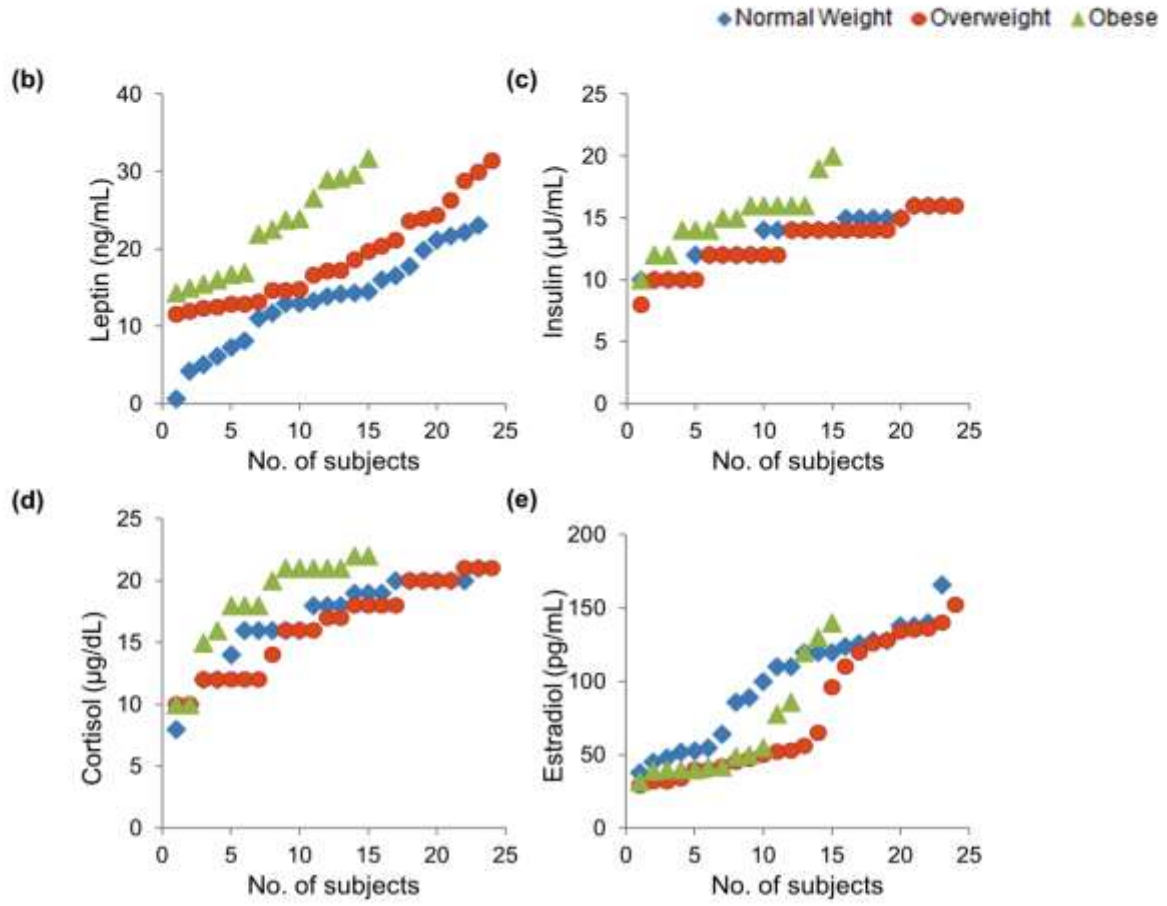

Fig. 1: Serum levels of Leptin, Insulin, Cortisol and Estradiol in normal weight, overweight and obese subjects. Values are represented as Mean±SEM. * $p \leq 0.05$ normal weight v/s overweight and obese subjects, # $p \leq 0.05$ overweight v/s obese subjects, Holm-Sidak method after one-way ANOVA.

Further, Western blot analyses also confirmed the upregulation in expression and secretion of TNFα, IL-1β and IL-6 along with increase in the expression of GFAP, a marker related to neurological disorders in overweight and obese subjects of our study [Fig. 2 (e-h)]. TNFα showed 4.6% and 13.5% increase in overweight and obese subjects respectively, whereas, IL-1β increased by 10.8% and 18.5% in overweight and obese subjects respectively as compared to normal weight subjects. On the other hand, the level of IL-6 and GFAPin serum was not different in overweight subjects as compared to normal weight subjects, however, obese subjects showed 25.5% increase ($p=0.054$) in IL-6 and 11.2% increase in serum GFAP as compared to normal weight subjects (Fig. 2e, g).The secretory isoforms of TNFα, IL-1β and IL-6 (Fig. 2f, h) also exhibited upregulation in the expression corresponding to BMI values. sTNFα increased by 4.4% and 12.2% in the overweight and obese subjects respectively. Similarly, sIL-1β showed significant increase by 47.6% and 49.5% in overweight and obese subjects respectively. However, the level of sIL-6was not different between overweight and normal weight subjects but obese subjects showed 7.4% increase in the level of sIL-6 as compared to normal weight subjects.

DISCUSSION

The current study investigated the correlation of BMI with various metabolic, hormonal and inflammatory markers in working perimenopausal women from Amritsar, Punjab (India). The elevated levels of triglycerides and LDL and low levels of HDL collectively reflect dyslipidemia profile indicating the 'atherogenic lipoprotein phenotype' (Musunuru, 2010). Dyslipidemia was more pronounced in obese subjects as compared to overweight individuals. Atherogenicdyslipidemia, a hallmark feature of obesity, has been attributed to increase in intra-abdominal fat (Nieves et al., 2003). In obesity, increased flux of free fatty acids to the liver causes hepatic accumulation of triglycerides leading to hypertriglyceridemia, which further increases the synthesis of LDL and VLDL in liver(Capell et al., 1996). The lipolysis is also impaired by reduced expression of lipoprotein lipase in obesity, which results in reduced concentration of HDL(Klop et al., 2013). Thus, higher BMI values of overweight and obese individuals, as observed in the current study, may be the outcome of increase in intra-abdominal fat deposition which may have resulted in atherogenic lipoprotein phenotype. Atherogenic dyslipidemia has been commonly used as an index for the evaluation of risk of cardiovascular diseases (Musunuru, 2010), rheumatoid arthritis (Singh et al., 2013) and polycystic ovary

syndrome (Valkenburget al., 2008). It is also associated with low bone mineral density in early postmenopausal overweight women (Orozco, 2004).

(a)

	Normal Weight (n=23)	Overweight (n=24)	Obese (n=15)
TNFα (pg/mL)	14.83±1.72	20.81±2.61	25.32±3.18*
IL-1β (pg/mL)	14.17±2.04	14.68±3.29	13.98±4.49
IL-6 (pg/mL)	14.52±0.41	14.76±0.56	16.52±1.42

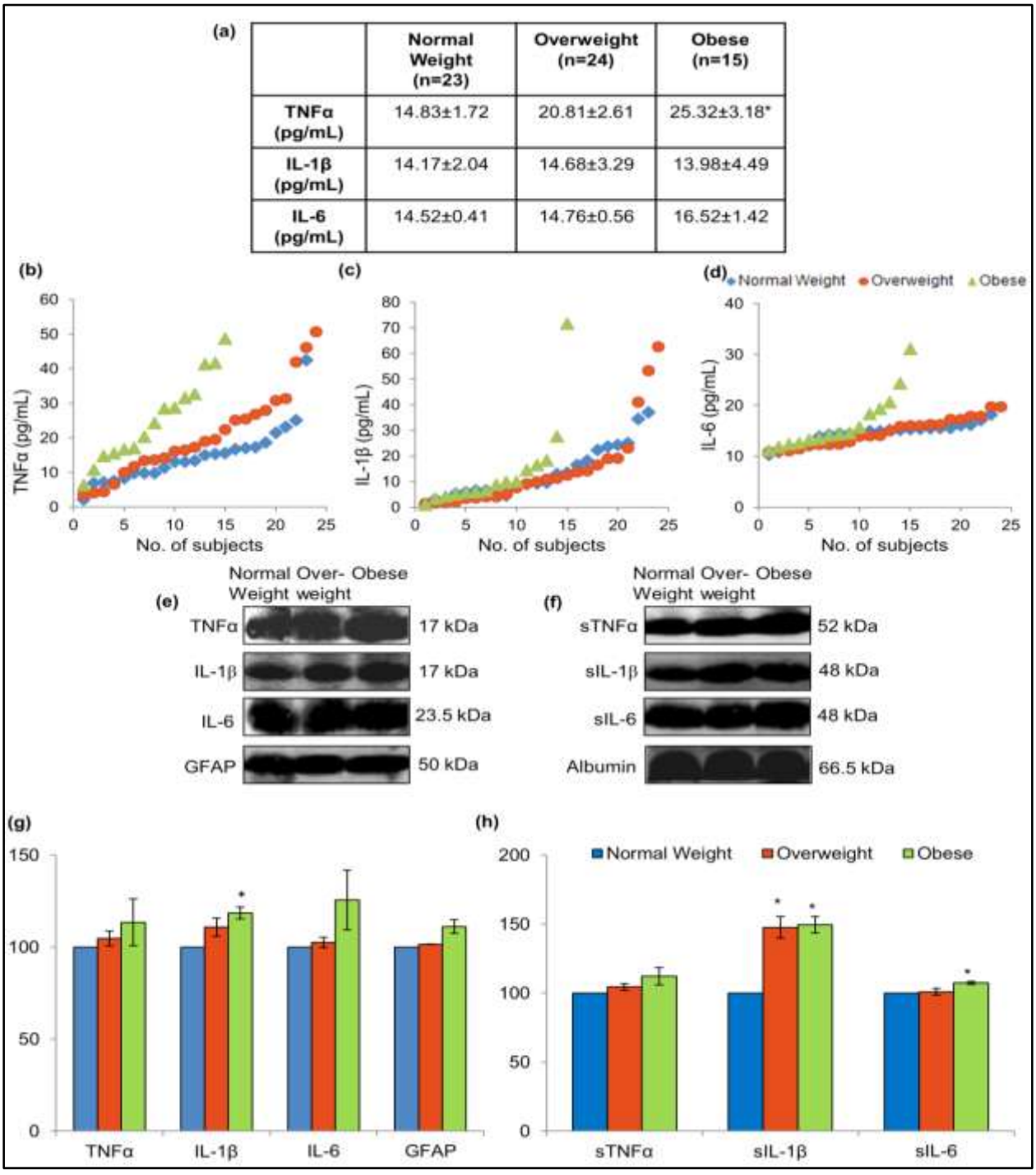

Fig. 2: (a-d) Serum levels of TNFα, IL-1β and IL-6 in normal weight, overweight and obese subjects by ELISA. **(e-h)** Representative Western blot analysis for pro-inflammatory cytokines TNFα, IL-1β and IL-6; and GFAP in serum of normal weight, overweight and obese subjects. Histograms represent percent change in intensity taking intensity in normal weight subjects as 100%. Values are represented as Mean±SEM. * $p \leq 0.05$ normal weight v/s overweight and obese subjects, Holm-Sidak method after one-way ANOVA.

Further, the production of cholesterol has been linearly correlated to the amount of body fat. 20 mg of additional cholesterol is synthesized for each kilogram of extra body fat(Bray, 2004). Thus, the higher level of cholesterol in the overweight and obese subjects of our study may be attributed to the extra amount of intra-abdominal fat. Hypercholesterolemia in obesity has been linked to arterial dysfunction and oxidative stress(Loffredoet al., 2012), which may lead to the disruption of hormone secretion by adipose tissue. The most affected hormones include leptin, adiponectin, visfatin and adipokines that play an indispensable role in numerous metabolic pathways responsible for regulation of basic physiological functions such as appetite and satiety(Aguilar and Fernandez, 2014).

Since the levels of leptin correlate linearly with the amount of body fat(Shah and Braverman, 2012),so the corresponding increase in the level of leptincorrelates to the higher content of body fat in these subjects irrespective of their menopausal status. Our lab has earlier reported that obese women in both premenopausal and postmenopausal phase have significantly higher leptin levels than their normal weight counterparts(Khokharet al., 2010). Increased serum concentrations of leptin have been suggested to be the result of leptin resistance. In obese individuals, increased adipose tissue requires an increase in vascular bed to maintain baseline circulation (Sierra-Honigmannet al., 1998). *In vivo* studies have reported increase in endothelial cell proliferation, a critical step for angiogenesis, after implantation of leptin infused discs subcutaneously (Anagnostoulis et al., 2008). The increase in the vascular endothelium may restrict the entry of leptin via Ob-Ra receptor located in the blood-brain barrier into the CNS. Saturation in this transport mechanism due to limited tissue access may cause leptin resistance(Martin et al., 2008). Chen et al. (2006) reported the presence of several serum leptin-interacting proteins (SLIPs) isolated by leptin affinity chromatography. One of the major SLIPs identified is C-reactive protein (CRP) that directly inhibits the binding of leptin to its receptor and prohibits the ability of leptin to activate STAT3, which is an absolute requirement for the effects of leptin intake of food and hepatic metabolism of glucose (Buettneret al., 2006). Binding of leptin by CRP may impede the flow of leptin into the CNS leading to leptin resistance.

Interestingly, the percentage change observed in serum leptin correlated with key inflammatory molecule TNFα. The corresponding increase in the expression of TNFα and leptin raises the possibility that their production might be interdependent and regulated by similar mechanisms. One of the known functions of TNFα is to directly on regulate the release of pre-

formed pool of leptin (Kirchgessner et al., 1997).The elevated expression of TNFα in overweight and obese subjects may be a contributing factor to hyperleptinemia.

Further, the increase in the level of fasting insulin in obese subjects may also be attributed to the elevated level of TNFα. Several studies have reported significant correlation between TNFα and fasting insulin(Nieto-Vazquez et al., 2008). The overexpression of TNFα activates proinflammatory pathways, which affect glucose uptake by adipocytes and myocytes and impair insulin signaling at the level of insulin receptor substrate (IRS) proteins(Nieto-Vazquez et al., 2008). High secretion of TNFα from human adipose tissue has been associated with decrease in [^{3}H]glucose incorporation into the lipids(Löfgrenet al., 2000). TNFα causes the activation of ERK and p38-MAPK pathways, which weaken the Tyr phosphorylation induced by insulin. Further, in obesity, the need for enhanced fatty acid β-oxidation causes overactivation of mitochondria which results in the generation of high amounts of ATP. This surplus energy, by negative feedback regulation, inactivates AMPK signalling pathway to reduce insulin-induced glucose uptake in order to decrease ATP production(Ye, 2013).Thus, the upregulation of TNFα and enhanced fatty acid β-oxidation may be linked to the elevated levels of insulin observed in the obese subjects. Insulin resistance may in turn be responsible for the higher levels of FBG observed in the overweight and obese subjects.

Marginal increase in cortisol was observed in obese group. A positive correlation between BMI and cortisol levels has been reported previously (Stalder et al., 2012).The production of cortisol depends on the functioning of HPA axis(Rus et al., 2016), which is, in turn, influenced by cytokines. Obese individuals have shown abnormalities in the regulation of HPA axis (Pasqualiand Vicennati, 2000). Adrenal cortex is stimulated by the pituitary gland to produce and release of cortisol, which exerts negative feedback regulation via glucocorticoid (GRs) and mineralocorticoid receptors (MRs). Upon binding of these receptors with cortisol, the activity of HPA axis is controlled (Rosmondet al., 2000). In mouse model of obesity, overexpression of 11-beta hydroxysteroid dehydrogenase 1 (HSD1) using adipose specific promoter has shown higher production of cortisol locally within adipose tissue, which further leads to adipocyte hypertrophy and expansion of visceral fat(Lee et al., 2014).High amount of GRs and MRs in obese individuals require higher amount of cortisol for the stabilization of HPA axis leading to the elevated levels of cortisol in obesity.

Further, the high amount of visceral adipose tissue in perimenopausal women has been earlier associated with decrease in circulating estradiol and increase in serum FSH(Lovejoy et al., 2008). High amount of body fat activates lipotoxicity in obese women, which may lead to perturbation of hormone balance intrigued by intracellular lipid accumulation, inflammatory response, oxidative stress and endoplasmic reticulum stress (Robkeret al., 2011). The normal weight, overweight and obese subjects were age-matched, so the possibility of decline in estradiol levels due to aging or menopausal transition is ruled out. Thus, the decline in estradiol may solely be attributed to the high amount of body fat in overweight and obese subjects, which is also supported by the fact that the level of estradiol showed further decrease from overweight to obese women. In a longitudinal study by Freeman et al. (2010), negative association between BMI and estradiol levels in premenopausal women has been reported, which was found to be reversed in post-menopause.The results of our study are in line with previous report(Pergola et al., 2006), wherein inhibition of estradiol secretion in obese fertile women has been attributed to hyperinsulinemia and has been negatively correlated to the BMI value.

Significantly high levels of TNFα in overweight and obese subjects, and increase in IL-6 in obese subjects confirms the induction of inflammation occurring due to obesity in these subjects. White adipose tissue in obese individuals has been reported to exhibit inflammation, which is defined by infiltration of macrophages, leukocytes, mast cells and $CD8^+$ T lymphocytes into the adipose tissue(Osborn and Olefsky, 2012). Paracrine interactions among macrophages and other cell types create an inflammatory milieu in adipose tissue. Lipolysis during obesity generates saturated fatty acids which activate TLR4 signalling and stimulate NFκB signalling pathway, which further activates transcription of proinflammatory genes such as COX-2, TNFα, IL-1β and IL-6(Howe et al., 2013), resulting in inflammation. Further, GFAP a marker for reactive gliosis, is a hallmark feature in stressful conditions. In humans, serum GFAP has been reported to be a highly specific biomarker for traumatic brain injury(Honda et al., 2010).Several animal studies have shown the induction of reactive gliosis and neuroinflammation in models of obesity (Dorfmanand Thaler, 2015)but no such data is available for human subjects. Thus, the increase in GFAP expression in obese subjects may be due to neuroinflammation and induction of astrogliosis occurring due to obesity.

The possible limitation of our study is the lack of measurement of some anthropometric parameters, like waist and hip circumference and body fat percentage. Although our study

groups contained relatively small number of subjects, the relationships observed between BMI and other parameters were strong and statistically significant. The strength of our study is the evaluation of large number of parameters in our study cohort.

CONCLUSIONS

The principal observation of this study was the relationship of leptin and estradiol with BMI and inflammatory cytokine TNFα in the context of inflammation in obese subjects. The association observed between overweight and obesity with dyslipidemia, serum leptin, cortisol, insulin and inflammatory cytokines seems to be independent of age and menopausal status and showed direct relationship with BMI values of these perimenopausal women as suggested in the schematic diagram (Fig. 3). Moreover, increase in BP in overweight and obese subjects along with FBG suggest the presence of subclinical hypertension(Han et al., 2015), which is further supported by more pronounced changes observed in obese subjects as compared to overweight subjects. Although obese subjects in the study did not report any disease history other than subclinical hypertension, but marked reduction in estradiol with significant increase in TNFα and leptin in these subjects seems to be significantly influenced by BMI and may be considered as important biomarkers for follow-up study of these subjects for obesity related co-morbid conditions.

LIST OF ABBREVIATIONS

AMPK	AMP Activated Protein Kinases
BMI	Body Mass Index
BP	Blood Pressure
CNS	Central Nervous System
COX2	Cyclooxygenase 2
ERK	Extracellular Signal-regulated Kinases
FBG	Fasting Blood Glucose
GFAP	Glial Fibrillary Acidic Protein
HDL	High Density Lipoprotein

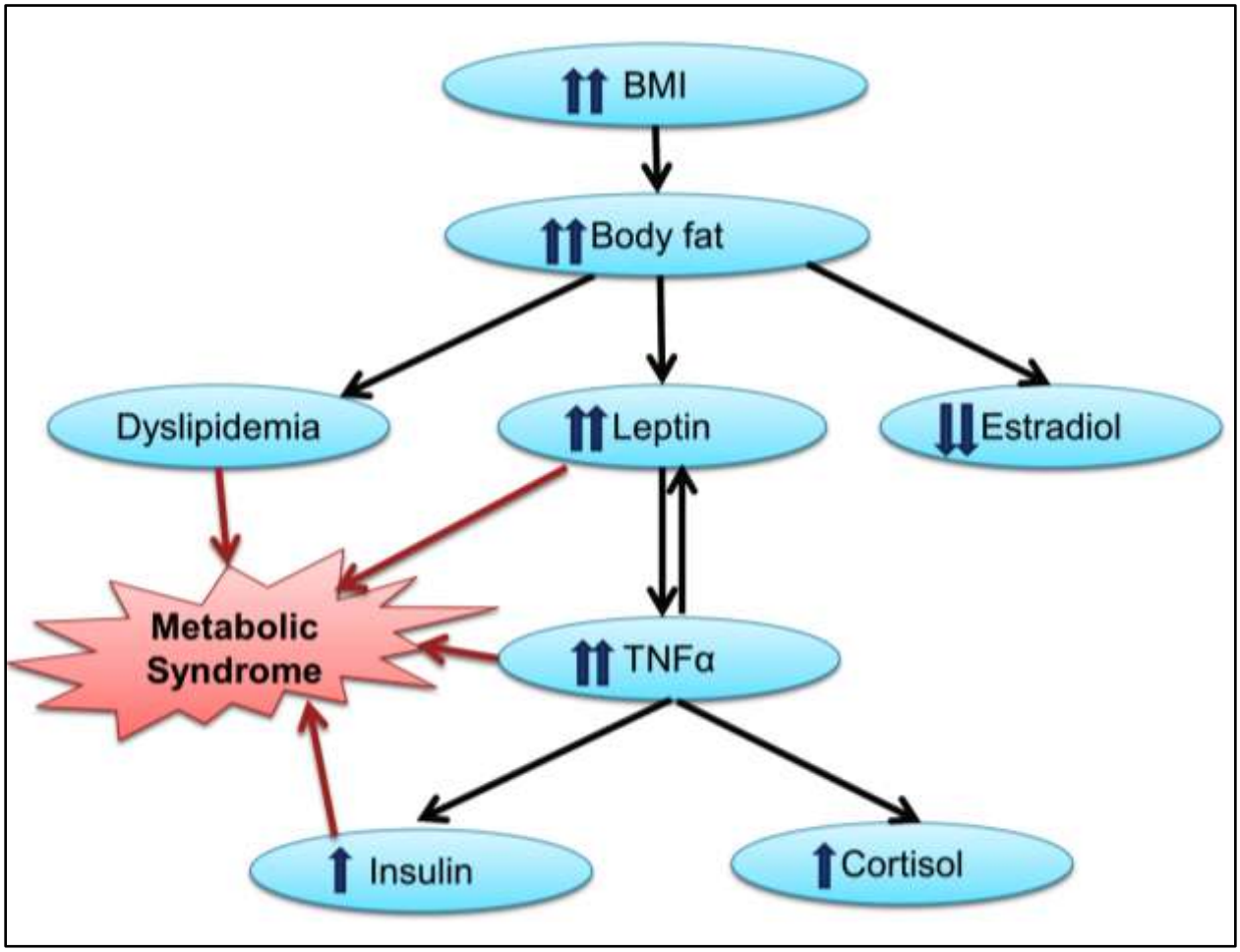

Fig. 3: Schematic representation of the study. BMI directly affects lipid profile, leptin, estradiol and TNFα levels, which further cause hyperinsulinemia and hypercortisolemia. These factors collectively contribute to the pathology of metabolic syndrome.

HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
HPA	Hypothalamic-Pituitary-Adrenal
IFN-γ	Interferon γ
IL-12	Interleukin - 12
IL-1β	Interleukin - 1β
IL-6	Interleukin - 6
IRS	Insulin Receptor Substrate
LDL	Low Density Lipoprotein
MAPK	Mitogen Activated Protein Kinases
SEM	Standard Error Mean

sIL-1β	Secreted form of Interleukin - 1β
sIL-6	Secreted form of Interleukin – 6
SLIP	Serum Leptin Interacting Protein
STAT3	Signal Transducer and Activator of Transcription 3
sTNFα	Secreted form of Tumor Necrosis Factor α
TLR4	Toll-like Receptor 4
TNFα	Tumor Necrosis Factor α
VLDL	Very Low Density Lipoprotein

DECLARATIONS

Ethics approval and consent to participate

All the subjects provided written informed consent before participating in the study. The study was approved by the Institutional Ethics Committee of Guru Nanak Dev University, Amritsar (Reference Number 656/HG dated March 29, 2016). All the experiments were performed in accordance with the institutional guidelines and ethical standards as laid down in the Declaration of Helsinki.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by the Department of Biotechnology, Ministry of Science and Technology, Government of India project grant (102/IFD/SAN/3330/2015-2016) to Gurcharan Kaur. The funding source had no role in study design; collection, analysis and interpretation of data; in writing of report; and in decision to submit the article for publication.

Authors' contributions

TK, SKG and GK conceived and designed the study. TK, BS and SKG acquired the data. TK, BS and GK analyzed and interpreted the data. TK and GK wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors are thankful to Ms. Vandana Khanna and Mr. Kuldeep Singh for their help and support in organizing the camp in health centre. Taranjeet Kaur is thankful to University Grants Commission (UGC), GOI for fellowship grant during the entire course of study. Infrastructure

provided by UGC under University with Potential for Excellence (UPE) and Centre with Potential for Excellence in Particular Area (CPEPA) schemes and Department of Biotechnology (DBT), India under DISC facility is highly acknowledged. Harpal Singh, Shaffi Manchanda, VedanganaSaini, Anuradha Sharma, Muskan Gupta and Rachana Mishra are deeply acknowledged for their help in initial screening and coordination with the subjects during the camp.

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