

# Comparative Study of Inflammatory and Oxidative Stress Biomarkers in Different Metabolically Healthy Obesity Phenotypes

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## Abstract

**Aims:** Obesity is the major contributor of the metabolic syndrome (MetS), but a unique phenotype of obesity known as metabolically healthy obese (MHO) shows healthier metabolic profile; however understanding of their biochemical correlates is poorly understood. Obesity is defined by Body mass index (BMI), but controversy exists regarding ethnic-specific BMI cut-offs. The present study used the Asian Indian BMI cut-offs to assess relationships of MHO phenotypes with oxidative stress (OS) and inflammation. **Methods:** In this case-control study, 299 metabolically-healthy (MH) respondents were divided into four groups as per Asian criteria for obesity: MH non-obese (MHNO), MH overweight (MHOW), MHO and MH severely obese (MHSO). Their oxidative stress and pro-inflammatory markers were measured. **Results:** Levels of hydroxyl radicals ( $\cdot\text{OH}$ ), fluorescent oxidation products (FLOP), MDA, PCO and inflammatory markers CRP, TNF- $\alpha$ , IL-6 were highest in MHSO phenotype followed by the MHO, MHOW and MHNO groups ( $p > 0.0001$ ), whereas antioxidant markers, CuZn-SOD, catalase, glutathione peroxidase and total antioxidant activity followed the reverse trend. The MHNO and MHOW groups showed significant difference with regard to ( $\cdot\text{OH}$ ) radicals and FLOP. Moreover,  $\cdot\text{OH}$  radicals, FLOP and inflammatory markers were significantly correlated to BMI in MHSO and MHO but not in MHNO and MHOW group. **Conclusion:** The MHO and MHSO phenotype display differences in terms of OS and inflammatory markers at lower BMI cut-offs, indicating that they may be on the way to becoming “unhealthy” obese. The lower BMI cut-offs proposed by Indian Consensus Group would help in understanding of manifestation of metabolic syndrome.

## Keywords

Metabolically Healthy Obesity, Metabolic Syndrome Risk Factors, Oxidative

## 1. Introduction

Obesity has been recognized as the major contributor to the global epidemic of metabolic syndrome, which has been defined as a cluster of conditions that occur together to increase the risk of heart disease, stroke and type 2 diabetes. However, these metabolic abnormalities do not affect all obese people and the concept of “healthy obesity” was suggested by Sims several decades ago, in 1985, as a subtype in the classification of obesity [1].

The issue is under more active investigation now, and those who are obese but are not affected by metabolic disturbances have been designated as “metabolically healthy obese” (MHO) phenotype [1]. They are, by definition, insulin sensitive, have normal blood pressure, favorable lipid profile, a lower proportion of visceral fat, less liver fat and normal glucose metabolism despite having an excessive amount of body fat [2] [3], and are reported to be associated with substantially lower risk of metabolic complications [4] and account for about 10% - 25% of obese people [5]. There is no definite classification criterion to describe MHO. However, the most acceptable criterion to define MHO in clinical practice is the absence of Metabolic Syndrome [3] [6], as defined by the NCEP-ATP III criteria [7] in overweight/obese subjects.

Biochemical differences have been suggested between “unhealthy obese” and “healthy obese”. The former, but not the latter, have been reported to benefit from weight loss [8], and MHO subjects have been reported to have lower oxidative stress, inflammatory markers and diminished adipose tissue macrophage infiltration in comparison to metabolically unhealthy obese individuals [9].

The currently most accepted categorization of obesity and overweight is body mass index (BMI), which is used within each population to identify the proportion of people with a high risk of an undesirable health state that warrants public health or clinical intervention. WHO categorizes those with  $BMI \leq 25 \text{ kg/m}^2$  as non-obese;  $25 - 30 \text{ kg/m}^2$  as overweight, and  $BMI > 30 \text{ kg/m}^2$  as obese. However, the last several years have witnessed a debate on whether ethnic-specific cut-off points for BMI for Asians are required in the light of scientific evidence that Asian populations have different associations between BMI, percentage of body fat, and health risks than European populations. In 2004, a WHO Expert Consultative Committee [10] addressed this debate and concluded that while the proportion of Asian people with a high risk of type 2 diabetes and cardiovascular disease is substantial at lower BMIs but available data do not necessarily indicate a clear BMI cut-off point for all Asians for overweight or obesity. The cut-off point for observed risk varies from  $22 \text{ kg/m}^2$  to  $25 \text{ kg/m}^2$  in different Asian populations; for high risk it varies from  $26 \text{ kg/m}^2$  to  $31 \text{ kg/m}^2$ , so the committee did not recommend redefining cut-off points for each population separately, and

agreed that the WHO BMI cut-off points should be retained as international classifications. However, they did identify a BMI of 23 as one of the potential public health action point.

The Indian Consensus Group (for Asian Indians residing in India) has persisted with its concerns and has published definitive guidelines recommending revised guidelines for Asians, particularly Indians, to classify a BMI of  $\geq 23$  kg/m<sup>2</sup> and  $\geq 25$  kg/m<sup>2</sup> as overweight and obese, respectively, because several studies have shown higher body fat, excess metabolic perturbations, and cardiovascular risk factors at lower value of BMI in Asian versus white populations [11].

In view of the foregoing, the present study has been undertaken to compare inflammatory and oxidative stress biomarkers in different metabolically healthy obesity phenotypes, which do not display the NCEP-ATP III risk factors for metabolic syndrome, and employing the BMI classifications for Asian Indians.

## 2. Material and Methods

### 2.1. Subjects

A large number of healthy respondents attending outpatient departments (OPD) in government hospitals and pathologies at Allahabad, India, during the years 2015 to 2017 were screened for metabolic syndrome (MetS) risk factors described by the US (NCEP) ATP III [6], according to which respondents suffering from any three of the following risk factors, namely, Central obesity (waist circumference  $\geq 102$  cm/40 inches (male),  $\geq 88$  cm/35 inches (female)), Dyslipidemia (TG  $\geq 150$  mg/dl, HDL-C  $< 40$  mg/dL (male),  $< 50$  mg/dL (female)) and Blood pressure  $\geq 130/85$  mmHg (or treated for hypertension) and Fasting plasma glucose  $\geq 110$  mg/dl) were excluded. Such respondents were included in the study and are termed “Metabolically Healthy (MH)” after ascertaining that they did not suffer from any infectious or non-communicable disease.

Their height and weight were measured following standard procedures, as recommended by WHO [12]. Their waist circumference (WC) was also measured, using a flexible measuring tape, at the natural waistline above the umbilicus and below the rib cage and recorded in centimeters. Body mass index (BMI) was computed as  $BMI = \text{weight (kg)}/[\text{height (m)}^2]$ . Their blood pressure was recorded following prescribed norms.

This case-control study was conducted on 299 respondents who fulfilled all inclusion and exclusion criteria. As described by guidelines by the Indian Consensus Group (for Asian Indians residing in India) [11], they were divided into the following four categories of metabolically healthy respondents:

- 1) Metabolically healthy non obese (MHNO) (BMI 18.5 - 22.9 kg/m<sup>2</sup> N = 96);
- 2) Metabolically healthy overweight (MHOW) (BMI 23 - 24.9 kg/m<sup>2</sup> N = 54);
- 3) Metabolically healthy obese (MHO) (BMI 25 - 29.9 kg/m<sup>2</sup> N = 147);
- 4) Metabolically healthy severe obese (MHSO) (BMI  $> 30$  kg/m<sup>2</sup> N = 102).

Respondents who gave their written informed consent were included in study. The study protocol was approved by the Institutional Ethics Committee of Pop-

ulation Resource & Research Centre (PRRC), Allahabad.

## 2.2. Blood Collection

Blood samples were collected, divided in sodium citrate anticoagulant-containing and plain vials, and processed to obtain packed red blood cells (RBCs), plasma and serum. RBCs were further processed to obtain hemolysate as described earlier [13], and stored at  $-80^{\circ}\text{C}$  until analysis.

## 2.3. Biochemical Parameters

### 2.3.1. Fasting Blood Glucose and Lipid Profile

The measurements of fasting blood glucose, total cholesterol, triglyceride and HDL cholesterol were performed with the autoanalyser kits manufactured by ERBA diagnostics Mannheim, Germany using semi-autoanalyser Chem-7, Erba Mannheim. The LDL-Cholesterol was calculated by using the Friedewald formula:  $\text{LDL-C} = \text{TC} - \text{HDL-C} - (\text{TG}/5)$ .

### 2.3.2. Oxidative Damage Markers

Hydroxyl radical ( $\cdot\text{OH}$ ), fluorescent oxidation product (FLOP) in plasma, and malondialdehyde (MDA), protein carbonyl (PCO) in erythrocytes were assessed by the methods of Korotkova, Misini *et al.* [14], Tianying *et al.* [15], Neihaus and Samuelsson [16] and Levine *et al.* [17] respectively, with appropriate modifications.

### 2.3.3. Enzymatic Antioxidants

Erythrocytic superoxide dismutase (CuZn-SOD) and catalase (CAT) and plasma glutathione peroxidase (GPx) were measured by the method of Marklund and Marklund [18], Sinha *et al.* [19] and Rotruck *et al.* [20] respectively.

### 2.3.4. Non Enzymatic Total Antioxidant Capacity (TAC) by Ferric Reducing Capacity of Plasma (FRAP) Assay

FRAP was estimated by the protocol of Benzie and Strain [21].

### 2.3.5. Estimation of Cytokines

C-reactive protein (CRP) was estimated by Automated Bioanalyzer kits (Accurax Biomedical). Serum Inflammatory markers, Human IL-6 and TNF- $\alpha$  were estimated by ELISA kits (Elabscience) according to the manufacturer's protocol.

### 2.3.6. Statistical Analysis

Data were analyzed using Microsoft Excel 2010, Prism Graph Pad 5 and JASP 0.8 software. All results are presented as mean  $\pm$  standard deviation. The statistical significance of the differences between groups was assessed using one way analysis of variance (ANOVA), followed by Tukey's honest significant difference post hoc test to assess all pairwise differences. Pearson correlation coefficients were obtained to see relationship between different variables. Unless stated otherwise, all values at 95% confidence with  $p < 0.05$  were considered statistically significant.

### 3. Results

The baseline characteristics of the study population, presented in **Table 1**, confirms that all groups, namely MHO, MHSO, MHNO and MHOW, based on their BMI are matched for gender, age and height.

NCEP-ATP III prescribed diagnostic and other clinical biochemical measures, revised for Asians, oxidative stress measures, and inflammatory markers in MHNO, MHOW, MHO and MHSO phenotypes are presented in **Table 2**.

The biochemical parameters including fasting plasma glucose, triglyceride, blood pressure were found to be significantly higher ( $p < 0.05$ ), and HDL-cholesterol, lower in MHSO and MHO phenotype as compared to MHOW and MHNO, who did not differ from each other (**Table 2**). Similar results were found for total cholesterol and LDL.

The concentrations of plasma/serum/erythrocytic oxidative stress markers and antioxidant markers were assessed in various phenotypes of obesity and again all OS markers, serum  $\cdot\text{OH}$  radicals and FLOP, Erythrocytic MDA and PCO were significantly higher at  $p < 0.0001$ , and antioxidant enzymes, CuZn-SOD, CAT, and total antioxidant capacity (FRAP) low as obesity became more pronounced, based on one-way ANOVA. Obesity influenced plasma GPx statistically significantly at  $p = 0.032$ . One-way ANOVA also confirmed that inflammatory markers, CRP, TNF- $\alpha$ , and IL6, increased significantly at  $p < 0.0001$  as obesity increased and followed the pattern MHSO > MHO > MHOW > MHNO.

Tukey's Honestly Significant Difference (HSD) post hoc test was performed to assess statistical significance of all pairwise differences between factor level means, and results are presented in **Table 3**.

Tukey's test compared various metabolically healthy phenotypes in pairs, which resulted in six permutations, namely: MHNO vs. MHOW, MHNO vs. MHO, MHNO vs. MHSO, MHOW vs. MHO, MHOW vs. MHSO and MHO vs. MHSO, for 10 parameters. No significant difference was found between the MHNO and MHOW groups with regard to any parameter, including all NCEP-ATPIII risk factors, OS, antioxidant markers and inflammatory markers. WC, BGF, SBP,

**Table 1.** General characteristics of metabolically healthy non obese control (MHNO), metabolically healthy overweight (MHOW), metabolically healthy obese (MHO) and metabolically healthy severe obese (MHSO).

Demographic Data	MHNO	MHOW	MHO	MHSO
N	96	54	147	102
Male/Female	52/44	33/21	74/73	59/43
Age (year)	51.4 $\pm$ 12.9	53 $\pm$ 9.5	52.83 $\pm$ 13.2	54.5 $\pm$ 12.9
Height (cm)	161.5 $\pm$ 6.9	160.6 $\pm$ 7.9	158.8 $\pm$ 6.6	159.6 $\pm$ 6.5
Weight (kilogram)	56.4 $\pm$ 4.7	61.5 $\pm$ 7.3	77.8 $\pm$ 6.8	82.8 $\pm$ 6.4
BMI (kg/m <sup>2</sup> )	21.3 $\pm$ 1.2	23.7 $\pm$ 0.5	26.8 $\pm$ 1.05	32.5 $\pm$ 1.3

All the values are expressed as mean  $\pm$  SD.

**Table 2.** NCEP-ATP III prescribed diagnostic and other clinical biochemical measures, revised for Asians, oxidative stress measures, and inflammatory markers in MHNO, MHOW, MHO and MHSO phenotypes.

MetS risk factors	MHNO	MHOW	MHO	MHSO	F value	p value
<b>Revised NCEP-ATP III Diagnostic Criteria For Mets</b>						
Waist circumference	72.2 ± 8.5	76.3 ± 6.5	85.6 ± 9.6	101 ± 11.9	163.2	<0.0001***
Fasting plasma glucose	83.5 ± 7	85.4 ± 8.6	93.2 ± 5.4	102.5 ± 9.2	126.8	<0.0001***
Triglyceride	126.8 ± 17.3	128.5 ± 18.8	131.5 ± 8.6	152 ± 7.5	76.1	<0.0001***
HDL-cholesterol	54.4 ± 6.9	54 ± 7.3	53.1 ± 5	48.5 ± 6.5	18.3	<0.0001***
Systolic blood pressure	115.1 ± 5.3	116.3 ± 5.3	119.9 ± 4.9	122.5 ± 4.9	76.1	<0.0001***
Diastolic blood pressure	75.7 ± 7.1	76.2 ± 6.4	79.1 ± 4.2	80.9 ± 4.8	18.4	<0.0001***
<b>Other clinical biochemical measures</b>						
Total cholesterol	174.9 ± 9.9	177.8 ± 10.3	181 ± 10.3	200 ± 14.1	97.5	<0.0001***
Low density lipoprotein-C	95.2 ± 12.3	98.1 ± 12.4	144.7 ± 9.7	158.4 ± 13.3	296.2	<0.0001***
Very low density lipoprotein-C	29.8 ± 10.3	29.5 ± 9.6	28.5 ± 4.9	20.8 ± 3.1	33	<0.0001***
<b>Oxidative stress markers</b>						
Serum OH radicals (µmol/L)	237 ± 22	268 ± 32	294 ± 54	413 ± 54	105.1	<0.0001***
Serum FLOP (FI\$/ml)	157 ± 18	162 ± 15	176 ± 31	193 ± 39	7.8	<0.0001***
Erythrocytic MDA (nmoles/g Hb)	0.8 ± 0.4	0.8 ± 0.3	1.5 ± 1	3.5 ± 1	185.1	<0.0001***
Erythrocytic PCO (nmole/g Hb)	0.4 ± 0.1	0.4 ± 0.1	0.9 ± 0.6	2.5 ± 0.8	314.7	<0.0001***
<b>Antioxidant Markers</b>						
CuZn-SOD (unit/g Hb)	3.9 ± 1.3	3.9 ± 1.2	3.2 ± 1.7	2.5 ± 1	15.4	<0.0001***
CAT (unit/g Hb)	2.9 ± 0.9	2.9 ± 1.9	2.7 ± 1	2 ± 0.6	8.6	<0.0001***
Plasma GPX (nmole/min/mg plasmaprotein)	4.9 ± 1.9	4.9 ± 1.9	4.7 ± 1.5	4.3 ± 1.8	2.9	0.032*
FRAP (µmole/ml of plasma)	4.3 ± 1.3	4.1 ± 1.7	3.7 ± 1.5	2.5 ± 0.9	19.8	<0.0001***
<b>Inflammatory markers</b>						
CRP (mg/ml)	1 ± 0.5	1 ± 0.5	1.4 ± 0.6	2.5 ± 0.8	124.2	<0.0001***
TNF-α (pg/ml)	32.6 ± 10.3	37.6 ± 8.2	119.2 ± 17.6	300.7 ± 63.7	276.8	<0.0001***
IL 6 (pg/ml)	6.1 ± 1.9	6.2 ± 1.5	10.5 ± 1.2	20.2 ± 6.7	66.8	<0.0001***

All the values are expressed as mean ± SD. (\*OH): Hydroxyl Radical, FLOPs: Fluorescent Oxidation Products, MDA: Malondialdehyde, PCO: Protein Carbonyl, SOD: Superoxide Dismutase, CAT: Catalase, GPX: Glutathione Peroxidase, FRAP: Ferric Reducing Ability of Plasma, CRP: C-Reactive Protein, TNF-α: Tumor Necrosis Factor alpha, IL 6: Interleukin 6. Cut-offs prescribed by NCEP-ATP III criteria: Waist circumference: Men: ≥90 cm, Women: ≥80 cm; Plasma fasting glucose: ≥100 mg/dl; Triglyceride: ≥150 mg/dl, HDL Cholesterol: Men: ≤40 mg/dl Women: ≤50 mg/dl; Blood pressure: Systolic ≥ 130 mmHg, Diastolic: >85 mmHg; Total cholesterol: 150 - 250 mg/dl, Low density lipoprotein: ≤150 mg/dl; Very low density lipoprotein: 5 - 40 mg/dl.

and all inflammatory markers were found to be significantly different in all other pairs of groups, namely, MHNO vs. MHO, MHNO vs. MHSO, MHOW vs. MHO, MHOW vs. MHSO and MHO vs. MHSO. TG, HDL, OH radicals and FLOP were not different between MHOW and MHO, but were significantly different for all other pairs of metabolically healthy obesity phenotypes.

**Table 3.** Results from Tukey's test for all parameters to assess pairwise significance between groups of metabolically healthy phenotypes.

	MHNO vs. MHOW	MHNO vs. MHO	MHNO vs. MHSO	MHOW vs. MHO	MHOW vs. MHSO	MHO vs. MHSO
WC	ns	***	***	***	***	***
BGF	ns	***	***	***	***	***
TG	ns	ns	***	ns	***	ns
HDL	ns	ns	***	ns	***	***
SBP	ns	***	***	***	***	***
OH Radicals	ns	***	***	ns	***	***
FLOP	ns	**	***	ns	***	***
CRP	ns	***	***	***	***	***
TNF alpha	ns	***	***	***	***	***
IL 6	ns	ns	***	ns	***	***

\*p < 0.05, \*\*p < 0.001, \*\*\*p < 0.0001. ns: not significant.

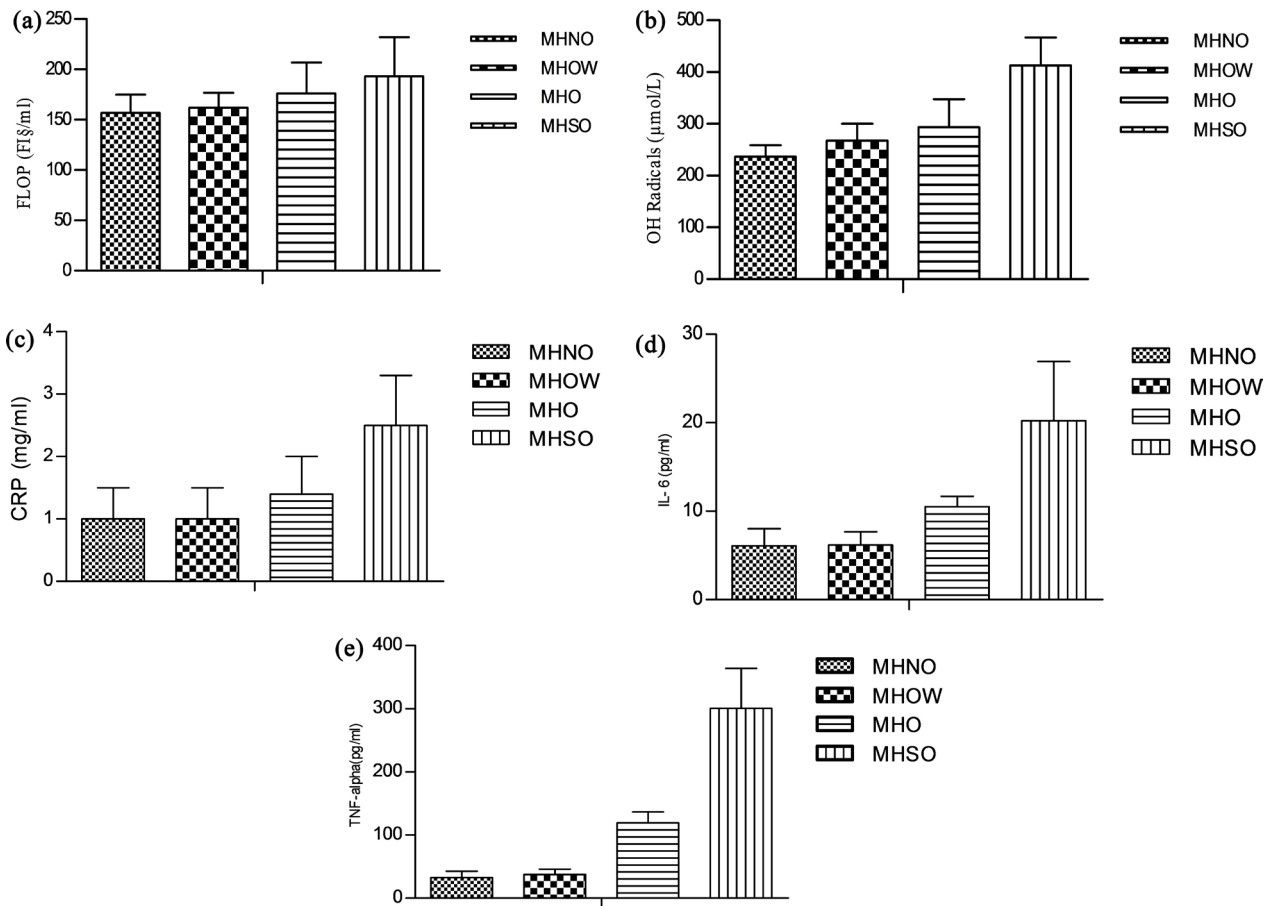
When the results were presented graphically (**Figure 1**), it was clear that the difference between the metabolically healthy severely obese and the obese is more than that between any other groups with regard to all parameters namely hydroxyl radicals, FLOP and all inflammatory markers, and the difference between the non-obese and the overweight is minimal for all these parameters. Although the difference between MHNO and MHOW was not found to be statistically significant, the graphs indicate that all parameters for MHNO show a healthier trend than MHOW.

Since groups were categorized based on their BMI, relationship of selected OS markers, OH Radicals, FLOP, and inflammatory markers, CRP, IL6 and TNF- $\alpha$  were assessed by calculating Pearson's correlation coefficients and all correlations were found to be highly statistically significant. It was also important to know whether this relationship was observed in all obesity groups studied hence the correlations were separately computed for each group and results are presented in **Table 4**.

It was interesting to find that none of the parameters were significantly related to BMI in the MHNO and MHOW phenotype, and were maximally correlated to BMI in the MHSO, followed by the MHO phenotype, indicating the significance of BMI > 25 used here as the cut-off for MHO according to Asian Guidelines but is the WHO cut-off for overweight.

#### 4. Discussion

Respondents were categorized as metabolically healthy because they had only a higher-than-normal BMI but no comorbidities. All risk factors for metabolic syndrome as prescribed by the NCEP-ATP III criteria were within the prescribed range. However, within this range, the fasting plasma glucose, triglyceride, blood



**Figure 1.** Graphical representation of oxidative stress markers (a) serum <sup>•</sup>OH radicals (b) serum FLOPs and inflammatory markers (c) CRP, (d) IL6, (e) TNF- $\alpha$  in MHNO, MHOW, MHO and MHSO phenotypes.

**Table 4.** Pearson’s correlation coefficients to assess relationships of BMI with OH Radicals, FLOP, CRP, IL6 and TNF- $\alpha$  for all respondents and for metabolically healthy non obese control (MHNO), metabolically healthy overweight (MHOW), metabolically healthy obese (MHO) and metabolically healthy severe obese (MHSO).

		OH Rad	FLOP	CRP	TNF a	IL 6
BMI	All groups combined	0.863*	0.457*	0.741*	0.818*	0.844*
	MHNO	0.135	0.129	0.090	0.027	-0.074
	MHOW	0.195	0.173	0.057	0.197	0.177
	MHO	0.463*	0.368*	0.211	0.375*	0.311*
	MHSO	0.585*	0.618*	0.324*	0.524*	0.630*

Values marked with asterisk are statistically significant at  $p \leq 0.05$ .

pressure were significantly higher, and HDL-cholesterol, lower as BMI increased from the non-obese to the overweight, obese and severely obese metabolically healthy groups in that order. Controversy has surrounded the idea that metabolically healthy obesity (MHO) may be considered really healthy [22] because MHO individuals are at increased (cardio) metabolic disease risk and type 2 diabetes [23], and may have other comorbidities. Consequently, they may be on



the way to becoming “unhealthy” obese. Detailed metabolic phenotyping of obese persons has been suggested for understanding the pathophysiology of metabolic disturbances to identify high-risk individuals or subgroups, which can help in optimization of prevention and treatment strategies to combat cardiometabolic diseases.

In a study designed to examine prevalence of the different metabolic phenotypes and to distinguish between unhealthy and healthy phenotypes of obesity [24], it was concluded that a healthy obese phenotype was associated with a better metabolic profile than observed in normal weight individuals with MetS, and increasing BMI had a significantly greater effect on estimates of liver fat and future CVD risk among those with MetS compared with participants without MetS.

The present study, conducted on respondents categorized into various obesity phenotypes based on Asian Guidelines [11], found that all OS markers, namely, serum hydroxyl radicals and FLOP, MDA, PCO, antioxidant markers CuZn-SOD, CAT, plasma GPx and total antioxidant activity, FRAP showed significant difference between MHNO and MHOW phenotypes, when assessed using one-way ANOVA, but when Tukey’s test for paired groups was calculated, no significant difference was found between the MHNO and their MHOW counterparts for any OS marker. Inflammatory markers, CRP, TNF- $\alpha$  and IL-6, which are known to be related to obesity, are also not different between the MHNO and MHOW phenotype, but are significantly higher in the MHO group and show a much larger value in the MHSO phenotype. Further, all these parameters were significantly positively or negatively correlated to BMI, as expected.

This is supported by a study designed to compare metabolically unhealthy overweight (MUO) and metabolically healthy overweight (MHO) phenotypes matched for BMI, total body fat percentages and lean body masses, which reported that the metabolomes in plasma revealed higher risks for oxidative and lipid-related tissue damage in MUO as measured by significantly higher levels of glycolic acid, 6-lysophosphatidylethanolamines (lysoPEs), and 12 lysophosphatidylcholines (lysoPCs) and lipoprotein-associated phospholipase A2 (Lp-PLA2) activity and higher levels of oxidized low-density lipoprotein (ox-LDL) and urinary 8-epi-prostaglandin F2 $\alpha$  (8-epi-PGF2 $\alpha$ ) [25]. On the other hand, in a study designed to compare selected oxidative stress markers in metabolically healthy morbidly obese (MHMO) with metabolically unhealthy morbidly obese (MUHMO) who had been diagnosed with MetS, no significant difference was found with regard to total oxidant status (TOS) and total antioxidant response (TAR) [26], in contradiction to the difference in FRAP observed in the present study between the metabolically healthy obese (MHO) and severely obese (MHSO) phenotypes.

Previous studies by our group have proposed the homeostatic mechanism in obesity with MetS which depends on antioxidant enzymes, CuZn-SOD, catalase and glutathione peroxidase which consequently modulate levels of oxidative stress

markers, malondialdehyde and protein carbonylation, and total antioxidant capacity. When these enzymes decrease, the entire system moves towards increased oxidative stress, and in some cases of mild disturbance, the overall oxidative stress is not visible due to the ability of the system to restore equilibrium [13]. The same pattern is visible in the present study, where neither the erythrocytic MDA and PCO, nor the antioxidant enzymes, CuZn-SOD, catalase or GPX varies between the MHNO and MHOW groups, leading to similar total antioxidant capacity, a pattern not found in the obese and more severely obese phenotypes. The significant reduction in antioxidant enzymes and consequent reduction in the total antioxidant capacity in obese, and further in the severely obese are indicative of the breakdown of this adaptation.

•OH radicals and FLOP are the only two oxidative stress markers that show a small but significant difference between MHNO and MHOW groups. This may be attributed to their higher sensitivity. In this connection, Goossens [22] reported that MHO phenotype is relatively better protected against chronic diseases but MHO should not be regarded as a harmless condition, because they may have a higher propensity for cardio-metabolic disease. Plasma FLOP has been reported to independently predict the risk of subsequent cardiovascular disease events in epidemiologic studies [27], but has not been investigated for possible differences with regard to obesity phenotypes, especially MHO. This is observed in the present study. The protective profile of metabolically healthy obese postmenopausal women has been reported and is attributed to their lower concentrations of hepatic circulating alanine transaminase, reflecting lower hepatic insulin resistance and lower liver fat content [28]. In another study [29], branched-chain amino acids, aromatic amino acids and orosomucoid have been identified as serum biomarkers through metabolomics approach to distinguish physically inactive overweight/obese women with metabolic syndrome from metabolically healthy ones, and this is independent of body weight and fat mass.

We also found that inflammatory markers, CRP, TNF- $\alpha$  and IL-6 were significantly higher in the MHO phenotype and maximum in the MHSO phenotype, as compared to the MHNO and MHOW phenotypes, which did not differ from each other. Inflammatory markers are known to be related to adipose tissue distribution even in metabolically healthy phenotypes [22]. Lowered serum adipocytokines, TNF- $\alpha$  and adipocyte fatty acid binding protein (A-FABP) in metabolically healthy obese and non-obese and were significantly associated with unhealthy metabolic profile in non-obese Korean individuals [30] and evidence has been reviewed that suggests that normal adipose tissue function contributes to the healthy obese phenotype [8].

Overall, there was little difference between the metabolically healthy normal weight and overweight in the biochemical markers related to oxidative stress as well as inflammatory markers respondents, but the impact of obesity and severe obesity in metabolically healthy respondents was highly significant on oxidative stress parameters as well as inflammatory markers. The pattern of OS markers

observed in this study highlights the homeostatic mechanisms in the metabolically healthy overweight respondents which helps their biochemistry to remain similar to the normal weight healthy respondents, indicating that they may actually belong to the same phenotype, even as the more sensitive markers, hydroxyl radicals and fluorescent products does indicate some small difference between these groups.

Further, it has been suggested that this healthier metabolic profile may not translate into a lower risk for mortality [9]. Mechanisms that could explain the favorable metabolic profile of MHO individuals are poorly understood, and may include differences in visceral fat accumulation, birth weight, adipose cell size and gene expression-encoding markers of adipose cell differentiation, which may favor the development of the MHO phenotype. It is important to make in depth study of the underlying cause effect relationships. Collectively, a greater understanding of the MHO individual has important implications for therapeutic decision making, the characterization of subjects in research protocols and medical education. Lack of difference in inflammatory markers between metabolically healthy non-obese and their overweight counterparts may be because of the location where the excessive calories are stored. Location and function of adipose tissue are linked and determine metabolic health. Adipose tissue inflammation may be an adaptive response that enables safe storage of excess nutrients in adipose tissue, thereby protecting against metabolic and inflammatory perturbations and consequent comorbidities (Goossens) [22]. For example, it has been demonstrated that in vivo IL-6 release from gluteofemoral adipose tissue was markedly lower than from the corresponding abdominal subcutaneous fat depot both in men and women [31], suggesting that lower body fat may have a more beneficial inflammatory phenotype. These differences in disease risk are due to strikingly divergent functional properties of these adipose tissue depots, explaining the disparity seen in various metabolically healthy groups in the present study. The difference was seen in various metabolically healthy groups in the present study. Accumulation of adipose tissue in the upper body (abdominal region) is associated with the development of obesity-related comorbidities and even all-cause mortality.

In this connection, it must be remembered that the Asian cut-offs for BMI were used in this study [11] which classify a BMI of  $\geq 23$  kg/m<sup>2</sup> and  $\geq 25$  kg/m<sup>2</sup> as overweight and obese. Asians display a greater proportion of body fat for a given BMI than Caucasians [32] and are found to be susceptible to develop type 2 diabetes and coronary artery disease in spite of lower BMI and the use of universal BMI cut-off points to classify subjects as normal weight, overweight and obese are not predictive for metabolic risk factors. Asian Indians display exclusive features of obesity like pattern of body fat distribution in abdomen, central obesity, ectopic fat deposition particularly in liver, pancreas, muscles etc. are major contributory factors to driven metabolic syndrome and type 2 diabetes mellitus. These metabolic perturbations are largely depending on combined effect of me-

tabolic profile and body fat. Although the use of BMI for categorizing metabolic health has been criticized for its limitations because it does not distinguish between lean and fat mass, nor does it indicate anything about fat distribution. However, in the present study, BMI has been used for categorizing phenotypes and its appropriateness can be confirmed because significant correlation of all parameters with BMI is found in the MHO and MHSO groups but not in the MHNO and MHOW phenotypes.

## 5. Conclusion

Our study highlights that the metabolically healthy obese phenotype exhibits altered metabolic profile in terms of oxidative stress and inflammation at Asian Indian BMI cut-offs, making this phenotype at high risk for metabolic syndrome, type 2 diabetes mellitus and cardiovascular diseases. Efforts should be made to prevent obesity and severe obesity phenotypes, because systemic inflammation and oxidative stress are visible in these groups, which are not evident in the overweight phenotype. More studies are required using the Asian guidelines of BMI to manifest clustering of metabolic syndrome risk factors in comparison to Europeans.

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## Ethical Approval and Informed Consent

The study designs were approved by Institutional Ethics Committee of Population Resource & Research Centre, Allahabad, India. An informed written consent was obtained from all participants.

## Conflicts of Interest

The authors declare no conflict of interest.

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