DE GRUYTER OPEN

Original Research

DOI: 10.1515/rrlm-2017-0020

Association of ischemia-modified albumin with oxidative stress status and insulin resistance in obese patients

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Abstract

***Objectives**: Obesity is associated with oxidative stress due to the overproduction of free radicals in some accompanying states, such as hyperglycemia, elevated lipid levels and chronic inflammation. Free radical accumulation may modify the structure of human serum albumin, generating ischemia-modified albumin (IMA), and increased serum levels of IMA have been linked to obesity-related diseases and oxidative damage. The association of IMA levels with oxidative stress and insulin resistance (IR) has not been evaluated in the context of obesity. The aim of this study is to determine IMA levels in the context of obesity and their relationship with oxidative status and insulin resistance.*

***Methods**: Sixty-one adult obese cases with body mass index (BMI) ≥ 30 were evaluated, with 30 healthy adults with 18.5 ≤ BMI ≤ 24.9 included in the control group. IMA, total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), total cholesterol, triglycerides, HDL and LDL-cholesterols were determined.*

***Results**: IMA, TAS, TOS, OSI, total cholesterol and LDL-cholesterol levels were not different between the control and obese groups (P-value >0.05), while triglyceride levels were determined to be higher and HDL-cholesterol levels were determined to be lower in the obese group (P-value <0.05). When IMA, TAS, TOS, OSI levels were compared between the control/IR(-), obese/IR(+) and obese/IR(-) groups, no statistically significant differences were detected (P-value >0.05), but the fasting blood glucose level was determined to be higher in the obese/IR(+) group than in the control group.*

***Conclusions**: We concluded that obesity and insulin resistance had no effect on IMA levels in the obese group, who showed no impairment in their oxidative balance.*

***Keywords**: obesity, ischemia-modified albumin, oxidative stress, insulin resistance*

Received: 1st February 2017; Accepted: 28th May 2017; Published: 17th July 2017

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Introduction

Obesity, an important metabolic disorder with rapidly increasing prevalence, is caused by impairment of the energy balance (1) and constitutes a risk factor for diabetes, hypertension, dyslipidemia and some cancers (2). Obesity can cause the overproduction of free radicals as a result of some accompanying states, such as hyperglycemia, chronic inflammation, increased muscle activity due to being overweight, increased lipid levels, deficient antioxidant mechanism, and the production of reactive oxygen species (ROS) (2,5). Oxidative stress, a state of imbalance between antioxidants and oxidants such as free radicals or reactive oxygen species (3) due to free radical overproduction, may be a unifying mechanism in the progress of obesity-based comorbidities, such as cardiovascular disease and diabetes mellitus (DM) (4). Free radical accumulation in obesity may modify the N-terminal region of human serum albumin (HSA) to generate ischemia-modified albumin (IMA). This structural change affects the binding capacity of albumin to the transition elements (6).

It is known that oxidative stress increases in obesity (14–17), and IMA levels are elevated in the context of abnormal conditions related to oxidative stress. Some studies have indicated that IMA levels are increased in obesity-related abnormalities, such as hypercholesterolemia (7), type 2 diabetes (8, 9), metabolic syndrome (10) and elevated free fatty acid (FFA) levels in the body (11). IMA is also increased in other conditions that are identified with free radical production, such as liver cirrhosis, acute infections and advanced cancer [12]. In this respect, IMA can be considered as a marker of oxidative stress in obesity and obesity-related abnormalities (6, 7, 13). Thus, the purpose of our study is to determine the IMA levels in the context of obesity and to investigate their relationship with oxidative status and insulin resistance (IR).

Materials and methods

Selection of cases

Study Group: Sixty-one patients (32 women and 29 men) aged 21- 72 (53.48 ± 10.55) with body mass index (BMI) ≥ 30 were enrolled as obese cases in the study group. Study group cases were chosen from among patients under regular medical control at Hıfzısıhha Institute-Izmir. The use of antioxidant agents (vitamins E and C), diagnoses of infectious diseases, and insufficient or inappropriate sampling were accepted as exclusion criteria.

Control Group: Thirty healthy cases (25 women and 5 men) aged 21- 68 (43.80 ± 14.00) with 18.5 ≤ BMI ≤ 24.9 kg/m^2 were included as a control group. Cases in the control group had taken no medications for at least 2 months. The exclusion criteria for the control group were same as those for the study group.

Weight classifications for all cases in the study and control groups were determined according to WHO criteria as normal-healthy weight 18.5 ≤ BMI ≤ 24.9 and obese BMI ≥ 30 kg/m^2 (18).

Sample collection

The study was evaluated and approved by the Ethics Committee of the Ministry of Health of Izmir Tepecik Education and Research Hospital (Protocol number: 67/9) in accordance with the Declaration of Helsinki. Written informed consents were obtained from all cases before the study was initialized.

Blood samples were collected at 08:00-10:00 in the morning with the participant in a sitting position following an overnight fast of 8-12 hours. Samples were collected in 8-mL vacuum gel sample tubes. Tubes were centrifuged at 1600 x g for 15 minutes, and serum samples were separated. Obtained serum samples were used to perform IR, T total antioxidant status (TAS), total oxidant status (TOS) and IMA anal-

yses. The [HOMA-IR]>2.5 insulin resistance was used as a base to categorize the control and obese groups.

Total antioxidant and oxidant analysis

Measurement of the total antioxidant status (TAS) of serum: The total antioxidant status of the serum was determined using a colorimetric measurement method in an automated analyzer (Abbott Aeroset, Illinois, USA) (19). The method is based on the production of the dianisidine radical (bright yellowish-brown) by reacting hydroxyl radicals (from the production of the Fenton reaction) with the substrate O-dianisidine (colorless). At the end of the reaction, the changed color intensity can be determined spectrophotometrically and is related to the total antioxidant status in the sample. The results are presented as mmol Trolox eq/L, and the intra- and interassay CVs were lower than 3% (20).

Measurement of the total oxidant status (TOS) of serum: The TOS of the serum was measured using a colorimetric measurement method in an automated analyzer (Abbott Aeroset, Illinois, USA) (21). In this method, ferric acid formed due to the oxidation of a ferrous ion-o-dianisidine complex to ferric ion by the effects of oxidants, which yielded a colored complex with Xylenol orange in an acidic medium. The difference in the color intensity was associated with the total number of oxidant molecules in the sample. The results are presented as the micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Eq/L), and the intra- and interassay CVs were lower than 3% (22).

Calculation of the oxidative stress index (OSI): The percent ratio of the total oxidation status level to the total antioxidant status was accepted as the OSI and as an indicator of the oxidative stress degree (23). The OSI value was calculated using the equation given below:

OSI= [(TOS, μmol H_2O_2 equivalent/l) / (TAS, μmol Trolox equivalent/l)] x100.

Ischemia-modified albumin analysis

The serum IMA was measured using the albumin cobalt binding test (24). The test principle is based on the colorimetric determination of the complex of dithiothreitol (DTT) with unbound cobalt. Cobalt chloride (50μl, 0.1%) was added to patient serum (200 μl), and incubated for 10 minutes to enable albumin-cobalt binding. DTT solution (50 μl, 1.5 mg/mL) was added to enable reaction of unbinded cobalt for 2 minutes. After 2 minutes, the reaction was terminated by adding NaCl (0.9%). The same method was followed for the sample blank, which used distilled water instead of DTT. At the end of the reaction, the absorbance values were determined at 470 nm using a spectrophotometer (Shimadzu UV-1201, Japan). The difference between the sample and the sample blank was recorded as the IMA value. The method's percent values for the intra- and interassay CVs were 3.20 and 3.91, respectively.

Other biochemical assays

Triglycerides, HDL-cholesterol and fasting blood glucose (FBG) levels were determined using routine methods (Konelab 60i/DIALAB GmBH, Wiener Neudorf, Austria). Insulin levels were determined using a chemiluminescence technique (Immulate 2000 analyzer, Bayer Diagnostics, Tarrytown, New York, USA). The equation (25): HOMA-IR = [fasting insulin (μU/mL) x fasting blood glucose (mmol/L)] /22.5 was used to calculate the HOMA-IR (Homeostasis Model for Assessment-Insulin Resistance) insulin resistance (25).

Statistics

The results of the study and control groups are expressed as the means ± standard deviations and 95% confidence intervals. Student's t-tests and ANOVA variance analyses (Bonferroni correction) were performed for comparisons of mean values of normal and obese cases and IR

(+) and IR (-) obese cases, respectively. The control and obese cases were balanced in terms of gender and age using covariance analysis. Pearson's correlation analysis (rho) was used to determine the relationships between biochemical parameters and BMI and IR. Statistical analyses were performed using SPSS 21 (SPSS for Windows, Chicago, Illinois, USA). Two-sided tests with a *P*-value <0.05 were accepted as significant.

Results

When IMA, TAS, TOS, OSI levels were compared between the control and obese groups, there was no significant difference was detected (*P*-value >0.05) (Table I). The imbalances between the groups in terms of gender and age were determined using covariance analysis.

When total cholesterol, triglycerides, and HDL- and LDL-cholesterol levels were compared between the control and obese groups, no significant alterations were detected for total cholesterol and LDL-cholesterol levels, while triglyceride levels were determined to be higher and HDL-cholesterol levels were determined to be lower in the obese group (*P*-value <0.05) (Table 2). BMI and HDL-cholesterol (r: -0.27, p < 0.01) and IMA and triglyceride levels (r:-0.24, *P*-value <0.01) showed weak negative correlations in all cases. FBG was found as higher in the obese group than in the control group (*P*-value <0.05) (Table II).

IMA, TAS, TOS, OSI and FBG levels were compared between control/IR (-) and obese/ IR (+) and obese/ IR (-) cases. No statistically significant differences were detected for IMA, TAS, TOS and OSI levels (Table III). The FBG level was determined to be higher in the obese/ IR (+) group than in the control group (*P*-value <0.05).

Table I. Mean values for IMA, TAS, TOS and OSI among control and obese groups

Biochemical parameters	Body composition	n	Mean	± SD	%95 Confidence Interval		p*
					Lower Bound	Upper Bound	
IMA (ABSU)	18.5 ≤ BMI ≤ 24.9	30	0.34	0.11	0.30	0.38	>0.05
	BMI ≥ 30	61	0.36	0.09	0.34	0.38	
TAS (mmol Trolox eq. /L)	18.5 ≤ BMI ≤ 24.9	30	1.91	0.59	1.69	2.14	>0.05
	BMI ≥ 30	61	1.86	0.43	1.75	1.97	
TOS (µmol H2O2eq./L)	18.5 ≤ BMI ≤ 24.9	30	13.51	4.78	11.72	15.29	>0.05
	BMI ≥ 30	61	12.68	2.44	12.05	13.30	
OSI (AU)	18.5 ≤ BMI ≤ 24.9	30	7.20	1.50	6.64	7.76	>0.05
	BMI ≥ 30	61	7.06	1.61	6.65	7.48	

Control group: 18.5 ≤ BMI ≤ 24.9 kg/m^2; Obese group: BMI ≥ 30 kg/m^2. Data are expressed as mean ± SD. Results were compared using the t test.

*P-value <0.05 for t test. Abbreviations: BMI, body mass index; SD, standart deviation; IMA, ischemia modified albumin; ABSU, absorbance units; TAS, total antioxidant response; TOS, total oxidant status; OSI, Oxidative stress index value; AU, Arbitrary unit

Table II. Mean values for lipid parameters and fasting blood glucose among control and obese groups

Lipid parameters	Body composition	n	Mean	± SD	%95 Confidence Interval		p*
					Lower Bound	Upper Bound	
Total cholesterol (mg/dl)	18.5 ≤ BMI ≤ 24.9	30	211.47	35.20	198.32	224.61	>0.05
	BMI ≥ 30	61	220.93	38.56	211.06	230.81	
Triglycerides (mg/dl)	18.5 ≤ BMI ≤ 24.9	30	106.70	42.13	90.97	122.43	<0.05
	BMI ≥ 30	61	154.43	111.30	125.92	182.93	
HDL-cholesterol (mg/dl)	18.5 ≤ BMI ≤ 24.9	30	55.13	11.64	50.79	59.48	<0.05
	BMI ≥ 30	61	47.84	9.98	45.28	50.39	
LDL-cholesterol (mg/dl)	18.5 ≤ BMI ≤ 24.9	30	136.70	29.65	125.63	147.77	>0.05
	BMI ≥ 30	61	141.82	32.204	133.50	150.14	
FBG (mg/dL)	18.5 ≤ BMI ≤ 24.9	30	90	1.4	87	93	<0.05
	BMI ≥ 30	61	100.23	2.13	93	106	

Control group: 18.5 ≤ BMI ≤ 24.9 kg/m^2; Obese group: BMI ≥ 30 kg/m^2. Data are expressed as mean ± SD. Results were compared using the t test.
*P-value <0.05 for t test. Abbreviations: BMI, body mass index; SD, standard deviation, HDL, high-density lipoprotein; LDL, low-density lipoprotein; FBG, fasting blood glucose.

Discussion and conclusion

Even if it is assumed that obesity increases oxidative stress (14-17), it is difficult to determine an obesity-associated oxidative stress response since most studies have incorporated different study designs with various oxidative stress biomarkers (2). In this study, there was no impairment of the oxidant-antioxidant balance in obese cases and IR (+) obese cases in comparison with the control group. Our study group consisted of outpatient individuals who attended regular check-ups. Reducing oxidative stress may be possible by various lifestyle modifications, such as changes in dietary intake (2), lowering insulin resistance and lipids via nutritional modifications (16), weight loss (14, 16, 26) and chronic exercise (27). However, the general consensus is that obesity is a cause of increased oxidative stress (14-17). Brown et al. (28) identified no statistically significant differences in TAS, superoxide dismutase and reduced glutathione levels, which are parameters of oxidative stress, among healthy normal-weight (as control) and obese groups.

Our study was the first to determine that IMA levels can be the same in obese individuals without oxidative stress. Although serum IMA levels were higher in the study group in comparison with the control group, there was no statistically significant difference. Only Piva et al. (29) focused on serum IMA levels in obese individuals. They indicated that elevated IMA levels were associated with oxidative stress. In our study group, IMA levels were higher than those of the control group, but there was no significant

Table III. Mean values for IMA, TAS, TOS, OSI and FBS among control and obese groups with or without insulin resistance

Biochemical parameters	Body composition	n	Mean	± SD	%95 Confidence Interval		p*
					Lower Bound	Upper Bound	
IMA (ABSU)	18.5 ≤ BMI ≤ 24.9 HOMA-IR ≤ 2.5	30	0.34	0.11	0.29	0.379	>0.05
	BMI ≥ 30 HOMA-IR ≤ 2.5	27	0.37	0.10	0.33	0.40	
	BMI ≥ 30 HOMA-IR > 2.5	34	0.36	0.10	0.32	0.39	
TAS (mmol Trolox eq. /L)	18.5 ≤ BMI ≤ 24.9 HOMA-IR ≤ 2.5	30	1.94	0.61	1.70	2.19	>0.05
	BMI ≥ 30 HOMA-IR ≤ 2.5	27	1.86	0.46	1.68	2.04	
	BMI ≥ 30 HOMA-IR > 2.5	34	1.85	0.40	1.71	1.99	
TOS (μmol H2O-2eq./L)	18.5 ≤ BMI ≤ 24.9 HOMA-IR ≤ 2.5	30	13.73	5.00	11.75	15.71	>0.05
	BMI ≥ 30 HOMA-IR ≤ 2.5	27	12.85	2.85	11.78	13.91	
	BMI ≥ 30 HOMA-IR > 2.5	34	12.43	1.92	11.75	13.10	
OSI (AU)	18.5 ≤ BMI ≤ 24.9 HOMA-IR ≤ 2.5	30	7.21	1.52	6.60	7.81	>0.05
	BMI ≥ 30 HOMA-IR ≤ 2.5	27	7.21	1.59	6.60	7.82	
	BMI ≥ 30 HOMA-IR > 2.5	34	6.95	1.62	6.38	7.51	
FBG (mg/dL)	18.5 ≤ BMI ≤ 24.9 HOMA-IR ≤ 2.5	30	90	1.4	87	93	<0.05**
	BMI ≥ 30 HOMA-IR ≤ 2.5	27	98	2.3	93	102	
	BMI ≥ 30 HOMA-IR > 2.5	34	102	2.0	97	106	

Control group: 18.5 ≤ BMI ≤ 24.9 kg/m^2; Obese group: BMI ≥ 30 kg/m^2. Data are expressed as mean ± SD. Results were compared using the Anova test followed by the Bonferroni-correction. *P-value <0.05 for the Anova test. **Statistical significance between Control group and obese/IR (+) group. Abbreviations: BMI, body mass index; SD, standart deviation; IR, insulin resistance; HOMA-IR, Homeostasis model of assessment-insulin resistance; IMA, ischemia modified albumin; ABSU, absorbance units; TAS, total antioxidant response; TOS, total oxidant status; OSI, Oxidative stress index value; AU, Arbitrary unit; FBG, fasting blood glucose.

difference between the two groups. A study can be performed with larger groups to achieve proper numbers for a statistical analysis of increased IMA levels in obese patients without oxidative stress. However, it can be said that the increase in IMA levels is associated with oxidative stress rather than obesity or obesity-related diseases.

Piwowar et al. (9) investigated the relationship between IMA levels and BMI in normal weight, overweight and obese type-II diabetic individuals and reported no relationship between IMA levels and BMI. In another study, Kaefer et al. (8) showed increased IMA and high-sensitivity C-reactive protein levels in type 2 diabetics, although no relationship between IMA and BMI was identified. These findings indicate that inflammatory processes are associated with IMA rather than visceral fat and obesity.

Obesity is directly related to IR and hyperglycemia (2). IR and hyperglycemia lead to oxidative stress via multiple biochemical processes (glucose auto-oxidation, stimulation of the polyol pathway, an imbalance between the amounts of reduced and oxidized coenzyme forms, non-enzymatic glycation and the generation of advanced glycation end-products-AGEs) (8, 9). In cases of oxidative stress, it has been reported that the capacity of albumin to bind exogenous cobalt is reduced (30). However, our study indicated that IR does not affect IMA levels. Although the individuals in the study group were obese and had insulin resistance, diabetes was not detected in these cases because they were chosen from among patients under regular medical control.

Piwovar et al. (9) investigated IMA levels depending on the glycemic control of type-II diabetic patients and reported that a correlation between IMA and HbA1c was only observed in patients with poor glycemic control (HbA1c > 7%). Another study by Kaefer et al. (8) reported that poor glycemic control effected IMA levels. This study indicated that levels of IMA were only higher in the highest quartile of glucose and hs-CRP levels.

Triglycerides were higher and HDL-cholesterol levels were lower in obese cases in comparison with the control group. Upon comparing obese cases with the control group, a statistically significant difference was observed only in triglyceride levels. As is well-known, dyslipidemic profiles in obesity include elevated triglycerides, lower HDL-cholesterol and elevated LDL-cholesterol levels (31). Triglycerides may be an important variable affecting IMA levels. In this study, we identified weak negative correlations between IMA and triglycerides in all cases ($r: -0.24$, $p<0.01$). However, in a previous study performed by Gidenne et al., there was no significant relationship between IMA levels and triglycerides (32). It is not clear whether this condition is related to interference or the incorrect lipid profiles observed in obesity.

IMA levels increase due to the structural modification of human albumin, which is caused by the accumulation and production of free radicals at extreme levels. Stress can also be indicated by this change, and it serves as a biomarker. In patients with higher hypertension and diabetes percentages, there is a correlation between BMI and IMA levels, as reported by Piva et al. The study suggested that increased serum albumin modification, which is related to ROS levels, together with insufficient peripheral oxygenation could be predicted by increases in IMA levels. These subjects exhibited IMA production. Another suggestion from the study was that alterations in inflammatory and oxidative stress biomarkers in obese subjects can be indicated by diabetes, which is a dependent predictor, according to an advanced statistical analysis on BMI (33). Regarding these results, it was concluded that the oxidative stress condition must be increased by a comorbidity, as IMA levels cannot increase merely due to obesity. Obese patients without insulin resistance and oxidative stress do

not differ in terms of IMA levels. Our results also support this consequence, which suggests that oxidative stress should be considered for evaluation of IMA levels in obesity and obesity-related clinical conditions. The comorbidities that may result in oxidative stress production can be used to classify a larger group of patients, which then can be used to prospectively assess IMA levels.

Study limitations

The number of cases in groups is small; this could be a reason for negative results. There were reported imbalances between the groups in terms of gender and age. Performing this study prospectively in a larger group would provide an improved balance between groups. A larger group of obese samples, classified according to comorbidities that may produce oxidative stress, should be used to prospectively assess IMA levels.

Conflict of interest

The authors report no conflicts of interest.

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