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Comparative Evaluation of Trimethylamine-N-Oxide, Ischemia Modified Albumin, Iron, and Copper among Normal Control, Obese Metabolically Healthy and Obese with Metabolic Syndrome: A Cross-Sectional Biochemical Analysis at Tertiary Care Hospital in Udupi District

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Abstract

Objective: Metabolic Syndrome is caused by obesity, hyperglycemia, high blood pressure, high triglycerides, and low high-density lipoprotein cholesterol (MetS). MetS has been related to increased risk of Type 2 Diabetes Mellitus (T2DM), cardiovascular disease, cardiovascular mortality, and all-cause mortality and are considered signi icant public health and clinical concern in developing and developed countries. This study was carried out to comparative evaluation of Trimethylamine-N-Oxide (TMAO), Ischemia Modi ied Albumin (IMA), Iron (Fe), and Copper (Cu).

Design: We conducted a cross-sectional biochemical analysis on 159 subjects, including 98 (61.63%) males and 61 (38.36%) females, from the OPD ward of Kasturba Hospital, which is a teaching hospital affiliated to Kasturba Medical College, Manipal, MAHE for regular health checkups as well as for illness related to metabolic syndrome from Feb 2021 to May 2021. The patient's medical records were followed up; the anthropometric measurements and clinical parameters were retrospectively collected. The subjects were categorized as subjects with and without MetS as per National Cholesterol Education Program Adult Treatment Panel (NCEPATPIII). The subjects with BMI more than 30 kg/m^2 were de ined as obese according to WHO classi ication

Results: Dunnett t (2-sided) test analysis indicates a signi icant difference for IMA among normal control and MetS group with mean values of 0.21 ± 0.409 and 0.62 ± 0.530 (P<0.005), respectively. Similarly, TMAO also shows signi icance compared to normal control, obese metabolically healthy, and obese with metabolic syndrome.

Conclusion: Our study strongly supports that TMAO is highly prevalent among the subjects, with MetS showing a signi icant positive association between obese metabolically healthy and obese with metabolic syndrome. Similarly, IMA also indicates a positive association with MetS compared with control but not with MHO.

Keywords: Hyperglycemia; Cardiovascular; Metabolic syndrome

Abbreviations: Metabolic Syndrome (MetS); Trimethylamine-N-Oxide (TMAO); Metabolically Healthy Obese (MHO); Ischemia Modified Albumin (IMA)

Introduction

Metabolic Syndrome is caused by obesity, hyperglycemia, high blood pressure, high triglycerides, and low high-density lipoprotein cholesterol (MetS). MetS has been related to increased risk of Type 2 Diabetes Mellitus (T2DM), cardiovascular disease, cardiovascular mortality, and all-cause mortality and are considered a major public health and clinical concern in developing and developed countries. In developing countries, the burden of non-communicable diseases is growing, resulting in high mortality rates.

Despite variations in the technique, diagnostic criteria, and the age of the subjects surveyed, the Asia-Pacific region is at high risk of the Mets epidemic. Metabolic syndrome affected about one-fifth of the adult population in most countries, with a secular rise in prevalence [1]. The prevalence of MetS is increasingly rising in India, in tandem with the aging of the population, evolving lifestyles and dietary habits, and the growing obesity epidemic: According to the most recent systematic review and meta-analysis on the adult population, the prevalence of MetS in India's adult population is 30%. From 13 percent (18-29 years) to 50 percent (50-59 years), the burden increased steadily across the age groups. They also discovered that people in urban areas had a higher prevalence of 32%, relative to tribal adults who had 28% and rural adults who had 22%. Obesity is a disorder in which fat accumulates in the body. It is a contributing factor or marker for various chronic diseases, including diabetes, Cardio Vascular Diseases (CVDs), cancer, and adverse health consequences. Commonly used screening methods to assess and characterize obesity are Body Mass Index

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(BMI), measured as weight in kilograms (kg)/by height in meters squared. A BMI of 25 kg/m^2 to 30 kg/m^2 is considered overweight, and a BMI of 30 kg/m^2 is considered obese. Obesity is responsible for a significant portion of healthcare costs as well as societal costs [2]. Obesity is the leading cause of MetS, which leads to atherosclerotic artery disorder and type 2 diabetes mellitus, according to a Hungarian cross-sectional analysis [3]. Obesity, on the other hand, is not necessarily associated with MetS. Some people are defined as Metabolically Healthy Obese (MHO) because they have a high degree of insulin sensitivity but don't have hypertension, hyperlipidemia, or other MetS symptoms. According to the epidemiological survey, MHO may account for a large portion of the obese population. As a result, preventing the progression of MetS-related diseases and lowering the global public health burden requires early detection and intervention [4].

Trimethylamine-N-Oxide (TMAO) is a minor, organic metabolite generated by the gut microbiota emerging as a new potential cause of atherosclerosis and cardiovascular danger [5]. Randrianarisoa, et al. found a positive correlation between TMAO and BMI, insulin resistance, visceral fat mass and liver fat content [6]. Other studies have found that TMAO levels rise in MetS [7]. According to a report, consumption of meat, eggs, or fish was not linked to plasma TMAO, choline, or betaine concentrations in a group of German adults. Higher plasma TMAO levels were associated with a substantially 3.0-fold increased 3-year significant adverse cardiac injury risk and a 3.6-fold increased 5-year mortality risk in patients with type-2 diabetes [8].

Ischemia-Modified Albumin (IMA) is a form of Human Serum Albumin (HAS) that cannot bind to transition metals at the N-terminus [9]. The serum IMA levels in the first hour of Acute Ischemic Stroke patients were significantly higher than in the control group. At 1 hour (108.9), 24 hours (94.2), 48 hours (82.1), 72 hours (77.6), and 144 hours (76.8) after admission, the IMA levels in patients showed a steady decline [10]. A recent study found that, while there is a correlation between IMA and cardiovascular disease, serum IMA levels were not associated with the condition's seriousness compared to controls [11]. IMA levels were statistically significantly higher in the metabolically unhealthy obese group than in the control group (Metabolically Healthy Obese) in a sample of children and adolescents aged 4 to 18 [12].

Iron is an element that is needed for life. Oxidative damage is a risk of iron overload. Iron levels are often elevated in MetS patients, according to previous research, and are related to the risk of complications [13]. A high serum ferritin level was associated with increased age, MetS incidence, BMI, WC, FBG, fasting insulin, HbA1c, TC, and TG levels in both men and women. In both men and women, higher serum ferritin levels were associated with lower HDL-C levels. In women only, increased serum ferritin levels were found to have substantial positive associations with systolic BP, diastolic BP, and LDL-C [14].

Copper is an important metal that is essential for the catalysis of many important cellular enzymes. In a study of Iranian adults with MetS, serum Cu levels were found to be significantly higher, but no major variations were found between mean serum Cu in individuals and different numbers of MetS components [15]. However, a study of Chinese adults found no connection between MetS and its constituents and copper [16]. Research into the link between dietary copper intake and MetS risk has found that the MetS-group, both men and women, consumes less of this trace ingredient [17]. Higher flavonoid intake was more closely associated with a lower risk of MetS when combined with high levels of Cu intake in a study [17].

Mets are a leading cause of non-communicable diseases such as cardiovascular disease and T2DM and associated morbidity and mortality. It is widespread throughout the world, with an exceptionally high prevalence in India. This research could develop a biomarker for MetS and its link to IMA and microelements like iron and copper.

To date, there is little evidence for the biomarkers TMAO and IMA, as well as essential trace elements such as copper and iron, concerning MetS components and MetS risk in the Indian population. As a result, this cross-sectional analysis aimed to look into the relationship between serum TMAO, IMA, Iron, and Copper in healthy obese people and obese people with MetS.

Materials and Methods

Study design and sampling method

The study design was Cross-sectional, prospective: a single-center while random sampling method was used for sample selection.

Study site

Patients for the study were obtained from Kasturba Hospital, a tertiary care hospital located in Manipal, Karnataka, India, a teaching hospital affiliated to Kasturba Medical College, and an associate hospital Manipal Academy of Higher Education, started in 1961. The research was conducted at Manipal, Kasturba Medical College, and Department of Biochemistry.

Study participants

A total of 159 patients scheduled for a regular checkup were enrolled. Out of the total, 98 (61.63%) were male, and 61 (38.36%) were female between the age group 30 to 70.

Anthropometric measurements and blood pressure

- All anthropometric measurements were taken without shoes and only wearing light clothing at the start of the research.
- Weight and height were measured in each subject to determine the BMI weight (Kg) divided by height squared (m), kg/m.
- Height was measured using a wall-mounted stadiometer. Bodyweight was determined using a calibrated balance beam scale.
- BMI was classified according to the World Health Organization (WHO)'s criteria with normal weight: 18.5 kg/m-24.9 kg/m; overweight, 25.0 kg/m-29.9 kg/m; grade I obesity, 30.0 kg/

m-34.9 kg/m; grade II obesity, 35.0 kg/m-39.9 kg/m; grade III obesity ≥ 40.0 kg/m [18].

Inclusion and exclusion criteria

- Patients coming for their regular checkup were included based on their BMI according to the World Health Organization (WHO)'s criteria, whereas metabolic syndrome is based on NCEP ATP3 2005 guidelines.
- Patients with heart or kidney failure, cancer patients, HIV patients, age<30 and >70 years, pregnant or breastfeeding females, refusal to participate were excluded.

Ethical consideration

All patients and their attendants gave written informed consent once the aims and objectives of the study were explained. The Institutional Ethics Committee approved the research (IEC: 711/2020), and it was registered with the Clinical Trial Registry of India (CTRI/2021/02/031478).

Study period

The total study period was six months; the enrollment period was from February to May 2021.

The procedure of sample collection

The remaining serum sample of patients received at the biochemistry lab, Kasturba Medical College Manipal, was pipetted into Eppendorf tubes after blood samples were collected by venous puncture, under standard aseptic conditions, into vacutainer tubes (BD Diagnostics). The serum samples were held at -80 degree until they were analyzed.

Biochemical analysis

- Estimation of serum Ischemia Modified Albumin (IMA)
- The method of Bar-Or, et al. was adapted for the analysis of serum IMA [19].
- Estimation of serum Trimethylamine-N-Oxide (TMAO)
- Methods of John C. Wekell and Harold Barnett [20] were adapted to estimate serum TMAO [20].
- Estimation of serum Iron and Copper was performed by using Kit (Agappe)

Statistical analysis

The results of the study and control groups are expressed as the means ± standard deviations and 95% confidence intervals. Dunnett t (2-sided) test and ANOVA variance analyses were performed to compare mean values of normal, obese metabolically healthy and obese with metabolic syndrome. SPSS version 26 was used for statistical analysis. A two-sided test with a P-value of less than 0.05 was considered significant.

Results

The study population consisted of 159 participants, 98 males (61.63%) and 61 females (38.36), aged 30-70 years, visiting Kasturba Hospital for regular checkups and illness related to metabolic syndrome.

Table 1 reports the number of participants, the mean and standard deviation of four parameters in each group healthy Non-obese healthy, metabolically healthy obese [2], and obese with metabolic syndrome [3].

(Descriptive)					
Group		**Iron (µg/dL)**	**Copper (µg/dL)**	**Ischemia modified albumin (ABSU)**	**Trimethylamine-N-oxide (µM)**
Non-obese healthy	Mean	102.85	197.74	0.21	1.08
	N	53	53	53	53
	Std. Deviation	90.847	139.392	0.409	0.267
Metabolically healthy obese	Mean	106.81	182.43	0.42	1.81
	N	53	53	53	53
	Std. Deviation	102.602	140.336	0.535	1.178
Obese with metabolic syndrome	Mean	107.19	153.23	0.62	2.17
	N	53	53	53	53
	Std. Deviation	87.271	103.113	0.53	1.424
Total	Mean	105.61	177.96	0.41	1.68
	N	159	159	159	159
	Std. Deviation	93.264	129.435	0.519	1.163

Table 1: Table showing the mean and SD values of serum level of Trimethylamine-N-oxide, Ischemia modified albumin, Iron, and Copper.

In **Table 2**, we report the one-way ANOVA analysis of all four parameters. In contrast, in **Table 3**, we made multiple comparisons between non-obese healthy, obese without metabolic syndrome, and obese with metabolic syndrome.

(ANOVA analysis)						
		Sum of Squares	**Degrees of Freedom (df)**	**Mean square**	**F**	**Sig.**
Iron (µg/dL)	Between groups	610.688	2	305.344	0.035	0.966
	Within groups	1365006.983	155	8806.497		
	Total	1365617.671	157			
Copper (µg/dL)	Between groups	53588.138	2	26794.069	1.612	0.203
	Within groups	2576696.552	155	16623.849		

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	Total	2630284.69	157			
IMA(ABSU)	Between groups	4.367	2	2.183	8.931	0
	Within groups	37.893	155	0.244		
	Total	42.259	157			
TMAO (μM)	Between groups	32.924	2	16.462	14.234	0
	Within groups	179.254	155	1.156		
	Total	212.177	157			

Table 2: Table showing the mean difference between groups.

(Dunnett t (2-sided)): Multiple comparisons							
Dependent Variable	**(I) group**	**(J) group**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
						Lower Bound	**Upper Bound**
Iron (μg/dL)	MHO	1	3.962	18.23	0.966	-36.74	44.66
	MetS	1	4.343	18.317	0.96	-36.55	45.24
Copper (μg/dL)	MHO	1	-15.302	25.046	0.766	-71.22	40.62
	MetS	1	-44.505	25.166	0.14	-100.69	11.68
IMA(ABSU)	MHO	1	0.208	0.096	0.059	-0.01	0.42
	MetS	1	0.408*	0.097	0	0.19	0.62
TMAO(μM)	MHO	1	0.736*	0.209	0.001	0.27	1.2
	MetS	1	1.098*	0.21	0	0.63	1.57

*The mean difference is significant at the 0.05 level. **Abbrevations:** IMA: Ischemia modified albumin; TMAO: Trimethylamine-N-oxide; 1: Non-obese control; MHO: Metabolically healthy obese; MetS: Obese with Metabolic syndrome.

Table 3: Table show the comparisons between non-obese control with obese metabolically healthy and obese with metabolic syndrome.

The present study showed that mean IMA levels in obese metabolically healthy and obese with metabolic syndrome patients were higher (0.42 ± 0.535) and (0.62 ± 0.530) respectively compared to (0.21 ± 0.409) in normal control (**Table 1**), with a p-value<0.005 indicating a significance. But in multiple comparisons of control with obese metabolically healthy doesn't show significant at P value<0.005, while obese with metabolic syndrome show significance.

Similarly, when the mean TMAO is compared with Non-obese healthy with metabolically healthy obese and metabolic syndrome, it shows a higher level (1.08 ± 0.267), (1.81 ± 1.178), and (2.17 ± 1.424), respectively. In contrast, Iron and Copper don't show significant (at p-value 0.005) with metabolically healthy obese and obese with metabolic syndrome compared to Non-obese healthy.

Discussion

Circulating levels of TMAO, IMA, Iron, and Copper in a sample of normal control adults, obese without metabolic syndrome, and obese with metabolic syndrome were assessed in this cross-sectional study. We found that serum TMAO and IMA circulating levels increase in obese and Mets in the current study. Moreover, we confirmed the positive association of circulating levels of TMAO with both obesity and MetS, whereas IMA with Mets. But Iron and copper don't show an association with obesity and metabolic syndrome.

A recent experimental study found that both antisense oligonucleotide-mediated knockdown and genetic deletion of the TMAO-producing enzyme FMO3 prevented mice from obesity caused by a high-fat diet Indicating a role for the gut microbe-driven TMA/FMO3/TMAO pathway in influencing complex transcriptional reprogramming in white adipocytes [21]. In this analysis, circulating levels of TMAO were positively associated with body weight, fat mass, mesenteric adiposity and subcutaneous adiposity across the various mice-inbred strains; in addition, the expression of FMO3 in liver was linked to BMI and waist-to-hip ratio in cohorts of overweight or obese subjects with metabolic features and other ethnicity [21]. Consistent with these data, the present study's findings show a clear positive association of circulating TMAO levels with MetS. Apart from its position as a risk factor for CVD and adverse events in high-risk individuals, new research indicates that TMAO derived from the gut microbiota may be a key environmental factor in obesity and obesity-related disorders [22]. In mice fed a high-fat diet, researchers discovered that a high urinary excretion of TMAO was linked to insulin resistance [23]. Hepatic insulin resistance is associated with elevated circulating TMAO levels and a high up-regulation of the TMAO-producing enzyme FMO3 in the liver, according to another report [24]. According to the proposed mechanism, TMAO may block the hepatic insulin signaling pathway, thereby exacerbating the impaired glucose tolerance and promoting the development of fatty liver [25]. According to human studies, Randrianarisoa, et al. reported a positive correlation between TMAO and MetS [6].

Reduced oxygen supply to the brain causes localized acidosis and the generation of free radicals. Ions like copper and zinc, generally bound to proteins in the plasma, are released from protein-binding sites and circulate in the free form [26]. The N-terminus of albumin, which binds transition metals typically, however, is susceptible to biochemical alteration. It is postulated that albumin acts as a "sacrificial" antioxidant to reduce injury during reperfusion [27]. The altered form is referred to as IMA. Following a period of ischemia, a reduction in the ability of albumin to bind cobalt is apparent, and hence the levels of IMA increase [28]. A recent study found a correlation between IMA

and cardiovascular disease, which is similar to our study 15. IMA levels were statistically significantly higher in the metabolically unhealthy obese group than in the control group (metabolically healthy obese) [16]. Our study showed a positive association between IMA and obesity with metabolic syndrome but not metabolically healthy.

Iron levels are often elevated in MetS patients, according to previous research, and are related to the risk of complications. But in our study, we didn't found any correlation between serum Iron with metabolic syndrome. Similarly, it shows serum ferritin levels were associated with MetS incidence and BMI in both men and women [14]

Cu intake at particular doses in healthy middle-aged volunteers showed an antioxidant effect in protecting red blood cell membranes from free-radical-mediated oxidation [29], but Simona Bo, et al. did not recommend Cu supplementation considering its association with inflammation and oxidative markers stress [30]. From our data, the lack of association between serum Cu levels and MetS is in line with the result reported in Iranian and Chinese subjects [16,31]. Similarly, in Croatian adults, plasma Cu levels are not linked to MetS, similar to ours. Still, many cross-sectional and prospective studies indicate that higher serum Cu levels are linked to MetS or its components.

Conclusion

In conclusion, our study demonstrated that TMAO might be a sensitive biomarker for screening of obese without metabolic syndrome and metabolic syndrome; similarly, IMA can also be used for obese patients. But in our study, we didn't found an association between copper and Iron with MetS and obesity. Further, well-designed validation studies are required to develop blood biomarkers to improve the care of patients with obesity and metabolic syndrome.

In conclusion, in general, Serum TMAO level was positively associated with obesity with metabolic syndrome and without metabolic syndrome as compared, whereas IMA was only related to metabolic syndrome. There was no positive association between Iron and Copper with both the group compared to control in our study. The key drawback of our research was that we did not examine individual's dietary patterns, which may have an effect on serum TMAO, IMA, Iron, and Copper levels.

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Conflicts of Interest

There are no conflicts of interest.

Reference

1. Ranasinghe P, Mathangasinghe Y, Jayawardena R, Hills AP, Misra A (2017) Prevalence and trends of metabolic syndrome among adults in the asia-pacific region: A systematic review. BMC Public Health 17: 101-105.
2. Krishnamoorthy Y, Rajaa S, Murali S, Rehman TI, Sahoo J, et al. (2020) Prevalence of metabolic syndrome among adult population in India: A systematic review and meta-analysis.
3. Lukács A, Horváth E, Máté Z, Szabó A, Virág K, et al. (2019) Abdominal obesity increases metabolic risk factors in non-obese adults: A Hungarian cross-sectional study. BMC Public Health 19: 1-8.
4. Duque AP, Luiz F, Rodrigues J, Mediano MFF, Tibiriça E, et al.(2020) Emerging concepts in metabolically healthy obesity. Am J Cardiovasc Dis 10: 48-61.
5. Barrea L, Annunziata G, Muscogiuri G, Di Somma C, Laudisio D, et al. (2018) Trimethylamine-N-oxide (TMAO) as novel potential biomarker of early predictors of metabolic syndrome. Nutrients 10: 1971-1975.
6. Randrianarisoa E, Lehn-Stefan A, Wang X, Hoene M, Peter A, et al. (2016) Relationship of serum Trimethylamine N-oxide (TMAO) levels with early atherosclerosis in humans.
7. Lent-Schochet D, Silva R, McLaughlin M, Huet B, Jialal I (2018) Changes to trimethylamine-N-oxide and its precursors in nascent metabolic syndrome. Horm Mol Biol Clin Investig 35: 1-8.
8. Subramaniam S, Fletcher C (2018) Trimethylamine N-oxide: Breathe new life. Br J Pharmacol 175: 1344-1353.
9. Su X, Zhang K, Guo F, Yuan B, Wang C, et al. (2013) Ischemia-modified albumin, a predictive marker of major adverse cardiovascular events in continuous ambulatory peritoneal dialysis patients. Clin Biochem 46: 1410-3.
10. Menon B, Ramalingam K, Krishna V (2019) Study of ischemia modified albumin as a biomarker in acute ischaemic stroke. Ann Neurosci 25: 187-90.
11. Etli M (2021) Investigation of serum ischemia-modified albumin levels in coronary artery disease patients. Indian J Thorac Cardiovasc Surg 37: 147-52.
12. Mengen E, Uçaktürk SA, Kocaay P, Kaymaz Ö, Neşelioğlu S, et al. (2020) The significance of thiol/disulfide homeostasis and ischemia-modified albumin levels in assessing oxidative stress in obese children and adolescents. JCRPE 12: 45-54.
13. Robberecht H, Bruyne TDe, Hermans N (2017) Biomarkers of the metabolic syndrome: Influence of minerals, oligo- and trace elements. J Trace Elem Med Biol 43: 23-28.
14. Cho MR, Park JK, Choi WJ, Cho AR, Lee YJ (2017) Serum ferritin level is positively associated with insulin resistance and metabolic syndrome in postmenopausal women: A nationwide population-based study. Maturitas 103: 3-7.
15. Darroudi S, Fereydouni N, Tayefi M, Esmaily H, Sadabadi F, et al. (2019) Altered serum Zinc and Copper in Iranian Adults who were of normal weight but metabolically obese. Sci Rep 9: 1-8.
16. Fang C, Wu W, Gu X, Dai S, Zhou Q, et al. (2019) Association of serum copper, zinc and selenium levels with risk of metabolic syndrome: A nested case-control study of middle-aged and older Chinese adults. J Trace Elem Med Biol 52: 209-215.

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17. Qu R, Jia Y, Liu J, Jin S, Han T, et al. (2018) Dietary flavonoids, copper intake, and risk of metabolic syndrome in chinese adults. 10:991-995.

18. World Health Organisation (2008) Waist Circumference and Waist-Hip Ratio. Report of a WHO Expert Consultation. Geneva : 8-11.

19. Bar-Or D, Lau E, Winkler J V (2000) A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia: A preliminary report. J Emerg Med 19: 311-315.

20. Wekell JC, Barnett H (1991) New method for analysis of trimethylamine oxide using ferrous sulfate and EDTA. J Food Sci 56: 132-5.

21. Schugar RC, Shih DM, Warrier M, Helsley RN, Ferguson D, et al. (2017) HHS Public Access. 19: 2451-61.

22. Amato MC, Giordano C, Galia M, Criscimanna A, Vitabile S, et al. (2010) Visceral adiposity index: A reliable indicator of visceral fat function associated with cardiometabolic risk. Diabetes Care 33: 920-922.

23. Dumas ME, Barton RH, Toye A, Cloarec O, Blancher C, et al. (2006) Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. Proc Natl Acad Sci USA 103: 12511-12516.

24. Miao J, Ling AV, Manthena PV, Gearing ME, Graham MJ, et al. (2015) Flavin-containing monooxygenase 3 as a potential player in diabetes-associated atherosclerosis. Nat Commun 6: 1-10.

25. Gao X, Liu X, Xu J, Xue C, Xue Y, et al. (2014) Dietary trimethylamine N-oxide exacerbates impaired glucose tolerance in mice fed a high fat diet. J Biosci Bioeng 118: 476-81.

26. Mosteller RD (1987) The New England Journal of Medicine Downloaded from nejm.org at DUKE MEDICAL CENTER LIBRARY From the NEJM Archive. Medical Society. N Engl J Med. 307:2012.

27. Gutierrez-Correa J, Stoppani AOM (1997) Inactivation of yeast glutathione reductase by fenton systems: Effect of metal chelators, catecholamines and thiol compounds. Free Radic Res 27: 543-55.

28. Can M, Demirtas S, Polat O, Yildiz A (2006) Evaluation of effects of ischaemia on the Albumin Cobalt Binding (ACB) assay in patients exposed to trauma. Emerg Med J 23: 537-539.

29. Rock E, Mazur A, O'Connor JM, Bonham MP, Rayssiguier Y, et al. (2000) The effect of copper supplementation on red blood cell oxidizability and plasma antioxidants in middle-aged healthy volunteers. Free Radic Biol Med 28: 324-329.

30. Bo S, Durazzo M, Gambino R, Berutti C, Milanesio N, et al. (2008) Associations of dietary and serum copper with inflammation, oxidative stress, and metabolic variables in adults. J Nutr 138: 305-10.

31. Ghayour MM, Shapouri MA, Azimi NM, Esmaeili H, Parizadeh SMR, et al. (2009) The relationship between established coronary risk factors and serum copper and zinc concentrations in a large Persian Cohort. J Trace Elem Med Biol 23: 167-75.