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[MCBS] Submission Acknowledgement

3 messages

MCBS Office <mcbs_office@cellbiopharm.com> Mon, May 11, 2020 at 9:58 PM
To: "Dr. Udin Bahrudin" <bahrudin00@gmail.com>

Dr. Udin Bahrudin:

Thank you for submitting the manuscript, "Body Fat Percentage, Waist Circumference and Body Mass Index are Correlated with Nitric Oxide Levels in Indonesian Young Adults with Central Obesity" to Molecular and Cellular Biomedical Sciences. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

Manuscript URL:
https://cellbiopharm.com/ojs/index.php/MCBS/author/submission/165
Username: udin_bahrudin

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

MCBS Office
Molecular and Cellular Biomedical Sciences

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bahrudin udin <bahrudin00@gmail.com> Mon, May 11, 2020 at 10:00 PM
To: feriyandi nauli <feriyandi.nauli@gmail.com>

FYI,
[Quoted text hidden]
--
Bahrudin, MD, PhD
Dept. of Cardiology and Vascular Medicine
Faculty of Medicine, Diponegoro Univ.
Jl. Dr. Sutomo 18 Semarang, Indonesia
Email: bahrudin00@gmail.com
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Feriyandi Nauli <feriyandi.nauli@gmail.com> Mon, May 11, 2020 at 10:01 PM
To: bahrudin udin <bahrudin00@gmail.com>

Terimakasih Dokter...
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Gmail bahrudin udin <bahrudin00@gmail.com>

[MCBS] #165 Manuscript - Initial Check

4 messages

MCBS Office <mcbs.office@gmail.com> Fri, May 15, 2020 at 12:21 PM
To: bahrudin00@gmail.com
Cc: feriyandi.nauli@gmail.com

Dear Dr. Udin Bahrudin,

Good day. Thank you for your submission of manuscript "**Body Fat Percentage, Waist Circumference and Body Mass Index are Correlated with Nitric Oxide Levels in Indonesian Young Adults with Central Obesity**" to Molecular and Celullar Biomedical Sciences.

Before your manuscript being sent to our reviewers, it has been initially checked. We need you to make some revisions according to the correction list. Find the attached manuscript for detail information.

Please send us an email of your revised manuscript before **May 20, 2020** so that we can proceed with the peer-reviewing process. Please let us if you have read this email.

Thank you. We wish you a nice day.

Best Regards,
MCBS Office

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bahrudin udin <bahrudin00@gmail.com> Sat, May 16, 2020 at 3:43 PM
To: MCBS Office <mcbs.office@gmail.com>
Cc: feriyandi nauli <feriyandi.nauli@gmail.com>

Dear Editor in Chief
MCBS

Thank you for your email and notification. Herewith in the attachment I send the revised manuscript. Please let us know if there is any requirement.

Sincerely yours,
Bahrudin
[Quoted text hidden]
--
Bahrudin, MD, PhD
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#165 Manuscript - Initial Check[1079] revised.docx
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MCBS Office <mcbs.office@gmail.com> Wed, May 20, 2020 at 10:36 AM

To: bahrudin udin <bahrudin00@gmail.com>

Dear Dr. Udin Bahrudin,

Good day. Thank you for your initial check revision. You manuscript has been sent to our reviewers for a peer-review process. One round of review might take around 3-4 weeks, we will contact you back after we receive the review result.

We wish you a nice day.

Best regards,
[Quoted text hidden]

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bahrudin udin <bahrudin00@gmail.com> Wed, May 20, 2020 at 10:43 AM
To: feriyandi nauli <feriyandi.nauli@gmail.com>

Fyi,
[Quoted text hidden]

Gmail **bahrudin udin <bahrudin00@gmail.com>**

[MCBS] #165 Editor Decision - Resubmit for Review

1 message

MCBS Office <mcbs.office@gmail.com> Wed, May 27, 2020 at 11:22 AM
To: bahrudin udin <bahrudin00@gmail.com>

Dear Dr. Udin Bahrudin,

Good day. We have reached a decision regarding your submission to Molecular and Cellular Biomedical Sciences, "**Body Fat Percentage, Waist Circumference and Body Mass Index are Correlated with Nitric Oxide Levels in Indonesian Young Adults with Central Obesity**".

Our decision is: **Resubmit for Review**.

Please find our reviewers comments in the file attached. Provide us a revised version of your manuscript and a response letter before **June 10, 2020**. Mark/highlighted the revised part of the manuscript, so that editor will notice the changes. In your response letter, please address your argument regarding the reviewer's questions.

Please let us know when you have read this email. Do not hesitate to contact us if you have any questions. Thank you, we wish you a nice day.

Best Regards,
MCBS Office

#165 Manuscript - Round 1 (Resubmit for Review).docx
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Body Fat Percentage, Waist Circumference and Body Mass Index are Correlated with Nitric Oxide Levels in Indonesian Young Adults with Central Obesity

Abstract

Background: Central obesity stands for the cornerstone of cardio-metabolic health and nitric oxide (NO) is a major regulator of cardiovascular function. Correlation between serum NO (NOx) and the obesity components in young adults remains elusive. Thus, this current study was conducted to know the correlation between serum nitric oxide levels and body fat percentage, waist circumference (WC) as well as body mass index (BMI) in Indonesian young adults with central obesity.

Materials and Methods: A cross-sectional study was conducted in Indonesian young adults ages 18-25 years, composing of 39 and 40 subjects with and without central obesity, respectively. Anthropometric measurements were performed to assess WC and BMI. Body fat percentages were measured using bioelectrical impedance analysis and NOx levels were assessed using Griess methods.

Results: Levels of NOx were significant higher in the subjects with central obesity than that of normal subjects, but the levels were no significant different between male and female subjects. NOx levels were strongly correlated with total body fat (r = 0.618, p<0.001), visceral fat (r = 0.733, p<0.001), subcutaneous fat (r = 0.547, p<0.001), WC (r = 0.717, p<0.001) and BMI (r = 0.788, p<0.001).

Conclusions: Levels of serum NO are higher in the central obesity group than that of the normal. Body fat percentage, waist circumference and body mass index are correlated with serum nitric oxide levels in Indonesian young adults with central obesity.

Keywords: nitric oxide, body fat percentage, young adults, central obesity.

Commented [11]: Judul perlu diganti, dan disebutkan di kota Riau. Karena sampling hanya di Universitas Riau tidak mewakili Indonesia. Penelitian sebelumnya sudah ada yg membahas di kota Padang.

Bisa lihat jurnal penelitian:
"Hubungan antara Indeks Massa Tubuh dengan Kadar Nitrit Oksid pada Masyarakat Etnik Minangkabau di Kota Padang" oleh Nidia Purwadianti, Fadil Oenzil, Delmi Sulastri

Commented [12]: Terdapat ketidakkonsistenan penulisan sampai akhir artikel, kadang NOx kadang NO.

Pertanyaan:
Kenapa menggunakan NOx bukan NO saja?
Karena NOx adalah istilah yg umum untuk polutan.

Introduction

Obesity is a buildup of abnormal or excessive adipose tissue which can interfere with the cardio-metabolic health of individuals.[1] A simple parameter commonly used to classify obesity is body mass index (BMI).[2] According to the World Health Organization (WHO) classification for Asia Pacific criteria, a person with a BMI of ≥23 kg/m^2 or ≥25 kg/m^2 is defined as overweight or obese, respectively.[1–3] Subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) are two main compartments of adipose tissue with different metabolic characteristics.[4] Central obesity is characterized by escalated VAT around intraabdominal organs.[4] This obesity is associated with various pathological disorders consisting of impaired lipid and glucose metabolisms, insulin resistance, and increased tendency for colon, breast and prostate cancers.[4]

Accurate measurements of body fat composition can carried out with sophisticated methods including magnetic resonance imaging (MRI), dual-energy X-ray absorptiometry (DXA) and computed tomography (CT).[4,5] CT-Scan is the gold standard for measuring quantitative tissue of intra-abdominal fat.[6] These methods in routine clinical practice cannot be applied since they are expensive, time-wasting, limited portability, requiring skilled staff, limited accessibility and radiation. Bioelectrical impedance analysis (BIA) is an alternative method for measuring body fat because it is fast, safe and non-invasive.[4,5,7,8] BIA showed a significant strong correlation with CT in measuring of VAT and SAT ($r = 0.89$ and $r = 0.85$, respectively)[6] and also with MRI in determining body fat and VAT ($r = 0.89$ and $r = 0.84$, respectively).[9]

Nitric oxide (NO) is a signaling and free radical molecule. It is synthetized from L-arginine by nitric oxide synthetase (NOS) in many cells in our body.[10,11] In normal conditions, it is synthesized by endothelial-NOS (eNOS) and neuronal-NOS (nNOS) and played an important regulator of cardiovascular and renal function.[12] Correlation between NO serum

(NOx) and the obesity components remain elusive. Several studies showed there were positive correlation between NOx and BMI[13], between NOx and body fat[14], between NOx and visceral fat[15], while others studies showed negative correlation between NOx and obesity[16], between NOx and abdominal adiposity.[17] Study about this correlation in young adults with central obesity is limited and have never been done in Indonesia. Thus, this study was conducted to know the correlation between serum nitric oxide levels and body fat percentage, waist circumference as well as body mass index in Indonesian young adults with central obesity.

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Mohon dibaca dan literatur ditambahkan:
"Gambaran Kadar Nitric Oxide (No) Pada Masyarakat Yogyakarta Profile Of Nitric Oxide (No) Levels In Yogyakarta Society" oleh Agustina Susilowati, Akrom, Endang Darmawan

Methods

Subjects

This cross-sectional was conducted in Riau University, Indonesia from December 2019 to February 2020. Subjects of study were college students aged 18-25 years old and selected by consecutive sampling. The WHO criteria for Asia Pacific was used to determine central obesity, i.e. waist circumference (WC) ≥90 cm and ≥80 cm for men and women, respectively. Subjects who, pregnant, taking drugs (either hormonal therapy, steroid or aspirin), suffer from ascites, abdominal tumors, cardiovascular disease, kidney disease, diabetes mellitus, allergic diseases or diarrhea were excluded. Informed consent for participating in this study was obtained. The investigation conformed to the principles outlined in the Declaration of Helsinki[18] and was approved by the institutional ethics committee of human research in University of Riau, number: B/227/UN.19.5.1.1.8/UEPKK/2019.

Anthropometric measurements

Anthropometric measurements were carried out to assess waist circumference (cm), height (cm) and weight (kg) in a standing position. WC was measured at the midpoint between the edge of the last rib and the iliac crest without pressing the skin at the end of normal

expiration using the met line. Weight and height were measured according to standard protocols. BMI was obtained from the calculation of body weight (kg) divided by the square of height (m^2). Examination of body fat compositions was carried out using a BIA (OMRON Karada Scan HBF-375) to measure the percentage of total body fat, visceral fat and subcutaneous fat.

Nitric oxide level measurement

Venous blood samples were taken in the morning after fasting for 12 hours. Serum NO levels were measured using the Griess methods by employing QuantiChrom™ Nitric Oxide Assay Kit (Catologue no. D2NO-100, BioAssay Systems, California, USA) at the GAKI Laboratory, Diponegoro University, Semarang, Indonesia. Briefly, serum samples were deproteinated by mixing 150 µL serum with 8 µL ZnSO4 and 8 µL NaOH, then they were centrifuged for 10 minutes at 14,000 rpm. A total of 100 µL of supernatant for each tube was transferred into another tube, and then 100 µL Reagent A, 4 µL Reagent B and 100 µL Reagent C were added. Those mixture were incubated for 10 minutes at 60^0 C prior transferring them into separated wells for detecting the signal density. The optical density was read at 540 nm using the enzyme-linked immunosorbent assay (ELISA) reader (ELx800, BioTek Instruments, Vermont, USA).

Statistical analysis

Data analysis was performed using a computer application. Test normality with Kolmogorov-Smirnov for total subjects and Shapiro-Wilk for the central obesity and without central obesity subgroup. The comparison of sex and age proportions between groups was performed chi-square test, the comparison of total fat variables between groups using the Independent-T-test, while the comparison of visceral fat, subcutaneous fat, BMI, WC and NOx

levels between groups using Mann-Whitney-U test and also the comparison of NOx levels between gender using Mann-Whitney-U. The correlation relationship between NO levels and percentage of body fat (total, visceral and subcutaneous fat), WC and BMI were measured using the Sperman's rho correlation test with $p<0.05$ significance value and 95% confidence interval. Differences with a p value of <0.05 were considered statistically significant.

Results

A total 79 subjects were recruited, consisting of 39 and 40 subjects with and without central obesity, respectively. Among them, 44 (55.7%) subjects were female. Table 1 shows the baseline clinical characteristics of the subjects with and without central obesity.

There were no significant differences ($p>0.05$) in the proportion of gender and age variables between both groups, while significant differences ($p<0.001$) were found for total body fat, visceral fat, subcutaneous fat, WC and BMI. The levels of NO were not significant differences between male and female in either the all subjects, ones with or without central obesity, with respectively p value 0.875, 0.135 and 0.712.

Table 2 shows that all variables had correlation with NOx levels, they were total body fat percentage ($r = 0.618, p<0.001$),visceral fat ($r = 0.733$, $p<0.001$), subcutaneous fat ($r = 0.547$, $p<0.001$), WC ($r = 0.717$, $p<0.001$) and BMI ($r = 0.788$, $p<0.001$).

Discussion

Correlation between NOx and the obesity components in young adults with central obesity remains elusive. This study was conducted to know the correlation between body fat percentage and NOx in those subjects and found that levels of the NO were strongly correlated with body fat percentage, WC and BMI. To the best of our knowledge, this is the first data about this issue reported from Indonesian subjects.

These results are in line with several previous studies that found higher levels of serum nitric oxide in obese subjects. Study of 313 children aged 8 to 9 years in Portugal concluded that the increase in NOx levels was related to the total body fat [12], as well as the study in Poland of 154 women grouped by BMI found positive correlation between the total body fat, BMI with NOx levels.[19] Similar research results were also obtained in a South Korean study of 363 subjects aged 14 to 19 years which concluded that obesity causes an increase in NO production in humans, the increasing in NOx levels is strongly correlated with obesity, total body fat and BMI.[14] The study of 110 children aged 7 to 14 years in Spain found higher and significant NOx levels in obese children compare to normal weight children.[20] Contradictory research results were reported in a study of 114 adolescents (aged 14.01 ± 3.19) in Austria, there was a decrease NOx levels in obese adolescents, NOx levels were negatively correlated with BMI.[16] Japanese study of 486 subjects aged 40-84 years concluded that there was an inverse relationship between NO bioactivity and accumulation of abdominal fat measured using WC and waist-hip circumference ratio.[17] Taken together, in obese young adults, the high levels of NOx are correlated positively with central obesity; but in adolescents, the correlation are inversely.

The high levels of NOx in obese subjects is caused by an increase of NO production in adipose tissue[19,21,22] as a compensatory mechanisms for changes related to obesity.[13,20] The presence of eNOS and inducible-NOS (iNOS) in the adipose cells induces the NO production.[15,23,24] Each of them produces NO with different rates.[23] eNOS generates a smaller amount of NO compared to iNOS which has the biggest capacity to ~~procude~~produce NO.[23,25] Under physiological conditions, eNOS is a dominant isoform in the adipose tissue compared to iNOS.[22,25] In obesity, accumulation of proinflammatory macrophages in adipose tissue is responsible for most of iNOS expressions by promoting its expression.[23] In patients with obesity, the hypertrophic adipocytes reduce a diffusion ability of oxygen and the reduction in vascularization promotes hypoxia.[24] Under the hypoxic conditions, endogenous NO production

Commented [14]: NO berasal dari L-carnitine. Apakah ada hub yg obese mengkonsumsi L-carnitine lbh banyak? Apakah bs ditambahkan di pembahasan?

increase due to the increased iNOS expression rather than the eNOS.[23–25] Thus, iNOS produces large amounts of NO as a defensive mechanism.[26]

In the population-based studies, the origin of NOx values in young obese subjects cannot be distinguished whether they are from eNOS or iNOS, since the difficulty in measuring of NO levels *in vivo*.[25,27] NO is a gaseous and has a very short half-life, ranging from 0.05 milliseconds to 30 seconds, depending on the environment and reactant availability.[23,24,26,27] Inability to distinguish the eNOS and iNOS components in NOx is a limitation of this study as well as other population-base studies. The existence of inflammation in the subject of this study could not be confirmed since we did not check any marker of inflammation.

NO production is divided into two independent pathways, i.e. NOS pathway and the NO3-NO2-NO pathway.[25] These pathways make the meaning of a high NOx levels more intricate, unless a well controlled food intake of nitrates and nitrites.[25] Nitrates do not have first-pass metabolism, so after consumption of food, their level increases rapidly within 15 minutes, peaking at 90 minutes with a half-life of NO3 5 to 8 hours.[24,25] Under normal conditions, both sources of NO contribute equally to the overall formation of NO.[28] During fasting condition, NOx level is weakly correlated with NO3 consumption in a regular diet.[25] Under physiological conditions, fasting plasma NO2 reflects up to 80% of endogenous NO production.[25] The current study did not control the diet of the subjects prior to the blood sample collection.

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Obesity with high NO levels may cause more deleterious effects.[12] The increased expression and function of iNOS causes the excessive NO production and the disturbed eNOS affects the decreased NO bioavailability. Both of them promote the growth of non-communicable diseases such as type 2 diabetes mellitus, hypertension, coronary heart disease, kidney disorders and various types of cancer.[25] Data of the current study that levels of NOx

were higher in obese subjects than that of the normal control indicated an alarm on the future disease for those subjects.

This study also found that there were no significant differences in NOx levels between men and women. These results are similar to data of other studies.[16,17] Although another study found inversely result.[29] This warrant for future study.

Conclusions

Finally, we conclude that levels of serum NO are higher in central obesity group than that of the normal. The body fat percentage, waist circumference and body mass index are correlated with serum nitric oxide levels in Indonesian young adults with central obesity.

References

1. WHO/IASO/IOTF. The Asia-Pacific Perspective : Redefining Obesity and its treatment. Australia: WHO; 2000. 15–21 p.
2. WHO. Obesity and overweight [Internet]. 2018 [cited 2019 Jul 21]. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
3. Lim JU, Lee JH, Kim JS, Hwang Y Il, Kim TH, Lim SY, et al. Comparison of World Health Organization and Asia-Pacific body mass index classifications in COPD patients. Int J COPD. 2017;12:2465–75.
4. Shuster A, Patlas M, Pinthus JH, Mourtzakis M. The clinical importance of visceral adiposity: A critical review of methods for visceral adipose tissue analysis. Br J Radiol. 2012;85(1009):1–10.
5. Ramírez-Vélez R, Correa-Bautista JE, Martínez-Torres J, González-Ruíz K, González-Jiménez E, Schmidt-RioValle J, et al. Performance of Two Bioelectrical Impedance Analyses in the Diagnosis of Overweight and Obesity in Children and Adolescents: The FUPRECOL Study. Nutrients. 2016;8(10):1–13.
6. Park KS, Lee DH, Lee J, Kim YJ, Jung KY, Kim KM, et al. Comparison between two methods of bioelectrical impedance analyses for accuracy in measuring abdominal visceral fat area. J Diabetes Complications. 2016;30(2):343–9.
7. Lu HK, Chen YY, Yeh C, Chuang CL, Chiang LM, Lai CL, et al. Discrepancies between leg-to-leg bioelectrical Impedance analysis and computerized tomography in abdominal visceral fat measurement. Sci Rep. 2017;7(1):1–8.
8. Day K, Kwok A, Evans A, Mata F, Verdejo-Garcia A, Hart K, et al. Comparison of a Bioelectrical Impedance Device against the Reference Method Dual Energy X-Ray Absorptiometry and Anthropometry for the Evaluation of Body Composition in Adults. Nutrients. 2018;10(10):1469.

9. Wang JG, Zhang Y, Chen HE, Li Y, Cheng XG, Xu L, et al. Comparison of two bioelectrical impedance analysis devices with dual energy x-ray absorptiometry and magnetic resonance imaging in the estimation of body composition. J Strength Cond Res. 2013;27(1):236–43.
10. Uppalapati A, Gogineni S, Espiritu JR. Association between Body Mass Index (BMI) and fraction of exhaled nitric oxide (FeNO) levels in the National Health and Nutrition Examination Survey (NHANES) 2007–2010. Obes Res Clin Pract. 2016;10(6):652–8.
11. Ahmad A, Dempsey S, Daneva Z, Azam M, Li N, Li P-L, et al. Role of Nitric Oxide in the Cardiovascular and Renal Systems. Int J Mol Sci. 2018;19(9):2605.
12. Correia-Costa L, Sousa T, Morato M, Cosme D, Afonso J, Areias JC, et al. Oxidative stress and nitric oxide are increased in obese children and correlate with cardiometabolic risk and renal function. Br J Nutr. 2016;116(5):805–15.
13. Ghasemi A, Zahediasl S, Azizi F. Elevated nitric oxide metabolites are associated with obesity in women. Arch Iran Med. 2013;16(9):521–5.
14. Choi JW, Pai SH, Kim SK, Ito M, Park CS, Cha YN. Increases in nitric oxide concentrations correlate strongly with body fat in obese humans. Clin Chem. 2001;47(6):1106–9.
15. Fujita K, Wada K, Nozaki Y, Yoneda M, Endo H, Takahashi H, et al. Serum nitric oxide metabolite as a biomarker of visceral fat accumulation: Clinical significance of measurement for nitrate/nitrite. Med Sci Monit. 2011;17(3):CR123–31.
16. Gruber HJ, Mayer C, Mangge H, Fauler G, Grandits N, Wilders-Truschnig M. Obesity reduces the bioavailability of nitric oxide in juveniles. Int J Obes. 2008;32(5):826–31.
17. Kondo T, Ueyama J, Imai R, Suzuki K, Ito Y. Association of abdominal circumference with serum nitric oxide concentration in healthy population. Environ Health Prev Med. 2006;11(6):321–5.

18. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191–4.
19. Olszanecka-Glinianowicz M, Zahorska-Markiewicz B, Janowska J, Zurakowski A. Serum concentrations of nitric oxide, tumor necrosis factor (TNF)-α and TNF soluble receptors in women with overweight and obesity. Metabolism. 2004;53(10):1268–73.
20. Codoñer-Franch P, Tavárez-Alonso S, Murria-Estal R, Megías-Vericat J, Tortajada-Girbés M, Alonso-Iglesias E. Nitric oxide production is increased in severely obese children and related to markers of oxidative stress and inflammation. Atherosclerosis. 2011;215(2):475–80.
21. Foroumandi E, Alizadeh M, Kheirouri S, Asghari Jafarabadi M. Exploring the role of body mass index in relationship of serum nitric oxide and advanced glycation end products in apparently healthy subjects. Chiang M-C, editor. PLoS One. 2019;14(3):e0213307.
22. Engeli S, Janke J, Gorzelniak K, Böhnke J, Ghose N, Lindschau C, et al. Regulation of the nitric oxide system in human adipose tissue. J Lipid Res. 2004;45(9):1640–8.
23. Sansbury BE, Hill BG. Regulation of obesity and insulin resistance by nitric oxide. Free Radic Biol Med. 2014;73:383–99.
24. Ghasemi A, Jeddi S. Anti-obesity and anti-diabetic effects of nitrate and nitrite. Nitric Oxide. 2017;70(24):9–24.
25. Bahadoran Z, Mirmiran P, Jeddi S, Carlström M, Azizi F, Ghasemi A. Circulating markers of nitric oxide homeostasis and cardiometabolic diseases: insights from population-based studies. Free Radic Res. 2019;53(4):359–76.
26. Khazan M, Hdayati M. The Role of Nitric Oxide in Health and Diseases. Scimetr. 2014;3(1):1–10.

27. Caimi G, Hopps E, Montana M, Carollo C, Calandrino V, Incalcaterra E, et al. Nitric oxide metabolites (nitrite and nitrate) in several clinical condition. Clin Hemorheol Microcirc. 2014;56(4):359–69.

28. Carlström M, Liu M, Yang T, Zollbrecht C, Huang L, Peleli M, et al. Cross-talk Between Nitrate-Nitrite-NO and NO Synthase Pathways in Control of Vascular NO Homeostasis. Antioxid Redox Signal. 2015;23(4):295–306.

29. Ghasemi A, Zahedi Asl S, Mehrabi Y, Saadat N, Azizi F. Serum nitric oxide metabolite levels in a general healthy population: relation to sex and age. Life Sci. 2008;83(9–10):326–31.

Tables

Table 1. Subjects characteristics according to groups

Variables	Central obesity Mean ± SD (Min - Max)	Without central obesity Mean ± SD (Min - Max)	*p*
Subjects (n)	39	40	0.058 [a]
Male	18	17	
Female	21	23	
Age (years)	18.7 ± 0.9	19.2 ± 1.3	0.147 [b]
	(18 – 21)	(18 – 22)	
BMI (kg/m^2)	30.55 ± 4.54	21.16 ± 2.61	<0.001 [b]
	(23.8 – 43.3)	(16.6 – 25.9)	
Waist circumference (cm)	96.50 ± 11.17	75.36 ± 6.73	<0.001 [b]
	(80.5 – 129.5)	(64.0 – 89.0)	
Total body fat (%)	33.23 ± 5.08	23.55 ± 7.21	<0.001 [c]
	(21.0 – 42.1)	(8.7 – 38.9)	
Visceral fat (%)	11.94 ± 5.52	3.68 ± 2.25	<0.001 [b]
	(3.5 – 29.0)	(0.5 – 8.5)	
Subcutaneous fat (%)	28.28 ± 7.60	18.66 ± 6.70	<0.001 [b]
	(15.0 – 41.1)	(6.4 – 30.3)	
Nitric oxide serum level (μmol/L)	168.41 ± 12.64	70.57 ± 44.99	<0.001 [b]
	(138.6 – 200.1)	(22.1 – 150.2)	

Note: [a] Chi-square test, [b] Mann Whitney U test, [c] Independent T test

Table 2. Correlation between serum NO levels and body fat percentage, WC as well as BMI

Variables	Serum nitric oxide levels					
	Central obesity		Without central obesity		Total subjects	
	r	*p*	r	*p*	r	*p*
Total body fat	0.370	0.020 [a]	0.064	0.695 [b]	0.618	<0.001 [b]
Visceral fat	0.257	0.115 [b]	0.109	0.504 [b]	0.733	<0.001 [b]
Subcutaneous fat	0.383	0.016 [b]	0.023	0.886 [b]	0.547	<0.001 [b]
Waist circumference	0.037	0.821 [b]	0.058	0.722 [b]	0.717	<0.001 [b]
Body mass index	0.352	0.028 [b]	0.105	0.520 [b]	0.788	<0.001 [b]

Note: [a] Pearson correlation test, [b] Spearman's Rho correlation test

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[MCBS] #162 Editor Decision Round 2 - Revisions Required

1 message

Nurrani Mustika Dewi <mcbs_office@cellbiopharm.com> Fri, Jun 12, 2020 at 9:18 AM
To: "Dr. Udin Bahrudin" <bahrudin00@gmail.com>

Dear Dr. Udin Bahrudin,

Good day, thank you for your revision in the previous round.

We have reached a decision regarding your submission to Molecular and Cellular Biomedical Sciences, "Body Fat Percentage, Waist Circumference, and Body Mass Index are Correlated with Nitric Oxide Levels in Young Adults with Central Obesity: An Observational Study in Riau, Indonesia".

Our decision is: Revisions Required.

This manuscript only needs some minor revisions. Please find our reviewers comments in the file attached, and revise it according to our reviewers' suggestions.

Provide us a revised version of your manuscript before June 22, 2020. Mark/highlighted the revised part of the manuscript, so that editor will notice the changes.

Please let us know when you have read this email. Do not hesitate to contact us if you have any questions. Thank you, we wish you a nice day.

Best Regards,
MCBS Office

#165 Manuscript - Round 2 (Revisions Required).docx
88K

Body Fat Percentage, Waist Circumference, and Body Mass Index are Correlated with Nitric Oxide Levels in Young Adults with Central Obesity: An Observational Study in Riau, Indonesia

Abstract

Background: Central obesity stands for the cornerstone of cardio-metabolic health and nitric oxide (NO) is a major regulator of cardiovascular function. Correlation between serum NO metabolites nitrate/nitrite and the obesity components in young adults remains elusive. Thus, this current study was conducted to know the correlation between serum NO metabolite levels and body fat percentage, waist circumference (WC) as well as body mass index (BMI) young adults with central obesity.

Materials and Methods: A cross-sectional study was conducted in Riau, Indonesia, involved 79 young adults ages 18-25 years, composing of 39 and 40 subjects with and without central obesity, respectively. Anthropometric measurements were performed to assess WC and BMI. Body fat percentages were measured using bioelectrical impedance analysis and serum NO metabolite levels were assessed using Griess methods.

Results: Levels of serum NO metabolites were significant higher in the subjects with central obesity than that of normal subjects (168.41±12.64 vs. 70.57±44.99 µmol/L, respectively, $p<0.001$), but the levels were no significant different between male and female subjects. Serum NO metabolite levels were strongly correlated with total body fat (r = 0.618, $p<0.001$), visceral fat (r = 0.733, $p<0.001$), subcutaneous fat (r = 0.547, $p<0.001$), WC (r = 0.717, $p<0.001$) and BMI (r = 0.788, $p<0.001$).

Conclusions: For ~~In~~ young adults ~~in of~~ Riau, Indonesia, levels of serum NO metabolites are higher in the central obesity group than that of the normal. In this population, body fat

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percentage, waist circumference, and body mass index are correlated with serum nitric oxide metabolite levels.

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Keywords: nitric oxide, body fat percentage, young adults, central obesity.

Introduction

Obesity is a buildup of abnormal or excessive adipose tissue which can interfere with the cardio-metabolic health of individuals.[1] A simple parameter commonly used to classify obesity is body mass index (BMI).[2] According to the World Health Organization (WHO) classification for Asia Pacific criteria, a person with a BMI of ≥23 kg/m^2 or ≥25 kg/m^2 is defined as overweight or obese, respectively.[1–3] Subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) are two main compartments of adipose tissue with different metabolic characteristics.[4] Central obesity is characterized by escalated VAT around intraabdominal organs.[4] This obesity is associated with various pathological disorders consisting of impaired lipid and glucose metabolisms, insulin resistance, and increased tendency for colon, breast and prostate cancers.[4]

Accurate measurements of body fat composition can carried out with sophisticated methods including magnetic resonance imaging (MRI), dual-energy X-ray absorptiometry (DXA) and computed tomography (CT).[4,5] CT-Scan is the gold standard for measuring quantitative tissue of intra-abdominal fat.[6] These methods in routine clinical practice cannot be applied since they are expensive, time-wasting, limited portability, requiring skilled staff, limited accessibility and radiation. Bioelectrical impedance analysis (BIA) is an alternative method for measuring body fat because it is fast, safe and non-invasive.[4,5,7,8] BIA showed a significant strong correlation with CT in measuring of VAT and SAT (r = 0.89 and r = 0.85, respectively)[6] and also with MRI in determining body fat and VAT (r = 0.89 and r = 0.84, respectively).[9]

Nitric oxide (NO) is a signaling and free radical molecule. It is synthetized from L-arginine by nitric oxide synthetase (NOS) in many cells in our body.[10,11] In normal conditions, it is synthesized by endothelial-NOS (eNOS) and neuronal-NOS (nNOS) and played an important regulator of cardiovascular and renal function.[12] Correlation between serum nitric

oxide metabolites, i.e., nitrate and nitrite, and the obesity components remain elusive. Several studies showed there were positive correlation between NO metabolites and BMI[13], between NO metabolites and body fat[14], between NO metabolites and visceral fat[15], while others studies showed negative correlation between NO metabolites and obesity[16], between NO metabolites and abdominal adiposity.[17] A few studies in Indonesia showed no statistical significant in those parameters. A study at Padang showed that NO metabolite levels were higher in obese subjects than in the normal, but there were no significant correlation between NO metabolites and BMI.[18] Another study in Yogyakarta found that NO metabolites were higher in male, aged more than 25 years, and obese subjects than that of the control, but there were no statistical significant differences.[19] Most population in the Padang study was adult, while in the Yogyakarta was young non-obese. Thus, this study was conducted in young adults with central obesity in Riau, Indonesia, to know the correlation between serum nitric oxide levels and body fat percentage, waist circumference as well as body mass index.

Methods

Subjects

This cross-sectional was conducted in Riau, Indonesia from December 2019 to February 2020. Subjects of study were college students aged 18-25 years old and selected by consecutive sampling. The WHO criteria for Asia Pacific was used to determine central obesity, i.e. waist circumference (WC) ≥90 cm and ≥80 cm for men and women, respectively. Subjects who~~,~~ pregnant, taking drugs (either hormonal therapy, steroid or aspirin), suffer from ascites, abdominal tumors, cardiovascular disease, kidney disease, diabetes mellitus, allergic diseases or diarrhea were excluded. Informed consent for participating in this study was obtained. The investigation conformed to the principles outlined in the Declaration of

Helsinki[20] and was approved by the institutional ethics committee of human research in University of Riau, number: B/227/UN.19.5.1.1.8/UEPKK/2019.

Anthropometric measurements

Anthropometric measurements were carried out to assess waist circumference (cm), height (cm) and weight (kg) in a standing position. WC was measured at the midpoint between the edge of the last rib and the iliac crest without pressing the skin at the end of normal expiration using the met line. Weight and height were measured according to standard protocols. BMI was obtained from the calculation of body weight (kg) divided by the square of height (m^2). Examination of body fat compositions was carried out using a BIA (OMRON Karada Scan HBF-375) to measure the percentage of total body fat, visceral fat and subcutaneous fat.

Nitric oxide level measurement

Measurement of NO is difficult technically since its rapid oxidation *in vivo*, therefore, we measured its stable metabolites, nitrate/nitrite, as indirect measurement of levels of NO production.[13,15,21] Venous blood samples were taken in the morning after fasting for 12 hours. Serum NO metabolite levels were measured using the Griess methods by employing QuantiChrom^{TM} Nitric Oxide Assay Kit (Catologue no. D2NO-100, BioAssay Systems, California, USA) at the GAKI Laboratory, Diponegoro University, Semarang, Indonesia. Briefly, serum samples were deproteinated by mixing 150 µL serum with 8 µL ZnSO4 and 8 µL NaOH, then they were centrifuged for 10 minutes at 14,000 rpm. A total of 100 µL of supernatant for each tube was transferred into another tube, and then 100 µL Reagent A, 4 µL Reagent B and 100 µL Reagent C were added for the reduction of nitrate to nitrite and the measurement of nitrite in a single step. Those mixture were incubated for 10 minutes at 60^0 C

prior transferring them into separated wells for detecting the signal density. The optical density was read at 540 nm using the enzyme-linked immunosorbent assay (ELISA) reader (ELx800, BioTek Instruments, Vermont, USA).

Statistical analysis

Data analysis was performed using a computer application. Test normality with Kolmogorov-Smirnov for total subjects and Shapiro-Wilk for the central obesity and without central obesity subgroup. The comparison of sex and age proportions between groups was performed chi-square test, the comparison of total fat variables between groups using the Independent-T-test, while the comparison of visceral fat, subcutaneous fat, BMI, WC and NO metabolite levels between groups using Mann-Whitney-U test and also the comparison of NO metabolite levels between gender using Mann-Whitney-U. The correlation relationship between NO metabolite levels and percentage of body fat (total, visceral and subcutaneous fat), WC and BMI were measured using the Sperman's rho correlation test with $p<0.05$ significance value and 95% confidence interval. Differences with a p value of <0.05 were considered statistically significant.

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Results

A total 79 subjects were recruited, consisting of 39 and 40 subjects with and without central obesity, respectively. Among them, 44 (55.7%) subjects were female. Table 1 shows the baseline clinical characteristics of the subjects with and without central obesity.

There were no significant differences ($p>0.05$) in the proportion of gender and age variables between both groups. Significant differences were found for serum NO metabolite levels between subjects with and without central obesity (168.41±12.64 vs. 70.57±44.99

µmol/L, respectively, p<0.001). Total body fat, visceral fat, subcutaneous fat, WC, and BMI were also significant differences between both groups.

Table 2 shows that there were no significant differences in the levels of serum NO metabolites between male and female, in either all subjects, ones with, or without central obesity, with respective p value 0.875, 0.135, and 0.712.

Table 3 shows that all variables had correlation with serum NO metabolite levels; they were total body fat percentage (r = 0.618, p<0.001), visceral fat (r = 0.733, p<0.001), subcutaneous fat (r = 0.547, p<0.001), WC (r = 0.717, p<0.001) and BMI (r = 0.788, p<0.001).

Discussion

Correlation between serum NO metabolites and the obesity components in young adults with central obesity remains elusive. This study was conducted to know the correlation between body fat percentage and serum NO metabolites in those subjects and found that levels of the serum NO metabolites were strongly correlated with body fat percentage, WC and BMI. To the best of our knowledge, this is the first data about this issue reported from particular population of Indonesian young adult subjects with obesity.

These results are in line with several previous studies that found higher levels of serum nitric oxide metabolites in obese subjects. Study of 313 children aged 8 to 9 years in Portugal concluded that the increase in serum NO metabolite levels was related to the total body fat,[12] as well as the study in Poland of 154 women grouped by BMI found positive correlation between the total body fat, BMI with serum NO metabolite levels.[22] Similar research results were also obtained in a South Korean study of 363 subjects aged 14 to 19 years which concluded that obesity causes an increase in NO production in humans, the increasing in serum NO metabolite levels is strongly correlated with obesity, total body fat and BMI.[14] The study of 110 children aged 7 to 14 years in Spain found higher and significant serum NO metabolite

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levels in obese children compare to normal weight children.[23] Contradictory research results were reported in a study of 114 adolescents (aged 14.01 ± 3.19) in Austria, there was a decrease serum NO metabolite levels in obese adolescents, serum NO metabolite levels were negatively correlated with BMI.[16] Japanese study of 486 subjects aged 40-84 years concluded that there was an inverse relationship between NO bioactivity and accumulation of abdominal fat measured using WC and waist-hip circumference ratio.[17] Taken together, in obese young adults, the high levels of serum NO metabolites are correlated positively with central obesity; but in adolescents, the correlation are inversely.

The subject criteria as well the result of this study were different from two previous studies conducted in Indonesia.[18,19] A study in Padang involved 130 subjects, 92.3% of them were adult aged 40 to 65 years.[18] Another study was conducted in Yogyakarta which recruited 44 subjects whom 81.8% were young aged 18 to 24 years, but 70.5% were non-obese.[19] Both the studies found no significant differences in NO metabolite levels between obese and non-obese subjects.[18,19] However, these studies did not mention the exclusion criteria and the preparation of subjects prior taking the blood samples. These are important since the levels of NO are affected by both diet and comorbid of the subjects.

The high levels of NO metabolites in obese subjects is caused by an increase of NO production in adipose tissue[22,24,25] as a compensatory mechanisms for changes related to obesity.[13,23] The presence of eNOS and inducible-NOS (iNOS) in the adipose cells induces the NO production.[15,26,27] Each of them produces NO with different rates.[26] eNOS generates a smaller amount of NO compared to iNOS which has the biggest capacity to produce NO.[21,26] Under physiological conditions, eNOS is a dominant isoform in the adipose tissue compared to iNOS.[21,25] In obesity, accumulation of proinflammatory macrophages in adipose tissue is responsible for most of iNOS expressions by promoting its expression.[26] In patients with obesity, the hypertrophic adipocytes reduce a diffusion ability of oxygen and the reduction in

vascularization promotes hypoxia.[27] Under the hypoxic conditions, endogenous NO production increase due to the increased iNOS expression rather than the eNOS.[21,26,27] Thus, iNOS produces large amounts of NO as a defensive mechanism.[28]

In the population-based studies, the origin of NO metabolite values in young obese subjects cannot be distinguished whether they are from eNOS or iNOS, since the difficulty in measuring of NO levels *in vivo*.[21,29] NO is a gaseous and has a very short half-life, ranging from 0.05 milliseconds to 30 seconds, depending on the environment and reactant availability.[26–29] Inability to distinguish the eNOS and iNOS components in serum NO metabolites is a limitation of this study as well as other population-base studies. The existence of inflammation in the subject of this study could not be confirmed since we did not check any marker of inflammation.

NO production is divided into two independent pathways, i.e. NOS pathway and the NO_3^--NO_2^--NO pathway.[21] These pathways make the meaning of a high serum NO metabolite levels more intricate, unless a well-controlled food intake of nitrates and nitrites.[21] Nitrates do not have first-pass metabolism, so after consumption of food, their level increases rapidly within 15 minutes, peaking at 90 minutes with a half-life of NO_3^- 5 to 8 hours.[21,27] Under normal conditions, both sources of NO contribute equally to the overall formation of NO.[30] During fasting condition, serum NO metabolite level is weakly correlated with NO_3^- consumption in a regular diet.[21] Under physiological conditions, fasting plasma NO_2^- reflects up to 80% of endogenous NO production.[21] The current study did not control the diet of the subjects prior to the blood sample collection except a fasting for overnight.

The association between high intake of L-arginine and NO synthesis remains unclear. An increase NO metabolite level was observed after oral supplementation of 6 g/day L-arginine for 2 months in diabetic patients with atherosclerotic peripheral arterial disease.[31] A high dietary intake of L-arginine was also strongly associated with serum NO metabolite levels in

overweight and obese subjects, but not in subjects with normal weight.[32] An acute oral supplementation of 6 g L-arginine in healthy subjects did not showed a significant change of NO metabolite level compared to the placebo group.[33,34] The mechanism how the supplementation of L-arginine increased endogenous NO production is not fully understood. This is due to normal L-arginine level greatly exceeds the Michaelis constant (K_m) of eNOS, therefore, increase of L-arginine concentration will not change the production of eNOS.[35] The current study did not calculate L-arginine dietary intake of the subjects prior blood sample collection.

Obesity with high NO metabolite levels may cause more deleterious effects.[12] The increased expression and function of iNOS causes the excessive NO production and the disturbed eNOS affects the decreased NO bioavailability. Both of them promote the growth of non-communicable diseases such as type 2 diabetes mellitus, hypertension, coronary heart disease, kidney disorders and various types of cancer.[21] Data of the current study that levels of serum NO metabolites were higher in obese subjects than that of the normal control indicated an alarm on the future disease for those subjects.

This study also found that there were no significant differences in serum NO metabolite levels between men and women. These results are similar to data of other studies.[16,17] Although another study found inversely result.[19,36] This warrant for future study.

Conclusions

~~Finally, we conclude that in~~For young adults ~~in of~~ Riau, Indonesia, the levels of serum NO metabolites are higher in the central obesity group than that of the normal. In this population, the body fat percentage, waist circumference, and body mass index are correlated with serum nitric oxide metabolite levels.

References

1. WHO/IASO/IOTF. The Asia-Pacific Perspective : Redefining Obesity and its treatment. Australia: WHO; 2000. 15–21 p.
2. WHO. Obesity and overweight [Internet]. 2018 [cited 2019 Jul 21]. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
3. Lim JU, Lee JH, Kim JS, Hwang Y Il, Kim TH, Lim SY, et al. Comparison of World Health Organization and Asia-Pacific body mass index classifications in COPD patients. Int J COPD. 2017;12:2465–75.
4. Shuster A, Patlas M, Pinthus JH, Mourtzakis M. The clinical importance of visceral adiposity: A critical review of methods for visceral adipose tissue analysis. Br J Radiol. 2012;85(1009):1–10.
5. Ramírez-Vélez R, Correa-Bautista JE, Martínez-Torres J, González-Ruíz K, González-Jiménez E, Schmidt-RioValle J, et al. Performance of Two Bioelectrical Impedance Analyses in the Diagnosis of Overweight and Obesity in Children and Adolescents: The FUPRECOL Study. Nutrients. 2016;8(10):1–13.
6. Park KS, Lee DH, Lee J, Kim YJ, Jung KY, Kim KM, et al. Comparison between two methods of bioelectrical impedance analyses for accuracy in measuring abdominal visceral fat area. J Diabetes Complications. 2016;30(2):343–9.
7. Lu HK, Chen YY, Yeh C, Chuang CL, Chiang LM, Lai CL, et al. Discrepancies between leg-to-leg bioelectrical Impedance analysis and computerized tomography in abdominal visceral fat measurement. Sci Rep. 2017;7(1):1–8.
8. Day K, Kwok A, Evans A, Mata F, Verdejo-Garcia A, Hart K, et al. Comparison of a Bioelectrical Impedance Device against the Reference Method Dual Energy X-Ray Absorptiometry and Anthropometry for the Evaluation of Body Composition in Adults. Nutrients. 2018;10(10):1469.

9. Wang JG, Zhang Y, Chen HE, Li Y, Cheng XG, Xu L, et al. Comparison of two bioelectrical impedance analysis devices with dual energy x-ray absorptiometry and magnetic resonance imaging in the estimation of body composition. J Strength Cond Res. 2013;27(1):236–43.
10. Uppalapati A, Gogineni S, Espiritu JR. Association between Body Mass Index (BMI) and fraction of exhaled nitric oxide (FeNO) levels in the National Health and Nutrition Examination Survey (NHANES) 2007–2010. Obes Res Clin Pract. 2016;10(6):652–8.
11. Ahmad A, Dempsey S, Daneva Z, Azam M, Li N, Li P-L, et al. Role of Nitric Oxide in the Cardiovascular and Renal Systems. Int J Mol Sci. 2018;19(9):2605.
12. Correia-Costa L, Sousa T, Morato M, Cosme D, Afonso J, Areias JC, et al. Oxidative stress and nitric oxide are increased in obese children and correlate with cardiometabolic risk and renal function. Br J Nutr. 2016;116(5):805–15.
13. Ghasemi A, Zahediasl S, Azizi F. Elevated nitric oxide metabolites are associated with obesity in women. Arch Iran Med. 2013;16(9):521–5.
14. Choi JW, Pai SH, Kim SK, Ito M, Park CS, Cha YN. Increases in nitric oxide concentrations correlate strongly with body fat in obese humans. Clin Chem. 2001;47(6):1106–9.
15. Fujita K, Wada K, Nozaki Y, Yoneda M, Endo H, Takahashi H, et al. Serum nitric oxide metabolite as a biomarker of visceral fat accumulation: Clinical significance of measurement for nitrate/nitrite. Med Sci Monit. 2011;17(3):CR123–31.
16. Gruber HJ, Mayer C, Mangge H, Fauler G, Grandits N, Wilders-Truschnig M. Obesity reduces the bioavailability of nitric oxide in juveniles. Int J Obes. 2008;32(5):826–31.
17. Kondo T, Ueyama J, Imai R, Suzuki K, Ito Y. Association of abdominal circumference with serum nitric oxide concentration in healthy population. Environ Health Prev Med. 2006;11(6):321–5.

18. Purwadianti N, Oenzil F, Sulastri D. Hubungan antara Indeks Massa Tubuh dengan Kadar Nitrit Oksid pada Masyarakat Etnik Minangkabau di Kota Padang. J Kesehat Andalas. 2015;4(2):364–8.
19. Susilowati A, Akrom, Darmawan E. Profile of Nitric Oxide (NO) Levels in Yogyakarta Society. J Kefarmasian Akfarindo. 2016;1(1):19–24.
20. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191–4.
21. Bahadoran Z, Mirmiran P, Jeddi S, Carlström M, Azizi F, Ghasemi A. Circulating markers of nitric oxide homeostasis and cardiometabolic diseases: insights from population-based studies. Free Radic Res. 2019;53(4):359–76.
22. Olszanecka-Glinianowicz M, Zahorska-Markiewicz B, Janowska J, Zurakowski A. Serum concentrations of nitric oxide, tumor necrosis factor (TNF)-α and TNF soluble receptors in women with overweight and obesity. Metabolism. 2004;53(10):1268–73.
23. Codoñer-Franch P, Tavárez-Alonso S, Murria-Estal R, Megías-Vericat J, Tortajada-Girbés M, Alonso-Iglesias E. Nitric oxide production is increased in severely obese children and related to markers of oxidative stress and inflammation. Atherosclerosis. 2011;215(2):475–80.
24. Foroumandi E, Alizadeh M, Kheirouri S, Asghari Jafarabadi M. Exploring the role of body mass index in relationship of serum nitric oxide and advanced glycation end products in apparently healthy subjects. Chiang M-C, editor. PLoS One. 2019;14(3):e0213307.
25. Engeli S, Janke J, Gorzelniak K, Böhnke J, Ghose N, Lindschau C, et al. Regulation of the nitric oxide system in human adipose tissue. J Lipid Res. 2004;45(9):1640–8.
26. Sansbury BE, Hill BG. Regulation of obesity and insulin resistance by nitric oxide.

Free Radic Biol Med. 2014;73:383–99.

27. Ghasemi A, Jeddi S. Anti-obesity and anti-diabetic effects of nitrate and nitrite. Nitric Oxide. 2017;70(24):9–24.

28. Khazan M, Hdayati M. The Role of Nitric Oxide in Health and Diseases. Scimetr. 2014;3(1):1–10.

29. Caimi G, Hopps E, Montana M, Carollo C, Calandrino V, Incalcaterra E, et al. Nitric oxide metabolites (nitrite and nitrate) in several clinical condition. Clin Hemorheol Microcirc. 2014;56(4):359–69.

30. Carlström M, Liu M, Yang T, Zollbrecht C, Huang L, Peleli M, et al. Cross-talk Between Nitrate-Nitrite-NO and NO Synthase Pathways in Control of Vascular NO Homeostasis. Antioxid Redox Signal. 2015;23(4):295–306.

31. Jabłecka A, Bogdański P, Balcer N, Cieślewicz A, Skołuda A, Musialik K. The effect of oral L-arginine supplementation on fasting glucose, HbA1c, nitric oxide and total antioxidant status in diabetic patients with atherosclerotic peripheral arterial disease of lower extremities. Eur Rev Med Pharmacol Sci. 2012;16(3):342–50.

32. Mirmiran P, Bahadoran Z, Ghasemi A, Azizi F. The Association of Dietary l-Arginine Intake and Serum Nitric Oxide Metabolites in Adults: A Population-Based Study. Nutrients. 2016;8(5):1–13.

33. Alvares TS, Conte-Junior CA, Silva JT, Paschoalin VMF. Acute L-Arginine supplementation does not increase nitric oxide production in healthy subjects. Nutr Metab (Lond). 2012;9(1):54.

34. Meirelles CM, Matsuura C. Acute supplementation of L-arginine affects neither strength performance nor nitric oxide production. J Sports Med Phys Fitness. 2018;58(3):216–20.

35. Khalaf D, Krüger M, Wehland M, Infanger M, Grimm D. The Effects of Oral l-

Arginine and l-Citrulline Supplementation on Blood Pressure. Nutrients. 2019;11(7):1679.

36. Ghasemi A, Zahedi Asl S, Mehrabi Y, Saadat N, Azizi F. Serum nitric oxide metabolite levels in a general healthy population: relation to sex and age. Life Sci. 2008;83(9–10):326–31.

Tables

Table 1. Subjects characteristics according to groups

Variables	Central obesity	Without central obesity	*p*
	Mean ± SD (Min - Max)	Mean ± SD (Min - Max)	
Subjects (n)	39	40	
Male	18	17	0.058 [a]
Female	21	23	
Age (years)	18.7 ± 0.9 (18 – 21)	19.2 ± 1.3 (18 – 22)	0.147 [b]
BMI (kg/m^2)	30.55 ± 4.54 (23.8 – 43.3)	21.16 ± 2.61 (16.6 – 25.9)	<0.001 [b]
Waist circumference (cm)	96.50 ± 11.17 (80.5 – 129.5)	75.36 ± 6.73 (64.0 – 89.0)	<0.001 [b]
Total body fat (%)	33.23 ± 5.08 (21.0 – 42.1)	23.55 ± 7.21 (8.7 – 38.9)	<0.001 [c]
Visceral fat (%)	11.94 ± 5.52 (3.5 – 29.0)	3.68 ± 2.25 (0.5 – 8.5)	<0.001 [b]
Subcutaneous fat (%)	28.28 ± 7.60 (15.0 – 41.1)	18.66 ± 6.70 (6.4 – 30.3)	<0.001 [b]
Serum nitric oxide metabolites level (µmol/L)	168.41 ± 12.64 (138.6 – 200.1)	70.57 ± 44.99 (22.1 – 150.2)	<0.001 [b]

Note: [a] Chi-square test, [b] Mann Whitney U test, [c] Independent T test

Table 2. Serum nitric oxide metabolite levels according to sex in the study subjects

Variables	Serum nitric oxide metabolites level					
	Central obesity		Without central obesity		Total subjects	
	Mean ± SD (Min - Max)	*p*	Mean ± SD (Min - Max)	*p*	Mean ± SD (Min - Max)	*p*
Male	164.79 ± 13.27 (138.6 – 184.7)	0.135 [a]	77.31 ± 48.78 (22.1 – 148.0)	0.712 [a]	122.30 ± 56.35 (22.1 – 184.7)	0.875 [a]
Female	171.50 ± 11.48 (153.6 – 200.1)		65.59 ± 42.38 (23.0 – 150.2)		116.14 ± 62.00 (23.00 – 200.1)	

Note: [a] Mann Whitney U test

339 **Table 3.** Correlation between serum NO metabolite levels and body fat percentage, WC as well as BMI

Variables	Serum nitric oxide metabolites level					
	Central obesity		Without central obesity		Total subjects	
	r	*p*	r	*p*	r	*p*
Total body fat	0.370	0.020 [a]	0.064	0.695 [b]	0.618	<0.001 [b]
Visceral fat	0.257	0.115 [b]	0.109	0.504 [b]	0.733	<0.001 [b]
Subcutaneous fat	0.383	0.016 [b]	0.023	0.886 [b]	0.547	<0.001 [b]
Waist circumference	0.037	0.821 [b]	0.058	0.722 [b]	0.717	<0.001 [b]
Body mass index	0.352	0.028 [b]	0.105	0.520 [b]	0.788	<0.001 [b]

340 Note: [a] Pearson correlation test, [b] Spearman's Rho correlation test

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MCBS Office <mcbs.office@gmail.com> Mon, Sep 14, 2020 at 9:42 AM
To: bahrudin udin <bahrudin00@gmail.com>

Dear Mr. Bahrudin, MD, PhD,

Good day. As for now, your manuscript is still waiting for the assignment schedule. We will let you know once it gets scheduled.
However, until your manuscript gets published, you can find your manuscript in our Article in Press section.

Hopefully this can answer your question. Thank you for your attention.

Best regards,
[Quoted text hidden]

Gmail bahrudin udin <bahrudin00@gmail.com>

[MCBS] #165 Article in Press & Proof Reading

7 messages

MCBS Office <mcbs.office@gmail.com> Mon, Sep 14, 2020 at 9:39 AM
To: bahrudin udin <bahrudin00@gmail.com>
Cc: feriyandi.nauli@gmail.com

Dear Authors,

Good day. Your manuscript **"Body Fat Percentage, Waist Circumference, and Body Mass Index are Correlated with Nitric Oxide Levels in Young Adults with Central Obesity: An Observational Study in Riau, Indonesia"** has been accepted for publication in Molecular and Cellular Biomedical Sciences, and is now available in the Article in Press section.

Herein we attach the final draft of your manuscript. We need your assistance to **check if there's any mistake in our printing** and please state your agreement. Please make sure you check every detail of the manuscript, because any mistake/misprint found in the article after proof reading done will become author's responsibility.

When you completed all your corrections/comments, please **fill the attached Proof Reading Form**. We will be waiting for your reply until **September 24, 2020**.

Thank you so much for your cooperation. We wish you a nice day.

Best Regards,
MCBS Office

2 attachments

In Press - Nauli F (Correlation of Serum Nitric Oxide in Central Obesity).pdf
2239K

#165 Manuscript - Initial Check.docx
79K

bahrudin udin <bahrudin00@gmail.com> Mon, Sep 14, 2020 at 9:24 PM
To: feriyandi nauli <feriyandi.nauli@gmail.com>

Ferri, bisa tolong dicek manuscript dengan teliti, dibuat tabel list koreksi
Halaman-alinea-baris...
Tertulis
Koreksi

Nanti yg kirim ke mcbs saya ya.

Bahrudin
[Quoted text hidden]

2 attachments

In Press - Nauli F (Correlation of Serum Nitric Oxide in Central Obesity).pdf
2239K

#165 Manuscript - Initial Check.docx
79K

Feriyandi Nauli <feriyandi.nauli@gmail.com> Mon, Sep 14, 2020 at 10:04 PM

To: bahrudin udin <bahrudin00@gmail.com>

Siap Dokter, insyaAllah saya kerjakan

[Quoted text hidden]

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bahrudin udin <bahrudin00@gmail.com> Tue, Oct 6, 2020 at 12:28 PM
To: MCBS Office <mcbs.office@gmail.com>
Cc: feriyandi nauli <feriyandi.nauli@gmail.com>

Dear MCBS Officer,

I apologize for the late response. Please find files in the attachment for our corrections.

Sincerely,
Udin Bahrudin
[Quoted text hidden]
--
Bahrudin, MD, PhD
Dept. of Cardiology and Vascular Medicine
Faculty of Medicine, Diponegoro Univ.
Jl. Dr. Sutomo 18 Semarang, Indonesia
Email: bahrudin00@gmail.com
Post mail: Jl. Kanfer Raya no. 4A Banyumanik, Semarang

2 attachments

CORRECTION TABLE of article in press & proof reading.docx
26K

In Press - Nauli F (Correlation of Serum Nitric Oxide in Central Obesity)-revision marked.pdf
1780K

MCBS Office <mcbs.office@gmail.com> Wed, Feb 10, 2021 at 10:41 AM
To: bahrudin udin <bahrudin00@gmail.com>
Cc: feriyandi nauli <feriyandi.nauli@gmail.com>

Dear Authors,

Thank you for your proofreading corrections, we have checked it and revised some of the mistakes according to your comments.
However there are some matters that are not supposed to be revised as the way you describe it, such as:
1. The affiliations: University's name should come later after the Faculty's name.
2. The affiliations: We decided to use "Universitas Diponegoro" and "Universitas Riau" and did not change it into English.
3. The references: DOI numbers are still written.

Herein we attach the final draft of your manuscript. Your manuscript is scheduled to be published in our next issue (Vol.5 No.1, March 2021).
We also need your assistance to fill the attached Proof Reading Form.

Thank you for your kind cooperation. Please do not hesitate to contact us if you have any questions.

Best Regards,
[Quoted text hidden]

2 attachments

#165 Proof Reading Form.doc
362K

MCBS V5N1A1 DRAFT.pdf
2004K

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bahrudin udin <bahrudin00@gmail.com> Mon, Feb 22, 2021 at 5:24 AM
To: MCBS Office <mcbs.office@gmail.com>
Cc: feriyandi nauli <feriyandi.nauli@gmail.com>

Dear MCBS Office,

Thank you for sending the manuscript. It can be published with some minor corrections.

Sincerely yours,
U Bahrudin
[Quoted text hidden]

3 attachments

#165 Proof Reading Form[1806].pdf
139K

CORRECTION TABLE of draft article.doc
36K

MCBS V5N1A1 DRAFT[1642]_revision highlighted.pdf
1651K

MCBS Office <mcbs.office@gmail.com> Tue, Feb 23, 2021 at 2:01 PM
To: bahrudin udin <bahrudin00@gmail.com>
Cc: feriyandi nauli <feriyandi.nauli@gmail.com>

Thank you for your response.
[Quoted text hidden]