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Inquiries about the current status of submitted paper

2 pesan

SONY WIBISONO <sony.wibisono@fk.unair.ac.id>
Kepada: alexander.kiss@savba.sk

20 Januari 2022 16.37

Dear Dr. Alexander Kiss

This is with regard to our submitted manuscript, titled **“The effects of aerobic exercise training on adiponectin level are potentially mediated by vitamin d in type 2 diabetic patients”** of authors Sony Wibisono Mudjanarko, Anugrahini Irawati, Damayanti Tinduh submitted to your journal on January, 7 2022 for consideration as an original article.

We have not received an update regarding the status of our manuscript since submission notification. Could you let us know when we can expect notice regarding the decision of the editorial board? We would be grateful if you could let us know whether there has been any further progress on our submission.

Thank you for your time and consideration. We look forward to hearing from you.

Sincerely,

Sony Wibisono Mudjanarko

Alexander kiss <alexander.kiss@savba.sk>
Kepada: SONY WIBISONO <sony.wibisono@fk.unair.ac.id>

20 Januari 2022 17.39

Dear dr. Mudjanarko,

Your manuscript is under the regular processing of the manuscripts sent for Endocrine Regulations publication. After getting the Reviewers responses, we will inform you about the status of acceptance of your manuscript.

Best regards

Alexander Kiss

From: "SONY WIBISONO" <sony.wibisono@fk.unair.ac.id>
To: "Alexander kiss" <alexander.kiss@savba.sk>
Sent: Thursday, January 20, 2022 10:37:30 AM
Subject: Inquiries about the current status of submitted paper

[Kutipan teks disembunyikan]



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Endocrine Regulations

1 pesan

Alexander kiss <alexander.kiss@savba.sk>
Kepada: sony.wibisono@fk.unair.ac.id

21 Maret 2022 15.36

Dear dr. Sony Wibisono MUDJANARKO,

I am glad to inform you that your article "Effects of aerobic exercise on adiponectin levels potentially mediated by vitamin D in type 2 diabetic patients" has been accepted for publication in Endocrine Regulations - see the attachment.

Alexander Kiss

 **acceptance_letter.pdf**
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endocrine regulations

international journal on experimental and clinical endocrinology

Institute of Experimental Endocrinology, Biomedical Research Center,
Slovak Academy of Sciences, Dubravská cesta 9, 845 05 Bratislava, Slovakia

Bratislava, March 20th, 2022

Dear dr. Sony Wibisono Mudjanarko,

I am pleased to inform you that your article "Effects of aerobic exercise on adiponectin levels potentially mediated by vitamin D in type 2 diabetic patients" by authors Sony Wibisono Mudjanarko, Anugrahini Irawati, Damayanti Tinduh, has been accepted for publication in Endocrine Regulations and will come up in the Endocrine Regulations Vol 56 No3 (July issue) 2022.

Thanks you for publishing in the Endocrine Regulations.

Yours sincerely,

Alexander Kiss
Editor-in-Chief

**SONY WIBISONO** <sony.wibisono@fk.unair.ac.id>

Endocrine Regulations

2 pesan

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Kepada: **SONY WIBISONO** <sony.wibisono@fk.unair.ac.id>

15 Juni 2022 14.43

Dear dr. Mudjanarko,

I am sending you the preproof form of your article in word format. Please, read it carefully and do corrections, if you need to do them, directly into the article and label them by yellow color. Return it soon.

With the best regards,

Alexander Kiss
Editor-in-Chief
Endocrine Regulations

**Mudjanarko_manuscript_final.doc**

74K

SONY WIBISONO <sony.wibisono@fk.unair.ac.id>
Kepada: Alexander kiss <alexander.kiss@savba.sk>

16 Juni 2022 00.36

Dear Dr. Alexander Kiss,

First of all, we would like to express our sincere gratitude and appreciation for accepting our article for final publication in Endocrine Regulations Journal. Thank you as well for sending us the pre-proof form of our article. We have read them and no further corrections are needed.

Sincerely,
Sony Wibisono Mudjanarko

[Kutipan teks disembunyikan]

Short title: Exercise impact on adiponectin potentially mediated by vitamin D

Effects of aerobic exercise on adiponectin levels potentially mediated by vitamin D in type 2 diabetic patients

Sony Wibisono MUDJANARKO¹, Anugrahini IRAWATI², Damayanti TINDUH³

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Objective. The positive effects of exercise on adiponectin and vitamin D have independently been reported. Recent studies have suggested that vitamin D increases adiponectin synthesis through inhibition of the rennin-angiotensin system in adipose tissue. However, studies evaluating the effects of an aerobic exercise on adiponectin and vitamin D simultaneously investigating the potential mechanism of vitamin D-dependent adiponectin pathways in patients with type 2 diabetes mellitus (T2DM) are still limited. This study was undertaken to examine the effects of aerobic exercise on adiponectin and its association with vitamin D in patients with T2DM.

Methods. Total twenty-two patients with T2DM were randomly divided into intervention and control group. The intervention group underwent a moderate intensity of a walking mode treadmill aerobic exercise for four weeks. The exercise protocol was adapted from modified Bruce test with a periodic speed and inclination increase. In both groups, body mass index (BMI), vitamin D, and adiponectin levels, were measured before and after four weeks of the lasting program.

Results. The mean of the increased adiponectin and vitamin D levels after exercise was significantly higher in the intervened than the control group, but statistically significant difference was only found in the adiponectin effect ($p=0.017$). There was a significant association found between vitamin D and adiponectin in the intervention group after data adjustments to age and BMI ($p=0.005$).

Conclusion. Moderate intensity of treadmill exercise with increased speed and inclination periodically increased adiponectin level in patients with T2DM. The increased adiponectin might potentially be mediated by increased vitamin D, but the level of their association impact was dependent on the age and BMI.

Key words: adiponectin, aerobic exercise, vitamin D, type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM) is a metabolic disease characterized by chronic hyperglycemia resulting from insulin resistance with relative insulin deficiency or insulin secretory defect with insulin resistance American Diabetes Association (ADA 2013). This type is the most prevalent form which accounts for 90–95% of all diabetic patients. The number of people with diabetes has quadrupled from 108 million in 1980 to 422 million in 2014 World Health Organization (WHO 2016). Diabetes can lead to complications in many organs and increase the mortality risk. WHO has reported that diabetes was the ninth leading cause of death worldwide in 2019 with estimation about 1.5 million deaths directly caused by diabetes (WHO 2021). T2DM became one of the major health problems in the world due to the high morbidity and mortality rates.

Previous studies have reported an association between low adiponectin level with increased risk of T2DM (Duncan et al. 2004; Li et al. 2009). Adiponectin is one of peptides secreted by adipocytes known to exert insulin sensitizer, anti-hyperglycemic, anti-inflammatory, and anti-atherogenic effects (Achari and Jain 2017). Adiponectin is known to have a protective effect on the diabetes progression through sensitizing insulin by the glucose uptake increase in the muscle cell (up regulate GLUT 4 translocation), reduction of the gluconeogenesis in the liver, and stimulation of glucose and fatty acid metabolism (Polito et al. 2020). Some researchers have been conducted to evaluate potential factors that increase circulating adiponectin as a promising therapeutic strategy in T2DM, one of which is physical exercise (Simpson and Singh 2008). Several studies have documented that exercise is positively associated with increased adiponectin level, but the results were inconsistent depending to age, gender, body mass index, health status, types, duration, and intensity of the exercise (Simpson and Singh 2008). Cnop et al. (2003) have suggested that the effect of exercise on the adiponectin levels was probably mediated by body composition change. However, other mechanisms may possibly link exercise to adiponectin level.

The US National Health and Nutrition Examination Survey (NHANES III 1997) report has suggested that physical exercise is positively correlated with increased serum vitamin D, which is an essential steroid metabolite with multiple metabolic effects including regulation of insulin secretion. Positive effects of exercise on serum vitamin D have been suggested to be directly related to enhanced of vitamin D mobilization from adipose tissue (Hengist et al. 2019). Vitamin D is also indicated to have a direct association with adiponectin varies with race, gender, and body mass index (BMI) (Bidulescu et al. 2014). Recent study has shown that serum vitamin D has a positive correlation with serum adiponectin in prediabetic and type 2 diabetic groups (Banerjee et al. 2016). The mechanisms proposed is negative regulation of rennin-angiotensin system in adipose tissue by vitamin D that conquers the inhibition of adiponectin secretion (Vaidya et al. 2011). However, it is not yet clear, whether exercise induced change in the adiponectin levels may directly or indirectly be mediated by vitamin D. To our knowledge, none of the studies carried out was centered to perform a simultaneous evaluation of the effects of aerobic exercise on adiponectin and vitamin D with aim to reveal the potential mechanism of vitamin D-dependent adiponectin pathways in the patients with T2DM. This present study was aimed to examine the effects of aerobic exercise training on adiponectin level and its association with the serum vitamin D in patients with T2DM.

Materials and methods

Study design and subjects. The current study was pretest-posttest control group design which subjects randomly assigned to intervention and control group with same assessment measures obtained before and after the treatment. The inclusion criteria included male T2DM patients who have been treated by standard medication, age between 35–55 years old, and systolic blood pressure within 110–130 mmHg. We excluded subjects with restrictive or obstructive respiratory tract disease, neuromusculoskeletal disease, peripheral diabetic neuropathy, had sign of inflammations, ulcer or gangrene on either one or both legs, history of heart, kidney, thyroid, and liver disease, vestibular or proprioceptive disturbance, use of long-term steroids and vitamin D supplements, and had routine aerobic exercise at least two times per week. Subjects with symptoms of chest pain, chest tightness, hypoglycemia or sign of ischemia identified by electrocardiogram during or after exercise was dropped out from the study. Twenty-two subjects were participating in the study, then divided into intervention and control group by randomized ballot. Written informed consent was obtained from all the subjects before their participation in the study. This study has been approved by Health Research Ethics Committee of Dr. Soetomo General Hospital Surabaya Indonesia with recommendation number 1266/KEPK/VI/2019.

Experimental protocols. The intervention group was given aerobic exercise with moderate intensity and frequency three sessions per week for four weeks. The training protocol was taken from a modified Bruce test with total seven stages with increasing speed and inclination periodically every 3 min to reach target heart rate (60–75% of maximum heart rate). Aerobic exercise was performed by walking exercise in Treadmill EN-Mill 2007. The heart rate of the subjects was monitored using a portable heart rate monitor throughout the entire session of exercise to maintain the exercise intensity. Treadmill exercise was lasted for about 30 min, included each 5 min for warm-up and cool-down. Vital signs (blood pressure, heart rate, oxygen saturation) and capillary blood glucose were assessed before training program. Subjects with normal vital sign and blood glucose level within 100–250 mg/dL were allowed to conduct the exercise. Borg scale was also assessed during treadmill to monitor perceived exertion. The subjects in the control group were instructed to maintain their lifestyle based on standards of medical care in diabetic patients, including medical nutrition therapy and exercise with duration min 150 min per week.

Anthropometric data and biochemical analysis. Clinical data (age, history of T2DM, the presences of comorbid, blood pressure) were obtained from all study subjects. Anthropometric data were measured in both groups before and after four-week program. Height and weight were examined to calculate body mass index [BMI=weight (kg)/height² (m²)]. Peripheral blood samples were collected in plain tube without any activator from all study subjects before and after four-week program. Blood samples, then stored at -80°C refrigerator until analysis done. Glycemic parameters (random plasma glucose, fasting plasma glucose, fasting insulin, HbA1C), serum vitamin D, and adiponectin were measured in both groups before the start of the intervention. Vitamin D and adiponectin were measured again after four-weeks program in both groups. Vitamin D levels were measured using Siemens ADVIA Centaur Vitamin D with chemiluminescent immunoassay (CLIA). Adiponectin concentration was measured as total adiponectin by ELISA kit (E-EL-H5811, Elabscience, USA). Change of BMI, vitamin D, and adiponectin after four-week program in both groups were determined by subtracting those variables post to pre intervention.

Statistical analysis. SPSS version 16.0 was used for statistical analysis. The results of the study were presented in tabular forms. Continuous variables were presented as mean and standard

deviation for normally distributed data. Continuous variables with skewed distributed data were presented as median and interquartile range. Categorical variables were presented as frequency and percentage. Comparison of clinical characteristics and biochemical parameters between intervention and control group were examined using independent sample t test for normally distributed data, Mann Whitney U test for skewed distributed data, and Chi square test for categorical variables. Bivariate correlation analysis using Pearson (for normally distributed data) or Spearman (for skewed distributed data) was also performed between the change of adiponectin level and the other studied parameters. Linear regression models were employed to investigate the relationship between change of vitamin D level and change of adiponectin level after four-week program in both groups with adjustment for their potentially confounding variables (age and BMI). P value < 0.05 was considered statistically significant.

Results

Among 22 subjects, one subject in the intervention group was dropped out from the study due to suffered from hypoglycemia during exercise program and one subject in the control group also could not participate until completion of the study. Total 20 subjects completed the study at the end. Table 1 showed that there were no significant statistical difference of baseline clinical characteristics and laboratory parameters between each group, except for median of age ($p=0.017$). All study subjects had low levels of serum vitamin D (below 30 ng/ml). There was no difference of BMI and change of BMI after four-week program in both groups. Adiponectin levels post four-week program were significantly higher in the intervention than control group ($p=0.003$). Adiponectin increased in both groups as shown in Table 2, but statistically significant difference was only found in the intervention group ($p=0.017$). Median of increased vitamin D level was higher in the intervention group, but statistically insignificant ($p=0.821$).

Bivariate correlation analysis showed that age, BMI, and change of vitamin D level independently were not predictor of change in adiponectin level after exercise as shown in Table 3. Linear regression model was employed to investigate the relationship among the change of adiponectin level with change of vitamin D level and other confounding variables. Age and BMI were included in the model to adjust for their potentially confounding effects. The results from multiple linear regression analysis are presented in Table 4 below. Increased of vitamin D level in exercise group was significantly associated with increased adiponectin level ($\beta=1.027$, $p=0.005$) after adjusting for age and BMI after exercise (fitted model: $F=6.79$, $p=0.023$, adjusted $R^2=0.659$). In this model, age ($\beta=0.852$, $p=0.018$) and BMI after exercise ($\beta=0.769$, $p=0.032$) were also found to be significant predictors for change of adiponectin level after exercise in the intervention group.

Discussion

This study demonstrates the effects of aerobic exercise on adiponectin levels in patients with T2DM and its correlation with change of vitamin D levels to investigate the potential mechanism of vitamin D-dependent adiponectin pathways. Our study suggests that aerobic exercise with moderate intensity for four weeks increased adiponectin levels in patients with T2DM with mean difference 130 ± 108 pg/ml. These findings complement the current knowledge regarding the effects of exercise on adiponectin level in diabetic patients. The results are in line with the previous systematic review and meta-analysis of 19 randomized controlled trials in prediabetic and diabetic adults that reported increased levels of adiponectin with mean difference

0.42 µg/ml (95% CI 0.23–0.60, $p < 0.00001$) after physical exercise, especially aerobic but neither resistance nor concurrent exercise (Becic et al. 2018). Exercise duration of the present study was shorter than recent study performed by Aly et al. (2014) that have shown an increased adiponectin in type 2 diabetic patients after moderate intensity of supervised aerobic training for 12 weeks. The benefits of exercise in our study may be resulted from increasing speed and inclination periodically on a walking mode treadmill that thought to have greater stimulation on the musculoskeletal system and energy metabolism compared with walking on a flat surface (Kim et al. 2020). Both BMI in each group of this study was increased after four-week program, but lower in the intervention group. The effects of exercise on adiponectin were suggested to determine by body composition change, especially reduction of intraabdominal fat mass (Cnop et al. 2003; Simpson and Singh 2008). Unfortunately, several indexes to estimate body fat (e.g., waist circumference, waist-hip ratio, or skin fold thickness) were not measured in the study though could affect on adiponectin. Correlation of increased adiponectin level with change of BMI after exercise in this study showed insignificant result. However, BMI is an index of lean body mass and cannot precisely measure body composition (muscle mass or body fat percentage). Other mechanisms could also possibly mediate the effects of exercise on adiponectin, e.g., via vitamin D pathways.

All study subjects in both groups had lower levels of baseline serum vitamin D (below 30 ng/ml). This data supports the results of previous research about the higher incidence of vitamin D deficiency or insufficiency in the diabetic population (Bayani et al. 2014). Deficient of vitamin D linked to abruption of insulin secretion and sensitivity that suggested to have a connection with the incidence or progression of T2DM (Mathieu and Gysemans 2005). Baseline serum vitamin D in the intervention group were lower than a control group that may be affected by an older median of age or diabetes duration. However, increased vitamin D levels in the intervention group after aerobic exercise were higher than the control group but statistically insignificant. Adipose tissue was known to act as a reservoir for vitamin D due to lipophilicity and exercise was suggested to strongly stimulate vitamin D mobilization from adipose tissue (Hengist et al. 2019). Previous studies reported that exercise caused the rise of glucagon, adrenaline, and atrial natriuretic peptide as stimulatory lipolytic hormones and suggested as a key mechanism of vitamin D release from adipose tissue (Moro et al. 2007; Hengist et al. 2019). The lipolytic response of adipose tissue to exercise in obesity was reported reduced compared with lean controls (Mittendorfer et al. 2004). This condition may explain the lower increased vitamin D levels in control group due to higher mean of baseline BMI. However, little is known about the effect of exercise on serum vitamin D in type 2 diabetic patients. Previous research conducted in rat experimentally-induced T2DM showed significant increase of serum vitamin D in diabetic group after moderate swimming exercise with duration 60 min a day and frequency five times a week for 4 weeks (Aly et al. 2016). These exercise protocols may suggest that higher frequency or duration of exercise is needed to increase vitamin D level significantly in patients with T2DM. This analysis supports the results from previous studies that reported higher vitamin D level in person with more intense physical activity (Chin et al. 2017).

We hypothesized that increased adiponectin level in type 2 diabetic patients might be potentially mediated by increased vitamin D level after aerobic exercise. However, the association between vitamin D and adiponectin in previous studies showed various results. Study in healthy non-diabetic adults indicating direct relationship between vitamin D and adiponectin but the association disappeared after adjustment to BMI (Gannage-Yared et al. 2009; Liu et al. 2009). Study in biracial population-based samples conducted by Bidulescu et al. (2014) reported that the association of vitamin D and adiponectin is dependent on race, gender, and BMI

category. However, these studies were carried out on a heterogeneous population that numerous confounding might affect adiponectin concentration, e.g., age, gender, race, BMI, diabetes and hypertension status, anti-hypertensive drug use, dietary electrolyte intake, dietary patterns, physical activity, alcohol intake, and menopausal status (Vaidya et al. 2012).

We found a significant association between vitamin D and adiponectin in the intervention group after adjusting to age and BMI ($p=0.005$). Recent studies have suggested that vitamin D affects adiponectin synthesis by adiponectin expression gene upregulation in visceral fat and down-regulation of TNF- α gene that inflicts the adiponectin synthesis (Gannage-Yared et al. 2009). Another study also proposed that vitamin D conquers the inhibition of adiponectin synthesis through negative regulation of rennin-angiotensin system in adipose tissue (Vaidya et al. 2011). In our linear regression model, age and BMI were also found to be significant predictors of increased adiponectin level after exercise in the intervention group. These results may indicate that the association of vitamin D and adiponectin after aerobic exercise is dependent on age and BMI. Age is positively correlated with increased adiponectin level after exercise ($p=0.018$). This result builds on existing evidence of higher adiponectin concentration on older age (Isobe et al. 2005). Decreased of adiponectin clearance by kidney was suggested as a possible cause for adiponectin increase with age (Isobe et al. 2005). Hence, BMI after exercise is inversely associated with increased adiponectin level in the intervention group ($p=0.032$). This result also similar with previous study in large samples revealed lower adiponectin level in subjects with higher BMI (Nielsen et al. 2020). This finding indicates that adipose tissue may exert a negative effect on adiponectin secretion as suggested in previous studies (Foula et al. 2020).

This study has certain limitations. First, the sample size was relatively small, but it was sufficient to reproduce the association of interest. Potential confounding variables that might affect vitamin D and adiponectin concentration (e.g., dietary intake of calcium, use of thiazolidinediones medication or insulin) were not controlled in the present study. Although our study suggested that the increased adiponectin levels after aerobic exercise might be affected by vitamin D, an association was examined using the observational analysis. Thus, the direct causal-effect relationship cannot be proven. Therefore, further research with longitudinal design is needed to establish the directionality of vitamin D on adiponectin level. Since our study indicated that vitamin D might have a potential mechanism to mediate the increased adiponectin, which known to exert many benefits in patients with T2DM, future research to evaluate the effect of vitamin D supplementation on adiponectin level in type 2 diabetic patients is warranted.

Conclusion

Moderate intensity of the treadmill exercise with increased speed and inclination periodically increased adiponectin levels in patients with T2DM. The increased adiponectin levels might potentially be mediated by increased vitamin D after aerobic exercise, but the association is dependent on the age and BMI.

Acknowledgement

The authors would like to thank to the whole staff of the Medical Rehabilitation Unit of Dr. Soetomo General Academic Hospital for their valuable contribution towards the study completion.

Conflict of interest: The authors declare no conflict of interest.

References

- Achari AE, Jain SK. Adiponectin, a therapeutic target for obesity, diabetes, and endothelial dysfunction. *Int J Mol Sci* 18, 1321, 2017.
- Aly FA, Alghadir AH, Gabr SA. Adiponectin response to supervised aerobic training in type II diabetic patients. *Asian Biomed* 8, 597–602, 2014.
- Aly YE, Abdou AS, Rashad MM, Nassef MM. Effect of exercise on serum vitamin D and tissue vitamin D receptors in experimentally induced type 2 diabetes mellitus. *J Adv Res* 7, 671–679, 2016.
- American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 36, 67–74, 2013.
- Banerjee A, Khemka V, Roy D, Poddar J, Roy TKS, Karnam SA. Role of serum adiponectin and vitamin D in prediabetes and diabetes mellitus. *Can J Diabetes* 41, 1–7, 2016.
- Bayani MA, Akbari R, Banasaz B, Saeedi F. Status of Vitamin-D in diabetic patients. *Caspian J Intern Med* 5, 40–42, 2014.
- Becic T, Studenik C, Hoffman G. Exercise increases adiponectin and reduces leptin levels in prediabetic and diabetic individuals: systematic review and meta-analysis of randomized controlled trials. *Med Sci* 6, 1–18, 2018.
- Bidulescu A, Morris AA, Stoyanova N, Meng Y, Voccarino V, Quyyumi AA. Association between vitamin d and adiponectin and its relationship with body mass index: the META health study. *Front Pub Health* 2, 1–6, 2014.
- Chin K, Zhao D, Tibuakuu M, Martin SS, Ndumele CE, Florido R. Physical activity, vitamin D, and incident atherosclerotic cardiovascular disease in whites and blacks: the ARIC study. *J Clin Endocrinol Metabol* 102, 1227–1236, 2017.
- Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, Retzlaff BM, Knopp RH, Brunzell JD, Kahn SE. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia* 46, 459–469, 2003.
- Duncan BB, Schmidt MI, Pankow JS, Bang H, Couper D, Ballantyne CM, Hoogeveen RC, Heiss G. Adiponectin and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes* 53, 2473–2478, 2004.
- Foula WH, Emara RH, Eldeeb MK, Mokhtar SA, El-Sahn FA. Effect of a weight loss program on serum adiponectin and insulin resistance among overweight and obese premenopausal females. *J Egypt Public Health Assoc* 95, 32, 2020.
- Gannage-Yared M, Chedid R, Khalife S, Azzi E, Zoghbi F, Halaby G. Vitamin D in relation to metabolic risk factors, insulin sensitivity and adiponectin in a young Middle-Eastern population. *Eur J Endocrinol* 160, 965–971, 2009.
- Hengist A, Perkin O, Gonzalez JT, Betts JA, Hewison M, Manolopoulos KN, Jones KS, Koulman A, Thompson D. Mobilising vitamin D from adipose tissue: The potential impact of exercise. *Nutrition Bull* 44, 25–35, 2019.
- Isobe T, Saitoh S, Takagi S, Takeuchi H, Chiba Y, Katoh N, Shimamoto K. Influence of gender, age, and renal function on plasma adiponectin level: the Tanno and Sobetsu study. *Eur J Endocrinol* 153, 91–98, 2005.
- Kim SJ, Yoon ES, Jung SY, Kim DY. Effect of uphill walking on browning factor and high molecular weight-adiponectin in postmenopausal women. *J Exerc Rehabil* 16, 265–271, 2020.

- Li S, Shin HJ, Ding EL, Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA* 302, 179–188, 2009.
- Liu E, Meigs JB, Pittas AG, McKeown NM, Economos CD, Booth SL, Jacques PF. Plasma 25-hydroxyvitamin D is associated with markers of the insulin resistant phenotype in nondiabetic adults. *J Nutrition* 139, 329–334, 2009.
- Mathieu C, Gysemans C. Vitamin D and diabetes. *Diabetologia* 48, 1247–1257, 2005.
- Mittendorfer B, Fields DA, Klein S. Excess body fat in men decreases plasma fatty acid availability and oxidation during endurance exercise. *Am J Physiol Endocrinol Metab* 286, 354–362, 2004.
- Moro C, Pillard F, de Glisezinski I, Crampes F, Thalamas C, Harant I, Marques MA, Lafontan M, Berlan M. Sex differences in lipolysis-regulating mechanisms in overweight subjects: effect of exercise intensity. *Obesity* 15, 2245–2255, 2007.
- National Health and Nutrition Examination Survey (U.S.). NHANES III plan and operations procedures manuals CD-ROM: draft. National Centers for Disease Control and Prevention, 1997.
- Nielsen MB, Colak Y, Benn M, Nordestgaard BG. Causal relationship between plasma adiponectin and body mass index: one- and two-sample bidirectional mendelian randomization analyses in 460 397 individuals. *Clin Chem* 66, 1548–1557, 2020.
- Polito R, Monda V, Nigro E, Messina A, Di Maio G, Giuliano MT, Orru S, Imperlini E, Calcagno G, Mosca L, Mollica MP, Trinchese G, Scarinci A, Sessa F, Salerno M, Marsala G, Buono P, Mancini A, Monda M, Daniele A, Messina G. The important role of adiponectin and orexin-A, two key proteins improving health status: focus on physical activity. *Front Physiol* 11, 1–17, 2020.
- Simpson KA, Singh MA. Effects of exercise on adiponectin: a systematic review. *Obesity* 16, 241–256, 2008.
- Vaidya A, Forman JP, Hopkins PN, Seely EW, Williams JS. 25-Hydroxyvitamin D is associated with plasma renin activity and the pressor response to dietary sodium intake in Caucasians. *J Renin Ang Aldoster Syst* 12, 311–320, 2011.
- Vaidya A, Williams JS, Forman JP. The independent association between 25-hydroxyvitamin D and adiponectin and its relation with BMI in two large cohorts: the NHS and the HPFS. *Obesity* 20, 86–191, 2012.
- World Health Organization. Global report on diabetes. Geneva, 2016.
- World Health Organization. Diabetes [Fact Sheet]. <https://www.who.int/news-room/fact-sheets/detail/diabetes>, 2021.



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Endocrine Regulations

2 pesan


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28 Juni 2022 15.53

Dear dr. Mudjnarko,

Please, check the PDF of your article.

Alexander Kiss

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Kepada: Alexander kiss <alexander.kiss@savba.sk>

30 Juni 2022 04.44

Dear Alexander Kiss,

After I read and looked at the PDF of my article. I have no changes and I agree with the PDF of my article. Thank you very much for accepting and publishing my article in Endocrine Regulations magazine.

regards

sony wibisono mudjanarko

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[Kutipan teks disembunyikan]

Effects of aerobic exercise on adiponectin levels potentially mediated by vitamin D in type 2 diabetic patients

Sony Wibisono MUDJANARKO¹, Anugrahini IRAWATI², Damayanti TINDUH³

¹Division of Endocrinology, Metabolism, and Diabetes, Department of Internal Medicine, Dr. Soetomo General Academic Hospital, Faculty of Medicine Universitas Airlangga, Indonesia; ²Department of Internal Medicine, Dr. Soetomo General Academic Hospital, Faculty of Medicine Universitas Airlangga, Indonesia; ³Department of Physical Medicine and Rehabilitation, Dr. Soetomo General Academic Hospital, Faculty of Medicine Universitas Airlangga, Indonesia
E-mail: sony.wibisono@fk.unair.ac.id

Objective. The positive effects of exercise on adiponectin and vitamin D have independently been reported. Recent studies have suggested that vitamin D increases adiponectin synthesis through inhibition of the rennin-angiotensin system in adipose tissue. However, studies evaluating the effects of an aerobic exercise on adiponectin and vitamin D simultaneously investigating the potential mechanism of vitamin D-dependent adiponectin pathways in patients with type 2 diabetes mellitus (T2DM) are still limited. This study was undertaken to examine the effects of aerobic exercise on adiponectin and its association with vitamin D in patients with T2DM.

Methods. Total twenty-two patients with T2DM were randomly divided into intervention and control group. The intervention group underwent a moderate intensity of a walking mode treadmill aerobic exercise for four weeks. The exercise protocol was adapted from modified Bruce test with a periodic speed and inclination increase. In both groups, body mass index (BMI), vitamin D, and adiponectin levels, were measured before and after four weeks of the lasting program.

Results. The mean of the increased adiponectin and vitamin D levels after exercise was significantly higher in the intervened than the control group, but statistically significant difference was only found in the adiponectin effect ($p=0.017$). There was a significant association found between vitamin D and adiponectin in the intervention group after data adjustments to age and BMI ($p=0.005$).

Conclusion. Moderate intensity of treadmill exercise with increased speed and inclination periodically increased adiponectin level in patients with T2DM. The increased adiponectin might potentially be mediated by increased vitamin D, but the level of their association impact was dependent on the age and BMI.

Key words: adiponectin, aerobic exercise, vitamin D, type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM) is a metabolic disease characterized by chronic hyperglycemia resulting from insulin resistance with relative insulin deficiency or insulin secretory defect with insulin resistance American Diabetes Association (ADA 2013). This type is the most prevalent form which accounts for 90–95% of all diabetic patients. The

number of people with diabetes has quadrupled from 108 million in 1980 to 422 million in 2014 World Health Organization (WHO 2016). Diabetes can lead to complications in many organs and increase the mortality risk. WHO has reported that diabetes was the ninth leading cause of death worldwide in 2019 with estimation about 1.5 million deaths directly

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1 caused by diabetes (WHO 2021). T2DM became one
2 of the major health problems in the world due to the
3 high morbidity and mortality rates.

4 Previous studies have reported an association
5 between low adiponectin level with increased risk of
6 T2DM (Duncan et al. 2004; Li et al. 2009). Adiponectin
7 is one of peptides secreted by adipocytes known to
8 exert insulin sensitizer, anti-hyperglycemic, anti-
9 inflammatory, and anti-atherogenic effects (Achari
10 and Jain 2017). Adiponectin is known to have a
11 protective effect on the diabetes progression through
12 sensitizing insulin by the glucose uptake increase in
13 the muscle cell (up regulate GLUT 4 translocation),
14 reduction of the gluconeogenesis in the liver, and
15 stimulation of glucose and fatty acid metabolism
16 (Polito et al. 2020). Some researchers have been
17 conducted to evaluate potential factors that increase
18 circulating adiponectin as a promising therapeutic
19 strategy in T2DM, one of which is physical exercise
20 (Simpson and Singh 2008). Several studies have
21 documented that exercise is positively associated
22 with increased adiponectin level, but the results were
23 inconsistent depending to age, gender, body mass
24 index, health status, types, duration, and intensity of
25 the exercise (Simpson and Singh 2008). Cnop et al.
26 (2003) have suggested that the effect of exercise on the
27 adiponectin levels was probably mediated by body
28 composition change. However, other mechanisms
29 may possibly link exercise to adiponectin level.

30 The US National Health and Nutrition Examination
31 Survey (NHANES III 1997) report has suggested
32 that physical exercise is positively correlated with
33 increased serum vitamin D, which is an essential
34 steroid metabolite with multiple metabolic effects
35 including regulation of insulin secretion. Positive
36 effects of exercise on serum vitamin D have been
37 suggested to be directly related to enhanced of
38 vitamin D mobilization from adipose tissue (Hengist
39 et al. 2019). Vitamin D is also indicated to have a
40 direct association with adiponectin varies with race,
41 gender, and body mass index (BMI) (Bidulescu et al.
42 2014). Recent study has shown that serum vitamin
43 D has a positive correlation with serum adiponectin
44 in prediabetic and type 2 diabetic groups (Banerjee
45 et al. 2016). The mechanisms proposed is negative
46 regulation of rennin-angiotensin system in adipose
47 tissue by vitamin D that conquers the inhibition of
48 adiponectin secretion (Vaidya et al. 2011). However,
49 it is not yet clear, whether exercise induced change in
50 the adiponectin levels may directly or indirectly be
51 mediated by vitamin D. To our knowledge, none of the
52 studies carried out was centered to perform a simul-
53 taneous evaluation of the effects of aerobic exercise

on adiponectin and vitamin D with aim to reveal 54
the potential mechanism of vitamin D-dependent 55
adiponectin pathways in the patients with T2DM. 56
This present study was aimed to examine the effects 57
of aerobic exercise training on adiponectin level and 58
its association with the serum vitamin D in patients 59
with T2DM. 60

61 **Materials and methods** 62

63
64 **Study design and subjects.** The current study was
65 pretest-posttest control group design which subjects
66 randomly assigned to intervention and control group
67 with same assessment measures obtained before and
68 after the treatment. The inclusion criteria included
69 male T2DM patients who have been treated by
70 standard medication, age between 35–55 years old,
71 and systolic blood pressure within 110–130 mmHg.
72 We excluded subjects with restrictive or obstructive
73 respiratory tract disease, neuromusculoskeletal
74 disease, peripheral diabetic neuropathy, had sign of
75 inflammations, ulcer or gangrene on either one or
76 both legs, history of heart, kidney, thyroid, and liver
77 disease, vestibular or proprioceptive disturbance, use
78 of long-term steroids and vitamin D supplements,
79 and had routine aerobic exercise at least two times
80 per week. Subjects with symptoms of chest pain,
81 chest tightness, hypoglycemia or sign of ischemia
82 identified by electrocardiogram during or after
83 exercise was dropped out from the study. Twenty-two
84 subjects were participating in the study, then divided
85 into intervention and control group by randomized
86 ballot. Written informed consent was obtained from
87 all the subjects before their participation in the study.
88 This study has been approved by Health Research
89 Ethics Committee of Dr. Soetomo General Hospital
90 Surabaya Indonesia with recommendation number
91 1266/KEPK/VI/2019.

92 **Experimental protocols.** The intervention group
93 was given aerobic exercise with moderate intensity
94 and frequency three sessions per week for four weeks.
95 The training protocol was taken from a modified
96 Bruce test with total seven stages with increasing
97 speed and inclination periodically every 3 min to
98 reach target heart rate (60–75% of maximum heart
99 rate). Aerobic exercise was performed by walking
100 exercise in Treadmill EN-Mill 2007. The heart rate
101 of the subjects was monitored using a portable
102 heart rate monitor throughout the entire session of
103 exercise to maintain the exercise intensity. Treadmill
104 exercise was lasted for about 30 min, included each 5
105 min for warm-up and cool-down. Vital signs (blood
106 pressure, heart rate, oxygen saturation) and capillary

1 blood glucose were assessed before training program.
 2 Subjects with normal vital sign and blood glucose
 3 level within 100–250 mg/dL were allowed to conduct
 4 the exercise. Borg scale was also assessed during
 5 treadmill to monitor perceived exertion. The subjects
 6 in the control group were instructed to maintain
 7 their lifestyle based on standards of medical care in
 8 diabetic patients, including medical nutrition therapy
 9 and exercise with duration min 150 min per week.

10 **Anthropometric data and biochemical analysis.**

11 Clinical data (age, history of T2DM, the presences
 12 of comorbid, blood pressure) were obtained from all
 13 study subjects. Anthropometric data were measured
 14 in both groups before and after four-week program.
 15 Height and weight were examined to calculate
 16 body mass index [BMI=weight (kg)/height² (m²)].
 17 Peripheral blood samples were collected in plain
 18 tube without any activator from all study subjects
 19 before and after four-week program. Blood samples,
 20 then stored at –80 °C refrigerator until analysis done.
 21 Glycemic parameters (random plasma glucose,
 22 fasting plasma glucose, fasting insulin, HbA1C),
 23 serum vitamin D, and adiponectin were measured
 24 in both groups before the start of the intervention.
 25 Vitamin D and adiponectin were measured again
 26 after four-weeks program in both groups. Vitamin D
 27 levels were measured using Siemens ADVIA Centaur
 28 Vitamin D with chemiluminescent immunoassay
 29 (CLIA). Adiponectin concentration was measured
 30 as total adiponectin by ELISA kit (E-EL-H5811,
 31 Elabscience, USA). Change of BMI, vitamin D, and
 32 adiponectin after four-week program in both groups
 33 were determined by subtracting those variables post
 34 to pre intervention.

35 **Statistical analysis.** SPSS version 16.0 was used
 36 for statistical analysis. The results of the study were
 37 presented in tabular forms. Continuous variables
 38 were presented as mean and standard deviation for
 39 normally distributed data. Continuous variables with
 40 skewed distributed data were presented as median
 41 and interquartile range. Categorical variables were
 42 presented as frequency and percentage. Comparison
 43 of clinical characteristics and biochemical
 44 parameters between intervention and control group
 45 were examined using independent sample t test for
 46 normally distributed data, Mann Whitney U test
 47 for skewed distributed data, and Chi square test for
 48 categorical variables. Bivariate correlation analysis
 49 using Pearson (for normally distributed data) or
 50 Spearman (for skewed distributed data) was also
 51 performed between the change of adiponectin level
 52 and the other studied parameters. Linear regression
 53 models were employed to investigate the relationship

between change of vitamin D level and change
 of adiponectin level after four-week program in
 both groups with adjustment for their potentially
 confounding variables (age and BMI). A p-value
 <0.05 was considered statistically significant.

Results

Among 22 subjects, one subject in the intervention
 group was dropped out from the study due to suffered
 from hypoglycemia during exercise program and one
 subject in the control group also could not participate
 until completion of the study. Total 20 subjects
 completed the study at the end. Table 1 showed
 that there were no significant statistical difference
 of baseline clinical characteristics and laboratory
 parameters between each group, except for median of
 age (p=0.017). All study subjects had low levels of serum
 vitamin D (below 30 ng/ml). There was no difference
 of BMI and change of BMI after four-week program
 in both groups. Adiponectin levels post four-week
 program were significantly higher in the intervention
 than control group (p=0.003). Adiponectin increased
 in both groups as shown in Table 2, but statistically
 significant difference was only found in the interven-
 tion group (p=0.017). Median of increased vitamin D
 level was higher in the intervention group, but statis-
 tically insignificant (p=0.821).

Bivariate correlation analysis showed that age,
 BMI, and change of vitamin D level independently
 were not predictor of change in adiponectin level
 after exercise as shown in Table 3. Linear regression
 model was employed to investigate the relation-
 ship among the change of adiponectin level with
 change of vitamin D level and other confounding
 variables. Age and BMI were included in the model
 to adjust for their potentially confounding effects.
 The results from multiple linear regression analysis
 are presented in Table 4 below. Increased of vitamin
 D level in exercise group was significantly associated
 with increased adiponectin level ($\beta=1.027$, p=0.005)
 after adjusting for age and BMI after exercise (fitted
 model: F=6.79, p=0.023, adjusted R² =0.659). In this
 model, age ($\beta=0.852$, p=0.018) and BMI after exercise
 ($\beta=0.769$, p=0.032) were also found to be significant
 predictors for change of adiponectin level after
 exercise in the intervention group.

Discussion

This study demonstrates the effects of aerobic
 exercise on adiponectin levels in patients with T2DM
 and its correlation with change of vitamin D levels

Table 1
Baseline characteristics of the study subjects in each group.

| Parameters | Intervention group (n=10) | Control group (n=10) | p-value |
|--------------------------------|------------------------------|-------------------------|--------------------|
| Age (years) | 51(49–55) | 47 (43–49) | 0.017 ^a |
| Diabetes duration (years) | 3 (2–10) | 2 (1–7) | 0.361 ^a |
| Presence of dyslipidemia (%) | 20 | 20 | 1.000 ^c |
| Presence of hypertension (%) | 40 | 20 | 0.628 ^c |
| Insulin use (%) | 40 | 20 | 0.628 ^c |
| BMI (kg/m ²) | 23.9±3.5 | 26.7±4.4 | 0.141 ^b |
| Systolic pressure (mmHg) | 115 (110–120) | 110 (110–120) | 0.435 ^a |
| Laboratory parameters: | | | |
| Random plasma glucose (mg/dL) | 180±50 | 159±32 | 0.272 ^b |
| Fasting plasma glucose (mg/dL) | 123 (102–179) | 106 (98–132) | 0.226 ^a |
| Fasting insulin (μ/ml) | 10.2 (7.3–12.0) | 8.5 (5.3–17.1) | 0.545 ^a |
| HbA1C (%) | 6.5±1.2 | 7.9±2.1 | 0.090 ^b |
| Vitamin D (ng/ml) | 18.3 (16.2–23.1) | 20.2 (18.8–25.4) | 0.226 ^a |
| Insufficiency vitamin D (%) | 40 | 60 | |
| Deficiency vitamin D (%) | 60 | 40 | |
| Adiponectin (pg/mL) | 456 (420–473) | 448 (418–482) | 0.597 ^a |

Abbreviations: BMI – body mass index; HbA1C – hemoglobin A1c. ^aMann Whitney U test was performed; ^bIndependent sample t test was performed; ^cChi-square test was performed.

Table 2
Comparison of clinical and laboratory parameters after four-week program between groups.

| Variables | Intervention group (n=10) | Control group (n=10) | p-value |
|-----------------------------|------------------------------|-------------------------|--------------------|
| BMI (kg/m ²) | | | |
| Baseline | 23.9±3.5 | 26.7±4.4 | |
| After 4 weeks | 24.9±4.0 | 27.4±5.3 | 0.250 ^b |
| Change of BMI | 0.28 (–0.45–1.88) | 0.35 (–0.002–1.8) | 1.000 ^a |
| Vitamin D (ng/ml) | | | |
| Baseline | 18.3 (16.2–23.1) | 20.16 (18.82–25.39) | |
| After 4 weeks | 21.7±4.5 | 23.26±5.47 | 0.510 ^b |
| Change of vitamin D level | 1.98 (–0.10–3.23) | 1.2 (–1.35–4.18) | 0.821 ^a |
| Adiponectin (pg/mL) | | | |
| Baseline | 456 (420–473) | 448 (418–482) | |
| After 4 weeks | 586±88 | 472±59 | 0.003 ^b |
| Change of adiponectin level | 130±108 | 5±105 | 0.017 ^b |

Abbreviations: BMI – body mass index. ^aMann Whitney U test was performed; ^bIndependent sample t test was performed.

to investigate the potential mechanism of vitamin D-dependent adiponectin pathways. Our study suggests that aerobic exercise with moderate intensity for four weeks increased adiponectin levels in patients with T2DM with mean difference 130±108 pg/ml. These findings complement the current knowledge

regarding the effects of exercise on adiponectin level in diabetic patients. The results are in line with the previous systematic review and meta-analysis of 19 randomized controlled trials in prediabetic and diabetic adults that reported increased levels of adiponectin with mean difference 0.42 μg/ml

Table 3
Correlation between clinical and metabolic parameters to adiponectin before adjustment.

| Variables | Change of adiponectin level | | | |
|---------------------------|-----------------------------|--------|---------------|--------|
| | Intervention group | | Control group | |
| | p-value | r | p-value | r |
| Age | 0.510 | 0.237 | 0.342 | -0.337 |
| Change of BMI | 0.187 | -0.455 | 0.595 | 0.192 |
| Change of vitamin D level | 0.603 | 0.188 | 0.450 | 0.270 |

Abbreviations: BMI – body mass index.

Table 4
Correlation between vitamin D and adiponectin with adjustment to age and BMI.

| Predictors | Change of adiponectin level | | | |
|---------------------------|-----------------------------|---------|---------------|---------|
| | Intervention group | | Control group | |
| | β | p-value | β | p-value |
| Age | 0.852 | 0.018 | -0.478 | 0.240 |
| BMI after 4 weeks | -0.769 | 0.032 | -0.129 | 0.726 |
| Change of vitamin D level | 1.027 | 0.005 | 0.422 | 0.292 |

Abbreviations: BMI – body mass index.

(95% CI 0.23–0.60, $p < 0.00001$) after physical exercise, especially aerobic but neither resistance nor concurrent exercise (Becic et al. 2018). Exercise duration of the present study was shorter than recent study performed by Aly et al. (2014) that have shown an increased adiponectin in type 2 diabetic patients after moderate intensity of supervised aerobic training for 12 weeks. The benefits of exercise in our study may be resulted from increasing speed and inclination periodically on a walking mode treadmill that thought to have greater stimulation on the musculoskeletal system and energy metabolism compared with walking on a flat surface (Kim et al. 2020). Both BMI in each group of this study was increased after four-week program, but lower in the intervention group. The effects of exercise on adiponectin were suggested to determine by body composition change, especially reduction of intraabdominal fat mass (Cnop et al. 2003; Simpson and Singh 2008). Unfortunately, several indexes to estimate body fat (e.g., waist circumference, waist-hip ratio, or skin fold thickness) were not measured in the study though could affect on adiponectin. Correlation of increased adiponectin level with change of BMI after exercise in this study showed insignificant result. However, BMI is an index of lean body mass and cannot precisely measure body composition (muscle mass or body fat percentage). Other mechanisms could also possibly mediate the effects of exercise on adiponectin, e.g., via vitamin D pathways.

All study subjects in both groups had lower levels of baseline serum vitamin D (below 30 ng/ml). This data supports the results of previous research about the higher incidence of vitamin D deficiency or insufficiency in the diabetic population (Bayani et al. 2014). Deficient of vitamin D linked to abruption of insulin secretion and sensitivity that suggested to have a connection with the incidence or progression of T2DM (Mathieu and Gysemans 2005). Baseline serum vitamin D in the intervention group were lower than a control group that may be affected by an older median of age or diabetes duration. However, increased vitamin D levels in the intervention group after aerobic exercise were higher than the control group but statistically insignificant. Adipose tissue was known to act as a reservoir for vitamin D due to lipophilicity and exercise was suggested to strongly stimulate vitamin D mobilization from adipose tissue (Hengist et al. 2019). Previous studies reported that exercise caused the rise of glucagon, adrenaline, and atrial natriuretic peptide as stimulatory lipolytic hormones and suggested as a key mechanism of vitamin D release from adipose tissue (Moro et al. 2007; Hengist et al. 2019). The lipolytic response of adipose tissue to exercise in obesity was reported reduced compared with lean controls (Mittendorfer et al. 2004). This condition may explain the lower increased vitamin D levels in control group due to higher mean of baseline BMI. However, little is known

1 about the effect of exercise on serum vitamin D in type
2 2 diabetic patients. Previous research conducted in rat
3 experimentally-induced T2DM showed significant
4 increase of serum vitamin D in diabetic group after
5 moderate swimming exercise with duration 60 min a
6 day and frequency five times a week for 4 weeks (Aly
7 et al. 2016). These exercise protocols may suggest that
8 higher frequency or duration of exercise is needed
9 to increase vitamin D level significantly in patients
10 with T2DM. This analysis supports the results from
11 previous studies that reported higher vitamin D level
12 in person with more intense physical activity (Chin
13 et al. 2017).

14 We hypothesized that increased adiponectin
15 level in type 2 diabetic patients might be potentially
16 mediated by increased vitamin D level after aerobic
17 exercise. However, the association between vitamin
18 D and adiponectin in previous studies showed
19 various results. Study in healthy non-diabetic adults
20 indicating direct relationship between vitamin D
21 and adiponectin but the association disappeared
22 after adjustment to BMI (Gannage-Yared et al. 2009;
23 Liu et al. 2009). Study in biracial population-based
24 samples conducted by Bidulescu et al. (2014) reported
25 that the association of vitamin D and adiponectin
26 is dependent on race, gender, and BMI category.
27 However, these studies were carried out on a hetero-
28 geneous population that numerous confounding
29 might affect adiponectin concentration, e.g., age,
30 gender, race, BMI, diabetes and hypertension status,
31 anti-hypertensive drug use, dietary electrolyte
32 intake, dietary patterns, physical activity, alcohol
33 intake, and menopausal status (Vaidya et al. 2012).

34 We found a significant association between
35 vitamin D and adiponectin in the intervention
36 group after adjusting to age and BMI ($p=0.005$).
37 Recent studies have suggested that vitamin D affects
38 adiponectin synthesis by adiponectin expression
39 gene upregulation in visceral fat and down-regu-
40 lation of TNF- α gene that inflicts the adiponectin
41 synthesis (Gannage-Yared et al. 2009). Another
42 study also proposed that vitamin D conquers
43 the inhibition of adiponectin synthesis through
44 negative regulation of rennin-angiotensin system
45 in adipose tissue (Vaidya et al. 2011). In our linear
46 regression model, age and BMI were also found to
47 be significant predictors of increased adiponectin
48 level after exercise in the intervention group. These
49 results may indicate that the association of vitamin D
50 and adiponectin after aerobic exercise is dependent
51 on age and BMI. Age is positively correlated with
52 increased adiponectin level after exercise ($p=0.018$).
53 This result builds on existing evidence of higher

adiponectin concentration on older age (Isobe et al. 54
2005). Decreased of adiponectin clearance by kidney 55
was suggested as a possible cause for adiponectin 56
increase with age (Isobe et al. 2005). Hence, BMI 57
after exercise is inversely associated with increased 58
adiponectin level in the intervention group 59
($p=0.032$). This result also similar with previous 60
study in large samples revealed lower adiponectin 61
level in subjects with higher BMI (Nielsen et al. 62
2020). This finding indicates that adipose tissue may 63
exert a negative effect on adiponectin secretion as 64
suggested in previous studies (Foula et al. 2020). 65

This study has certain limitations. First, the 66
sample size was relatively small, but it was sufficient 67
to reproduce the association of interest. Potential 68
confounding variables that might affect vitamin D 69
and adiponectin concentration (e.g., dietary intake 70
of calcium, use of thiazolidinediones medication 71
or insulin) were not controlled in the present study. 72
Although our study suggested that the increased 73
adiponectin levels after aerobic exercise might be 74
affected by vitamin D, an association was examined 75
using the observational analysis. Thus, the direct 76
causal-effect relationship cannot be proven. 77
Therefore, further research with longitudinal design 78
is needed to establish the directionality of vitamin 79
D on adiponectin level. Since our study indicated 80
that vitamin D might have a potential mechanism to 81
mediate the increased adiponectin, which known to 82
exert many benefits in patients with T2DM, future 83
research to evaluate the effect of vitamin D supple- 84
mentation on adiponectin level in type 2 diabetic 85
patients is warranted. 86

87 Conclusion 88

89
90 Moderate intensity of the treadmill exercise
91 with increased speed and inclination periodically
92 increased adiponectin levels in patients with T2DM.
93 The increased adiponectin levels might potentially
94 be mediated by increased vitamin D after aerobic
95 exercise, but the association is dependent on the age
96 and BMI. 97

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106 of interest.

References

- 1
2
3 Achari AE, Jain SK. Adiponectin, a therapeutic target for obesity, diabetes, and endothelial dysfunction. *Int J*
4 *Mol Sci* 18, 1321, 2017. 54
5 Aly FA, Alghadir AH, Gabr SA. Adiponectin response to supervised aerobic training in type II diabetic patients.
6 *Asian Biomed* 8, 597–602, 2014. 55
7 Aly YE, Abdou AS, Rashad MM, Nassef MM. Effect of exercise on serum vitamin D and tissue vitamin D recep-
8 tors in experimentally induced type 2 diabetes mellitus. *J Adv Res* 7, 671–679, 2016. 56
9 American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 36, 67–74, 2013. 57
10 Banerjee A, Khemka V, Roy D, Poddar J, Roy TKS, Karnam SA. Role of serum adiponectin and vitamin D in
11 prediabetes and diabetes mellitus. *Can J Diabetes* 41, 1–7, 2016. 58
12 Bayani MA, Akbari R, Banasaz B, Saeedi F. Status of Vitamin-D in diabetic patients. *Caspian J Intern Med* 5,
13 40–42, 2014. 59
14 Becic T, Studenik C, Hoffman G. Exercise increases adiponectin and reduces leptin levels in prediabetic and
15 diabetic individuals: systematic review and meta-analysis of randomized controlled trials. *Med Sci* 6,
16 1–18, 2018. 60
17 Bidulescu A, Morris AA, Stoyanova N, Meng Y, Voccarino V, Quyyumi AA. Association between vitamin d
18 and adiponectin and its relationship with body mass index: the META health study. *Front Pub Health*
19 2, 1–6, 2014. 61
20 Chin K, Zhao D, Tibuakuu M, Martin SS, Ndumele CE, Florido R. Physical activity, vitamin D, and incident
21 atherosclerotic cardiovascular disease in whites and blacks: the ARIC study. *J Clin Endocrinol Metabol*
22 102, 1227–1236, 2017. 62
23 Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, Retzlaff BM, Knopp RH, Brunzell JD,
24 Kahn SE. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipopro-
25 teins: evidence for independent roles of age and sex. *Diabetologia* 46, 459–469, 2003. 63
26 Duncan BB, Schmidt MI, Pankow JS, Bang H, Couper D, Ballantyne CM, Hoogeveen RC, Heiss G. Adiponec-
27 tin and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes* 53,
28 2473–2478, 2004. 64
29 Foula WH, Emara RH, Eldeeb MK, Mokhtar SA, El-Sahn FA. Effect of a weight loss program on serum adi-
30 ponectin and insulin resistance among overweight and obese premenopausal females. *J Egypt Public*
31 *Health Assoc* 95, 32, 2020. 65
32 Gannage-Yared M, Chedid R, Khalife S, Azzi E, Zoghbi F, Halaby G. Vitamin D in relation to metabolic risk
33 factors, insulin sensitivity and adiponectin in a young Middle-Eastern population. *Eur J Endocrinol*
34 160, 965–971, 2009. 66
35 Hengist A, Perkin O, Gonzalez JT, Betts JA, Hewison M, Manolopoulos KN, Jones KS, Koulman A, Thompson
36 D. Mobilising vitamin D from adipose tissue: The potential impact of exercise. *Nutrition Bull* 44, 25–35,
37 2019. 67
38 Isobe T, Saitoh S, Takagi S, Takeuchi H, Chiba Y, Katoh N, Shimamoto K. Influence of gender, age, and renal
39 function on plasma adiponectin level: the Tanno and Sobetsu study. *Eur J Endocrinol* 153, 91–98, 2005. 68
40 Kim SJ, Yoon ES, Jung SY, Kim DY. Effect of uphill walking on browning factor and high molecular weight-
41 adiponectin in postmenopausal women. *J Exerc Rehabil* 16, 265–271, 2020. 69
42 Li S, Shin HJ, Ding EL, Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-
43 analysis. *JAMA* 302, 179–188, 2009. 70
44 Liu E, Meigs JB, Pittas AG, McKeown NM, Economos CD, Booth SL, Jacques PF. Plasma 25-hydroxyvitamin
45 d is associated with markers of the insulin resistant phenotype in nondiabetic adults. *J Nutrition* 139,
46 329–334, 2009. 71
47 Mathieu C, Gysemans C. Vitamin D and diabetes. *Diabetologia* 48, 1247–1257, 2005. 72
48 Mittendorfer B, Fields DA, Klein S. Excess body fat in men decreases plasma fatty acid availability and oxidation
49 during endurance exercise. *Am J Physiol Endocrinol Metab* 286, 354–362, 2004. 73
50 Moro C, Pillard F, de Glisezinski I, Crampes F, Thalamos C, Harant I, Marques MA, Lafontan M, Berlan M.
51 Sex differences in lipolysis-regulating mechanisms in overweight subjects: effect of exercise intensity. *Obesity*
52 15, 2245–2255, 2007. 74
53 National Health and Nutrition Examination Survey (U.S.). NHANES III plan and operations procedures manu-
als CD-ROM: draft. National Centers for Disease Control and Prevention, 1997. 75

| | | |
|----|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| 1 | Nielsen MB, Colak Y, Benn M, Nordestgaard BG. Causal relationship between plasma adiponectin and body | 54 |
| 2 | mass index: one- and two-sample bidirectional mendelian randomization analyses in 460 397 individu- | 55 |
| 3 | als. <i>Clin Chem</i> 66, 1548–1557, 2020. | 56 |
| 4 | Polito R, Monda V, Nigro E, Messina A, Di Maio G, Giuliano MT, Orru S, Imperlini E, Calcagno G, Mosca L, | 57 |
| 5 | Mollica MP, Trinchese G, Scarinci A, Sessa F, Salerno M, Marsala G, Buono P, Mancini A, Monda M, | 58 |
| 6 | Daniele A, Messina G. The important role of adiponectin and orexin-A, two key proteins improving | 59 |
| 7 | health status: focus on physical activity. <i>Front Physiol</i> 11, 1–17, 2020. | 60 |
| 8 | Simpson KA, Singh MA. Effects of exercise on adiponectin: a systematic review. <i>Obesity</i> 16, 241–256, 2008. | 61 |
| 9 | Vaidya A, Forman JP, Hopkins PN, Seely EW, Williams JS. 25-Hydroxyvitamin D is associated with plasma | 62 |
| 10 | renin activity and the pressor response to dietary sodium intake in Caucasians. <i>J Renin Ang Aldoster</i> | 63 |
| 11 | <i>Syst</i> 12, 311–320, 2011. | 64 |
| 12 | Vaidya A, Williams JS, Forman JP. The independent association between 25-hydroxyvitamin D and adiponec- | 65 |
| 13 | tin and its relation with BMI in two large cohorts: the NHS and the HPFS. <i>Obesity</i> 20, 86–191, 2012. | 66 |
| 14 | World Health Organization. Global report on diabetes. Geneva, 2016. | 67 |
| 15 | World Health Organization. Diabetes [Fact Sheet]. https://www.who.int/news-room/fact-sheets/detail/diabetes , | 68 |
| 16 | 2021. | 69 |
| 17 | | 70 |
| 18 | | 71 |
| 19 | | 72 |
| 20 | | 73 |
| 21 | | 74 |
| 22 | | 75 |
| 23 | | 76 |
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SONY WIBISONO <sony.wibisono@fk.unair.ac.id>

Manuscript Submission for Publication in Endocrine Regulations Journal

2 pesan

SONY WIBISONO <sony.wibisono@fk.unair.ac.id>

7 Januari 2022 22.36




Kepada: endocrine.regulations@savba.sk

Dear Editor-in-Chief
Endocrine Regulations Journal,

Here are required documents for manuscript submission in order to publication in *Endocrine Regulations*. Thank you for your consideration in this manuscript.

Sincerely,
Sony Wibisono Mudjanarko, MD, PhD

3 lampiran

-  **Manuscript for Endocrine Regulation_Exercise on adiponectin potentially mediated by vitamin D.docx**
60K
-  **Cover Letter for Endocrine Regulations.docx**
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-  **Suggestion Reviewer for Endocrine Regulations.docx**
29K

Endocrine Regulations <endocrine.regulations@savba.sk>

8 Januari 2022 01.21

Kepada: SONY WIBISONO <sony.wibisono@fk.unair.ac.id>

Dear dr. Mudjanarko,

Thank you for submitting your manuscript entitled as "**The Effects of Aerobic Exercise Training on Adiponectin Level are Potentially Mediated by Vitamin D in Type 2 Diabetic Patients**" of authors Sony Wibisono Mudjanarko, Anugrahini Irawati, Damayanti Tinduh to *Endocrine Regulations*.

Alexander Kiss
Editor-in-Chief



SONY WIBISONO <sony.wibisono@fk.unair.ac.id>

Inquiries about the current status of submitted paper

2 pesan

SONY WIBISONO <sony.wibisono@fk.unair.ac.id>
Kepada: alexander.kiss@savba.sk

20 Januari 2022 16.37

Dear Dr. Alexander Kiss

This is with regard to our submitted manuscript, titled "**The effects of aerobic exercise training on adiponectin level are potentially mediated by vitamin d in type 2 diabetic patients**" of authors Sony Wibisono Mudjanarko, Anugrahini Irawati, Damayanti Tinduh submitted to your journal on January, 7 2022 for consideration as an original article.

We have not received an update regarding the status of our manuscript since submission notification. Could you let us know when we can expect notice regarding the decision of the editorial board? We would be grateful if you could let us know whether there has been any further progress on our submission.

Thank you for your time and consideration. We look forward to hearing from you.

Sincerely,

Sony Wibisono Mudjanarko

Alexander kiss <alexander.kiss@savba.sk>
Kepada: SONY WIBISONO <sony.wibisono@fk.unair.ac.id>

20 Januari 2022 17.39

Dear dr. Mudjanarko,

Your manuscript is under the regular processing of the manuscripts sent for Endocrine Regulations publication. After getting the Reviewers responses, we will inform you about the status of acceptance of your manuscript.

Best regards

Alexander Kiss

From: "SONY WIBISONO" <sony.wibisono@fk.unair.ac.id>
To: "Alexander kiss" <alexander.kiss@savba.sk>
Sent: Thursday, January 20, 2022 10:37:30 AM
Subject: Inquiries about the current status of submitted paper

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SONY WIBISONO <sony.wibisono@fk.unair.ac.id>

Inquiries about the current status of submitted paper

2 pesan

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Best regards

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Subject: Inquiries about the current status of submitted paper

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SONY WIBISONO <sony.wibisono@fk.unair.ac.id>

Endocrine Regulations

1 pesan

Alexander kiss <alexander.kiss@savba.sk>
Kepada: sony.wibisono@fk.unair.ac.id

21 Maret 2022 15.36

Dear dr. Sony Wibisono MUDJANARKO,

I am glad to inform you that your article "Effects of aerobic exercise on adiponectin levels potentially mediated by vitamin D in type 2 diabetic patients" has been accepted for publication in Endocrine Regulations - see the attachment.

Alexander Kiss

 **acceptance_letter.pdf**
8K

endocrine regulations

international journal on experimental and clinical endocrinology

Institute of Experimental Endocrinology, Biomedical Research Center,
Slovak Academy of Sciences, Dubravská cesta 9, 845 05 Bratislava, Slovakia

Bratislava, March 20th, 2022

Dear dr. Sony Wibisono Mudjanarko,

I am pleased to inform you that your article "Effects of aerobic exercise on adiponectin levels potentially mediated by vitamin D in type 2 diabetic patients" by authors Sony Wibisono Mudjanarko, Anugrahini Irawati, Damayanti Tinduh, has been accepted for publication in Endocrine Regulations and will come up in the Endocrine Regulations Vol 56 No3 (July issue) 2022.

Thanks you for publishing in the Endocrine Regulations.

Yours sincerely,

Alexander Kiss
Editor-in-Chief

**SONY WIBISONO** <sony.wibisono@fk.unair.ac.id>

Endocrine Regulations

2 pesan

Alexander kiss <alexander.kiss@savba.sk>
Kepada: **SONY WIBISONO** <sony.wibisono@fk.unair.ac.id>

15 Juni 2022 14.43

Dear dr. Mudjanarko,

I am sending you the preproof form of your article in word format. Please, read it carefully and do corrections, if you need to do them, directly into the article and label them by yellow color. Return it soon.

With the best regards,

Alexander Kiss
Editor-in-Chief
Endocrine Regulations

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SONY WIBISONO <sony.wibisono@fk.unair.ac.id>
Kepada: Alexander kiss <alexander.kiss@savba.sk>

16 Juni 2022 00.36

Dear Dr. Alexander Kiss,

First of all, we would like to express our sincere gratitude and appreciation for accepting our article for final publication in Endocrine Regulations Journal. Thank you as well for sending us the pre-proof form of our article. We have read them and no further corrections are needed.

Sincerely,
Sony Wibisono Mudjanarko

[Kutipan teks disembunyikan]



SONY WIBISONO <sony.wibisono@fk.unair.ac.id>

Endocrine Regulations

2 pesan


Alexander kiss <alexander.kiss@savba.sk>
Kepada: SONY WIBISONO <sony.wibisono@fk.unair.ac.id>

28 Juni 2022 15.53

Dear dr. Mudjnarko,

Please, check the PDF of your article.

Alexander Kiss

 **EndoReg_2022_56_03_5_Mudjanarko_000 (1).pdf**
324K

sony mudjanarko <sony.wibisono@fk.unair.ac.id>
Kepada: Alexander kiss <alexander.kiss@savba.sk>

30 Juni 2022 04.44

Dear Alexander Kiss,

After I read and looked at the PDF of my article. I have no changes and I agree with the PDF of my article. Thank you very much for accepting and publishing my article in Endocrine Regulations magazine.

regards

sony wibisono mudjanarko

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Effects of aerobic exercise on adiponectin levels potentially mediated by vitamin D in type 2 diabetic patients

Sony Wibisono MUDJANARKO¹, Anugrahini IRAWATI², Damayanti TINDUH³

¹Division of Endocrinology, Metabolism, and Diabetes, Department of Internal Medicine, Dr. Soetomo General Academic Hospital, Faculty of Medicine Universitas Airlangga, Indonesia; ²Department of Internal Medicine, Dr. Soetomo General Academic Hospital, Faculty of Medicine Universitas Airlangga, Indonesia; ³Department of Physical Medicine and Rehabilitation, Dr. Soetomo General Academic Hospital, Faculty of Medicine Universitas Airlangga, Indonesia
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Objective. The positive effects of exercise on adiponectin and vitamin D have independently been reported. Recent studies have suggested that vitamin D increases adiponectin synthesis through inhibition of the rennin-angiotensin system in adipose tissue. However, studies evaluating the effects of an aerobic exercise on adiponectin and vitamin D simultaneously investigating the potential mechanism of vitamin D-dependent adiponectin pathways in patients with type 2 diabetes mellitus (T2DM) are still limited. This study was undertaken to examine the effects of aerobic exercise on adiponectin and its association with vitamin D in patients with T2DM.

Methods. Total twenty-two patients with T2DM were randomly divided into intervention and control group. The intervention group underwent a moderate intensity of a walking mode treadmill aerobic exercise for four weeks. The exercise protocol was adapted from modified Bruce test with a periodic speed and inclination increase. In both groups, body mass index (BMI), vitamin D, and adiponectin levels, were measured before and after four weeks of the lasting program.

Results. The mean of the increased adiponectin and vitamin D levels after exercise was significantly higher in the intervened than the control group, but statistically significant difference was only found in the adiponectin effect ($p=0.017$). There was a significant association found between vitamin D and adiponectin in the intervention group after data adjustments to age and BMI ($p=0.005$).

Conclusion. Moderate intensity of treadmill exercise with increased speed and inclination periodically increased adiponectin level in patients with T2DM. The increased adiponectin might potentially be mediated by increased vitamin D, but the level of their association impact was dependent on the age and BMI.

Key words: adiponectin, aerobic exercise, vitamin D, type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM) is a metabolic disease characterized by chronic hyperglycemia resulting from insulin resistance with relative insulin deficiency or insulin secretory defect with insulin resistance American Diabetes Association (ADA 2013). This type is the most prevalent form which accounts for 90–95% of all diabetic patients. The

number of people with diabetes has quadrupled from 108 million in 1980 to 422 million in 2014 World Health Organization (WHO 2016). Diabetes can lead to complications in many organs and increase the mortality risk. WHO has reported that diabetes was the ninth leading cause of death worldwide in 2019 with estimation about 1.5 million deaths directly

Corresponding author: Sony Wibisono Mudjanarko, MD, PhD, 57 Mayjend Prof. Moestopo Street Pacar Kembang Tambak Sari, Surabaya, Indonesia, 60132; phone/fax: +6231-502-3865; e-mail: sony.wibisono@fk.unair.ac.id.

1 caused by diabetes (WHO 2021). T2DM became one
2 of the major health problems in the world due to the
3 high morbidity and mortality rates.

4 Previous studies have reported an association
5 between low adiponectin level with increased risk of
6 T2DM (Duncan et al. 2004; Li et al. 2009). Adiponectin
7 is one of peptides secreted by adipocytes known to
8 exert insulin sensitizer, anti-hyperglycemic, anti-
9 inflammatory, and anti-atherogenic effects (Achari
10 and Jain 2017). Adiponectin is known to have a
11 protective effect on the diabetes progression through
12 sensitizing insulin by the glucose uptake increase in
13 the muscle cell (up regulate GLUT 4 translocation),
14 reduction of the gluconeogenesis in the liver, and
15 stimulation of glucose and fatty acid metabolism
16 (Polito et al. 2020). Some researchers have been
17 conducted to evaluate potential factors that increase
18 circulating adiponectin as a promising therapeutic
19 strategy in T2DM, one of which is physical exercise
20 (Simpson and Singh 2008). Several studies have
21 documented that exercise is positively associated
22 with increased adiponectin level, but the results were
23 inconsistent depending to age, gender, body mass
24 index, health status, types, duration, and intensity of
25 the exercise (Simpson and Singh 2008). Cnop et al.
26 (2003) have suggested that the effect of exercise on the
27 adiponectin levels was probably mediated by body
28 composition change. However, other mechanisms
29 may possibly link exercise to adiponectin level.

30 The US National Health and Nutrition Examination
31 Survey (NHANES III 1997) report has suggested
32 that physical exercise is positively correlated with
33 increased serum vitamin D, which is an essential
34 steroid metabolite with multiple metabolic effects
35 including regulation of insulin secretion. Positive
36 effects of exercise on serum vitamin D have been
37 suggested to be directly related to enhanced of
38 vitamin D mobilization from adipose tissue (Hengist
39 et al. 2019). Vitamin D is also indicated to have a
40 direct association with adiponectin varies with race,
41 gender, and body mass index (BMI) (Bidulescu et al.
42 2014). Recent study has shown that serum vitamin
43 D has a positive correlation with serum adiponectin
44 in prediabetic and type 2 diabetic groups (Banerjee
45 et al. 2016). The mechanisms proposed is negative
46 regulation of rennin-angiotensin system in adipose
47 tissue by vitamin D that conquers the inhibition of
48 adiponectin secretion (Vaidya et al. 2011). However,
49 it is not yet clear, whether exercise induced change in
50 the adiponectin levels may directly or indirectly be
51 mediated by vitamin D. To our knowledge, none of the
52 studies carried out was centered to perform a simul-
53 taneous evaluation of the effects of aerobic exercise

on adiponectin and vitamin D with aim to reveal 54
the potential mechanism of vitamin D-dependent 55
adiponectin pathways in the patients with T2DM. 56
This present study was aimed to examine the effects 57
of aerobic exercise training on adiponectin level and 58
its association with the serum vitamin D in patients 59
with T2DM. 60

61 **Materials and methods** 62

63
64 **Study design and subjects.** The current study was
65 pretest-posttest control group design which subjects
66 randomly assigned to intervention and control group
67 with same assessment measures obtained before and
68 after the treatment. The inclusion criteria included
69 male T2DM patients who have been treated by
70 standard medication, age between 35–55 years old,
71 and systolic blood pressure within 110–130 mmHg.
72 We excluded subjects with restrictive or obstructive
73 respiratory tract disease, neuromusculoskeletal
74 disease, peripheral diabetic neuropathy, had sign of
75 inflammations, ulcer or gangrene on either one or
76 both legs, history of heart, kidney, thyroid, and liver
77 disease, vestibular or proprioceptive disturbance, use
78 of long-term steroids and vitamin D supplements,
79 and had routine aerobic exercise at least two times
80 per week. Subjects with symptoms of chest pain,
81 chest tightness, hypoglycemia or sign of ischemia
82 identified by electrocardiogram during or after
83 exercise was dropped out from the study. Twenty-two
84 subjects were participating in the study, then divided
85 into intervention and control group by randomized
86 ballot. Written informed consent was obtained from
87 all the subjects before their participation in the study.
88 This study has been approved by Health Research
89 Ethics Committee of Dr. Soetomo General Hospital
90 Surabaya Indonesia with recommendation number
91 1266/KEPK/VI/2019.

92 **Experimental protocols.** The intervention group
93 was given aerobic exercise with moderate intensity
94 and frequency three sessions per week for four weeks.
95 The training protocol was taken from a modified
96 Bruce test with total seven stages with increasing
97 speed and inclination periodically every 3 min to
98 reach target heart rate (60–75% of maximum heart
99 rate). Aerobic exercise was performed by walking
100 exercise in Treadmill EN-Mill 2007. The heart rate
101 of the subjects was monitored using a portable
102 heart rate monitor throughout the entire session of
103 exercise to maintain the exercise intensity. Treadmill
104 exercise was lasted for about 30 min, included each 5
105 min for warm-up and cool-down. Vital signs (blood
106 pressure, heart rate, oxygen saturation) and capillary

1 blood glucose were assessed before training program.
 2 Subjects with normal vital sign and blood glucose
 3 level within 100–250 mg/dL were allowed to conduct
 4 the exercise. Borg scale was also assessed during
 5 treadmill to monitor perceived exertion. The subjects
 6 in the control group were instructed to maintain
 7 their lifestyle based on standards of medical care in
 8 diabetic patients, including medical nutrition therapy
 9 and exercise with duration min 150 min per week.

10 **Anthropometric data and biochemical analysis.**

11 Clinical data (age, history of T2DM, the presences
 12 of comorbid, blood pressure) were obtained from all
 13 study subjects. Anthropometric data were measured
 14 in both groups before and after four-week program.
 15 Height and weight were examined to calculate
 16 body mass index [BMI=weight (kg)/height² (m²)].
 17 Peripheral blood samples were collected in plain
 18 tube without any activator from all study subjects
 19 before and after four-week program. Blood samples,
 20 then stored at –80 °C refrigerator until analysis done.
 21 Glycemic parameters (random plasma glucose,
 22 fasting plasma glucose, fasting insulin, HbA1C),
 23 serum vitamin D, and adiponectin were measured
 24 in both groups before the start of the intervention.
 25 Vitamin D and adiponectin were measured again
 26 after four-weeks program in both groups. Vitamin D
 27 levels were measured using Siemens ADVIA Centaur
 28 Vitamin D with chemiluminescent immunoassay
 29 (CLIA). Adiponectin concentration was measured
 30 as total adiponectin by ELISA kit (E-EL-H5811,
 31 Elabscience, USA). Change of BMI, vitamin D, and
 32 adiponectin after four-week program in both groups
 33 were determined by subtracting those variables post
 34 to pre intervention.

35 **Statistical analysis.** SPSS version 16.0 was used
 36 for statistical analysis. The results of the study were
 37 presented in tabular forms. Continuous variables
 38 were presented as mean and standard deviation for
 39 normally distributed data. Continuous variables with
 40 skewed distributed data were presented as median
 41 and interquartile range. Categorical variables were
 42 presented as frequency and percentage. Comparison
 43 of clinical characteristics and biochemical
 44 parameters between intervention and control group
 45 were examined using independent sample t test for
 46 normally distributed data, Mann Whitney U test
 47 for skewed distributed data, and Chi square test for
 48 categorical variables. Bivariate correlation analysis
 49 using Pearson (for normally distributed data) or
 50 Spearman (for skewed distributed data) was also
 51 performed between the change of adiponectin level
 52 and the other studied parameters. Linear regression
 53 models were employed to investigate the relationship

between change of vitamin D level and change
 of adiponectin level after four-week program in
 both groups with adjustment for their potentially
 confounding variables (age and BMI). A p-value
 <0.05 was considered statistically significant.

Results

Among 22 subjects, one subject in the intervention
 group was dropped out from the study due to suffered
 from hypoglycemia during exercise program and one
 subject in the control group also could not participate
 until completion of the study. Total 20 subjects
 completed the study at the end. Table 1 showed
 that there were no significant statistical difference
 of baseline clinical characteristics and laboratory
 parameters between each group, except for median of
 age (p=0.017). All study subjects had low levels of serum
 vitamin D (below 30 ng/ml). There was no difference
 of BMI and change of BMI after four-week program
 in both groups. Adiponectin levels post four-week
 program were significantly higher in the intervention
 than control group (p=0.003). Adiponectin increased
 in both groups as shown in Table 2, but statistically
 significant difference was only found in the interven-
 tion group (p=0.017). Median of increased vitamin D
 level was higher in the intervention group, but statis-
 tically insignificant (p=0.821).

Bivariate correlation analysis showed that age,
 BMI, and change of vitamin D level independently
 were not predictor of change in adiponectin level
 after exercise as shown in Table 3. Linear regression
 model was employed to investigate the relation-
 ship among the change of adiponectin level with
 change of vitamin D level and other confounding
 variables. Age and BMI were included in the model
 to adjust for their potentially confounding effects.
 The results from multiple linear regression analysis
 are presented in Table 4 below. Increased of vitamin
 D level in exercise group was significantly associated
 with increased adiponectin level ($\beta=1.027$, p=0.005)
 after adjusting for age and BMI after exercise (fitted
 model: F=6.79, p=0.023, adjusted R² =0.659). In this
 model, age ($\beta=0.852$, p=0.018) and BMI after exercise
 ($\beta=0.769$, p=0.032) were also found to be significant
 predictors for change of adiponectin level after
 exercise in the intervention group.

Discussion

This study demonstrates the effects of aerobic
 exercise on adiponectin levels in patients with T2DM
 and its correlation with change of vitamin D levels

Table 1
Baseline characteristics of the study subjects in each group.

| Parameters | Intervention group (n=10) | Control group (n=10) | p-value |
|--------------------------------|------------------------------|-------------------------|--------------------|
| Age (years) | 51(49–55) | 47 (43–49) | 0.017 ^a |
| Diabetes duration (years) | 3 (2–10) | 2 (1–7) | 0.361 ^a |
| Presence of dyslipidemia (%) | 20 | 20 | 1.000 ^c |
| Presence of hypertension (%) | 40 | 20 | 0.628 ^c |
| Insulin use (%) | 40 | 20 | 0.628 ^c |
| BMI (kg/m ²) | 23.9±3.5 | 26.7±4.4 | 0.141 ^b |
| Systolic pressure (mmHg) | 115 (110–120) | 110 (110–120) | 0.435 ^a |
| Laboratory parameters: | | | |
| Random plasma glucose (mg/dL) | 180±50 | 159±32 | 0.272 ^b |
| Fasting plasma glucose (mg/dL) | 123 (102–179) | 106 (98–132) | 0.226 ^a |
| Fasting insulin (μ/ml) | 10.2 (7.3–12.0) | 8.5 (5.3–17.1) | 0.545 ^a |
| HbA1C (%) | 6.5±1.2 | 7.9±2.1 | 0.090 ^b |
| Vitamin D (ng/ml) | 18.3 (16.2–23.1) | 20.2 (18.8–25.4) | 0.226 ^a |
| Insufficiency vitamin D (%) | 40 | 60 | |
| Deficiency vitamin D (%) | 60 | 40 | |
| Adiponectin (pg/mL) | 456 (420–473) | 448 (418–482) | 0.597 ^a |

Abbreviations: BMI – body mass index; HbA1C – hemoglobin A1c. ^aMann Whitney U test was performed; ^bIndependent sample t test was performed; ^cChi-square test was performed.

Table 2
Comparison of clinical and laboratory parameters after four-week program between groups.

| Variables | Intervention group (n=10) | Control group (n=10) | p-value |
|-----------------------------|------------------------------|-------------------------|--------------------|
| BMI (kg/m ²) | | | |
| Baseline | 23.9±3.5 | 26.7±4.4 | |
| After 4 weeks | 24.9±4.0 | 27.4±5.3 | 0.250 ^b |
| Change of BMI | 0.28 (–0.45–1.88) | 0.35 (–0.002–1.8) | 1.000 ^a |
| Vitamin D (ng/ml) | | | |
| Baseline | 18.3 (16.2–23.1) | 20.16 (18.82–25.39) | |
| After 4 weeks | 21.7±4.5 | 23.26±5.47 | 0.510 ^b |
| Change of vitamin D level | 1.98 (–0.10–3.23) | 1.2 (–1.35–4.18) | 0.821 ^a |
| Adiponectin (pg/mL) | | | |
| Baseline | 456 (420–473) | 448 (418–482) | |
| After 4 weeks | 586±88 | 472±59 | 0.003 ^b |
| Change of adiponectin level | 130±108 | 5±105 | 0.017 ^b |

Abbreviations: BMI – body mass index. ^aMann Whitney U test was performed; ^bIndependent sample t test was performed.

to investigate the potential mechanism of vitamin D-dependent adiponectin pathways. Our study suggests that aerobic exercise with moderate intensity for four weeks increased adiponectin levels in patients with T2DM with mean difference 130±108 pg/ml. These findings complement the current knowledge

regarding the effects of exercise on adiponectin level in diabetic patients. The results are in line with the previous systematic review and meta-analysis of 19 randomized controlled trials in prediabetic and diabetic adults that reported increased levels of adiponectin with mean difference 0.42 μg/ml

Table 3
Correlation between clinical and metabolic parameters to adiponectin before adjustment.

| Variables | Change of adiponectin level | | | |
|---------------------------|-----------------------------|--------|---------------|--------|
| | Intervention group | | Control group | |
| | p-value | r | p-value | r |
| Age | 0.510 | 0.237 | 0.342 | -0.337 |
| Change of BMI | 0.187 | -0.455 | 0.595 | 0.192 |
| Change of vitamin D level | 0.603 | 0.188 | 0.450 | 0.270 |

Abbreviations: BMI – body mass index.

Table 4
Correlation between vitamin D and adiponectin with adjustment to age and BMI.

| Predictors | Change of adiponectin level | | | |
|---------------------------|-----------------------------|---------|---------------|---------|
| | Intervention group | | Control group | |
| | β | p-value | β | p-value |
| Age | 0.852 | 0.018 | -0.478 | 0.240 |
| BMI after 4 weeks | -0.769 | 0.032 | -0.129 | 0.726 |
| Change of vitamin D level | 1.027 | 0.005 | 0.422 | 0.292 |

Abbreviations: BMI – body mass index.

(95% CI 0.23–0.60, $p < 0.00001$) after physical exercise, especially aerobic but neither resistance nor concurrent exercise (Becic et al. 2018). Exercise duration of the present study was shorter than recent study performed by Aly et al. (2014) that have shown an increased adiponectin in type 2 diabetic patients after moderate intensity of supervised aerobic training for 12 weeks. The benefits of exercise in our study may be resulted from increasing speed and inclination periodically on a walking mode treadmill that thought to have greater stimulation on the musculoskeletal system and energy metabolism compared with walking on a flat surface (Kim et al. 2020). Both BMI in each group of this study was increased after four-week program, but lower in the intervention group. The effects of exercise on adiponectin were suggested to determine by body composition change, especially reduction of intraabdominal fat mass (Cnop et al. 2003; Simpson and Singh 2008). Unfortunately, several indexes to estimate body fat (e.g., waist circumference, waist-hip ratio, or skin fold thickness) were not measured in the study though could affect on adiponectin. Correlation of increased adiponectin level with change of BMI after exercise in this study showed insignificant result. However, BMI is an index of lean body mass and cannot precisely measure body composition (muscle mass or body fat percentage). Other mechanisms could also possibly mediate the effects of exercise on adiponectin, e.g., via vitamin D pathways.

All study subjects in both groups had lower levels of baseline serum vitamin D (below 30 ng/ml). This data supports the results of previous research about the higher incidence of vitamin D deficiency or insufficiency in the diabetic population (Bayani et al. 2014). Deficient of vitamin D linked to abruption of insulin secretion and sensitivity that suggested to have a connection with the incidence or progression of T2DM (Mathieu and Gysemans 2005). Baseline serum vitamin D in the intervention group were lower than a control group that may be affected by an older median of age or diabetes duration. However, increased vitamin D levels in the intervention group after aerobic exercise were higher than the control group but statistically insignificant. Adipose tissue was known to act as a reservoir for vitamin D due to lipophilicity and exercise was suggested to strongly stimulate vitamin D mobilization from adipose tissue (Hengist et al. 2019). Previous studies reported that exercise caused the rise of glucagon, adrenaline, and atrial natriuretic peptide as stimulatory lipolytic hormones and suggested as a key mechanism of vitamin D release from adipose tissue (Moro et al. 2007; Hengist et al. 2019). The lipolytic response of adipose tissue to exercise in obesity was reported reduced compared with lean controls (Mittendorfer et al. 2004). This condition may explain the lower increased vitamin D levels in control group due to higher mean of baseline BMI. However, little is known

1 about the effect of exercise on serum vitamin D in type
2 2 diabetic patients. Previous research conducted in rat
3 experimentally-induced T2DM showed significant
4 increase of serum vitamin D in diabetic group after
5 moderate swimming exercise with duration 60 min a
6 day and frequency five times a week for 4 weeks (Aly
7 et al. 2016). These exercise protocols may suggest that
8 higher frequency or duration of exercise is needed
9 to increase vitamin D level significantly in patients
10 with T2DM. This analysis supports the results from
11 previous studies that reported higher vitamin D level
12 in person with more intense physical activity (Chin
13 et al. 2017).

14 We hypothesized that increased adiponectin
15 level in type 2 diabetic patients might be potentially
16 mediated by increased vitamin D level after aerobic
17 exercise. However, the association between vitamin
18 D and adiponectin in previous studies showed
19 various results. Study in healthy non-diabetic adults
20 indicating direct relationship between vitamin D
21 and adiponectin but the association disappeared
22 after adjustment to BMI (Gannage-Yared et al. 2009;
23 Liu et al. 2009). Study in biracial population-based
24 samples conducted by Bidulescu et al. (2014) reported
25 that the association of vitamin D and adiponectin
26 is dependent on race, gender, and BMI category.
27 However, these studies were carried out on a hetero-
28 geneous population that numerous confounding
29 might affect adiponectin concentration, e.g., age,
30 gender, race, BMI, diabetes and hypertension status,
31 anti-hypertensive drug use, dietary electrolyte
32 intake, dietary patterns, physical activity, alcohol
33 intake, and menopausal status (Vaidya et al. 2012).

34 We found a significant association between
35 vitamin D and adiponectin in the intervention
36 group after adjusting to age and BMI ($p=0.005$).
37 Recent studies have suggested that vitamin D affects
38 adiponectin synthesis by adiponectin expression
39 gene upregulation in visceral fat and down-regu-
40 lation of TNF- α gene that inflicts the adiponectin
41 synthesis (Gannage-Yared et al. 2009). Another
42 study also proposed that vitamin D conquers
43 the inhibition of adiponectin synthesis through
44 negative regulation of rennin-angiotensin system
45 in adipose tissue (Vaidya et al. 2011). In our linear
46 regression model, age and BMI were also found to
47 be significant predictors of increased adiponectin
48 level after exercise in the intervention group. These
49 results may indicate that the association of vitamin D
50 and adiponectin after aerobic exercise is dependent
51 on age and BMI. Age is positively correlated with
52 increased adiponectin level after exercise ($p=0.018$).
53 This result builds on existing evidence of higher

adiponectin concentration on older age (Isobe et al. 54
2005). Decreased of adiponectin clearance by kidney 55
was suggested as a possible cause for adiponectin 56
increase with age (Isobe et al. 2005). Hence, BMI 57
after exercise is inversely associated with increased 58
adiponectin level in the intervention group 59
($p=0.032$). This result also similar with previous 60
study in large samples revealed lower adiponectin 61
level in subjects with higher BMI (Nielsen et al. 62
2020). This finding indicates that adipose tissue may 63
exert a negative effect on adiponectin secretion as 64
suggested in previous studies (Foula et al. 2020). 65

This study has certain limitations. First, the 66
sample size was relatively small, but it was sufficient 67
to reproduce the association of interest. Potential 68
confounding variables that might affect vitamin D 69
and adiponectin concentration (e.g., dietary intake 70
of calcium, use of thiazolidinediones medication 71
or insulin) were not controlled in the present study. 72
Although our study suggested that the increased 73
adiponectin levels after aerobic exercise might be 74
affected by vitamin D, an association was examined 75
using the observational analysis. Thus, the direct 76
causal-effect relationship cannot be proven. 77
Therefore, further research with longitudinal design 78
is needed to establish the directionality of vitamin 79
D on adiponectin level. Since our study indicated 80
that vitamin D might have a potential mechanism to 81
mediate the increased adiponectin, which known to 82
exert many benefits in patients with T2DM, future 83
research to evaluate the effect of vitamin D supple- 84
mentation on adiponectin level in type 2 diabetic 85
patients is warranted. 86

87 Conclusion 88

89
90 Moderate intensity of the treadmill exercise
91 with increased speed and inclination periodically
92 increased adiponectin levels in patients with T2DM.
93 The increased adiponectin levels might potentially
94 be mediated by increased vitamin D after aerobic
95 exercise, but the association is dependent on the age
96 and BMI. 97

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References

- 1
2
3 Achari AE, Jain SK. Adiponectin, a therapeutic target for obesity, diabetes, and endothelial dysfunction. *Int J*
4 *Mol Sci* 18, 1321, 2017. 54
5 Aly FA, Alghadir AH, Gabr SA. Adiponectin response to supervised aerobic training in type II diabetic patients.
6 *Asian Biomed* 8, 597–602, 2014. 55
7 Aly YE, Abdou AS, Rashad MM, Nassef MM. Effect of exercise on serum vitamin D and tissue vitamin D recep-
8 tors in experimentally induced type 2 diabetes mellitus. *J Adv Res* 7, 671–679, 2016. 56
9 American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 36, 67–74, 2013. 57
10 Banerjee A, Khemka V, Roy D, Poddar J, Roy TKS, Karnam SA. Role of serum adiponectin and vitamin D in
11 prediabetes and diabetes mellitus. *Can J Diabetes* 41, 1–7, 2016. 58
12 Bayani MA, Akbari R, Banasaz B, Saeedi F. Status of Vitamin-D in diabetic patients. *Caspian J Intern Med* 5,
13 40–42, 2014. 59
14 Becic T, Studenik C, Hoffman G. Exercise increases adiponectin and reduces leptin levels in prediabetic and
15 diabetic individuals: systematic review and meta-analysis of randomized controlled trials. *Med Sci* 6,
16 1–18, 2018. 60
17 Bidulescu A, Morris AA, Stoyanova N, Meng Y, Voccarino V, Quyyumi AA. Association between vitamin d
18 and adiponectin and its relationship with body mass index: the META health study. *Front Pub Health*
19 2, 1–6, 2014. 61
20 Chin K, Zhao D, Tibuakuu M, Martin SS, Ndumele CE, Florido R. Physical activity, vitamin D, and incident
21 atherosclerotic cardiovascular disease in whites and blacks: the ARIC study. *J Clin Endocrinol Metabol*
22 102, 1227–1236, 2017. 62
23 Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, Retzlaff BM, Knopp RH, Brunzell JD,
24 Kahn SE. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipopro-
25 teins: evidence for independent roles of age and sex. *Diabetologia* 46, 459–469, 2003. 63
26 Duncan BB, Schmidt MI, Pankow JS, Bang H, Couper D, Ballantyne CM, Hoogeveen RC, Heiss G. Adiponec-
27 tin and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes* 53,
28 2473–2478, 2004. 64
29 Foula WH, Emara RH, Eldeeb MK, Mokhtar SA, El-Sahn FA. Effect of a weight loss program on serum adi-
30 ponectin and insulin resistance among overweight and obese premenopausal females. *J Egypt Public*
31 *Health Assoc* 95, 32, 2020. 65
32 Gannage-Yared M, Chedid R, Khalife S, Azzi E, Zoghbi F, Halaby G. Vitamin D in relation to metabolic risk
33 factors, insulin sensitivity and adiponectin in a young Middle-Eastern population. *Eur J Endocrinol*
34 160, 965–971, 2009. 66
35 Hengist A, Perkin O, Gonzalez JT, Betts JA, Hewison M, Manolopoulos KN, Jones KS, Koulman A, Thompson
36 D. Mobilising vitamin D from adipose tissue: The potential impact of exercise. *Nutrition Bull* 44, 25–35,
37 2019. 67
38 Isobe T, Saitoh S, Takagi S, Takeuchi H, Chiba Y, Katoh N, Shimamoto K. Influence of gender, age, and renal
39 function on plasma adiponectin level: the Tanno and Sobetsu study. *Eur J Endocrinol* 153, 91–98, 2005. 68
40 Kim SJ, Yoon ES, Jung SY, Kim DY. Effect of uphill walking on browning factor and high molecular weight-
41 adiponectin in postmenopausal women. *J Exerc Rehabil* 16, 265–271, 2020. 69
42 Li S, Shin HJ, Ding EL, Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-
43 analysis. *JAMA* 302, 179–188, 2009. 70
44 Liu E, Meigs JB, Pittas AG, McKeown NM, Economos CD, Booth SL, Jacques PF. Plasma 25-hydroxyvitamin
45 d is associated with markers of the insulin resistant phenotype in nondiabetic adults. *J Nutrition* 139,
46 329–334, 2009. 71
47 Mathieu C, Gysemans C. Vitamin D and diabetes. *Diabetologia* 48, 1247–1257, 2005. 72
48 Mittendorfer B, Fields DA, Klein S. Excess body fat in men decreases plasma fatty acid availability and oxidation
49 during endurance exercise. *Am J Physiol Endocrinol Metab* 286, 354–362, 2004. 73
50 Moro C, Pillard F, de Glisezinski I, Crampes F, Thalamos C, Harant I, Marques MA, Lafontan M, Berlan M.
51 Sex differences in lipolysis-regulating mechanisms in overweight subjects: effect of exercise intensity.
52 *Obesity* 15, 2245–2255, 2007. 74
53 National Health and Nutrition Examination Survey (U.S.). NHANES III plan and operations procedures manu-
als CD-ROM: draft. National Centers for Disease Control and Prevention, 1997. 75

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|----|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| 1 | Nielsen MB, Colak Y, Benn M, Nordestgaard BG. Causal relationship between plasma adiponectin and body | 54 |
| 2 | mass index: one- and two-sample bidirectional mendelian randomization analyses in 460 397 individu- | 55 |
| 3 | als. <i>Clin Chem</i> 66, 1548–1557, 2020. | 56 |
| 4 | Polito R, Monda V, Nigro E, Messina A, Di Maio G, Giuliano MT, Orru S, Imperlini E, Calcagno G, Mosca L, | 57 |
| 5 | Mollica MP, Trinchese G, Scarinci A, Sessa F, Salerno M, Marsala G, Buono P, Mancini A, Monda M, | 58 |
| 6 | Daniele A, Messina G. The important role of adiponectin and orexin-A, two key proteins improving | 59 |
| 7 | health status: focus on physical activity. <i>Front Physiol</i> 11, 1–17, 2020. | 60 |
| 8 | Simpson KA, Singh MA. Effects of exercise on adiponectin: a systematic review. <i>Obesity</i> 16, 241–256, 2008. | 61 |
| 9 | Vaidya A, Forman JP, Hopkins PN, Seely EW, Williams JS. 25-Hydroxyvitamin D is associated with plasma | 62 |
| 10 | renin activity and the pressor response to dietary sodium intake in Caucasians. <i>J Renin Ang Aldoster</i> | 63 |
| 11 | <i>Syst</i> 12, 311–320, 2011. | 64 |
| 12 | Vaidya A, Williams JS, Forman JP. The independent association between 25-hydroxyvitamin D and adiponec- | 65 |
| 13 | tin and its relation with BMI in two large cohorts: the NHS and the HPFS. <i>Obesity</i> 20, 86–191, 2012. | 66 |
| 14 | World Health Organization. Global report on diabetes. Geneva, 2016. | 67 |
| 15 | World Health Organization. Diabetes [Fact Sheet]. https://www.who.int/news-room/fact-sheets/detail/diabetes , | 68 |
| 16 | 2021. | 69 |
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Effects of aerobic exercise on adiponectin levels potentially mediated by vitamin D in type 2 diabetic patients

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Objective. The positive effects of exercise on adiponectin and vitamin D have independently been reported. Recent studies have suggested that vitamin D increases adiponectin synthesis through inhibition of the rennin-angiotensin system in adipose tissue. However, studies evaluating the effects of an aerobic exercise on adiponectin and vitamin D simultaneously investigating the potential mechanism of vitamin D-dependent adiponectin pathways in patients with type 2 diabetes mellitus (T2DM) are still limited. This study was undertaken to examine the effects of aerobic exercise on adiponectin and its association with vitamin D in patients with T2DM.

Methods. Total twenty-two patients with T2DM were randomly divided into intervention and control group. The intervention group underwent a moderate intensity of a walking mode treadmill aerobic exercise for four weeks. The exercise protocol was adapted from modified Bruce test with a periodic speed and inclination increase. In both groups, body mass index (BMI), vitamin D, and adiponectin levels, were measured before and after four weeks of the lasting program.

Results. The mean of the increased adiponectin and vitamin D levels after exercise was significantly higher in the intervened than the control group, but statistically significant difference was only found in the adiponectin effect ($p=0.017$). There was a significant association found between vitamin D and adiponectin in the intervention group after data adjustments to age and BMI ($p=0.005$).

Conclusion. Moderate intensity of treadmill exercise with increased speed and inclination periodically increased adiponectin level in patients with T2DM. The increased adiponectin might potentially be mediated by increased vitamin D, but the level of their association impact was dependent on the age and BMI.

Key words: adiponectin, aerobic exercise, vitamin D, type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM) is a metabolic disease characterized by chronic hyperglycemia resulting from insulin resistance with relative insulin deficiency or insulin secretory defect with insulin resistance American Diabetes Association (ADA 2013). This type is the most prevalent form which accounts for 90–95% of all diabetic patients. The

number of people with diabetes has quadrupled from 108 million in 1980 to 422 million in 2014 World Health Organization (WHO 2016). Diabetes can lead to complications in many organs and increase the mortality risk. WHO has reported that diabetes was the ninth leading cause of death worldwide in 2019 with estimation about 1.5 million deaths directly

caused by diabetes (WHO 2021). T2DM became one of the major health problems in the world due to the high morbidity and mortality rates.

Previous studies have reported an association between low adiponectin level with increased risk of T2DM (Duncan et al. 2004; Li et al. 2009). Adiponectin is one of peptides secreted by adipocytes known to exert insulin sensitizer, anti-hyperglycemic, anti-inflammatory, and anti-atherogenic effects (Achari and Jain 2017). Adiponectin is known to have a protective effect on the diabetes progression through sensitizing insulin by the glucose uptake increase in the muscle cell (up regulate GLUT 4 translocation), reduction of the gluconeogenesis in the liver, and stimulation of glucose and fatty acid metabolism (Polito et al. 2020). Some researchers have been conducted to evaluate potential factors that increase circulating adiponectin as a promising therapeutic strategy in T2DM, one of which is physical exercise (Simpson and Singh 2008). Several studies have documented that exercise is positively associated with increased adiponectin level, but the results were inconsistent depending to age, gender, body mass index, health status, types, duration, and intensity of the exercise (Simpson and Singh 2008). Cnop et al. (2003) have suggested that the effect of exercise on the adiponectin levels was probably mediated by body composition change. However, other mechanisms may possibly link exercise to adiponectin level.

The US National Health and Nutrition Examination Survey (NHANES III 1997) report has suggested that physical exercise is positively correlated with increased serum vitamin D, which is an essential steroid metabolite with multiple metabolic effects including regulation of insulin secretion. Positive effects of exercise on serum vitamin D have been suggested to be directly related to enhanced of vitamin D mobilization from adipose tissue (Hengist et al. 2019). Vitamin D is also indicated to have a direct association with adiponectin varies with race, gender, and body mass index (BMI) (Bidulescu et al. 2014). Recent study has shown that serum vitamin D has a positive correlation with serum adiponectin in prediabetic and type 2 diabetic groups (Banerjee et al. 2016). The mechanisms proposed is negative regulation of rennin-angiotensin system in adipose tissue by vitamin D that conquers the inhibition of adiponectin secretion (Vaidya et al. 2011). However, it is not yet clear, whether exercise induced change in the adiponectin levels may directly or indirectly be mediated by vitamin D. To our knowledge, none of the studies carried out was centered to perform a simultaneous evaluation of the effects of aerobic exercise

on adiponectin and vitamin D with aim to reveal the potential mechanism of vitamin D-dependent adiponectin pathways in the patients with T2DM. This present study was aimed to examine the effects of aerobic exercise training on adiponectin level and its association with the serum vitamin D in patients with T2DM.

Materials and methods

Study design and subjects. The current study was pretest-posttest control group design which subjects randomly assigned to intervention and control group with same assessment measures obtained before and after the treatment. The inclusion criteria included male T2DM patients who have been treated by standard medication, age between 35–55 years old, and systolic blood pressure within 110–130 mmHg. We excluded subjects with restrictive or obstructive respiratory tract disease, neuromusculoskeletal disease, peripheral diabetic neuropathy, had sign of inflammations, ulcer or gangrene on either one or both legs, history of heart, kidney, thyroid, and liver disease, vestibular or proprioceptive disturbance, use of long-term steroids and vitamin D supplements, and had routine aerobic exercise at least two times per week. Subjects with symptoms of chest pain, chest tightness, hypoglycemia or sign of ischemia identified by electrocardiogram during or after exercise was dropped out from the study. Twenty-two subjects were participating in the study, then divided into intervention and control group by randomized ballot. Written informed consent was obtained from all the subjects before their participation in the study. This study has been approved by Health Research Ethics Committee of Dr. Soetomo General Hospital Surabaya Indonesia with recommendation number 1266/KEPK/VI/2019.

Experimental protocols. The intervention group was given aerobic exercise with moderate intensity and frequency three sessions per week for four weeks. The training protocol was taken from a modified Bruce test with total seven stages with increasing speed and inclination periodically every 3 min to reach target heart rate (60–75% of maximum heart rate). Aerobic exercise was performed by walking exercise in Treadmill EN-Mill 2007. The heart rate of the subjects was monitored using a portable heart rate monitor throughout the entire session of exercise to maintain the exercise intensity. Treadmill exercise was lasted for about 30 min, included each 5 min for warm-up and cool-down. Vital signs (blood pressure, heart rate, oxygen saturation) and capillary

blood glucose were assessed before training program. Subjects with normal vital sign and blood glucose level within 100–250 mg/dL were allowed to conduct the exercise. Borg scale was also assessed during treadmill to monitor perceived exertion. The subjects in the control group were instructed to maintain their lifestyle based on standards of medical care in diabetic patients, including medical nutrition therapy and exercise with duration min 150 min per week.

Anthropometric data and biochemical analysis. Clinical data (age, history of T2DM, the presences of comorbid, blood pressure) were obtained from all study subjects. Anthropometric data were measured in both groups before and after four-week program. Height and weight were examined to calculate body mass index [BMI=weight (kg)/height² (m²)]. Peripheral blood samples were collected in plain tube without any activator from all study subjects before and after four-week program. Blood samples, then stored at –80 °C refrigerator until analysis done. Glycemic parameters (random plasma glucose, fasting plasma glucose, fasting insulin, HbA1C), serum vitamin D, and adiponectin were measured in both groups before the start of the intervention. Vitamin D and adiponectin were measured again after four-weeks program in both groups. Vitamin D levels were measured using Siemens ADVIA Centaur Vitamin D with chemiluminescent immunoassay (CLIA). Adiponectin concentration was measured as total adiponectin by ELISA kit (E-EL-H5811, Elabscience, USA). Change of BMI, vitamin D, and adiponectin after four-week program in both groups were determined by subtracting those variables post to pre intervention.

Statistical analysis. SPSS version 16.0 was used for statistical analysis. The results of the study were presented in tabular forms. Continuous variables were presented as mean and standard deviation for normally distributed data. Continuous variables with skewed distributed data were presented as median and interquartile range. Categorical variables were presented as frequency and percentage. Comparison of clinical characteristics and biochemical parameters between intervention and control group were examined using independent sample t test for normally distributed data, Mann Whitney U test for skewed distributed data, and Chi square test for categorical variables. Bivariate correlation analysis using Pearson (for normally distributed data) or Spearman (for skewed distributed data) was also performed between the change of adiponectin level and the other studied parameters. Linear regression models were employed to investigate the relationship

between change of vitamin D level and change of adiponectin level after four-week program in both groups with adjustment for their potentially confounding variables (age and BMI). A p-value <0.05 was considered statistically significant.

Results

Among 22 subjects, one subject in the intervention group was dropped out from the study due to suffered from hypoglycemia during exercise program and one subject in the control group also could not participate until completion of the study. Total 20 subjects completed the study at the end. Table 1 showed that there were no significant statistical difference of baseline clinical characteristics and laboratory parameters between each group, except for median of age (p=0.017). All study subjects had low levels of serum vitamin D (below 30 ng/ml). There was no difference of BMI and change of BMI after four-week program in both groups. Adiponectin levels post four-week program were significantly higher in the intervention than control group (p=0.003). Adiponectin increased in both groups as shown in Table 2, but statistically significant difference was only found in the intervention group (p=0.017). Median of increased vitamin D level was higher in the intervention group, but statistically insignificant (p=0.821).

Bivariate correlation analysis showed that age, BMI, and change of vitamin D level independently were not predictor of change in adiponectin level after exercise as shown in Table 3. Linear regression model was employed to investigate the relationship among the change of adiponectin level with change of vitamin D level and other confounding variables. Age and BMI were included in the model to adjust for their potentially confounding effects. The results from multiple linear regression analysis are presented in Table 4 below. Increased of vitamin D level in exercise group was significantly associated with increased adiponectin level ($\beta=1.027$, p=0.005) after adjusting for age and BMI after exercise (fitted model: F=6.79, p=0.023, adjusted R² =0.659). In this model, age ($\beta=0.852$, p=0.018) and BMI after exercise ($\beta=0.769$, p=0.032) were also found to be significant predictors for change of adiponectin level after exercise in the intervention group.

Discussion

This study demonstrates the effects of aerobic exercise on adiponectin levels in patients with T2DM and its correlation with change of vitamin D levels

Table 1
Baseline characteristics of the study subjects in each group.

| Parameters | Intervention group (n=10) | Control group (n=10) | p-value |
|--------------------------------|------------------------------|-------------------------|--------------------|
| Age (years) | 51(49–55) | 47 (43–49) | 0.017 ^a |
| Diabetes duration (years) | 3 (2–10) | 2 (1–7) | 0.361 ^a |
| Presence of dyslipidemia (%) | 20 | 20 | 1.000 ^c |
| Presence of hypertension (%) | 40 | 20 | 0.628 ^c |
| Insulin use (%) | 40 | 20 | 0.628 ^c |
| BMI (kg/m ²) | 23.9±3.5 | 26.7±4.4 | 0.141 ^b |
| Systolic pressure (mmHg) | 115 (110–120) | 110 (110–120) | 0.435 ^a |
| Laboratory parameters: | | | |
| Random plasma glucose (mg/dL) | 180±50 | 159±32 | 0.272 ^b |
| Fasting plasma glucose (mg/dL) | 123 (102–179) | 106 (98–132) | 0.226 ^a |
| Fasting insulin (μ/ml) | 10.2 (7.3–12.0) | 8.5 (5.3–17.1) | 0.545 ^a |
| HbA1C (%) | 6.5±1.2 | 7.9±2.1 | 0.090 ^b |
| Vitamin D (ng/ml) | 18.3 (16.2–23.1) | 20.2 (18.8–25.4) | 0.226 ^a |
| Insufficiency vitamin D (%) | 40 | 60 | |
| Deficiency vitamin D (%) | 60 | 40 | |
| Adiponectin (pg/mL) | 456 (420–473) | 448 (418–482) | 0.597 ^a |

Abbreviations: BMI – body mass index; HbA1C – hemoglobin A1c. ^aMann Whitney U test was performed; ^bIndependent sample t test was performed; ^cChi-square test was performed.

Table 2
Comparison of clinical and laboratory parameters after four-week program between groups.

| Variables | Intervention group (n=10) | Control group (n=10) | p-value |
|-----------------------------|------------------------------|-------------------------|--------------------|
| BMI (kg/m ²) | | | |
| Baseline | 23.9±3.5 | 26.7±4.4 | |
| After 4 weeks | 24.9±4.0 | 27.4±5.3 | 0.250 ^b |
| Change of BMI | 0.28 (–0.45–1.88) | 0.35 (–0.002–1.8) | 1.000 ^a |
| Vitamin D (ng/ml) | | | |
| Baseline | 18.3 (16.2–23.1) | 20.16 (18.82–25.39) | |
| After 4 weeks | 21.7±4.5 | 23.26±5.47 | 0.510 ^b |
| Change of vitamin D level | 1.98 (–0.10–3.23) | 1.2 (–1.35–4.18) | 0.821 ^a |
| Adiponectin (pg/mL) | | | |
| Baseline | 456 (420–473) | 448 (418–482) | |
| After 4 weeks | 586±88 | 472±59 | 0.003 ^b |
| Change of adiponectin level | 130±108 | 5±105 | 0.017 ^b |

Abbreviations: BMI – body mass index. ^aMann Whitney U test was performed; ^bIndependent sample t test was performed.

to investigate the potential mechanism of vitamin D-dependent adiponectin pathways. Our study suggests that aerobic exercise with moderate intensity for four weeks increased adiponectin levels in patients with T2DM with mean difference 130±108 pg/ml. These findings complement the current knowledge

regarding the effects of exercise on adiponectin level in diabetic patients. The results are in line with the previous systematic review and meta-analysis of 19 randomized controlled trials in prediabetic and diabetic adults that reported increased levels of adiponectin with mean difference 0.42 μg/ml

Table 3
Correlation between clinical and metabolic parameters to adiponectin before adjustment.

| Variables | Change of adiponectin level | | | |
|---------------------------|-----------------------------|--------|---------------|--------|
| | Intervention group | | Control group | |
| | p-value | r | p-value | r |
| Age | 0.510 | 0.237 | 0.342 | -0.337 |
| Change of BMI | 0.187 | -0.455 | 0.595 | 0.192 |
| Change of vitamin D level | 0.603 | 0.188 | 0.450 | 0.270 |

Abbreviations: BMI – body mass index.

Table 4
Correlation between vitamin D and adiponectin with adjustment to age and BMI.

| Predictors | Change of adiponectin level | | | |
|---------------------------|-----------------------------|---------|---------------|---------|
| | Intervention group | | Control group | |
| | β | p-value | β | p-value |
| Age | 0.852 | 0.018 | -0.478 | 0.240 |
| BMI after 4 weeks | -0.769 | 0.032 | -0.129 | 0.726 |
| Change of vitamin D level | 1.027 | 0.005 | 0.422 | 0.292 |

Abbreviations: BMI – body mass index.

(95% CI 0.23–0.60, $p < 0.00001$) after physical exercise, especially aerobic but neither resistance nor concurrent exercise (Becic et al. 2018). Exercise duration of the present study was shorter than recent study performed by Aly et al. (2014) that have shown an increased adiponectin in type 2 diabetic patients after moderate intensity of supervised aerobic training for 12 weeks. The benefits of exercise in our study may be resulted from increasing speed and inclination periodically on a walking mode treadmill that thought to have greater stimulation on the musculoskeletal system and energy metabolism compared with walking on a flat surface (Kim et al. 2020). Both BMI in each group of this study was increased after four-week program, but lower in the intervention group. The effects of exercise on adiponectin were suggested to determine by body composition change, especially reduction of intraabdominal fat mass (Cnop et al. 2003; Simpson and Singh 2008). Unfortunately, several indexes to estimate body fat (e.g., waist circumference, waist-hip ratio, or skin fold thickness) were not measured in the study though could affect on adiponectin. Correlation of increased adiponectin level with change of BMI after exercise in this study showed insignificant result. However, BMI is an index of lean body mass and cannot precisely measure body composition (muscle mass or body fat percentage). Other mechanisms could also possibly mediate the effects of exercise on adiponectin, e.g., via vitamin D pathways.

All study subjects in both groups had lower levels of baseline serum vitamin D (below 30 ng/ml). This data supports the results of previous research about the higher incidence of vitamin D deficiency or insufficiency in the diabetic population (Bayani et al. 2014). Deficient of vitamin D linked to abruption of insulin secretion and sensitivity that suggested to have a connection with the incidence or progression of T2DM (Mathieu and Gysemans 2005). Baseline serum vitamin D in the intervention group were lower than a control group that may be affected by an older median of age or diabetes duration. However, increased vitamin D levels in the intervention group after aerobic exercise were higher than the control group but statistically insignificant. Adipose tissue was known to act as a reservoir for vitamin D due to lipophilicity and exercise was suggested to strongly stimulate vitamin D mobilization from adipose tissue (Hengist et al. 2019). Previous studies reported that exercise caused the rise of glucagon, adrenaline, and atrial natriuretic peptide as stimulatory lipolytic hormones and suggested as a key mechanism of vitamin D release from adipose tissue (Moro et al. 2007; Hengist et al. 2019). The lipolytic response of adipose tissue to exercise in obesity was reported reduced compared with lean controls (Mittendorfer et al. 2004). This condition may explain the lower increased vitamin D levels in control group due to higher mean of baseline BMI. However, little is known

about the effect of exercise on serum vitamin D in type 2 diabetic patients. Previous research conducted in rat experimentally-induced T2DM showed significant increase of serum vitamin D in diabetic group after moderate swimming exercise with duration 60 min a day and frequency five times a week for 4 weeks (Aly et al. 2016). These exercise protocols may suggest that higher frequency or duration of exercise is needed to increase vitamin D level significantly in patients with T2DM. This analysis supports the results from previous studies that reported higher vitamin D level in person with more intense physical activity (Chin et al. 2017).

We hypothesized that increased adiponectin level in type 2 diabetic patients might be potentially mediated by increased vitamin D level after aerobic exercise. However, the association between vitamin D and adiponectin in previous studies showed various results. Study in healthy non-diabetic adults indicating direct relationship between vitamin D and adiponectin but the association disappeared after adjustment to BMI (Gannage-Yared et al. 2009; Liu et al. 2009). Study in biracial population-based samples conducted by Bidulescu et al. (2014) reported that the association of vitamin D and adiponectin is dependent on race, gender, and BMI category. However, these studies were carried out on a heterogeneous population that numerous confounding might affect adiponectin concentration, e.g., age, gender, race, BMI, diabetes and hypertension status, anti-hypertensive drug use, dietary electrolyte intake, dietary patterns, physical activity, alcohol intake, and menopausal status (Vaidya et al. 2012).

We found a significant association between vitamin D and adiponectin in the intervention group after adjusting to age and BMI ($p=0.005$). Recent studies have suggested that vitamin D affects adiponectin synthesis by adiponectin expression gene upregulation in visceral fat and down-regulation of TNF- α gene that inflicts the adiponectin synthesis (Gannage-Yared et al. 2009). Another study also proposed that vitamin D conquers the inhibition of adiponectin synthesis through negative regulation of rennin-angiotensin system in adipose tissue (Vaidya et al. 2011). In our linear regression model, age and BMI were also found to be significant predictors of increased adiponectin level after exercise in the intervention group. These results may indicate that the association of vitamin D and adiponectin after aerobic exercise is dependent on age and BMI. Age is positively correlated with increased adiponectin level after exercise ($p=0.018$). This result builds on existing evidence of higher

adiponectin concentration on older age (Isobe et al. 2005). Decreased of adiponectin clearance by kidney was suggested as a possible cause for adiponectin increase with age (Isobe et al. 2005). Hence, BMI after exercise is inversely associated with increased adiponectin level in the intervention group ($p=0.032$). This result also similar with previous study in large samples revealed lower adiponectin level in subjects with higher BMI (Nielsen et al. 2020). This finding indicates that adipose tissue may exert a negative effect on adiponectin secretion as suggested in previous studies (Foula et al. 2020).

This study has certain limitations. First, the sample size was relatively small, but it was sufficient to reproduce the association of interest. Potential confounding variables that might affect vitamin D and adiponectin concentration (e.g., dietary intake of calcium, use of thiazolidinediones medication or insulin) were not controlled in the present study. Although our study suggested that the increased adiponectin levels after aerobic exercise might be affected by vitamin D, an association was examined using the observational analysis. Thus, the direct causal-effect relationship cannot be proven. Therefore, further research with longitudinal design is needed to establish the directionality of vitamin D on adiponectin level. Since our study indicated that vitamin D might have a potential mechanism to mediate the increased adiponectin, which known to exert many benefits in patients with T2DM, future research to evaluate the effect of vitamin D supplementation on adiponectin level in type 2 diabetic patients is warranted.

Conclusion

Moderate intensity of the treadmill exercise with increased speed and inclination periodically increased adiponectin levels in patients with T2DM. The increased adiponectin levels might potentially be mediated by increased vitamin D after aerobic exercise, but the association is dependent on the age and BMI.

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References

- Achhari AE, Jain SK. Adiponectin, a therapeutic target for obesity, diabetes, and endothelial dysfunction. *Int J Mol Sci* 18, 1321, 2017.
- Aly FA, Alghadir AH, Gabr SA. Adiponectin response to supervised aerobic training in type II diabetic patients. *Asian Biomed* 8, 597–602, 2014.
- Aly YE, Abdou AS, Rashad MM, Nassef MM. Effect of exercise on serum vitamin D and tissue vitamin D receptors in experimentally induced type 2 diabetes mellitus. *J Adv Res* 7, 671–679, 2016.
- American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 36, 67–74, 2013.
- Banerjee A, Khemka V, Roy D, Poddar J, Roy TKS, Karnam SA. Role of serum adiponectin and vitamin D in prediabetes and diabetes mellitus. *Can J Diabetes* 41, 1–7, 2016.
- Bayani MA, Akbari R, Banasaz B, Saeedi F. Status of Vitamin-D in diabetic patients. *Caspian J Intern Med* 5, 40–42, 2014.
- Becic T, Studenik C, Hoffman G. Exercise increases adiponectin and reduces leptin levels in prediabetic and diabetic individuals: systematic review and meta-analysis of randomized controlled trials. *Med Sci* 6, 1–18, 2018.
- Bidulescu A, Morris AA, Stoyanova N, Meng Y, Voccarino V, Quyyumi AA. Association between vitamin d and adiponectin and its relationship with body mass index: the META health study. *Front Pub Health* 2, 1–6, 2014.
- Chin K, Zhao D, Tibuakuu M, Martin SS, Ndumele CE, Florido R. Physical activity, vitamin D, and incident atherosclerotic cardiovascular disease in whites and blacks: the ARIC study. *J Clin Endocrinol Metabol* 102, 1227–1236, 2017.
- Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, Retzlaff BM, Knopp RH, Brunzell JD, Kahn SE. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia* 46, 459–469, 2003.
- Duncan BB, Schmidt MI, Pankow JS, Bang H, Couper D, Ballantyne CM, Hoogeveen RC, Heiss G. Adiponectin and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes* 53, 2473–2478, 2004.
- Foula WH, Emara RH, Eldeeb MK, Mokhtar SA, El-Sahn FA. Effect of a weight loss program on serum adiponectin and insulin resistance among overweight and obese premenopausal females. *J Egypt Public Health Assoc* 95, 32, 2020.
- Gannage-Yared M, Chedid R, Khalife S, Azzi E, Zoghbi F, Halaby G. Vitamin D in relation to metabolic risk factors, insulin sensitivity and adiponectin in a young Middle-Eastern population. *Eur J Endocrinol* 160, 965–971, 2009.
- Hengist A, Perkin O, Gonzalez JT, Betts JA, Hewison M, Manolopoulos KN, Jones KS, Koulman A, Thompson D. Mobilising vitamin D from adipose tissue: The potential impact of exercise. *Nutrition Bull* 44, 25–35, 2019.
- Isobe T, Saitoh S, Takagi S, Takeuchi H, Chiba Y, Katoh N, Shimamoto K. Influence of gender, age, and renal function on plasma adiponectin level: the Tanno and Sobetsu study. *Eur J Endocrinol* 153, 91–98, 2005.
- Kim SJ, Yoon ES, Jung SY, Kim DY. Effect of uphill walking on browning factor and high molecular weight-adiponectin in postmenopausal women. *J Exerc Rehabil* 16, 265–271, 2020.
- Li S, Shin HJ, Ding EL, Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA* 302, 179–188, 2009.
- Liu E, Meigs JB, Pittas AG, McKeown NM, Economos CD, Booth SL, Jacques PF. Plasma 25-hydroxyvitamin d is associated with markers of the insulin resistant phenotype in nondiabetic adults. *J Nutrition* 139, 329–334, 2009.
- Mathieu C, Gysemans C. Vitamin D and diabetes. *Diabetologia* 48, 1247–1257, 2005.
- Mittendorfer B, Fields DA, Klein S. Excess body fat in men decreases plasma fatty acid availability and oxidation during endurance exercise. *Am J Physiol Endocrinol Metab* 286, 354–362, 2004.
- Moro C, Pillard F, de Glisezinski I, Crampes F, Thalamos C, Harant I, Marques MA, Lafontan M, Berlan M. Sex differences in lipolysis-regulating mechanisms in overweight subjects: effect of exercise intensity. *Obesity* 15, 2245–2255, 2007.
- National Health and Nutrition Examination Survey (U.S.). NHANES III plan and operations procedures manuals CD-ROM: draft. National Centers for Disease Control and Prevention, 1997.

- Nielsen MB, Colak Y, Benn M, Nordestgaard BG. Causal relationship between plasma adiponectin and body mass index: one- and two-sample bidirectional mendelian randomization analyses in 460 397 individuals. *Clin Chem* 66, 1548–1557, 2020.
- Polito R, Monda V, Nigro E, Messina A, Di Maio G, Giuliano MT, Orru S, Imperlini E, Calcagno G, Mosca L, Mollica MP, Trinchese G, Scarinci A, Sessa F, Salerno M, Marsala G, Buono P, Mancini A, Monda M, Daniele A, Messina G. The important role of adiponectin and orexin-A, two key proteins improving health status: focus on physical activity. *Front Physiol* 11, 1–17, 2020.
- Simpson KA, Singh MA. Effects of exercise on adiponectin: a systematic review. *Obesity* 16, 241–256, 2008.
- Vaidya A, Forman JP, Hopkins PN, Seely EW, Williams JS. 25-Hydroxyvitamin D is associated with plasma renin activity and the pressor response to dietary sodium intake in Caucasians. *J Renin Ang Aldoster Syst* 12, 311–320, 2011.
- Vaidya A, Williams JS, Forman JP. The independent association between 25-hydroxyvitamin D and adiponectin and its relation with BMI in two large cohorts: the NHS and the HPFS. *Obesity* 20, 86–191, 2012.
- World Health Organization. Global report on diabetes. Geneva, 2016.
- World Health Organization. Diabetes [Fact Sheet]. <https://www.who.int/news-room/fact-sheets/detail/diabetes>, 2021.

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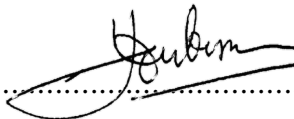
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