The Positive Effect of Moderate Walking Exercise on Chemerin Levels in Portuguese Patients With Type 2 Diabetes Mellitus

Maria João Neuparth, PhD,† Jorge Brandão Proença, PhD,† Alice Santos-Silva, PhD,‡§ and Susana Coimbra, PhD†§*

Background: Physical exercise intervention is known to be crucial in the management of type 2 diabetes mellitus (T2DM). We aimed to evaluate, in patients with T2DM, the effect of regular moderate walking exercise on markers of oxidative stress, lipid metabolism, and inflammation.

Methods: We studied 30 patients with T2DM who walked regularly during the last year and 53 patients with T2DM who did not perform any type of exercise. The patients were evaluated for chemerin, adiponectin, leptin, oxidized low-density lipoprotein, and C-reactive protein (CRP) levels.

Results: The active T2DM patients showed significantly lower body mass index, as compared with the inactive patients. The active T2DM patients showed significantly lower levels of chemerin and CRP than those of the inactive T2DM patients (CRP lost significance after adjustment for body mass index). The active patients, compared with the inactive, presented a trend toward higher levels of adiponectin and lower values of oxidized low-density lipoprotein. Leptin differed significantly between sexes, and the active women presented a trend toward lower levels as compared with the inactive women.

Conclusions: In the patients with T2DM, the practice of moderate walking in a regular basis was sufficient to reduce chemerin levels, which suggests that practice of regular physical exercise should be encouraged.

Key Words: physical exercise, adipokines, body mass index, inflammation, lipid peroxidation

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Type 2 diabetes mellitus (T2DM) is known to be associated with obesity, suggesting that adipose tissue may play an important role in its development.[1] Adipose tissue secretes several adipokines, such as adiponectin and leptin, which interfere with insulin resistance, glucose and lipid metabolism, lipid peroxidation, and the inflammatory process.[2–4] Physical activity seems to have a positive effect on adiponectin and leptin levels in patients with T2DM.[5]

From the *Centro de Investigação em Actividade Física, Saúde e Lazer (CIAFEL), Universidade do Porto, Porto, Portugal; †CESPU, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Gandra-PRD, Portugal; ‡Laboratório de Bioquímica, Departamento de Ciências Biológicas, Faculdade de Farmácia (FFUP); and §Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, Porto, Portugal.

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Reprints: Maria João Neuparth, PhD, and Susana Coimbra, PhD, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, CESPU, Rua Central da Gandra 1317, 4585-116 Gandra-PRD, Portugal. E-mail: mneuparth@hotmail.com; ssn.coimbra@gmail.com.

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Chemerin is a novel adipokine associated with inflammation, adipogenesis, and glucose and lipid metabolism.[6,7] A decrease in chemerin levels was observed both in obesity and impaired glucose regulation after the practice of intensive physical exercise.[8] As far as we know, the effect of practicing moderate walking on a regular basis on chemerin and other adipokine levels has been poorly clarified.

Our aim was to study, in T2DM patients, the effect of walking on chemerin, adiponectin, leptin, oxidized low-density lipoprotein (oxLDL), and C-reactive protein (CRP) levels. With that purpose, we studied T2DM patients who performed regular moderate walking during the last year and T2DM patients who did not perform any kind of exercise.

MATERIALS AND METHODS

The protocol used was approved by the Committee on Ethics of the Instituto Superior das Ciências da Saúde Norte, CESPU, Gandra-Paredes, Portugal.

We performed a cross-sectional and retrospective study in T2DM patients. These patients were selected from the general population and invited to participate in this study. Afterward, an accurate and detailed interview with the patients was performed to collect and record the clinical characteristics of the disease; the sociodemographic data; and the retrospective habits, especially those concerning their physical activity during the last year. Only the patients reporting that their only type of physical activity in the last year was moderate walking exercise, practiced for at least 30 minutes, 3 times a week, and the patients who did not perform any kind of physical exercise were included in this study after informed consent. Patients whose statements were confusing, unclear, and inconsistent and those who performed intensive activity or a physical activity lower than our defined criteria were excluded from this study. Patients presenting inflammatory or infectious diseases and liver or kidney diseases were also excluded from this study. The selected active patients reported to walk continuously, without interruptions, for 30 minutes up to 1 hour a day, 3 to 5 times per week, during the last year, with rapid and steady step but without running. Two groups were established: one, the active group (AG), including 30 Portuguese adult patients with T2DM who performed walking training on a regular basis during the last year, and the other, the sedentary group (SG), including 53 adult patients with T2DM who did not perform any type of exercise during the last year. Sociodemographic, clinical, and analytical data of the 2 studied groups are presented in Table 1.

The diet of the T2DM patients was low in carbohydrates and fat, and all were treated with oral hypoglycemic drugs. Leisure time activities performed by the patients did not imply physical exercise. The type of medication, nutrition intake, other leisure activities besides walking, diastolic and systolic blood

TABLE 1. Sociodemographic, Clinical, and Analytical Data in the Portuguese T2DM Patients Practicing Moderate Walking Exercise on a Regular Basis During the Last Year (AG) and in the Patients Who Did Not Practice Physical Exercise (SG)

	AG (n = 30)	SG (n = 53)	*P*
Sex (F/M)	13/17	28/25	0.412
Age, y	62.2 ± 11.1	63.1 ± 9.8	0.682
Disease length, y	8.8 ± 6.3	9.4 ± 6.9	0.758
ATD, y	53.4 ± 10.0	53.8 ± 9.7	0.808
BMI, kg/m^2	26.6 ± 4.4	28.1 ± 3.6	0.006
Weight, kg	68.6 ± 12.9	76.3 ± 11.5	0.006
Height, m	1.6 ± 0.1	1.7 ± 0.1	0.597
Glucose, mg/dL	126 (99–181)	128 (105–158)	0.974
Glycated hemoglobin, %	7.05 (6.10–7.85)	6.80 (6.25–7.90)	0.861
Chemerin, ng/mL	134 (102–181)	181 (156–199)	0.001
Adiponectin, ng/mL	6706 (3642–9636)	5425 (4213–7158)	0.206
Leptin, ng/L	14.8 (6.9–24.9)	20.2 (5.6–52.5)	0.251
Female	23.1 (19.1–47.1)	49.6 (20.8–61.3)	0.159
Male	7.5 (3.1–24.9)	6.2 (3.2–18.6)	0.980
oxLDL, U/L	36.8 ± 12.5	42.8 ± 14.0	0.056
CRP, mg/L	1.67 (0.61–2.67)	2.98 (0.88–6.57)	0.006*

Measurements are expressed as mean ± SD or as median values (interquartile ranges) according to Gaussian distribution of the substances; comparisons between the groups used the Student unpaired *t* test or the Mann-Whitney *U* test, according to Gaussian distribution.

*Loss of significance after adjustment for BMI using analysis of covariance.

ATD indicates age at time of diagnosis; F, female; M, male.

pressure, and smoking and alcohol drinking habits were similar in both groups of T2DM patients. Besides the oral hypoglycemic therapy, none of the patients were receiving any medication that could interfere with our results (eg, antioxidants, anti-inflammatory drugs, and anti-obesity therapies).

Blood was collected by venipuncture into tubes containing EDTA as anticoagulant, to obtain plasma. We evaluated the plasmatic levels of chemerin, adiponectin, leptin, oxLDL (enzyme immunoassays: Chemerin ELISA, Biovendor R&DP, Heidelberg, Germany [intra-assay coefficient of variation (CV), 2.82%]; Adiponectin, R&D Systems, Minneapolis [intra-assay CV, 2.95%]; and Leptin and Oxidized LDL ELISA, Mercodia, Uppsala, Sweden [intra-assay CV, 4.78% and 3.73%, respectively]), CRP (immunoturbidimetry: Prestige 24i CRP-Ultra (HS); P.Z. Cormay, Lublin, Poland), glucose (enzymatic colorimetric method: Prestige; P.Z. Cormay, Lublin, Poland), and glycated hemoglobin (spectrophotometric method: Prestige 24i HbA_{1C}; P.Z. Cormay, Lublin, Poland).

We used the Statistical Package for the Social Sciences (version 17 for Windows, Chicago, IL) for the statistical analysis. A *P* value of lower than 0.05 was considered as statistically significant. Measurements are expressed as mean ± SD, when presenting a Gaussian distribution, or as median values (interquartile ranges) for those with a non-Gaussian distribution. The correlation analysis was performed by calculating the Spearman correlation coefficient. Comparisons between the groups used the Student unpaired *t* test or the Mann-Whitney *U* test, according to Gaussian distribution of the substances. Adjustment for confounding factors (eg, body mass index [BMI], weight) used analysis of covariance, after transformation of variables (those with a non-Gaussian distribution, eg, chemerin, adiponectin, leptin, CRP); variables were linearized by logarithmic transformation and, afterward, checked for a Gaussian distribution. Analysis of covariance allows to evaluate whether the significance of the differences between the groups are independent of the confounding factors.

RESULTS

Both groups of patients with T2DM were matched for sex, age at the time of diagnosis, age at the time of the present study, and disease length. Glucose and glycated hemoglobin presented similar values for both groups (Table 1).

When considering all T2DM patients, the practice of regular walking exercise correlated significantly and inversely with chemerin ($r = -0.644$; $P \leq 0.001$), oxLDL ($r = -0.218$; $P = 0.047$), CRP ($r = -0.301$; $P = 0.006$), and BMI ($r = -0.313$; $P = 0.004$) values.

The AG of the T2DM patients presented significantly lower BMI and weight values than those of the SG of the T2DM patients. The AG of the T2DM patients also presented significantly lower levels of chemerin and CRP, as compared with the SG patients. After adjustment for the confounding factor BMI, CRP values lost statistical significance ($P = 0.103$), whereas the differences in chemerin levels remained significantly different for the 2 groups ($P = 0.012$). The AG of the T2DM patients also showed, as compared with the SG of the T2DM patients, a trend toward higher levels of adiponectin and lower values of oxLDL. Concerning leptin values, we found a significant difference between sexes and a trend toward lower levels in the female active patients, when compared with the inactive ones (Table 1).

DISCUSSION

The practice of physical exercise is known to have an important role in T2DM management. Patients with T2DM often have functional limitations and physical disabilities that do not allow the practice of intensive physical exercise. Thus, an

alternative is the practice of moderate physical activity, such as regular walking, one of the most simple, basic, and accessible forms of exercise.

According to our results, the regular practice of moderate walking by T2DM patients has positive effects on adipose metabolism, lipid peroxidation, and inflammation. The T2DM patients who performed moderate walking, when compared with those who did not practice physical exercise, presented lower weight and BMI values, suggesting that physical activity leads to less adipose mass, with an improvement in adipokine production, namely, in chemerin.

Chemerin has been associated with changes in inflammation, glucose, and lipid metabolism.[7] This adipokine plays a vital role in adipocyte differentiation and development and seems to be a modulator of different metabolic pathways in the mature adipocyte.[7] Raised chemerin levels were found in diabetes and obesity.[9] Intensive and acute physical training has shown to have a positive effect, by reducing chemerin levels, in the impairment of glucose regulation and weight loss.[8,10] Saremi et al.[11] found that the levels of chemerin decreased in men performing 50 to 60 minutes, 5 days a week, for 12 weeks, of aerobic exercise, which included exercises and 15 to 50 minutes of walking-running, increasing weekly the exercise intensity. The influence of the practice of merely regular moderate walking exercise, without running, a simple, light, and easy form of exercise, in its levels is not well clarified. Our data show that this type of physical activity performed by T2DM patients was associated with chemerin levels lower than those presented by the sedentary T2DM patients. In accordance with our results, Venojärvi et al.[12] found that Nordic walking, fitness walking with specially designed poles, contributes to decreased chemerin levels in middle-aged men.

Considering that chemerin is involved in the pathophysiology of insulin resistance, oxidative stress, and inflammation,[9,13,14] the regular practice of physical exercise, even a moderate form of exercise, by contributing to decreased chemerin values may favor insulin sensitivity and improve the lipid profile in T2DM patients. A lifestyle modification of T2DM patients, through practice of regular physical exercise, even in a moderate rate, and healthy diet habits should be encouraged. Indeed, according to Kim et al.,[15] 12 weeks of intensive lifestyle modification, which included supervised exercise sessions, significantly decreased serum chemerin level, which was associated with an improvement in insulin sensitivity.

Adiponectin and leptin levels are known to be altered in obesity.[16–18] Adiponectin has anti-inflammatory activity and protects against metabolic and cardiovascular diseases.[19] An inhibitory feedback mechanism that is triggered when adipose mass increases has been suggested.[2] This feedback mechanism is, probably, a consequence of the increased secretion of other adipokines or of a reduced adipocyte metabolic function, inducing a reduction in adiponectin levels. A deficient activity of leptin leads to severe insulin resistance and vascular dysfunction. A resistance to leptin effects has been also proposed to explain the reduced activity found in obesity.[3] A study by Hopps et al.[5] showed a significant positive effect of physical exercise practice in adiponectin and leptin levels in T2DM patients, with a rise in adiponectin and a decrease in leptin levels. In accordance, we found a trend toward lower leptin levels, at least in the women, and toward higher adiponectin concentrations in the active T2DM patients. The performance of a moderate form of physical exercise by our AG of T2DM patients may explain the less marked effect in these adipokines; indeed, most of the reported studies used intensive and acute exercise protocols.

The practice of exercise by T2DM patients has been associated with an increase in oxLDL levels.[20] In the present study, we observed that the AG of the T2DM patients presented a trend toward lower values of oxLDL, as compared with the SG of the T2DM patients. Again, the use of a protocol of exercise that was moderate and performed regularly and not an intensive and acute exercise protocol may account for the differences observed.

The lower CRP levels found in the AG of the T2DM patients seem to result from an associated effect of exercise practice and lower body mass and not only from physical exercise practice because the statistical significance was lost after adjustment for BMI.

To confirm our results, further studies in a larger population are unwarranted, namely, longitudinal studies that might strengthen our results.

In summary, in T2DM patients, the practice of moderate walking on a regular basis was associated with lower levels of chemerin, which suggests that practice of regular physical exercise should be encouraged.

REFERENCES

1. Sell H, Eckel J. Chemotactic cytokines, obesity and type 2 diabetes: in vivo and in vitro evidence for a possible causal correlation? *Proc Nutr Soc*. 2009;68:378–384.
2. Oh DK, Ciaraldi T, Henry RR. Adiponectin in health and disease. *Diabetes Obes Metab*. 2007;9:282–289.
3. Enriori PJ, Evans AE, Sinnayah P, et al. Leptin resistance and obesity. *Obesity (Silver Spring)*. 2006;14(suppl 5):254S–258S.
4. Rondinone CM. Adipocyte-derived hormones, cytokines, and mediators. *Endocrine*. 2006;29:81–90.
5. Hopps E, Canino B, Caimi G. Effects of exercise on inflammation markers in type 2 diabetic subjects. *Acta Diabetol*. 2011;48:183–189.
6. Bozaoglu K, Bolton K, McMillan J, et al. Chemerin is a novel adipokine associated with obesity and metabolic syndrome. *Endocrinology*. 2007;148:4687–4694.
7. Goralski KB, McCarthy TC, Hanniman EA, et al. Chemerin, a novel adipokine that regulates adipogenesis and adipocyte metabolism. *J Biol Chem*. 2007;282:28175–28188.
8. Chakaroun R, Raschpichler M, Kloting N, et al. Effects of weight loss and exercise on chemerin serum concentrations and adipose tissue expression in human obesity. *Metabolism*. 2012;61:706–714.
9. Ernst MC, Issa M, Goralski KB, et al. Chemerin exacerbates glucose intolerance in mouse models of obesity and diabetes. *Endocrinology*. 2010;151:1998–2007.
10. Stefanov T, Blüher M, Vekova A, et al. Circulating chemerin decreases in response to a combined strength and endurance training. *Endocrine*. 2013 Jun 20. [Epub ahead of print].
11. Saremi A, Shavandi N, Parastesh M, et al. Twelve-week aerobic training decreases chemerin level and improves cardiometabolic risk factors in overweight and obese men. *Asian J Sports Med*. 2010;1:151–158.
12. Venojärvi M, Wasenius N, Manderoos S, et al. Nordic walking decreased circulating chemerin and leptin concentrations in middle-aged men with impaired glucose regulation. *Ann Med*. 2013;45:162–170.
13. Sell H, Laurencikiene J, Taube A, et al. Chemerin is a novel adipocyte-derived factor inducing insulin resistance in primary human skeletal muscle cells. *Diabetes*. 2009;58:2731–2740.
14. Lehrke M, Becker A, Greif M, et al. Chemerin is associated with markers of inflammation and components of the metabolic syndrome

but does not predict coronary atherosclerosis. *Eur J Endocrinol*. 2009;161:339–344.

15. Kim SH, Lee SH, Ahn KY, et al. Effect of lifestyle modification on serum chemerin concentration and its association with insulin sensitivity in overweight and obese adults with type 2 diabetes. *Clin Endocrinol (Oxf)*. 2013 May 18. [Epub ahead of print].
16. Ricci R, Bevilacqua F. The potential role of leptin and adiponectin in obesity: a comparative review. *Vet J*. 2012;191:292–298.
17. Hotta K, Funahashi T, Arita Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol*. 2000;20:1595–1599.
18. Bahceci M, Gokalp D, Bahceci S, et al. The correlation between adiposity and adiponectin, tumor necrosis factor alpha, interleukin-6 and high sensitivity C-reactive protein levels. Is adipocyte size associated with inflammation in adults? *J Endocrinol Invest*. 2007;30:210–214.
19. Tilg H, Moschen AR. Role of adiponectin and PBEF/visfatin as regulators of inflammation: involvement in obesity-associated diseases. *Clin Sci (Lond)*. 2008;114:275–288.
20. Kostic N, Caparavic Z, Ilic S, et al. Exercise-induced oxidative stress and antioxidant enzyme activity in type 2 diabetic patients with and without diastolic dysfunction and hypertension. *Srp Arh Celok Lek*. 2009;137:146–151.

