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Original research

A comparison of dicarbonyl stress and advanced glycation endproducts in lifelong endurance athletes *vs.* sedentary controls

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ABSTRACT

Objectives: Dicarbonyl stress and high concentrations of advanced glycation endproducts (AGEs) relate to an elevated risk for cardiovascular diseases (CVD). Exercise training lowers the risk for future CVD. We tested the hypothesis that lifelong endurance athletes have lower dicarbonyl stress and AGEs compared to sedentary controls and that these differences relate to a better cardiovascular health profile.
Design: Cross-sectional study.
Methods: We included 18 lifelong endurance athletes (ATH, 61 ± 7 years) and 18 sedentary controls (SED, 58 ± 7 years) and measured circulating glyoxal (GO), methylglyoxal (MGO) and 3-deoxyglucosone (3DG) as markers of dicarbonyl stress. Furthermore, we measured serum levels of protein-bound AGEs N^{ϵ}-(carboxymethyl)lysine (CML), N^{ϵ}-(carboxyethyl)lysine (CEL), methylglyoxal-derived hydroimidazolone-1 (MG-H1), and pentosidine. Additionally, we measured cardiorespiratory fitness (VO_2peak) and cardiovascular health markers.
Results: ATH had lower concentrations of MGO (196 [180–246] *vs.* 242 [207–292] nmol/mmol lysine, $p = 0.043$) and 3DG (927 [868–972] *vs.* 1061 [982–1114] nmol/mmol lysine, $p < 0.01$), but no GO compared to SED. ATH demonstrated higher concentrations CML and CEL compared to SED. Pentosidine did not differ across groups and MG-H1 was significantly lower in ATH compared to SED. Concentrations of MGO en 3DG were inversely correlated with cardiovascular health markers, whereas CML and CEL were positively correlated with VO_2peak and cardiovascular health markers.
Conclusion: Lifelong exercise training relates to lower dicarbonyl stress (MGO and 3DG) and the AGE MG-H1. The underlying mechanism and (clinical) relevance of higher CML and CEL concentrations among lifelong athletes warrants future research, since it conflicts with the idea that higher AGE concentrations relate to poor cardiovascular health outcomes.

1. Introduction

Advanced glycation endproducts (AGEs) are a complex group of modified proteins or lipids that are formed by a process of non-enzymatically glycation and oxidation. AGEs formation is a slow process (*i.e.,* weeks to months) and depends on the extent of oxidative stress, degree of hyperglycemia, and turnover rate of proteins.[1,2] The formation of AGEs is irreversible and AGEs accumulate with increasing age. Highly reactive dicarbonyls (α-oxoaldehydes) are involved in the fast formation of AGEs and accumulation of dicarbonyls is known as dicarbonyl stress.[1,2] Dicarbonyls are precursors for AGEs[3] and the most important dicarbonyl marker is the highly reactive methylglyoxal (MGO).[4] Dicarbonyl stress and a high concentration of AGEs are linked to the development of cardiovascular diseases.[4–6]

Higher levels of circulating AGEs are also related to higher vascular stiffness.[7–9] There are several mechanisms proposed how AGEs may affect the vascular wall properties, such as binding to receptor AGEs (RAGEs) and cross-linking matrix proteins in the vessel wall.[2,10] AGE-binding to RAGEs leads to an upregulation of inflammation and production of reactive oxygen species.[11,12] These processes augment vascular dysfunction and may promote vascu-

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lar stiffness.[11,12] Alternatively, AGEs can also bind to collagen and elastin to form crosslinks with matrix proteins, which promotes vascular stiffness.[12] Strategies to lower the burden of high levels of AGEs may improve cardiovascular health and need to be explored.

Regular exercise training is part of a healthy lifestyle and is an effective strategy to reduce the risk for cardiovascular morbidity and mortality.[13,14] Exercise training attenuates the age-associated decline in cardiovascular function,[15,16] and improves glucose[17] and lipid metabolism.[18] Findings from animal studies suggest that these health benefits of exercise training may relate to a reduction of dicarbonyl stress and AGEs concentrations.[19,20] Clinical studies linking exercise training with dicarbonyl stress or AGEs are, however, sparse and conflicting.[21–23] A previous study demonstrated that 12 months of Tai Chi training for 2 sessions/week significantly reduced serum AGEs concentrations in asymptomatic middle-aged adults.[23] However, another study found no effect on serum AGEs concentrations in middle-aged overweight or obese men after a 3-month aerobic moderate intensity exercise training program.[21] Variation in study outcomes may partially relate to the training duration (3 *vs.* 12 months), exercise intensity (light *vs.* moderate), or study population (asymptomatic *vs.* overweight/obese). Lifelong endurance athletes may provide better insight to what extent exercise is related to attenuated AGEs formation.

Therefore, we tested the hypothesis that lifelong endurance athletes have lower dicarbonyl stress and a lower concentration of AGEs compared to sedentary controls. Additionally, we explored whether lower dicarbonyl stress and lower concentration of AGEs relate to a better cardiovascular health profile.

2. Methods

Thirty-six male participants aged >45 years were included and stratified into 2 groups based on their lifelong exercise patterns: (1) lifelong endurance athletes (ATH, n = 18), (2) sedentary controls (SED, n = 18). ATH had to perform ≥20 years of endurance exercise training (*e.g.*, running or cycling) for ≥4 h/week, whereas SED had to report ≥20 years of habitual physical activity <2 h/week. Current smokers, participants with a history of diabetes mellitus or cardiovascular disease, or participants not able to perform an incremental maximal cycling test were not included in the study. The Local Committee on Research Involving Human Subjects of the region Arnhem and Nijmegen approved the study. All participants gave their written informed consent prior to study participation.

During this cross-sectional study, participants visited our laboratory on 2 separate days. On day 1, participants were medically screened for eligibility, followed by an incremental maximal cycling test to determine their physical fitness. On day 2, pulse wave velocity was measured as an index of vascular stiffness and blood samples were obtained under fasting conditions. Both testing days were scheduled within a 14-day time-frame, with at least 1 recovery day between measurement day 1 and 2.

A physician medically screened the participants by taking a detailed medical history, physical examination, and 12-lead electrocardiogram. After screening, participants performed an incremental maximal cycling test to determine the cardiorespiratory fitness and peak oxygen uptake (VO_2peak, mLO_2/min). The test took place in a temperature-controlled room (18–19° C) and under the supervision of a physician. Participants cycled with 60–80 rotations per minute while the workload increased with 20 W/min for ATH and 10 W/min for CON. Heart rate was continuously measured *via* a 12 lead-electrocardiogram. Oxygen uptake (VO_2 [mL/min]), carbon dioxide output (VCO_2 [mL/min]), and respiratory exchange ratio (RER) were continuously measured *via* a gas analyser (CPET, Cosmed v9.1b, Rome, Italy). Lactate concentration (mmol/L) was measured (Lactate Pro™ 2, Arkray, type LT-1730, Kyoto, Japan) *via* a capillary blood sample taken 1.5 min after cessation of the exercise test. The incremental maximal cycling test was considered successful when 2 of the 4 criteria were met: (I) RER ≥ 1.05, (II) achievement of at least 85% of age-predicted maximal heart rate (220-age), (III) blood lactate ≥ 6.00 mmol/L, or (IV) flattening of VO_2 uptake curve (≤150 mL increase during the last minute).[24,25]

Lifelong exercise patterns were queried *via* an exercise history questionnaire, distinguishing 5 age-periods: (I) 20–29 years, (II) 30–39 years, (III) 40–49 years, (IV) 50–59 years and (V) >60 years. Each category consisted of 2 queries: (1) type of activity (*e.g.*, running, cycling, etc., or nothing) and (2) exercise time (hours) per activity per week. Based on the Compendium of Physical Activities,[26] the corresponding metabolic equivalent of task (MET) score per exercise activity was determined. Vigorous exercise activities were defined as a MET score >6. Subsequently, exercise volume (MET-h/week) was calculated by multiplying exercise time with accompanying MET score. The average exercise time and dose were calculated over the last 2 decades.

Before the second testing day, participants were asked to abstain from (I) (vigorous) physical activities for 24 h, (II) caffeine, alcohol, or vitamin supplement intake for at least 18 h, and (III) food intake for ≥6 h. Central and peripheral pulse wave velocity was assessed with a three-lead electrocardiogram and an echo-Doppler ultrasound machine (WakiLoki Doppler, 4 MHz, Atys) at the left carotid artery, right common femoral artery, and radial artery. The distances between sternal notch and site of measurement for the carotid artery and between radial artery and common femoral artery *via* the umbilicus were measured.[27] At least 10 cardiac cycles were recorded for analyses. Based on the R–R interval and onset of the Doppler waveform, central and peripheral pulse wave velocities were calculated in Matlab R2014 (The MathWorks Inc., United States).

Following vascular measurements, a fasting blood sample (8 mL) was obtained from an antecubital vein for the assessment of concentrations of dicarbonyl stress and AGEs. Additionally, lysine and traditional cardiovascular risk factors (total-, high-density lipoproteins [HDL]-, low-density lipoproteins [LDL]-cholesterol, triglycerides, glycated hemoglobin [HbA1C], and glucose) were determined. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated based on glucose and insulin concentrations (IR = (fasting insulin [mU/L] × fasting glucose [mmol/L])/22.5).[28] To gain insight in the cardiovascular (risk) profile of ATH and SED, the 10-year CVD risk was calculated *via* the Framingham Risk Score (FRS).[29]

For measurement of serum levels of diarbonyl components and AGEs, we used ultra-performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS, Waters, Milford Massachusetts, USA). UPLC–MS/MS combines liquid chromatography for separation and tandem mass spectrometry for specific detection.

Whole blood samples in serum-separating tubes were centrifuged after collection (10 min, 4 °C, 3,000 × *g*) and supernatant was stored at −80 °C until analysis. Serum levels of dicarbonyl compounds glyoxal (GO), MGO, and 3-deoxyglucosone (3DG) were analysed following a previously described protocol.[3] Briefly, serum samples were deproteinized using perchloric acid and subsequently derivatized with *o*-phenylenediamine. GO, MGO, and 3DG concentrations were measured using stable isotope-dilution UPLC–MS/MS (Waters, Milford Massachusetts, USA) with a run-to-run time of 8 min. Intra-run and inter-run variations were 4.3% and 14.3% for GO, 2.9% and 7.3% for MGO, and 2.4% and 12.0% for 3DG, respectively.[3]

Protein-bound serum AGEs N^{ϵ}-(carboxymethyl)lysine (CML), N^{ϵ}-(carboxyethyl)lysine (CEL), methylglyoxal-derived hydroimidazolone-1 (MG-H1), and lysine were measured with UPLC–MS/MS (Waters, Milford Massachusetts, USA), as previously described.[30,31] Pentosidine was measured with high-performance

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