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A comparison of dicarbonyl stress and advanced glycation endproducts in lifelong endurance athletes *versus* 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.

Key words: oxidative stress; cardiovascular disease; physical activity; exercise physiology

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 vascular 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.

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 hours/week, whereas SED had to report ≥20 years of habitual physical activity <2 hours/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 Watt/min for ATH and 10 Watt/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 minute 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-hours/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 hours, II) caffeine, alcohol, or vitamin supplement intake for at least 18 hours, and III) food intake for ≥6 hours. 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] X 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 liquid chromatography and fluorescent detection. [31] Intra-run and inter-run variations were 2.8% and 7.1% for CML, 3.7% and 6.4% for CEL, 3.7% and 5.1% for MG-H1, and 2.0% and 3.1% for pentosidine. [30, 31] All serum AGEs were adjusted for lysine concentrations as a marker of total protein concentration.

Participant characteristics were summarized with means and standard deviations or median and interquartile range (IQR), when appropriate. Categorical data were analysed using the *Fisher's exact*

test. Parameters were checked for normality using a *Shapiro-Wilk* test and Q-Q plots. Skewed variables were $\log_e$-transformed before statistical analyses were conducted. Differences in participant characteristics, lifelong exercise patterns, and cardiovascular health markers between ATH and SED were analysed using an independent *Student's t* test. As an overall measure of pulse wave velocity, z-scores of central and peripheral pulse wave velocities were averaged. Correlations between markers for dicarbonyl stress or AGEs and markers for cardiovascular health (BMI, pulse wave velocity, cardiorespiratory fitness, Framingham risk score, and glucose metabolism) were evaluated using *Spearman's rank* test. All statistical analyses were performed using SPSS 21.0 software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). Statistical significance was assumed at $p<0.05$ (two-sided).

Results

Age, height, mean arterial pressure, and smoking history did not differ between groups, but ATH demonstrated a lower body weight and Body Mass Index compared to SED (Table 1). HbA1c, total cholesterol, and glucose concentrations did not differ between groups, but ATH demonstrated a higher HDL cholesterol concentration and lower LDL cholesterol, triglycerides, and HOMA-IR compared to SED (Table 1). The median time between smoking cessation and study participation was 28 years (Q_{25}: 12 to Q_{75}: 40) in ATH *versus* 25 years (Q_{25}: 15 to Q_{75}: 37) in SED (P=0.78).

ATH showed a significantly higher weekly exercise time and dose compared to SED (Table 1). ATH mostly performed vigorous-intensity exercise activities (e.g. running or road cycling). We observed a higher VO_2peak in ATH (3544±651 mL/min) compared to SED (2843±519 mL/min, p<0.01). Likewise, ATH reached a higher power output during the incremental exercise test compared to SED (p<0.01, Table 1).

Central pulse wave velocity was significantly lower in ATH (7.0±2.2 m/s) compared to SED (9.2±2.3 m/s, P<0.01). Peripheral pulse wave velocity was significantly lower in ATH (8.1±1.5 m/s) compared to SED (9.4±1.6 m/s, p=0.017).

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) concentrations were lower in ATH compared to SED (Figure 1). Glyoxal concentrations did not differ between ATH *vs.* SED (314 [202-451] *vs.* 342 [266-388] nmol/mmol lysine, p=0.86, Figure 1).

CML was significantly higher in ATH (80 [73-89] nmol/mmol lysine) *vs.* SED (68 [56-76] nmol/mmol lysine, p<0.01, Figure 2). Similarly, CEL was significantly higher in ATH (35 [28-41] nmol/mmol lysine) compared to SED (28 [24-34] nmol/mmol lysine, p=0.035). Pentosidine (0.63 [0.59-0.86] *vs.* 0.56 [0.48-0.67] nmol/mmol lysine, p=0.11) did not differ between groups (Figure 2).

MG-H1 concentration was significantly lower in ATH (363 [288-468] nmol/mmol lysine) compared to SED (460 [340-536] nmol/mmol lysine, p=0.043, Figure 2).

MGO was positively correlated with BMI, central PWV, and FRS. (Table 2). 3DG was negatively correlated with VO_2peak, but positively correlated with BMI, central and peripheral PWV, FRS, and glucose (Table 2). GO did not correlate with cardiovascular health parameters (Table 2).

CML was negatively correlated with BMI and peripheral PWV, but positively correlated with VO_2peak. MG-H1 was negatively correlated with VO_2peak (Table 2). Pentosidine was negatively correlated with peripheral PWV and glucose (Table 2). CEL did not correlate with cardiovascular health parameters (Table 2).

Discussion

This study aimed to compare markers of dicarbonyl stress and circulating AGEs between lifelong endurance athletes and sedentary controls. MGO and 3DG were significantly lower in ATH compared to SED, and were related to a better cardiovascular health profile. However, we also found that CML and CEL were significantly higher in ATH compared to SED.

The benefits of exercise training on cardiovascular health are indisputable [14-16], but underlying mechanisms explaining the lower risk for cardiovascular events in physically active individuals are not fully understood [16]. Our results suggest that benefits of exercise training relate to a lower concentration of MGO and 3DG. These findings are in line with a recent study in rats, which demonstrated that running exercise was associated with a reduction in dicarbonyl stress [19]. In general, we found that markers of dicarbonyl stress showed a moderate, yet significant correlation with cardiovascular health or metabolic markers. For example, lower concentration MGO and 3DG were correlated to low Framingham risk score, lower insulin concentration, and better HOMA-IR. Reducing hyperglycaemia and improving insulin sensitivity may be a first step to reduce accumulation of MGO [4, 32] and 3DG [32]. High levels of dicarbonyl stress, and especially MGO, increase morbidity risk [4-6]. MGO is highly reactive and is mainly catabolized via glyoxalase I of the glyoxalase system. The activity of the glyoxalase system depends on concentrations of reduced glutathione (GSH) [4, 33]. Biosynthesis of GSH is heavily dependent of the antioxidant response element-nuclear respiratory factor (ARE-Nrf) pathway. Animal and human studies demonstrated that an acute bout of swimming or moderate intensity endurance exercise training upregulate the ARE-Nrf pathway and GSH biosynthesis. This led to the hypothesis that exercise training enhances the glyoxalase system and may lower MGO and MG-H1 concentrations. [34] Based on our data, it can be speculated that exercise training possibly lowers the levels of MGO and MG-H1 via an upregulation of the glyoxalase system. Further research is warranted to explore these pathways. Taken together, our data demonstrated that exercise training is related to lower levels of MGO, 3DG, and MG-H1.

In contrast to our hypothesis, we found that 2 of the 4 AGEs (CML and CEL) were significantly higher in ATH, whereas MG-H1 was significantly lower in ATH compared to SED. Although MG-H1 is a AGE, it is produced in a much shorter timeframe and is less stable than CML, CEL or pentosidine. [35] MG-H1 may, therefore, better relate to abnormal accumulation of dicarbonyl stress. [35] This could explain why MG-H1 showed opposite results compared to the other AGEs, since dicarbonyl stress was lower in ATH compared to SED.

Previous studies indicated that an increase in AGEs concentration relates to poor health outcomes. [4-6] Our findings are contradictory to this concept, as we found an inverse relation between circulating CML and pulse wave velocity, BMI, and cardiorespiratory fitness. A potential explanation for this finding could be that exercise enhances collagen turnover rate, which breaks and prevents AGE cross-links in the vessel wall. [12, 36, 37] This may contribute to higher levels of circulating AGEs, but this hypothesis needs to be reinforced with future studies. Alternatively, a recent animal study demonstrated that a 12-week running exercise training leads to suppressed RAGEs activation in the aorta of aged rats. [38] It could be speculated that attenuated RAGEs activity limits the uptake of AGEs from the circulation to the surrounding tissue, [39] leading to increased levels of circulating AGEs. Thus, the observation of higher AGEs in lifelong endurance athletes may relate to a higher collagen turnover and/or suppression of RAGEs due to long-term exercise training.

Another possible explanation for the higher AGEs concentrations in ATH *vs.* SED may relate to the (vigorous) exercise training regimes of our lifelong endurance athletes. Acute exercise induces a transient increase in oxidative stress, [40] which upregulates the formation of AGEs. [1, 2] Mice deficient in NADPH (nicotinamide adenine dinucleotide phosphate) oxidase, a pathway involved in the generation of reactive oxygen species, showed an impaired CML generation, which suggests that oxidative stress is a potential stimulus to generate CML. [41] Although the sudden increase in oxidative stress is a necessary stimulus to enhance the anti-oxidative defence mechanism (*i.e.,* glyoxalase system), [42] it is possible that the formation of AGEs is simultaneously upregulated. The positive relation between exercise dose / time and CML concentrations found in the present study (Table 2) may relate to the

effects of sustained exposure to vigorous exercise training. Hence, lifelong and repetitive exposure to vigorous exercise increases oxidative stress and may boost the accumulation of circulating AGEs in the blood. Future research is warranted to elucidate the underlying mechanisms and (clinical) impact of higher AGEs (CML and CEL) concentrations in athletes, as this observation contradicts with the general believe that high concentrations of circulating AGEs relate to CVD.

This cross-sectional study is inherent to some limitations. First, the comparison between athletes and sedentary individuals does not prove that exercise can attenuate the formation of dicarbonyl stress. A randomized clinical trial would be needed to confirm causation. However, our results indicate that exercise training is related to lower dicarbonyl stress. Unfortunately, we do not have information about the dietary habits of the participants. The absorption, bioavailability, and effects of dietary AGEs are poorly understood in vivo, [43] and it could be that diet patterns may contribute to the differences in AGEs between ATH and SED. AGE-rich food intake has been associated with higher levels of serum AGEs, whereas an AGE-restricted diet has been associated with lower serum AGEs. [21] However, whether food AGEs influence protein bound AGEs, as measured in this study, is not clear. Free AGEs may be relatively quickly absorbed, biotransformed, and excreted. On the other hand, high molecule weight AGEs, such as protein bound AGEs, may not be very extensively absorbed due to insufficient degradation by gastrointestinal enzymes. [43] Further research is warranted to establish a direct relation between dietary AGEs and protein-bound AGEs. Finally, all the participants of the study were men and the lifelong athletes performed endurance exercise activities only, which limits the generalizability of the present study.

Conclusion

Findings of the present study indicate that lifelong exercise training is associated with lower dicarbonyl stress (MGO and 3DG), which is related to improved cardiovascular health. Although MG-H1 was lower in lifelong endurance athletes compared to sedentary controls, AGEs concentrations of CML and CEL were significantly higher in athletes compared to sedentary controls. 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 AGEs concentrations relate to poor cardiovascular health.

Practical Implications

- Results of our study support the cardiovascular health benefits of lifelong exercise training, as lifelong endurance athletes demonstrated a better cardiovascular risk profile compared to sedentary controls.
- Lifelong exercise training is related to lower dicarbonyl stress, as veteran athletes had lower concentrations of methylglyoxal and 3-deoxyglucosone compared to sedentary controls.
- Lifelong exercise training is related to higher concentrations of advanced glycation endproducts (N^{ε}-(carboxymethyl)lysine and N^{ε}-(carboxyethyl)lysine). Although previous studies indicated that higher concentrations of advanced glycation endproducts were associated with adverse outcomes, the clinical significance of our findings in a highly active population is unknown.

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426 # Figure legend

Figure 1. Individual and average values of markers for (A) dicarbonyl stress and (B) advanced glycation endproducts in lifelong athletes (circles) and sedentary controls (squares). For dicarbonyl markers, GO concentrations did not differ between groups, whereas MGO and 3DG were significantly lower in athletes compared to controls. For advanced glycation endproducts, CML and CEL concentrations were higher in athletes compared to controls. Concentrations of pentosidine did not differ between groups. Concentrations of MG-H1 were lower in athletes compared to controls. P-value refers to an *independent Student's t* or (¥) *Mann-Whitney U* test. Group averages are presented as median and interquartile range.

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Table_1

Table 1. Participants' characteristics of lifelong endurance athletes (ATH, *n*=18) and sedentary controls (SED, *n*=18). Data is presented as mean and standard deviation or median and interquartile range (IQR). P-value refers to an *independent Student's t* test or *Mann-Whitney U* (*) test.

	ATH	**SED**	***p*-value**
n	18	18	
CHARACTERISTICS			
Age (years)	61±7	58±7	0.29
Height (m)	179±8	181±6	0.31
Weight (kg)	74±8	87±10	<0.01
Body Mass Index (kg/m^2) ¥	23.6 (21.1-24.9)	26.7 (25.0-27.4)	<0.01
Mean arterial pressure (mmHg) *	98 (90-106)	103 (93-107)	0.70
Systolic blood pressure (mmHg)	134±17	137±16	0.53
Diastolic blood pressure (mmHg)	84±10	84±10	0.92
Smoking history (%yes [n])	10 (56)	15 (83)	0.15
CARDIOVASCULAR HEALTH PARAMETERS			
Pulse Wave Velocity			
Central PWV (m/s)	7.0±2.2	9.2±2.3	<0.01
Peripheral PWV (m/s)	8.1±1.5	9.4±1.6	0.017
Framingham Risk Score (%) *	10.1 (7.5-20.3)	16.5 (10.1-19.5)	0.12
VO_2peak (mL/min)	3544±651	2843±519	<0.01
Fasting blood levels			
HbA1c (mmol/mol) ¥	35.5 (34.4-38.3)	35.5 (35.5-38.3)	0.53
Cholesterol (mmol/L)	5.4±0.8	5.9±0.9	0.07
LDL (mmol/L)	3.3±0.8	4.0±0.8	0.012
HDL (mmol/L)	1.8±0.3	1.4±0.3	<0.01
Triglycerides (mmol/L) *	0.8 (0.7-1.2)	1.3 (1.0-2.4)	<0.01
Glucose (mmol/L) *	4.6 (4.4-5.0)	4.7 (4.4-4.9)	0.66
Insulin (mU/L)	2.8±1.8	6.8±2.9	<0.01
HOMA-IR *	0.5 (0.3-0.9)	1.3 (0.8-2.2)	<0.01
LIFELONG EXERCISE PATTERNS			
Exercise time (hours/week) ¥	7.1 (5.8-11.9)	0.5 (0.0-1.4)	<0.01
Exercise dose (MET-hours/week) ¥	60 (47-110)	4 (0-12)	<0.01
INCREMENTAL EXERCISE TEST			
Maximal heart rate (beats/min)	165±13	171±15	0.29
RER (ratio: VCO_2 / VO_2) *	1.13 (1.06-1.17)	1.08 (1.05-1.14)	0.029
Lactate (mmol/L) *	11.6 (8.9-12.3)	11.1 (9.4-12.8)	0.77
Power Output (W)	319±58	209±46	<0.01

HbA1c: Glycated haemoglobin; HDL: High-density lipoprotein; HOMA-IR: homeostasis model assessment of insulin resistance; LDL: low-density lipoprotein; MET: Metabolic Equivalent of Task; PWV: pulse wave velocity; RER: respiratory exchange ratio; VO_2peak: peak oxygen uptake;

* Data were $\log_e$-transformed before statistical analysis

¥ non-parametrically tested via Mann-Whitney U

Table_2

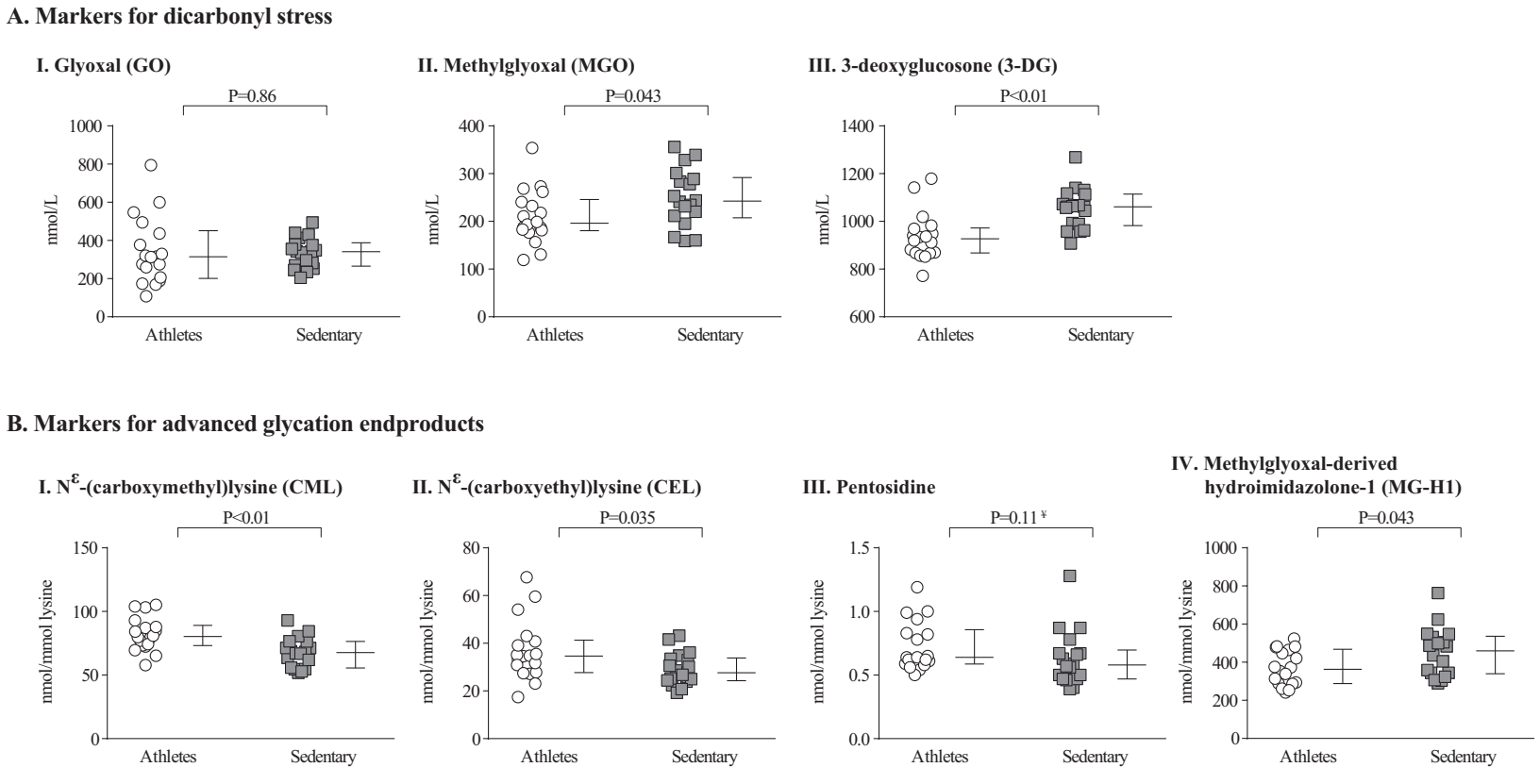
Table 2. *Spearman's Rank* (ρ) correlations between dicarbonyl stress, advanced glycation endproducts, and cardiovascular health parameters

	Dicarbonyl stress			Advanced glycation endproducts			
	GO	MGO	3DG	CML	CEL	Pentosidine	MG-H1
CARDIOVASCULAR HEALTH MARKERS							
BMI	0.02	0.35*	0.40*	-0.53**	-0.13	-0.31	0.19
Average PWV	0.12	0.35*	0.55**	-0.54**	0.10	-0.31	0.04
Central PWV	0.24	0.51**	0.46**	-0.30	0.07	-0.10	0.03
Peripheral PWV	-0.05	0.10	0.44**	-0.58**	0.11	-0.43*	0.02
VO_2peak (mL/min)	0.01	-0.32	-0.47**	0.34*	0.33	0.18	-0.55**
FRS	0.24	0.52**	0.43**	-0.23	-0.06	-0.09	0.11
Glucose	-0.19	0.15	0.46**	-0.13	0.13	-0.41*	-0.09
Insulin	0.04	0.36*	0.44**	-0.36*	-0.24	-0.12	0.35*
HOMA-IR	-0.01	0.34	0.49**	-0.36*	-0.21	-0.16	0.33
LIFELONG EXERCISE PATTERNS							
Exercise time	-0.04	-0.34*	-0.53**	0.46**	0.32	0.28	-0.36*
Exercise dose	-0.04	-0.34*	-0.53**	0.45**	0.36*	0.30	-0.37*

3DG: 3-deoxyglucosone; CEL: N_ε-(carboxyethyl)lysine; CML: N_ε-(carboxymethyl)lysine; FRS: Framingham risk score; GO: glyoxal; HOMA-IR: homeostasis model assessment of insulin resistance; MG-H1: Methylglyoxal-derived hydroimidazolone-1; MGO: methylglyoxal; VO_2peak: peak oxygen uptake (cardiorespiratory fitness); Average PWV: average pulse wave velocity, the average of the z-scores of central and peripheral PWV;

Correlation is significant at *0.05 or **0.01 level (two-sided).

Figure_1


A. Markers for dicarbonyl stress
I. Glyoxal (GO)
P=0.86
nmol/L
1000
800
600
400
200
0
Athletes
Sedentary
II. Methylglyoxal (MGO)
P=0.043
nmol/L
400
300
200
100
0
Athletes
Sedentary
III. 3-deoxyglucosone (3-DG)
P<0.01
nmol/L
1400
1200
1000
800
600
Athletes
Sedentary
B. Markers for advanced glycation endproducts
I. Nε-(carboxymethyl)lysine (CML)
P<0.01
nmol/mmol lysine
150
100
50
0
Athletes
Sedentary
II. Nε-(carboxyethyl)lysine (CEL)
P=0.035
nmol/mmol lysine
80
60
40
20
0
Athletes
Sedentary
III. Pentosidine
P=0.11 ¥
nmol/mmol lysine
1.5
1.0
0.5
0.0
Athletes
Sedentary
IV. Methylglyoxal-derived hydroimidazolone-1 (MG-H1)
P=0.043
nmol/mmol lysine
1000
800
600
400
200
0
Athletes
Sedentary