Physical activity and markers of glycation in older individuals

Clinical Science (2020) **134** 1095–1105
https://doi.org/10.1042/CS20200255

PORTLAND PRESS

Research Article

Physical activity and markers of glycation in older individuals: data from a combined cross-sectional and randomized controlled trial (EXAMIN AGE)

Mathias D.G. Van den Eynde[1,2,*], Lukas Streese[3,*], Alfons J.H.M. Houben[1,2], Coen D.A. Stehouwer[1,2], Jean L.J.M. Scheijen[1,2], Casper G. Schalkwijk[1,2], Nordin M.J. Hanssen[1,2] and Henner Hanssen[3]

[1]Department of Internal Medicine, Maastricht University Medical Centre, Maastricht, The Netherlands; [2]CARIM School for Cardiovascular Diseases, Maastricht, The Netherlands; [3]Division of Sports and Exercise Medicine, Department of Sport, Exercise and Health, University of Basel, Basel, Switzerland

Correspondence: Casper Schalkwijk (c.schalkwijk@maastrichtuniversity.nl)

Background: Advanced glycation end products (AGEs) are protein modifications that are predominantly formed from dicarbonyl compounds that arise from glucose and lipid metabolism. AGEs and sedentary behavior have been identified as a driver of accelerated (vascular) aging. The effect of physical activity on AGE accumulation is unknown. Therefore, we investigated whether plasma AGEs and dicarbonyl levels are different across older individuals that were active or sedentary and whether plasma AGEs are affected by high-intensity interval training (HIIT).

Methods: We included healthy older active (HA, n=38, 44.7% female, 60.1 ± 7.7 years old) and healthy older sedentary (HS, n=36, 72.2% female, 60.0 ± 7.3 years old) individuals as well as older sedentary individuals with increased cardiovascular risk (SR, n=84, 50% female, 58.7 ± 6.6 years old). The SR group was randomized into a 12-week walking-based HIIT program or control group. We measured protein-bound and free plasma AGEs and dicarbonyls by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) at baseline and after the HIIT intervention.

Results: Protein-bound AGE N^{ε}-(carboxymethyl)lysine (CML) was lower in SR (2.6 ± 0.5 μmol/l) and HS (3.1 ± 0.5 μmol/l) than in HA (3.6 ± 0.6 μmol/l; P<0.05) and remained significantly lower after adjustment for several potential confounders. None of the other glycation markers were different between HS and HA. HIIT did not change plasma AGEs and dicarbonyls in SR.

Discussion: Although lifestyle interventions may act as important modulators of cardiovascular risk, HIIT is not a potent short-term intervention to reduce glycation in older individuals, underlining the need for other approaches, such as pharmacological agents, to reduce AGEs and lower cardiovascular risk in this population.

Introduction

Advanced glycation end products (AGEs) are linked to a plethora of age-related conditions including cardiovascular disease (CVD) [1–4] and vascular diseases associated with diabetes [5,6]. As such, this heterogeneous family of sugar–protein modifications has been identified as a potential driver of the aging process [6]. The formation of AGEs is complex, but recent data have demonstrated that glycolysis- and lipid oxidation-derived dicarbonyl compounds such as methylglyoxal (MGO), glyoxal (GO) and 3-deoxyglucosone (3-DG) are important precursors in the rapid formation of AGEs such as N^{ε}-(carboxymethyl)lysine (CML), N^{ε}-(carboxyethyl)lysine (CEL) and 5-hydro-5-methylimidazolone (MG-H1) [7]. These toxic dicarbonyls and their derived AGEs are thought to be central players in the

*These authors contributed equally to this work.

Received: 04 March 2020
Revised: 23 April 2020
Accepted: 01 May 2020

Accepted Manuscript online: 01 May 2020
Version of Record published: 12 May 2020

Clinical Science (2020) **134** 1095–1105
https://doi.org/10.1042/CS20200255

development of age-related diseases like diabetes mellitus and CVD [8–10]. Therefore, strategies to lower the burden of high levels of AGEs may improve cardiovascular health. However, clinical studies targeting the formation of dicarbonyls and AGEs with specific inhibitors did not achieve clinical implementation due to safety concerns and/or limited potency to effectively lower AGEs *in vivo* [11].

Lifestyle intervention can be employed to effectively lower the burden of glycation in humans [12]. Physical activity has a profound impact on insulin sensitivity and the rate of glycolysis and lipid oxidation [13]. Furthermore, physical exercise has been shown to attenuate the age-related decline of cardiovascular function [14–16] and reduces both cardiovascular morbidity and mortality [17,18]. These positive effects of physical exercise are believed to be largely due to an improvement of metabolic control, and may therefore be in part due to a reduction in AGEs and dicarbonyls. Indeed, animal studies have suggested that physical exercise may reduce dicarbonyl stress and AGEs [19]. However, human studies linking exercise training with dicarbonyl stress or AGEs are sparse and have yielded conflicting outcomes with either positive, [20–22] negative, [23] or no effects [24,25] on AGE levels, presumably due to heterogeneities between study populations and techniques used to measure AGEs. In the current study, we tested the hypothesis that sedentary behavior is associated with increased levels of glycation, and that an exercise intervention reduces the rate of glycation. Specifically, we investigated whether AGE and dicarbonyl levels differed between older active and sedentary individuals with and without cardiovascular risk factors. Additionally, we assessed the effect of high-intensity interval training (HIIT) in a randomized controlled trial in older sedentary individuals at increased cardiovascular risk.

Methods

Study design and procedures

The Exercise, Arterial Crosstalk Modulation, and Inflammation in an Aging population (EXAMIN AGE) study was designed to investigate the influence of physical activity on healthy aging and the effects of high intensity training as described in detail recently [16,26,27]. In the cross-sectional part, we examined the association between physical activity and fitness, and plasma AGEs and dicarbonyls in healthy older active (HA, n=38) and healthy sedentary (HS, n=36) individuals, as well as older sedentary individuals with increased cardiovascular risk (SR, n=84). For the interventional part, the SR group was randomized into a 12-week walking-based HIIT group (n=44) or a control group with standard physical activity recommendations based on current guidelines (n=40), [28] in order to examine the effects of HIIT on AGE and dicarbonyl levels in older adults (see flowchart, Supplementary Figure S1). All procedures were performed as previously described [26].

Briefly, HA and HS as well as SR participants were recruited and enrolled in the cross-sectional study based on data from the first visit. Recruitment of the SR group was based on the agreement to take part in the exercise program following the cross-sectional assessment (which served as a baseline examination for the consecutive intervention study). At the first visit, anthropometric measurements were performed, physical activity was assessed, and 24-h blood pressure monitoring and blood sampling were performed. On a separate visit cardiopulmonary exercise testing was performed to assess maximal oxygen uptake (VO_2 max) as an estimate of peak endurance performance. For the intervention study, all measurements were repeated during the follow-up visit 12 weeks later, n=40 of the HIIT group and n=34 of the control group completed the intervention study.

VO_2 max was measured using the Cortex Metalyzer R 3B metabolic test system (Cortex Biophysik GmbH, Leipzig, Germany). All participants wore an Aipermotion 440 accelerometer (Aipermon GmbH, Munich, Germany) for six consecutive days on their left hip. From the five most active days, we calculated walking and fast walking in minutes per day, distance in meter per day and total steps per day using the AiperView 440 and ActiCoach MPAT2Viewer Software (Aipermon GmbH, Munich, Germany). The Freiburg Questionnaire of Physical Activity (FQPA) was used to assess self-reported sport activities in metabolic equivalents (METs) per week based on the Ainsworth Compendium [29].

All participants were medically examined by the study physician before inclusion, and written informed consent was obtained from all eligible individuals. All study procedures and ethical considerations have been previously described [26]. The present study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Northwestern and Central Switzerland (EKNZ-2015-351). The EXAMIN AGE study was registered on ClinicalTrials.gov (NCT02796976) in June 2016.

Study population and in-/exclusion criteria

Both the healthy active and healthy sedentary group included healthy men and women aged 50–80 years without cardiovascular risk factors, with an active lifestyle (>9 MET/week) or a sedentary lifestyle (≤3 MET/week), respectively. Individuals with a history of cardiovascular, pulmonary or chronic inflammatory disease, a blood pressure ≥ 140/90 mmHg during 24-h monitoring, macular degeneration or glaucoma, a history of smoking or any additional risk factors, were excluded.

The SR group was aged 50–80 years, and had at least two additional cardiovascular risk factors (being either obesity, elevated triglyceride or low-density lipoprotein (LDL) levels, decreased high-density lipoprotein (HDL) levels, elevated blood pressure, elevated plasma glucose, or current smoking; the risk factor distribution is shown in Supplementary Table S1) [26]. Individuals with decompensated cardiovascular, pulmonary or chronic inflammatory disease, macular degeneration, glaucoma or compromising orthopedic problems were excluded from the study.

Exercise intervention

The SR group were randomized to the intervention or control group by an independent research assistant. The physical exercise intervention comprised of a 12-week supervised Nordic Walking-based HIIT, performed three times per week. In the first week, the participants trained with an intensity of 75% of their maximum heart rate to get familiarized with a continuous walking-based training. In the second week, a stepwise increase in the intensity up to 80–90% of their maximum heart rate was performed. In the following 10 weeks, the participants performed the HIIT based on the following protocol with a total duration of 45 min per session: warm-up for 10 min at 60–70% of maximum heart rate, followed by a high-intensity interval consisting of 4 × 4 min at 80–90% with 3 min of active recovery at 60–70%, and a cool-down of 10 min at 60–70%. Heart rate was monitored during training by standard heart rate sensors. The control group received physical activity recommendations based on the European Guidelines on Cardiovascular Disease Prevention in Clinical Practice [28].

Measurement of plasma dicarbonyls and AGEs

All plasma samples were stored at −80° C prior to analyses. Plasma levels of dicarbonyls and AGEs were measured in EDTA plasma samples. Ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) was used to determine plasma levels of the free and protein-bound AGEs CML, CEL, MG-H1, [30] and the dicarbonyls MGO, GO and 3-DG [31]. Coefficients of variation were all below 10%. To infer influence of plasma protein content on protein-bound AGE levels, we measured total plasma protein with the Bradford reaction.

Statistics

Baseline characteristics were presented as mean ± standard deviation or as interquartile ranges when applicable. Differences at baseline between the HA, HS and SR groups were tested with one-way ANOVA. We used linear regression to assess associations between physical activity classification (determinant) and plasma AGE levels or plasma dicarbonyls (outcome). Betas were expressed as standardized regression coefficients. We adjusted for potential confounders: Model 1: crude analysis, Model 2: crude analysis + confounders (age, sex, fat and muscle mass, LDL- and glucose levels). The associations with protein-bound AGEs were also adjusted for total plasma protein levels. Next, we assessed the effect of the HIIT intervention on the plasma glycation markers, using a one-way ANCOVA with correction for baseline values. We Ln-transformed protein-bound MG-H1 and the free AGEs to achieve a normal distribution of the residuals in these analyses.

Results

Table 1 shows the baseline characteristics of the cross-sectional EXAMIN AGE study comparing HA, HS and SR groups.

Plasma glycation levels in older active and sedentary subjects

To investigate the influence of active and sedentary behavior on plasma levels of glycation, we first compared crude plasma AGE and dicarbonyl levels across the HA, HS, SR groups. We found that plasma protein-bound CML levels were significantly lower in HS compared with HA, and still lower in SR (Table 1, Figure 1). GO, a major dicarbonyl precursor for CML, was also significantly lower in HS and SR. We made similar observations for MGO and MGO-derived MG-H1, although this was significant only for MG-H1 in the SR group (Figure 1). In line with plasma glucose levels (Table 1), levels of the glucose-derived dicarbonyl 3-DG were significantly higher in SR, but not in HS.

Clinical Science (2020) **134** 1095–1105
https://doi.org/10.1042/CS20200255

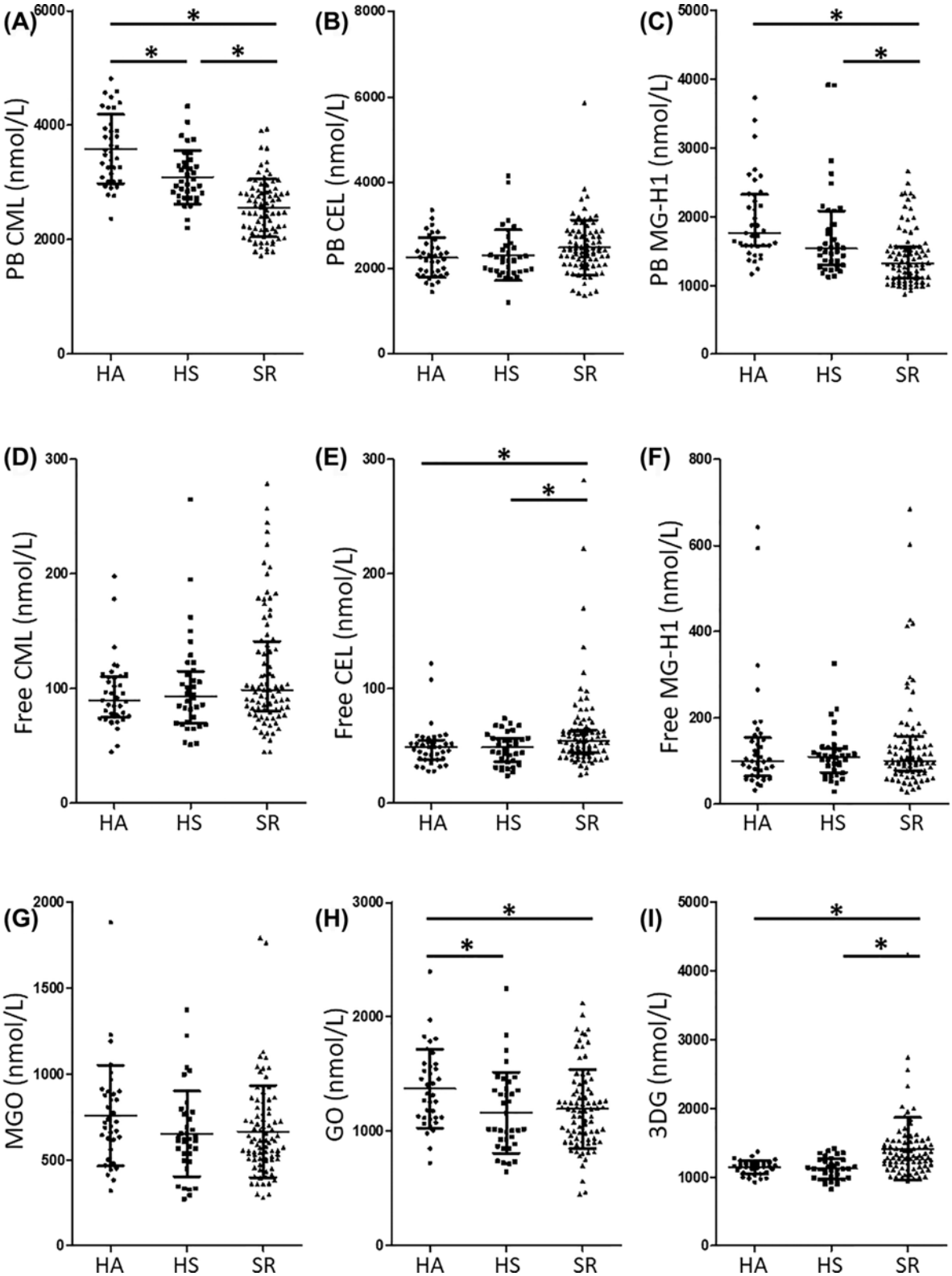

Figure 1. Plasma levels of AGEs and dicarbonyl compounds in HA subjects (*n*=38), HS subjects (*n*=36) and SR (*n*=84)

Using UPLC-MS/MS we measured levels of protein-bound CML (**A**), CEL (**B**), MG-H1 (**C**), free CML (**D**), CEL (**E**) and MG-H1 (**F**), and the dicarbonyl compounds MGO (**G**), GO (**H**) and 3-DG (**I**). Represented data are mean ± SD (A,B,G–I) or median and interquartile ranges (C–F), as appropriate. Differences were compared with ANOVA with Bonferroni correction. * = $P<0.05$. Skewed variables were Ln-transformed.

Table 1 Participants' characteristics

	HA (*n*=38)	HS (*n*=36)	SR (*n*=84)
Sex (female, %)	44.7	72.2	50.0
Age (years)	60.1 ± 7.7	60.0 ± 7.3	58.7 ± 6.6
BMI (kg/m^2)	22.1 ± 1.7	24.8 ± 2.4	33.2 ± 4.1
Fat mass (kg)	13.0 ± 3.8	22.8 ± 5.9	37.9 ± 9.7
Muscle mass (kg)	28.6 ± 4.3	25.9 ± 4.8	31.6 ± 6.9
Current smoker (%)	0	0	33.0
Systolic BP (mmHg)	127.8 ± 15.3	127.2 ± 14.9	132.3 ± 14.3
Diastolic BP (mmHg)	77.8 ± 8.1	81.0 ± 8.2	87.3 ± 9.7
Fasting glucose (mmol/l)	4.7 ± 0.4	4.7 ± 0.5	5.8 ± 1.8
LDL cholesterol (mmol/l)	2.9 ± 0.7	3.1 ± 0.8	3.2 ± 0.8
HDL cholesterol (mmol/l)	2.0 ± 0.4	1.7 ± 0.4	1.3 ± 0.3
Triglycerides (mmol/l)	1.0 (0.7–1.1)	1.0 (0.8–1.3)	1.6 (1.1–1.8)
VO_2 max (ml/min/kg)	42.5 ± 8.3	29.9 ± 4.3	26.0 ± 4.3
Accelerometer			
Walking (min/day)	108 ± 45	104 ± 39	91 ± 39
Fast walking (min/day)	29 (23–41)	16 (6–28)	11 (4–20)
Distance (meter/day)	9310 ± 3385	6424 ± 2696	5568 ± 2397
Steps (*n*)	13267 ± 4870	10105 ± 3828	8712 ± 3588
Glycation markers			
Protein-bound CML (nmol/l)	3586 ± 606.5	3091 ± 466.1	2556 ± 504.0
Protein-bound CEL (nmol/l)	2254 ± 467.2	2315 ± 592.2	2493 ± 642.1
Protein-bound MG-H1 (nmol/l)	1762 (1572–2324)	1535 (1294–2085)	1325 (1112–1565)
Free CML (nmol/l)	90.0 (75–110.5)	93.5 (70.3–115.3)	99.0 (80.5–141.0)
Free CEL (nmol/l)	49.0 (38.5–55.0)	49.0 (36.3–57.0)	54.5 (44.0–64.0)
Free MG-H1 (nmol/l)	100.0 (65.5–155.0)	109.5 (73.8–130)	100.5 (77.0–157.3)
MGO (nmol/l)	757.9 ± 292.6	652.1 ± 249.7	665.0 ± 267.8
GO (nmol/l)	1372 ± 343.5	1163 ± 354.7	1195 ± 345.9
3-DG (nmol/l)	1143 ± 101.7	1121 ± 154.0	1412 ± 455.6

Abbreviations: HA subjects, HS subjects and SR. Blood pressure (BP), LDL, HDL. Glycation markers: protein-bound and free CML, CEL, MG-H1 and the dicarbonyl compounds MGO, GO and 3-DG. Represented data are mean ± SD or median and interquartile ranges, as appropriate.

Next, we performed linear regression analyses to address potential confounding with regard to the associations between the sedentary groups and plasma AGE and dicarbonyl levels. Adjustment for potential confounders (sex, age, fat and muscle mass, LDL- and glucose levels) strongly attenuated the association between lower CML levels and the HS and SR groups relative to HA (Table 2, Model 2). The point estimates of the association between lower plasma MG-H1 levels and SR, relative to HA, were slightly attenuated after adjustment for potential confounders (Table 2, Models 1–2). Adjustment for total plasma protein did not influence any of the associations with the protein-bound AGEs (data not shown).

The association between lower GO levels and SR was attenuated and lost statistical significance after adjustment for potential confounders (Table 2, Model 2), while the association with healthy older sedentary (HS) remained largely unaffected. The association between SR and higher 3-DG levels reversed when we adjusted for potential confounders (Table 2, Model 2).

Associations between plasma glycation levels and VO_2 max

Next, we pooled all subjects (HA and HS and SR groups) to study the associations between physical fitness and plasma AGEs and dicarbonyls. In line with the lower plasma CML levels in HS and SR, we found that a higher VO_2 max, as a marker of physical fitness, was associated with higher plasma CML levels (Table 3, Model 1). This association was attenuated after adjustment for potential confounders (sex, age and fat and muscle mass, LDL- and glucose levels (Table 3, Model 2). Although a higher VO_2 max was also associated with MG-H1 levels in a crude analysis, this association lost statistical significance after adjustment for potential confounders (Table 3, Models 1–2). Likewise, the association between a higher VO_2 max and lower 3-DG levels was no longer significant after adjustment for potential confounders (Table 3, Models 1–2).

Clinical Science (2020) **134** 1095–1105
https://doi.org/10.1042/CS20200255

Table 2 Associations between plasma AGE and dicarbonyl levels of HS subjects and older sedentary subjects at risk (SR) compared with HA subjects, adjusted for potential confounders

Model	HS	SR	HS	SR	HS	SR
	PB CML		PB CEL		Ln-PB MG-H1	
1	**−0.74 (−1.09 to −0.38)**	**−1.51 (−1.82 to −1.21)**	0.10 (−0.36 to 0.56)	**0.40 (0.01 to 0.79)**	−0.27 (−0.69 to 0.16)	**−0.96 (−1.32 to −0.60)**
2	**−0.55 (−0.93 to −0.16)**	**−0.87 (−1.39 to −0.35)**	0.17 (−0.33 to 0.66)	0.23 (−0.44 to 0.90)	−0.22 (−0.68 to 0.25)	−0.93 (−1.56 to 0.30)
	Ln-free CML		Ln-free CEL		Ln-free Ln-MG-H1	
1	0.09 (−0.36 to 0.54)	0.33 (−0.06 to 0.71)	−0.07 (−0.49 to 0.36)	**0.38 (0.02 to 0.74)**	−0.11 (−0.56 to 0.34)	−0.05 (−0.42 to 0.34)
2	0.16 (−0.32 to 0.63)	0.34 (−0.29 to 0.98)	−0.13 (−0.54 to 0.27)	**−0.04 (0.58 to 0.51)**	−0.11 (−0.56 to 0.34)	−0.13 (−0.73 to 0.47)
	MGO		GO		3-DG	
1	−0.39 (−0.86 to 0.08)	−0.36 (−0.76 to 0.03)	**−0.59 (−1.04 to −0.14)**	**−0.52 (−0.91 to −0.14)**	**−0.06 (−0.39 to 0.27)**	**0.64 (0.36 to 0.92)**
2	−0.44 (−0.94 to 0.07)	−0.40 (−1.08 to 0.28)	**−0.51 (−1.01 to −0.02)**	−0.28 (−0.94 to 0.39)	**−0.17 (−0.34 to 0.00)**	−0.12 (−0.34 to 0.11)

β's are expressed per standard deviation plasma AGE, healthy older sedentary (HS, *n*=36) and SR (*n*=84) versus HA (*n*=38) as the reference group. The free AGEs and protein-bound MG-H1 were Ln transformed prior to analyses.
Model 1: Crude
Model 2: 1+ age, sex, fat mass and muscle mass, fasting LDL and glucose levels.

Table 3 Associations between VO_2 max and plasma AGEs and dicarbonyl levels

Model	PB CML	PB CEL	Ln-PB MG-H1
1	**0.48 (0.34 to 0.62)**	−0.06 (−0.23 to 0.10)	**0.29 (0.14 to 0.44)**
2	**0.27 (0.05 to 0.49)**	−0.07 (−0.35 to 0.22)	0.17 (−0.10 to 0.44)
	Ln-free CML	**Ln-free CEL**	**Ln-free MG-H1**
1	−0.08 (−0.23 to 0.07)	−0.07 (−0.21 to 0.07)	0.10 (−0.05 to 0.25)
2	−0.14 (−0.40 to 0.12)	0.01 (−0.22 to 0.23)	0.06 (−0.20 to 0.31)
	MGO	**GO**	**3-DG**
1	0.08 (−0.08 to 0.24)	0.13 (−0.03 to 0.29)	**−0.21 (−0.33 to −0.09)**
2	0.09 (−0.20 to 0.38)	0.11 (−0.18 to 0.39)	0.03 (−0.07 to 0.13)

Pooled analysis of all subjects from the HA (*n*=38), HS (*n*=36) and SR (*n*=84) group combined. Glycation markers: protein-bound (PB) and free CML, CEL, MG-H1, and the dicarbonyl compounds MGO, GO and 3-DG. β expressed as standard deviation plasma AGE per standard deviation VO_2 max. The free AGEs and protein-bound MG-H1 were Ln transformed prior to analyses.
Model 1: Crude
Model 2: 1+ age, sex, fat mass and muscle mass, fasting LDL and glucose levels.

A 12-week HIIT and plasma glycation markers in sedentary individuals at increased cardiovascular risk

Next, we studied whether HIIT can reduce plasma AGEs and dicarbonyls. Although the intervention significantly increased VO_2 max and muscle mass and decreased weight, fat mass, BMI, and LDL after 12 weeks (Table 4), [16] we found no statistically significant effects on plasma protein-bound or free CML, CEL and MG-H1 levels or plasma MGO, GO and 3-DG levels by the HIIT intervention (Figure 2). There were no significant mean differences between control and HIIT for any of the glycation markers when we adjusted for the baseline values (Table 5). Additionally, we analyzed the trial excluding individuals using glucose-lowering medication (*n*=6 in the control and *n*=6 in the HIIT group), this did not alter any of the results (data not shown).

Discussion

The main finding from the cross-sectional part of the present study is that plasma levels of glycation were not higher in sedentary subjects (either the HS or SR groups) as compared with active subjects (the HA group). Protein-bound plasma levels of CML in fact appeared to be lower in the HS and SR groups. In line, higher CML levels were associated with a higher VO_2 max, but no other consistent associations were found between markers of glycation and sedentary behavior or VO_2 max. A second main finding is that a 12-week HIIT intervention did not influence any of the plasma glycation markers.

Our current cross-sectional findings are in line with our previous study showing higher CML concentrations in lifelong endurance athletes compared with sedentary individuals [23]. We also confirm a positive association between

Table 4 Participants' characteristics of SR before and after intervention

	Control (n=34)		Intervention (n=40)	
	Baseline	**Follow-up**	**Baseline**	**Follow-up**
BMI	32.9 ± 4.8	32.5 ± 4.7	32.9 ± 3.3	32.5 ± 3.4
Current smoker (%)	32.4	35.4	30.0	25.0
Systolic BP (mmHg)	128.7 ± 13.5	134.2 ± 14.3	134.2 ± 14.3	133.8 ± 11.9
Diastolic BP (mmHg)	85.4 ± 10.3	88.2 ± 9.5	88.2 ± 9.5	86.9 ± 7.1
Fasting glucose (mmol/l)	5.8 ± 1.4	5.6 ± 1.2	5.8 ± 2.1	5.7 ± 1.7
LDL cholesterol (mmol/l)	3.0 ± 0.7	2.9 ± 0.8	3.3 ± 0.8	3.0 ± 0.8
HDL cholesterol (mmol/l)	1.4 ± 0.3	1.4 ± 0.4	1.3 ± 0.3	1.3 ± 0.3
Triglycerides (mmol/l)	1.6 (1.1–1.8)	1.5 (1.1–2.3)	1.5 (1.2–1.8)	1.4 (1.2–1.70)
VO_2 max (ml/min/kg)	26.1 ± 5.0	25.0 ± 4.0	26.4 ± 3.8	28.7 ± 4.1*
Accelerometer				
Walking (min/day)	97 ± 39	94 ± 42	89 ± 41	92 ± 39
Fast walking (min/day)	10 (5–20)	8 (3–20)	12 (4–21)	14 (7–24)
Distance (meters/day)	5868 ± 2495	5615 ± 2652	5556 ± 2428	5796 ± 2207
Steps (n)	9256 ± 3648	8920 ± 4108	8591 ± 3628	9065 ± 3497
Glycation markers				
Protein-bound CML (nmol/l)	2647.5 ± 547.1	2618.6 ± 607.8	2587.2 ± 457.0	2602.8 ± 429.4
Protein-bound CEL (nmol/l)	2435.5 ± 515.9	2220.9 ± 540.5	2471.0 ± 521.3	2446.5 ± 561.5
Protein-bound MG-H1 (nmol/l)	1273 (1056–1499)	1435 (1212–1663)	1481 (1121–1618)	1337 (1202–1536)
Free CML (nmol/l)	95.5 (76.8–149.3)	103.9 (78.2–142.4)	102.0 (83.0–132.0)	105.2 (82.5–126.7)
Free CEL (nmol/l)	57.0 (41.0–71.8)	54.7 (38.6–67.8)	54.0 (44.0–61.8)	57.0 (43.2–78.7)
Free MG-H1 (nmol/l)	104.0 (62.75–138.5)	120.6 (85.0–186.7)	100.0 (77.0–159.0)	126.1 (82.0–180.9)
MGO (nmol/l)	643.1 ± 276.0	830.3 ± 346.4	684.7 ± 203.1	1013.8 ± 627.9
GO (nmol/l)	1218.9 ± 335.9	1411.5 ± 453.7	1227.6 ± 362.0	1439.2 ± 437.1
3-DG (nmol/l)	1368.1 ± 301.4	1368.5 ± 273.8	1455.5 ± 550.8	1378.2 ± 454.3

Blood pressure (BP), LDL, HDL. Data are represented as mean ± standard deviation or as interquartile ranges, as appropriate. *P<0.05, versus control differences were compared across group allocation at follow-up with ANCOVA adjusting for baseline values. Glycation markers: protein-bound and free CML, CEL, MG-H1, and the dicarbonyl compounds MGO, GO and 3-DG.

Table 5 Mean differences between control and HITT for plasma AGEs and dicarbonyls

Glycation markers (nmol/l)	% change control (ratio geom. means FU-BL values)	% change intervention (ratio geom. means FU-BL values)	Mean difference (control – intervention)	Lower bound 95% CI	Upper bound 95% CI
Protein-bound CML	−1.5	−0.8	−20.17	−167.71	127.37
Protein-bound CEL	−9.3	−1.9	−213.48	−459.97	33.01
Protein-bound MG-H1	11.1	−4.3	0.082	−0.028	0.19
Free CML	2.6	−2.4	0.040	−0.103	0.183
Free CEL	−6.5	6.8	−0.097	−0.242	0.047
Free MG-H1	9.6	10.5	0.013	−0.192	0.218
MGO	24.4	34.1	−161.70	−398.12	74.72
GO	10.3	17.0	−25.45	−229.42	178.53
3-DG	0.5	−2.1	54.51	−33.33	142.34

Mean differences and % change of plasma AGEs and dycarbonyls, between the HIIT intervention (n=40) and control group (n=34) in SR. In individuals randomized to either control or HIIT intervention, we measured at baseline and follow-up levels of protein-bound CML, CEL, MG-H1, free CML, free CEL, free MG-H1, and the dicarbonyl compounds MGO, GO and 3-DG with UPLC-MS/MS. Differences were compared across group allocation at follow-up with ANCOVA adjusting for baseline values. Represented data are mean differences between the control and intervention group with 95% confidence intervals. Protein-bound MG-H1 and free AGEs were ln transformed to achieve a normal distribution of the residuals and therefore presented on the Ln scale.

Clinical Science (2020) **134** 1095–1105
https://doi.org/10.1042/CS20200255

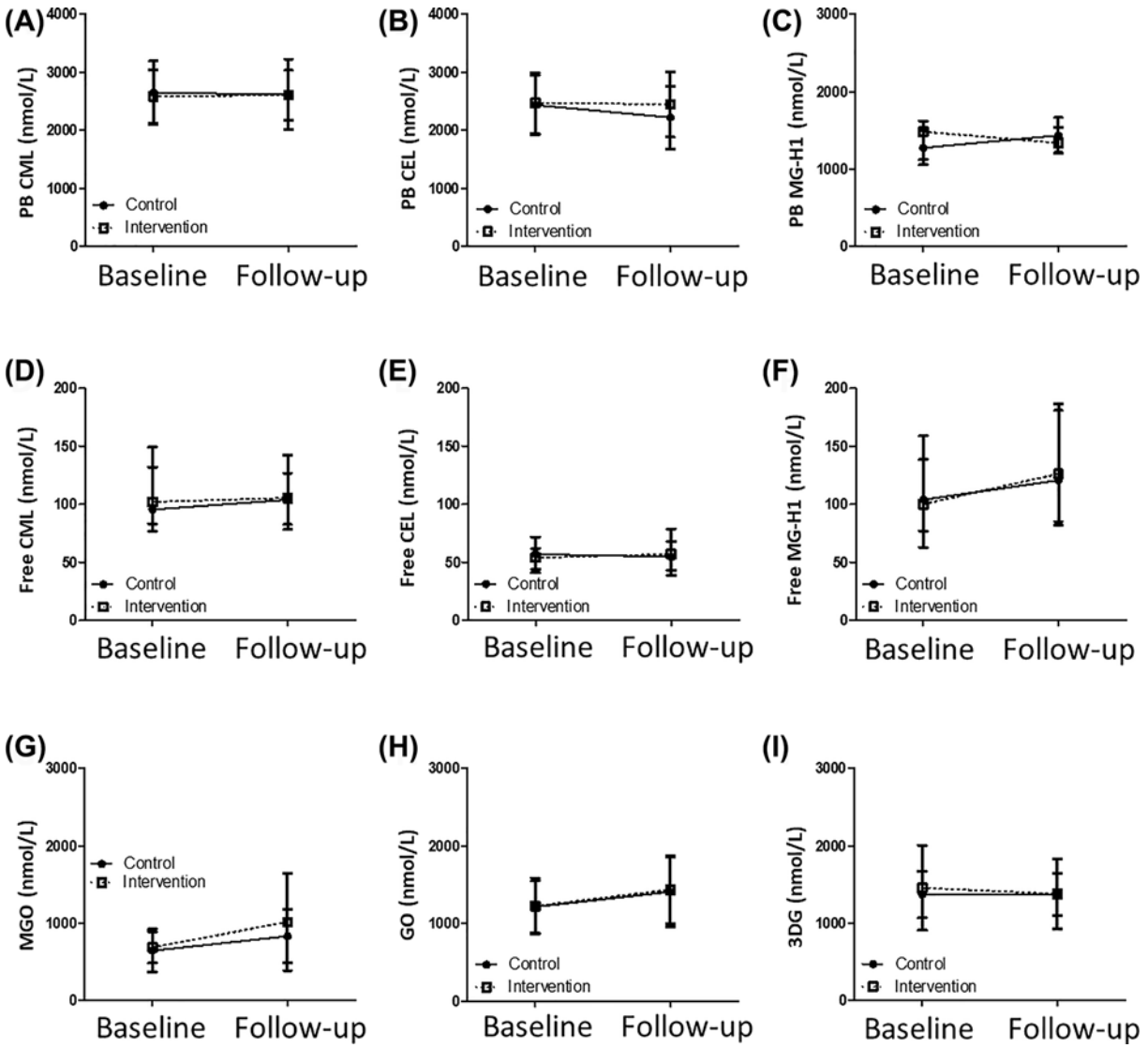

Figure 2. Effect of HIIT on plasma AGE and dycarbonyl compounds in SR

In individuals randomized to either control (n=34) or HIIT (n=40) intervention, we measured at baseline and follow-up levels of protein-bound CML (**A**), CEL (**B**), MG-H1 (**C**), and free CML (**D**), CEL (**E**) and MG-H1 (**F**), and the dicarbonyl compounds MGO (**G**), GO (**H**) and 3-DG (**I**) with UPLC-MS/MS. Represented data are mean ± SD (A,B,G–I) or median and interquartile ranges (C–F), as appropriate. Differences were compared across group allocation at follow-up with ANCOVA adjusting for baseline values.

CML and VO_2 max. Although we do not have a clear explanation for the finding of higher plasma CML levels in more active individuals, exercise generally promotes tissue repair, turnover of matrix proteins and the breakdown of cross-links in the vessel wall, [7,32,33], which may lead to higher levels of circulating protein-bound AGEs. Another explanation for the higher AGEs levels in HA individuals could be a higher metabolic rate, resulting in an increased formation of AGEs [34,35]. Furthermore, greater physical activity is associated with a higher caloric intake and we recently demonstrated that higher energy intake (kcal/day) is associated with a higher intake of dietary AGEs [36]. Therefore, higher energy intake in active individuals may, at least in part, explain the increased levels of CML in these individuals. Unfortunately, we could not take potential confounding by dietary factors fully into account in the current study.

So far, human studies linking exercise training with plasma AGE levels have yielded conflicting results. It has been demonstrated that a 12-month Tai Chi intervention reduced plasma AGEs in healthy middle-aged adults [20] and that physical training in patients with either HIV [21] or breast cancer [22] also reduced circulating glycation levels. In

agreement with these studies, an animal study showed a reduction in plasma dicarbonyls and CML in rats subjected to physical exercise (treadmill running), [19] although the interventions and metabolic effects in animals are, in general, more severe than in human studies. In contrast with the reduction in pre(AGEs) by physical activity found in the abovementioned studies, a clinical study with middle-aged overweight and obese men did not show any effect on serum AGEs after a 3-month aerobic moderate intensity exercise intervention [25]. We previously found, using state-of-the-art measurements of dicarbonyls and AGEs with UPLC tandem MS, that lifelong exercise training was linked to reduced plasma levels of the AGE MG-H1 and reduced dicarbonyl stress while plasma CML and CEL were higher [23]. These mixed outcomes of physical activity on (pre)AGEs are presumably due to heterogeneities between study populations, duration of intervention, the intensity of physical activity and techniques used to measure AGEs. Our current study adds to previous work by including a large array of protein-bound and free AGEs as well as the major dicarbonyls, providing a relatively large sample size of older adults and applying a HIIT intervention in the context of plasma glycation for the first time.

The present study has a few important implications. First, the current study suggests that it is unlikely that glycation in older individuals can easily or quickly be targeted by interventions that improve physical fitness through HIIT. This is relevant as we previously found that caloric restriction is a potent intervention to reduce dicarbonyl stress [37]. In addition, scavenging compounds that reduce glycation may therefore still be needed to lower glycation *in vivo*. This is challenging, as some of these compounds such as aminoguanidine were either linked to potential toxicity, or had limited efficacy when tested in humans [38]. However, some compounds such as pyridoxamine, carnosine or carnosinol and the trans-resveratrol/hesperetin co-formulation remain under active investigation with potential efficacy and a more favorable pharmacologic profile [39–41].

The present study has several limitations. We showed previously that free plasma AGEs are associated with dietary intake of AGEs [36] and in our study, we cannot exclude the confounding influence of dietary AGEs. Moreover, we do not know if the current findings were in any way confounded by caloric intake. Furthermore, our intervention, although quite intense for older individuals, had a duration of 12 weeks, and longer interventions may have had more profound effects on plasma glycation markers. Finally, we cannot rule out that exercise lowers tissue AGEs and dicarbonyls, but that this change is not reflected by plasma measurements. It should also be noted that our results cannot be generalized to a younger population.

Clinical perspectives

- The effect of physical activity on AGE accumulation is still unknown and human studies linking exercise training with dicarbonyl stress or AGEs have yielded conflicting results.
- Plasma markers of glycation are not increased in sedentary individuals or lower in physically active older individuals. Protein-bound CML levels were positively associated with VO_2 max, and appeared to be associated with physical fitness.
- These findings imply the need for other approaches, such as pharmacological agents, directed at reducing age-related diseases and cardiovascular risk through reduction in the glycation pathway.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

This work was supported by the Dr E. Dekker grant by the Dutch Heart Foundation [grant number 2017T039 (to N.M.J.H.)]; A Junior Post-Doc grant from the Dutch Diabetes Foundation [grant number 2017.85.005 (to N.M.J.H.)]; and the Swiss National Science Foundation, SNSF [grant number 32003B_159518/1 (to H.H.)].

Author Contribution

M.D.G.v.d.E. and N.H. analyzed data and wrote the manuscript. L.S. performed the clinical trial. H.H. supervised the clinical trial. J.L.J.M.S. conducted glycation analysis. C.G.S., A.J.H.M.H., C.D.A.S. and H.H. supervised this research. All authors read and approved the final manuscript.

Clinical Science (2020) **134** 1095–1105
https://doi.org/10.1042/CS20200255

Abbreviations

3-DG, 3-deoxyglucosone; AGE, advanced glycation endproduct; CEL, N^{ε}-(carboxyethyl)lysine; CML, N^{ε}-(carboxymethyl)lysine; CVD, cardiovascular disease; EXAMIN AGE, The Exercise, Arterial Crosstalk Modulation, and Inflammation in an Aging population; GO, glyoxal; HA, healthy older active; HIIT, high-intensity interval training; HS, healthy older sedentary; LDL, low-density lipoprotein; MGO, methylglyoxal; MG-H1, 5-hydro-5-methylimidazolone; SR, older sedentary individuals with increased cardiovascular risk; VO_2 max, maximal oxygen uptake.

References

1 Forbes, J.M. and Cooper, M.E. (2013) Mechanisms of diabetic complications. *Physiol. Rev.* **93**, 137–188, https://doi.org/10.1152/physrev.00045.2011
2 Rabbani, N. and Thornalley, P.J. (2015) Dicarbonyl stress in cell and tissue dysfunction contributing to ageing and disease. *Biochem. Biophys. Res. Commun.* **458**, 221–226, https://doi.org/10.1016/j.bbrc.2015.01.140
3 Hanssen, N.M., Beulens, J.W., van Dieren, S., Scheijen, J.L., van der, A.D., Spijkerman, A.M. et al. (2015) Plasma advanced glycation end products are associated with incident cardiovascular events in individuals with type 2 diabetes: a case-cohort study with a median follow-up of 10 years (epic-nl). *Diabetes* **64**, 257–265, https://doi.org/10.2337/db13-1864
4 Hanssen, N.M., Wouters, K., Huijberts, M.S., Gijbels, M.J., Sluimer, J.C., Scheijen, J.L. et al. (2014) Higher levels of advanced glycation endproducts in human carotid atherosclerotic plaques are associated with a rupture-prone phenotype. *Eur. Heart J.* **35**, 1137–1146, https://doi.org/10.1093/eurheartj/eht402
5 Goldin, A., Beckman, J.A., Schmidt, A.M. and Creager, M.A. (2006) Advanced glycation end products: sparking the development of diabetic vascular injury. *Circulation* **114**, 597–605, https://doi.org/10.1161/CIRCULATIONAHA.106.621854
6 Maessen, D.E., Stehouwer, C.D. and Schalkwijk, C.G. (2015) The role of methylglyoxal and the glyoxalase system in diabetes and other age-related diseases. *Clin. Sci. (Lond.)* **128**, 839–861, https://doi.org/10.1042/CS20140683
7 Sell, D.R. and Monnier, V.M. (2012) Molecular basis of arterial stiffening: role of glycation - a mini-review. *Gerontology* **58**, 227–237, https://doi.org/10.1159/000334668
8 Hanssen, N.M.J., Westerink, J., Scheijen, J., van der Graaf, Y., Stehouwer, C.D.A., Schalkwijk, C.G. et al. (2018) Higher plasma methylglyoxal levels are associated with incident cardiovascular disease and mortality in individuals with type 2 diabetes. *Diabetes Care* **41**, 1689–1695, https://doi.org/10.2337/dc18-0159
9 Brownlee, M. (2001) Biochemistry and molecular cell biology of diabetic complications. *Nature* **414**, 813–820, https://doi.org/10.1038/414813a
10 Schalkwijk, C. and Stehouwer, C.D. (2020) Methylglyoxal, a highly reactive dicarbonyl compound, in diabetes, its vascular complications and other age-related diseases. *Physiol. Rev.* **100**, 407–461
11 Engelen, L., Stehouwer, C.D. and Schalkwijk, C.G. (2013) Current therapeutic interventions in the glycation pathway: Evidence from clinical studies. *Diabetes Obes. Metab.* **15**, 677–689, https://doi.org/10.1111/dom.12058
12 Maessen, D.E., Hanssen, N.M., Scheijen, J.L., van der Kallen, C.J., van Greevenbroek, M.M., Stehouwer, C.D. et al. (2015) Post-glucose load plasma alpha-dicarbonyl concentrations are increased in individuals with impaired glucose metabolism and type 2 diabetes: The codam study. *Diabetes Care* **38**, 913–920, https://doi.org/10.2337/dc14-2605
13 Cartee, G.D., Hepple, R.T., Bamman, M.M. and Zierath, J.R. (2016) Exercise promotes healthy aging of skeletal muscle. *Cell Metab.* **23**, 1034–1047, https://doi.org/10.1016/j.cmet.2016.05.007
14 Eijsvogels, T.M.H., Molossi, S., Lee, D.-C., Emery, M.S. and Thompson, P.D. (2016) Exercise at the extremes: the amount of exercise to reduce cardiovascular events. *J. Am. Coll. Cardiol.* **67**, 316–329, https://doi.org/10.1016/j.jacc.2015.11.034
15 Mora, S., Cook, N., Buring Julie, E., Ridker Paul, M. and Lee, I.M. (2007) Physical activity and reduced risk of cardiovascular events. *Circulation* **116**, 2110–2118, https://doi.org/10.1161/CIRCULATIONAHA.107.729939
16 Streese, L., Khan, A.W., Deiseroth, A., Hussain, S., Suades, R., Tiaden, A. et al. (2019) High-intensity interval training modulates retinal microvascular phenotype and DNA methylation of p66shc gene: a randomized controlled trial (examin age). *Eur. Heart J.* **41**, 1514–1519, https://doi.org/10.1093/eurheartj/ehz196
17 Blair, S.N., Kampert, J.B., Kohl, III, H.W., Barlow, C.E., Macera, C.A., Paffenbarger, R.S. et al. (1996) Influences of cardiorespiratory fitness and other precursors on cardiovascular disease and all-cause mortality in men and women. *JAMA* **276**, 205–210, https://doi.org/10.1001/jama.1996.03540030039029
18 Maessen, M.F.H., Verbeek, A.L.M., Bakker, E.A., Thompson, P.D., Hopman, M.T.E. and Eijsvogels, T.M.H. (2016) Lifelong exercise patterns and cardiovascular health. *Mayo Clin. Proc.* **91**, 745–754, https://doi.org/10.1016/j.mayocp.2016.02.028
19 Boor, P., Celec, P., Behuliak, M., Grancic, P., Kebis, A., Kukan, M. et al. (2009) Regular moderate exercise reduces advanced glycation and ameliorates early diabetic nephropathy in obese zucker rats. *Metabolism* **58**, 1669–1677, https://doi.org/10.1016/j.metabol.2009.05.025
20 Goon, J.A., Aini, A.H., Musalmah, M., Anum, M.Y., Nazaimoon, W.M. and Ngah, W.Z. (2009) Effect of tai chi exercise on DNA damage, antioxidant enzymes, and oxidative stress in middle-age adults. *J. Phys. Activity Health* **6**, 43–54
21 Rodrigues, K.L., Borges, J.P., Lopes, G.O., Pereira, E., Mediano, M.F.F., Farinatti, P. et al. (2018) Influence of physical exercise on advanced glycation end products levels in patients living with the human immunodeficiency virus. *Front. Physiol.* **9**, 1641, https://doi.org/10.3389/fphys.2018.01641
22 Walter, K.R., Ford, M.E., Gregoski, M.J., Kramer, R.M., Knight, K.D., Spruill, L. et al. (2019) Advanced glycation end products are elevated in estrogen receptor-positive breast cancer patients, alter response to therapy, and can be targeted by lifestyle intervention. *Breast Cancer Res. Treat.* **173**, 559–571, https://doi.org/10.1007/s10549-018-4992-7

23 Maessen, M.F.H., Schalkwijk, C.G., Verheggen, R., Aengevaeren, V.L., Hopman, M.T.E. and Eijsvogels, T.M.H. (2017) A comparison of dicarbonyl stress and advanced glycation endproducts in lifelong endurance athletes vs. sedentary controls. *J. Sci. Med. Sport* **20**, 921–926, https://doi.org/10.1016/j.jsams.2017.03.011
24 Oudegeest-Sander, M.H., Olde Rikkert, M.G., Smits, P., Thijssen, D.H., van Dijk, A.P., Levine, B.D. et al. (2013) The effect of an advanced glycation end-product crosslink breaker and exercise training on vascular function in older individuals: a randomized factorial design trial. *Exp. Gerontol.* **48**, 1509–1517, https://doi.org/10.1016/j.exger.2013.10.009
25 Macias-Cervantes, M.H., Rodriguez-Soto, J.M., Uribarri, J., Diaz-Cisneros, F.J., Cai, W. and Garay-Sevilla, M.E. (2015) Effect of an advanced glycation end product-restricted diet and exercise on metabolic parameters in adult overweight men. *Nutrition* **31**, 446–451, https://doi.org/10.1016/j.nut.2014.10.004
26 Streese, L., Deiseroth, A., Schafer, J., Schmidt-Trucksass, A. and Hanssen, H. (2018) Exercise, arterial crosstalk-modulation, and inflammation in an aging population: the examin age study. *Front. Physiol.* **9**, 116, https://doi.org/10.3389/fphys.2018.00116
27 Deiseroth, A., Streese, L., Kochli, S., Wust, R.S., Infanger, D., Schmidt-Trucksass, A. et al. (2019) Exercise and arterial stiffness in the elderly: A combined cross-sectional and randomized controlled trial (examin age). *Front. Physiol.* **10**, 1119, https://doi.org/10.3389/fphys.2019.01119
28 Piepoli, M.F., Hoes, A.W., Agewall, S., Albus, C., Brotons, C., Catapano, A.L. et al. (2016) 2016 european guidelines on cardiovascular disease prevention in clinical practice: The sixth joint task force of the european society of cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of 10 societies and by invited experts)developed with the special contribution of the european association for cardiovascular prevention & rehabilitation (EACPR). *Eur. Heart J.* **37**, 2315–2381
29 Ainsworth, B.E., Haskell, W.L., Herrmann, S.D., Meckes, N., Bassett, Jr, D.R., Tudor-Locke, C. et al. (2011) Compendium of physical activities: a second update of codes and MET values. *Med. Sci. Sports Exerc.* **43**, 1575–1581, https://doi.org/10.1249/MSS.0b013e31821ece12
30 Martens, R.J.H., Broers, N.J.H., Canaud, B., Christiaans, M.H.L., Cornelis, T., Gauly, A. et al. (2019) Relations of advanced glycation endproducts and dicarbonyls with endothelial dysfunction and low-grade inflammation in individuals with end-stage renal disease in the transition to renal replacement therapy: a cross-sectional observational study. *PLoS ONE* **14**, https://doi.org/10.1371/journal.pone.0221058
31 Scheijen, J.L. and Schalkwijk, C.G. (2014) Quantification of glyoxal, methylglyoxal and 3-deoxyglucosone in blood and plasma by ultra performance liquid chromatography tandem mass spectrometry: evaluation of blood specimen. *Clin. Chem. Lab. Med.* **52**, 85–91
32 Couppe, C., Svensson, R.B., Grosset, J.F., Kovanen, V., Nielsen, R.H., Olsen, M.R. et al. (2014) Life-long endurance running is associated with reduced glycation and mechanical stress in connective tissue. *Age* **36**, 9665, https://doi.org/10.1007/s11357-014-9665-9
33 Zieman, S.J., Melenovsky, V. and Kass, D.A. (2005) Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler. Thromb. Vasc. Biol.* **25**, 932–943, https://doi.org/10.1161/01.ATV.0000160548.78317.29
34 Powers, S.K. and Jackson, M.J. (2008) Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiol. Rev.* **88**, 1243–1276, https://doi.org/10.1152/physrev.00031.2007
35 Anderson, M.M. and Heinecke, J.W. (2003) Production of n(epsilon)-(carboxymethyl)lysine is impaired in mice deficient in nadph oxidase: a role for phagocyte-derived oxidants in the formation of advanced glycation end products during inflammation. *Diabetes* **52**, 2137–2143, https://doi.org/10.2337/diabetes.52.8.2137
36 Scheijen, J., Hanssen, N.M.J., van Greevenbroek, M.M., Van der Kallen, C.J., Feskens, E.J.M., Stehouwer, C.D.A. et al. (2018) Dietary intake of advanced glycation endproducts is associated with higher levels of advanced glycation endproducts in plasma and urine: The codam study. *Clin. Nutr.* **37**, 919–925, https://doi.org/10.1016/j.clnu.2017.03.019
37 Maessen, D.E., Hanssen, N.M., Lips, M.A., Scheijen, J.L., Willems van Dijk, K., Pijl, H. et al. (2016) Energy restriction and roux-en-y gastric bypass reduce postprandial alpha-dicarbonyl stress in obese women with type 2 diabetes. *Diabetologia* **59**, 2013–2017, https://doi.org/10.1007/s00125-016-4009-1
38 Borg, D.J. and Forbes, J.M. (2016) Targeting advanced glycation with pharmaceutical agents: where are we now. *Glycoconj. J.* **33**, 653–670, https://doi.org/10.1007/s10719-016-9691-1
39 Xue, M., Weickert, M.O., Qureshi, S., Kandala, N.B., Anwar, A., Waldron, M. et al. (2016) Improved glycemic control and vascular function in overweight and obese subjects by glyoxalase 1 inducer formulation. *Diabetes* **65**, 2282–2294, https://doi.org/10.2337/db16-0153
40 Maessen, D.E., Brouwers, O., Gaens, K.H., Wouters, K., Cleutjens, J.P., Janssen, B.J. et al. (2016) Delayed intervention with pyridoxamine improves metabolic function and prevents adipose tissue inflammation and insulin resistance in high-fat diet-induced obese mice. *Diabetes* **65**, 956–966, https://doi.org/10.2337/db15-1390
41 Anderson, E.J., Vistoli, G., Katunga, L.A., Funai, K., Regazzoni, L., Monroe, T.B. et al. (2018) A carnosine analog mitigates metabolic disorders of obesity by reducing carbonyl stress. *J. Clin. Invest.* **128**, 5280–5293, https://doi.org/10.1172/JCI94307