Original article

A physically active lifestyle is related to a lower level of skin autofluorescence in a large population with chronic-disease (LifeLines cohort)

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Abstract

Background: Physical activity (PA) has substantial health benefits and is important in combatting chronic diseases, which have been associated with elevated levels of advanced glycation endproducts (AGEs). AGEs play a role in the aging process, and an association between PA and AGEs has been reported. We aimed to investigate the relationship between PA and AGE accumulation in a general population and in a population with chronic diseases.

Methods: This large cross-sectional population study used data from adult participants in the LifeLines project, with participant information drawn from the LifeLines database as well data from patients with diabetes mellitus or renal and/or cardiovascular diseases. Tissue AGE accumulation was assessed non-invasively by skin-autofluorescence (SAF) using an AGE reader (DiagnOptics Technologies BV, Groningen, the Netherlands). PA was assessed using the short questionnaire to assess health-enhancing physical activity (SQUASH). Multivariate linear regression analyses were adjusted for age, body mass index, sex, and smoking status.

Results: Data from 63,452 participants (general population $n = 59,177$, chronic disease $n = 4275$) were analyzed. The general population was significantly younger (43.58 ± 11.77 years, mean $\pm$ SD) and had significantly lower SAF (1.90 ± 0.42 arbitrary units (AU)) compared to the population with chronic disease (age: 55.51 ± 12.07 years; SAF: 2.27 ± 0.51 AU). In the group with chronic disease, more hours of moderate to vigorous physical activities per week were associated with lower SAF ($\beta = -0.002$, 95% confidence interval (95%CI): -0.002 to -0.001). For the general population, there was no association between hours of moderate to vigorous activity and SAF ($\beta = 3.2 \times 10^{-5}$, 95%CI: $0.000-0.001$, $p = 0.742$). However, there was an association in the general population between total hours of PA per week and SAF ($\beta = 4.2 \times 10^{-4}$, 95%CI: $0.000-0.001$, $p < 0.001$), but this association was not found in the chronic disease population ($\beta = -3.2 \times 10^{-4}$, 95%CI: -0.001 to 0.000, $p = 0.347$).

Conclusion: Our study demonstrates that an inverse relationship exists between PA and AGE accumulation in the population with chronic disease. More hours of moderate to vigorous activity is associated with a significantly decreased SAF. More PA is associated with a lower SAF, even after adjusting for the established predictors (age, body mass index, smoking status, and sex). Our findings could help to promote health and prolong longevity.

Keywords: Advanced glycation endproducts; Chronic disease population; General population; Physical activity; Skin autofluorescence

1. Introduction

Regular physical activity (PA) has substantial health benefits and is a component of chronic-disease prevention and management.[1−3] Conversely, physical inactivity is comparable to smoking and obesity[4] in that it is associated with negative effects on health. The amount of PA needed to convey health benefits ranges from >15 min/day (or 90 min/week[5]) to 150 min/week spread out over 1 or 2 days/week.[6] The current Dutch Health Council recommendation, based on the international guidelines of the World Health Organization, is

to be active at a moderate or vigorous intensity level for at least 150 min/week spread out over several days.[7]

Accumulation of advanced glycation endproducts (AGEs) in long-lived tissues is partially a nonenzymatic, constitutional process, and the level of AGEs increases with age.[8] AGEs are also rapidly formed and accumulated in the human body during glycemic and oxidative stress.[9−11] Chronic diseases, such as diabetes mellitus (DM), chronic kidney disease (CKD), and cardiovascular diseases (CVDs), have been associated with elevated levels of AGEs[8,12−16] and with higher levels of oxidative stress.[17−19] Accumulation of AGEs is associated with negative effects on health, as was first shown in relation to DM[8,14,20] and later extended to CKD.[13]

Skin autofluorescence (SAF) has been proposed as a non-invasive, simple way to assess tissue accumulation due to AGEs. In patients with DM, pre-existing CVD, or renal disease, as well as in the general population, a raised SAF level is a predictor of disease and mortality.[20−23] Furthermore, a higher SAF level is associated with cardiovascular events and mortality in patients with peripheral artery disease.[24] A positive association between SAF and smoking, body mass index (BMI), and glycated hemoglobin levels has been shown in a multi-disciplinary prospective population-based cohort study (the LifeLines cohort).[25] However, the effect of PA on SAF was not taken into account, despite the fact that physical inactivity is a risk factor for DM, CKD, and CVDs.[4]

So far, relatively few studies have analyzed the effect of regular PA on AGE accumulation or SAF and, likewise, few studies have analyzed the association between SAF and life-style-related diseases in a large population. Lower SAF was observed in a small subgroup of 226 healthy persons in a population study of Slovakians who performed physical exercise >30 min/day more than 3 times a week.[26] Also, a decline in serum AGE level was shown for a population of healthy, sedentary, non-smoking, middle-aged women ($n = 47$) after they participated a 12-week lifestyle modification program that included an exercise component.[27] Furthermore, an independent association was found between more training years and lower SAF values in healthy athletes ($n = 182$) compared to sedentary controls ($n = 34$),[28] and life-long endurance training was also associated with lower SAF levels in athletes ($n = 15$) compared to older untrained persons ($n = 12$).[29] A positive association was found between PA and SAF in people with type 1 DM ($n = 119$).[30] However, another cross-sectional study was unable to demonstrate a relationship between PA level and sitting time and SAF in healthy adults ($n = 256$).[31] This finding was in line with the study of Sánchez et al.,[32] who also did not find an association between PA and SAF in middle-aged subjects ($n = 2646$). Because there are discrepancies in the findings of the few previously conducted studies, the association between PA and SAF remains unclear.

The aim of the current study was to investigate the association between PA and SAF in a large population, adjusted for age, BMI, and smoking. We hypothesized that there is an inverse relationship between the level of PA and SAF.

2. Methods

LifeLines is a multi-disciplinary, prospective, population-based cohort study examining, in a unique 3-generation design, the health and health-related behaviors of 167,729 persons living in the north of the Netherlands.[33] It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioral, physical, and psychological factors that contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics.[34] All LifeLines participants provided written informed consent before participating in the study. The Medical Ethical Review Committee of the University Medical Center Groningen approved the LifeLines study.

2.1. Participants

For the current study, we evaluated the data for all participants ≥18 years old, for whom the self-reported short questionnaire to assess health-enhancing physical activity (SQUASH) data on PA, SAF measurements were available, and information about DM, CKD, and CVD was available ($n = 63,452$). Sex, BMI (calculated as kg/m^2), self-reported smoking behavior (*yes* or *no*) and pack-years were retrieved from the LifeLines database. Height was measured to the nearest 0.5 cm and weight to the nearest 0.5 kg. We divided the cohort into 2 groups, one consisting of the general LifeLines population and the other consisting of participants with a chronic disease (we included DM, CKD, and/or CVD). We defined the presence of DM as self-reported and/or based on a fasting blood glucose level of ≥7.0 mmol/L, and/or a glycated hmoglobin level of ≥6.5% (48 mmol/L, and/or the use of glucose-lowering drugs). The presence of CKD was defined as an estimated glomerular filtration rate <60 mL/min, as calculated by the Modification of Diet in Renal Disease formula.[35] The presence of CVD was defined as a self-reported history of heart attack, stroke, aortic aneurysm, balloon angioplasty, and/or bypass surgery, heart failure, and narrowing in one or both carotid arteries. The response "I don't know" to the question regarding heart failure was considered negative.

2.2. SQUASH questionnaire

In the LifeLines cohort, the SQUASH questionnaire was used to estimate the activity level of the participants. This Dutch questionnaire is considered a reliable and valid questionnaire for measuring the level of PA in an adult population.[36] The questionnaire consists of various domains of being physically active: commuting, work, household activities, and leisure time (including walking, biking, gardening, doing odd jobs, and sports). When filling in the questionnaire, participants were asked to keep in mind an average week during the past month. The results of the SQUASH questionnaire were processed as described previously.[36] For our analysis, the total hours per week of PA was used as the main outcome measure. Furthermore, the total hours of moderate-to-vigorous physical activity (MVPA) per week were used in the analysis.

2.3. SAF analysis

SAF was assessed non-invasively by using the AGE Reader (DiagnOptics Technologies BV, Groningen, the Netherlands).

A detailed description and reference values for this method have been reported earlier.[37,38] In short, a skin surface of approximately 4 cm^2 is illuminated by the AGE Reader (DiagnOptics Technologies BV) and guarded against surrounding light, with an excitation light source with a wavelength between 300 nm and 420 nm (peak intensity at 370 nm). An internal spectrometer in the range of 300−600 nm measures the emission light and reflected excitation light from the skin. Measurements were performed by trained staff on the volar site of the left and right forearm, 10 cm below the elbow, at room temperature. To calculate the SAF, the average emitted light intensity per nm (range 420−600 nm) is divided by the average excitation light intensity per nm (range 300−420 nm) and multiplied by 100. SAF is expressed in arbitrary units (AU). An error of approximately 5%, when repeated SAF measurements were taken over 1 day, was found in previous studies.[37] A mean linear increase of 0.023 AU per year of aging was observed in men and women.[38]

2.4. Statistical analyses

Patients' characteristics and outcome measures are shown as mean ± SD, number (%), or median (interquartile range). To assess differences between the general population group and the chronic disease group, an independent t test was performed for age and SAF. A χ^2 test was conducted for sex and smoking behavior, and a Mann-Whitney U test was performed for BMI, total hours of PA per week, and total hours of MVPA per week because of non-normality. A multivariate linear regression analysis was performed to examine the association between PA and SAF, corrected for age, BMI, smoking, and sex. These covariates were selected based on the relation with AGEs, as shown in earlier research.[25] We did not include waist and hip circumference as covariates because BMI was a stronger predictor for SAF. For statistical analysis, IBM SPSS (Version 22.0; IBM Corp., Armonk, NY, USA) was used.

3. Results

A total of 63,452 participants was included for analysis. The general population consisted of 59,177 participants. DM occurred in $n = 2013$, CKD in $n = 696$, and CVD in $n = 1947$ participants, for a total of 4656 diseased participants. More than 1 chronic

disease condition could be present in an individual, but participants were included only once in the chronic disease group, resulting in 4275 unique participants in the chronic disease group. Table 1 provides the characteristics of the study's population. The participants with chronic disease were significantly older compared to the general population (55.51 years old $vs.$ 43.58 years old, $p < 0.001$), had higher BMIs (27.9 kg/m^2 $vs.$ 25.2 kg/m^2, $p < 0.001$), and consisted of more men (52.3% $vs.$ 40.3%, $p < 0.001$). The general population had a significantly lower SAF compared to the chronic disease population (1.90 AU $vs.$ 2.27 AU, $p < 0.001$). The general population had significantly more total hours PA (48.3 h/week $vs.$ 40.0 h/week, $p < 0.001$); however, the chronic disease group had significantly more total hours of MVPA (10.4 h/week $vs.$ 6.3 h/week, $p < 0.001$). The chronic disease population had a significantly higher amount of packyears (6.5 packyears $vs.$ 0.4 packyears, $p < 0.001$). There were no relevant differences in the characteristics between those with and without an available SQUASH questionnaire and SAF measurement.

The results of the linear regression analysis showed that SAF was independently associated with total hours of PA per week ($\beta = 4.2 \times 10^{-4}$, 95% confidence interval (95%CI): 0.000−0.001, $p < 0.001$) after adjusting for age, sex, BMI, and packyears (Table 2). In the chronic disease group, there was no independent association between SAF and total hours of PA per week ($\beta = -3.2 \times 10^{-4}$, 95%CI: −0.001 to 0.000, $p = 0.347$). When looking at the MVPA (Table 3), it is clear that there was no independent association between SAF and MVPA hours per week for the general population ($\beta = 3.2 \times 10^{-5}$, 95%CI: 0.000−0.001, $p = 0.742$). However, for the chronic disease group there was an independent association between SAF and MVPA hours per week ($\beta = -0.002$, 95%CI: −0.002 to −0.001). Also, sex showed no independent association with SAF ($p = 0.192$).

4. Discussion

This is the first study to address the possible relationship between PA and SAF as an accepted marker of AGE accumulation in a very large and representative population cohort. This study shows an association between MVPA and SAF, after adjusting for the established predictors (age, BMI, packyears, and sex) for the chronic disease group. A lower SAF is

Table 1
Characteristics of the study population.

	Total	General	Disease
n	63,452	59,177	4275
Age (year)	44.38 ± 12.61	43.58 ± 11.77	55.51 ± 12.07*
Male (%)	41.1	40.3	52.3*
Body mass index (kg/m^2)[‡]	25.4 (23.1−28.1)	25.2 (23.0−27.9)	27.9 (25.2−31.0)*
Packyears[‡]	0.60 (0.00−9.00)	0.40 (0.00−8.50)	6.50 (0.00−19.50)*
Smoking status (packyears)[‡]			
Current smoker	14.77 ± 11.38	14.26 ± 11.02	22.72 ± 13.80
Former smoker	9.81 ± 9.98	9.10 ± 9.05	16.34 ± 14.70
Total PA (h/week)	48.0 (32.3−59.8)	48.3 (33.3−60.0)	40.0 (23.0−54.8)*
MVPA (h/week)	6.5 (2.5−18.8)	6.3 (2.5−18.0)	10.4 (3.8−24.8)*
Skin autofluorescence (AU)	1.92 ± 0.44	1.90 ± 0.42	2.27 ± 0.51*

Note: Data are presented as mean ± SD, or median (interquartile range), or percentage (%).
[‡] Missing values for body mass index ($n = 12$), smoking status ($n = 2212$), packyears ($n = 2138$).
* $p < 0.001$ for differences between general and disease groups.
Abbreviations: AU = arbitrary units; MVPA= moderate-to-vigorous physical activity; PA = physical activity.

Table 2
Multivariate linear regression with SAF as the dependent variable and total hours of PA as a covariate for PA.

	General group			Chronic disease group		
	β	SE	95%CI	β	SE	95%CI
Constant	1.002	0.011	0.981−1.023	1.043	0.057	0.930−1.155
Age	0.018*	0.000	0.018−0.018	0.017*	0.001	0.016−0.019
Male	REF			REF		
Female	−0.039*	0.003	−0.044 to −0.033	−0.007	0.014	−0.034 to 0.020
BMI	0.003*	0.000	0.002−0.003	0.007*	0.001	0.004−0.009
Packyears	0.009*	0.000	0.009−0.010	0.007*	0.000	0.006−0.008
Total PA	4.2×10^{-4}*	0.000	0.000−0.001	-3.2×10^{-4}	0.000	−0.001 to 0.000

Notes: For the general population: adjusted R square = 0.358, $p < 0.001$. For the chronic disease population: adjusted R square = 0.255, $p < 0.001$.
* $p < 0.001$ indicates a significant contribution to the regression model.
Abbreviations: 95%CI = 95% confidence interval; BMI = body mass index; PA = physical activity; REF = reference; SAF = skin autofluorescence.

seen when people perform more hours of moderate-to-vigorous activities per week. This study also showed an association between total hours of PA per week and SAF for the general population; however, the contribution of total hours of PA was small ($\beta = 4.2 \times 10^{-4}$). Because the mean SAF increases by 0.02 AU per year of aging,[38] an increase of 4.2×10^{-4} cannot be considered clinically relevant.

Our findings in the general population confirm the earlier results of 2 smaller cross-sectional studies in adults ($n = 256$) and in people who are at risk for a CVD ($n = 2636$). Neither of these 2 studies found an association between SAF and PA.[31,32] In these 2 studies, PA was assessed by a questionnaire and measured in metabolic equivalent of task-minutes per week, which is in line with the MVPA hours per week used in our study. Another study of an elderly population ($n = 4188$) also did not find an association between PA energy expenditure and skin AGEs.[39]

Other studies, however, have found an association between PA and SAF. A smaller cross-sectional study of young healthy non-smokers observed a lower SAF in subjects who performed physical exercise for more than 30 min/day more than 3 times a week compared to people who exercised 1−2 days a week or not at all.[26] Furthermore, there was a decline in serum AGEs levels among healthy sedentary non-smoking middle-aged women who participated in a 12-week lifestyle-modification program that had an exercise program as a component.[27]

These contradictory findings might be caused by the different measures of PA used in the studies. Our study used total hours of PA per week and total hours of MVPA per week. These variables do not provide information about the division

of the active hours over a week. This might be essential because studies have shown that the so-called weekend warrior, who is physically active for only 1 or 2 days a week, already has a reduced risk of disease and mortality.[6,40] The SQUASH questionnaire we used contains a question about how many days per week a person is active for at least 30 min of moderate intensity; however, this question is harder to answer when individuals are asked to estimate accurately the number of hours spent per activity. More research is needed to provide detailed information about the hours of activities per day that individuals participate in to determine whether the division of active hours influences SAF.

The results of our study for the chronic disease group extend the findings of previous studies over a wider age range and across multiple chronic diseases (not only in type 1 DM, but also type 2 DM, renal disease, and CVD). One study found lower SAF in a small group ($n = 119$) of people with type 1 DM who performed more PA.[30] In another study involving elderly participants (≥65 years old), higher SAF was found in those with lower PA.[41] In that study, the high-SAF group (≥2.56 AU) had fewer active days per week (4.53 days) of activity compared to the low-SAF group (≤2.19 AU; 4.94 days). Accumulation of AGEs as measured by SAF has been shown to be an independent predictor of mortality and/or cardiovascular morbidity in persons with DM, renal failure, or pre-existing CVD[13,21,22,24,42] and also in the general population.[23] Thus, reduction of AGE accumulation through PA, even if the effect on SAF is small, might be beneficial and contribute to a better health status. More research is needed to

Table 3
Multivariate linear regression with SAF as the dependent variable and MVPA as a covariate for PA.

	General group			Chronic disease group		
	β	SE	95%CI	β	SE	95%CI
Constant	1.025	0.010	1.005−1.044	1.030	0.051	0.930−1.130
Age	0.018*	0.000	0.018−0.018	0.018*	0.001	0.017−0.019
Male	REF			REF		
Female	−0.039*	0.003	−0.045 to −0.034	−0.019	0.014	−0.047 to 0.009
BMI	0.003*	0.000	0.002−0.003	0.007*	0.001	0.004−0.009
Packyears	0.009*	0.000	0.009−0.010	0.007*	0.000	0.006−0.008
MVPA	3.2×10^{-5}	0.000	0.000−0.001	−0.002*	0.000	−0.002 to −0.001

Notes: For the general population: adjusted R square = 0.357, $p < 0.001$. For the chronic disease population: adjusted R square = 0.257, $p < 0.001$.
* $p < 0.001$ indicates a significant contribution to the regression model.
Abbreviations: 95%CI = 95% confidence interval; BMI = body mass index; MVPA = moderate-to-vigorous physical activity; PA = physical activity; REF = reference; SAF = skin autofluorescence.

investigate whether reduction of AGE accumulation through PA could (partially) explain the demonstrated effects of exercise as medicine in people with chronic diseases.[43]

5. Strengths and limitations

LifeLines is a large cohort, consisting of a general population as well as diseased people, from all age groups. The data collection is robust and extensive. PA was measured with the validated SQUASH questionnaire. To our knowledge, LifeLines is the largest cohort in which AGE accumulation by SAF has been assessed. Therefore, this is an excellent cohort to test associations between AGE accumulation and health behaviors and the presence of chronic diseases. The LifeLines dataset contains reliable data on numerous confounders, of which the most relevant and well-known have been used in our study.

In our study, the general population consists of people without DM, CKD, or CVD. However, this does not mean that participants do not have any other diseases. DM, CKD, and CVD have been associated with elevated levels of AGEs,[8,12,13] but we cannot rule out the influence of other diseases. Another limitation in our study is that we cannot exclude the possibility that PA may be related to differences in dietary patterns and, thus, be a factor influencing SAF.

Self-reported PA itself is prone to the provision of socially acceptable answers and, therefore, may overestimate an individual's level of activity. This possibility, however, would have led to an underestimation of the true effect of PA on SAF. Because the SQUASH questionnaire is short and easy to fill in, it is the most cost-effective and feasible way to measure PA in a large population and is reliable and valid,[36] we consider the results from the SQUASH questionnaire to be a good representation of the PA patterns among participants in the LifeLines project.

Our cross-sectional design has obvious limitations. It allows associations only between SAF and PA to be identified, as was the case in previously mentioned literature.[26,30,31,41] The decades-long lag time between (un) healthy behaviors and AGE accumulation renders interventional research challenging, if not impossible. Because SAF measures the AGE accumulation in the skin from the past 15 years,[44] previous behaviors that are not reflected in the LifeLines data could have influenced AGE accumulation significantly. The level of PA that was used for our model is a snapshot of the current activity level of participants, which has not necessarily been the same for the previous 15 years. Future research should incorporate the history of PA and sitting time in order to further investigate the effect of PA on the accumulation of AGEs.

6. Conclusion

This study demonstrates, in a very large and representative population cohort, an inverse relation between PA and AGE accumulation in a population with chronic disease. More PA is associated with a lower SAF, even after adjusting for the established predictors (age, BMI, smoking status, and sex) and could help to promote health and prolong longevity. Our study shows that more hours of moderate-to-vigorous activity is associated with a significantly decreased SAF for the group with chronic disease.

Acknowledgments

Authors' contributions

SCZ contributed to the study design, analyzed the data, and drafted the manuscript; AJS and JZ contributed to the study design; JKV analyzed the data and drafted the manuscript; IAS helped in analyzing the data. All authors contributed to the interpretation of the results and revised the manuscript for important intellectual content. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Competing interests

AJS is founder and shareholder of Diagnoptics Technologies, the company that develops the AGE Reader. Diagnoptics Technologies had no role in the design of the LifeLines project or in the analyses conducted for this study. It provided no funding and exerted no restrictions of any sort in the publication of information concerning the AGE Reader.

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