



# Formation of Advanced Glycated End-Products can be Counteracted by Daily Physical Activity, an Observational Study in Dutch Elementary School Children



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## Abstract

High consumption of carbohydrates is linked to metabolic syndrome, possibly via the endogenous formation of advanced glycated end-products. Many Dutch elementary school children have a carbohydrate intake of >130g/day, the estimated minimum requirement.

In this observational study, 126 Dutch elementary school children (5-12y of age) from two schools differing in frequency of gym lessons (2 or 5 times a week) were included.

In all participants, height, weight, waist circumference, autofluorescence of skin glycated end-products (AGE-score), sports activity and carbohydrate consumption were recorded once. Sports activities in leisure time differentiated participants in 'sportsmen' and 'non-sportsmen'.

Carbohydrate intake and AGE score were positively associated in non-sportsmen ( $p < 0.003$ ), but negatively in sportsmen ( $p < 0.002$ ). In sportsmen, but not in non-sportsmen ( $p > 0.50$ ), a positive association was found ( $p < 0.002$ ) between carbohydrate intake and subject age. The intake of total carbohydrate and carbohydrates from juices and soft drinks was lower ( $p < 0.001$ ) at the Wassenberg School relative to the Alexander School. Based on waist to height ratio, >95% of the children had normal fat mass. No correlations were found between waist to height ratio or BMI and carbohydrate intake. Waist to height ratio was positively associated with BMI ( $p < 0.001$ ) and subject age ( $p < 0.001$ ). Of all principal parameters, AGE score is most affected by being sportsmen or not ( $p < 0.001$ ).

This study indicates that an increased intake of carbohydrates can be counteracted by sufficient physical activity (>2.5 hours per week). This implies that skin autofluorescence is a fast and non-invasive method to screen children for life style.

**Keywords:** School children; Auto fluorescence; Physical activity; Carbohydrate intake; Anthropometry

**Abbreviations:** SSB: Sugar Sweetened Beverages; EFSA: European Food Safety Authority; IOM: US Institute of Medicine; AGEs: Advanced Glycation End-Products

## Introduction

High consumption of carbohydrates, and in particular free sugars (monosaccharides and disaccharides added to food or those naturally present in honey, syrups and fruit juices [1]), is linked to metabolic syndrome [2]. For that reason alone, the steep increase in carbohydrate consumption during the last 200 years is frightening. In England, sucrose consumption rose from 6.8kg in 1815 to 54.5kg per capita in 1970. In the US, the intake of total refined carbohydrates (sucrose, high fructose corn syrup and glucose) increased from 55.5kg in 1970 to 69.1kg per capita in 2000. Similar trends have been described for other countries that went through this Industrial Era [3]. For Dutch children (2-6 years of age), the Food Consumption Survey [4] indicated a mean intake of 75kg of carbohydrates

per year of which 46.7kg consisted of mono and disaccharides. The mean contribution to total energy intake was calculated to be 57%. Based on a food survey from 2007-2010 [5] amongst Dutch children aged 7-11 years, about 17.5kg of mono- and disaccharides are consumed via sweetened drinks and pure fruit juices, with the majority coming from the sweetened drinks.

These sugar sweetened beverages (SSB) are commonly consumed in many countries in rather high volumes [6]. In children, a high consumption of SSB is associated with higher asthma prevalence, increased levels of high-sensitivity C-reactive protein, increased risk of type 2 diabetes, and obesity [6-8]. The average consumption of SSB in a study in

Dutch school children (n=322, 3-7 years of age) was about 800ml per day according to the parents [9], and replacement of one SSB serving per school day over a period of 18 months with a non-caloric drink reduced weight gain by 1kg [10] as compared to the control group.

Dietary guidelines set by the European Food Safety Authority (EFSA) and US Institute of Medicine (IOM), suggest that no more than 20-25% of the total daily energy intake should come from added sugars (sucrose, fructose, glucose, starch hydrolysates, i.e. glucose syrup and high-fructose syrup, and other isolated sugar preparations used as such or added during food preparation and manufacturing) [11,12]. Thus, children 7-10 years of age with an average daily energy requirement of 2000kcal [13] are recommended to not consume more than 100-125g of these added sugars per day. This amount is also close to the estimated glucose requirement of the brain, 130g/day, which is considered to represent the minimum carbohydrate requirement for 97.5% of children from 1 year of age and onwards [11]. The latest WHO [1] recommendation limits the intake of free sugars (more detailed than added sugars) to only 5-10% of total daily calories (25-50g per day). In other words, overconsumption of carbohydrates beyond the minimum requirements, and in particular of added or free sugars, is apparent in The Netherlands.

High or increased carbohydrate intake results in increased blood glucose concentrations (glycemic index and load dependent) and the subsequent release of insulin. Long term (chronic) frequent increases in blood glucose concentrations induce insulin resistance being the primary cause for metabolic syndrome [3]. High blood glucose levels are also associated with increased non-enzymatic glycation of proteins finally leading to the advanced glycation end-products (AGEs). Formation of AGEs is accelerated by oxidative stress, and accumulation occurs in areas rich in extracellular matrix proteins, including the skin. Interaction of AGE with its cell receptor (RAGE) may stimulate inflammation and growth factor production that, among others, sustains the development of atherosclerotic plaques [14-16]. AGEs are also linked to degenerative diseases, including diabetes and Alzheimer, and may lead to serious microvascular complications [17,18].

Blood glucose is used as an energy source in muscles. Uptake of glucose by muscle tissue follows insulin and glycogen dependent routes [19,20]. Physical activity (glycogen depletion) is known to upregulate the plasma membrane glucose transporter GLUT4 protein in an insulin independent fashion. This leads to a higher uptake of glucose in the cell and in the longer term to an increase in insulin sensitivity [21,22]. Therefore, with increasing physical activity, the body should be able to cope with higher intakes of carbohydrates without stimulating AGE formation. In contrast, a sedentary lifestyle negatively affects mitochondrial activity and increases oxidative stress leading to AGE formation [23].

Therefore, the level of AGEs formation/accumulation can be used as a risk marker for the deleterious effects of hyperglycemia and oxidative stress. It has been found that AGEs in the skin, as measured by autofluorescence, are predictive for the progression of microvascular complications in type-1 diabetes patients, including children, and are positively correlated with glycated hemoglobin (HbA1c) [18]. In other words, skin AGEs might be a perfect non-invasive parameter to screen subjects for their glycation status and related risks, and correlates with N-carboxymethyl lysine and collagen fluorescence (328/378 nm and 370/440nm excitation/emission) as measured in skin biopsies [15,24]. Skin autofluorescence can be measured using a portable device (AGE Reader) that uses an excitation light source of 300-420nm (peak excitation ~350nm), in combination with spectrophotometric measurement (300-600nm) [24]. The read-out of the AGE Reader is referred to as the AGE score.

In the present study, the effects of carbohydrate intake and physical activity on autofluorescence (AGE-score) of the ventral site of the lower arm and on anthropometric parameters of fat mass were studied in Dutch school children (5-12y) recruited from two elementary schools. The schools differed in mean carbohydrate intake as well as in the level of daily physical activity. It is hypothesized that daily sports activity can counteract AGE formation, due to carbohydrate intake, in the skin of children.

## Material and Methods

### Study design

The present observational study was conducted in 126 Dutch elementary schoolchildren [5-12 y] from two different schools, one studied in autumn 2015 (Alexander School, n=61) and the other in autumn 2016 (Wassenberg School, n=65). Both schools are located in Leeuwarden, The Netherlands, with a comparable target population in terms of social economic status. For both schools, the same equipment and methodology were used and measurements were performed by the same researcher. Schools were different in total carbohydrate intake (lowest at the Wassenberg School) and frequency of gym classes (45 minutes each class; daily at the Wassenberg School versus twice weekly at the Alexander School).

No selection criteria were applied; however, non-Caucasian children could not be included in data evaluation as the Age Reader is not capable of measuring AGE in dark skin. Children 5-12y of age, received an informative letter for the parents as well as an informed consent form. When parents agreed on the participation of their child, the signed informed consent form was collected at school and the child was registered as a participant. From all registered participants, anthropometric data (height, weight, and waist circumference) were collected and skin AGE scores were measured. Information on sports activity and carbohydrate consumption was collected using a

questionnaire. The anthropometric data and AGE score were measured in a single session at a secluded location at school during regular school days. A questionnaire on food habits, carbohydrate intake and physical activity was filled out by the researcher at school in a face-to-face interview with the child. Parents were asked to fill out the questionnaire at home. Per child, it took about 20-30 minutes to complete the data collection. Based on the information on physical activity, those who were involved in sports activities outside of school were called 'sportsmen', whereas those who were exclusively subjected to sports activities during gym classes at school were referred to as 'non-sportsmen'.

As a pilot, a sub group of children  $\geq 9$  y of age at the Wassenberg School was asked to wear an Actiwatch (accelerometer) to obtain data on their sleep quality. Higher physical activity should lead to better sleep, measured as sleep efficiency.

This study was carried out in accordance with the Helsinki Declaration as revised in 2013 [25]. Registration in a publicly accessible database did not take place as this was an observational study.

### AGE-reader

Skin autofluorescence as a parameter of skin AGEs was measured with the AGE Reader (DiagnOptics Technologies BV, Groningen, The Netherlands), a portable advanced spectrophotometer. The outcome of this measurement is called the AGE score. The AGE reader uses an excitation light source of 300-420nm and spectrophotometric (300-600nm) measurement of autofluorescence of skin AGEs [26] (up till the papillary region of the dermis) at the ventral site of the lower arm. The measurement is fully automated and only takes 30seconds [15]. However, if too much melanin is present in the skin, or the skin has imperfections, such as moles or eczema, the AGE-reader cannot perform an optimal scan due to excessive reflection. Thus, only light skinned children with healthy/clear skin can be evaluated. In the present study, the child was asked to sit down, roll up the right arm sleeve (if needed), and to remove any obstructive elements from the arm. To prevent a disturbance of ointments or body lotion, the skin was cleaned with a paper tissue and 70% ethanol, and air-dried. In a fully relaxed position the ventral site of the right lower arm was placed on the scanning side of the AGE-reader. To compensate for the small width of the arms of the children and to prevent accidental light contamination, a black cloth was draped over the arm during measurements. Relaxed state was checked by making sure the child's hand was in a limb state and the body didn't appear to be in any kind of physical distress. The AGE-score was calculated by taking the average of three independent AGE-reader scans rounded to the nearest decimal.

### Anthropometric measurements

Children were weighed on a non-medical grade scale in their casual clothing, but with the removal of heavy objects from their pockets and or heavy over clothing when possible. Weight was recorded to the nearest 100g. Standing height was obtained by measuring the child without shoes, using a cm tape measure attached vertically to the wall. Subjects stood on a flat surface with their heels, buttocks, upper back and head against the wall and with their arms hanging loosely by the side. To prevent misreading of height due to the child's hair, a hardcover atlas was gently placed on top of the head under a 90degree angle with the wall. Height was rounded to the nearest cm. Waist circumference was measured by using an auto retracting body measuring tape (Body Tape Measure, Gima S.p.A., Gessate (M), Italy). The tape was placed over the clothing around the thinnest part of the waist between the ribs and the hips, secured and retraced and the result rounded to the nearest 0.1cm. Waist to height ratio (cm/cm) and BMI (kg/m<sup>2</sup>) were calculated. For classification, calculated BMIs and waist to height ratios were compared with the reference BMI values of Dutch children [27] and reference waist to height values as reported by Schneider et al. [28]. In this way, children were classified as severe underweight, underweight, healthy weight, overweight and severe overweight based on BMI, and extremely slim, healthy slim, healthy, overweight or very overweight based on waist to height ratio.

### Questionnaires

Two questionnaires, a child and an adult (parents') version, for food intake, physical activity and sleep were designed for this study. Both questionnaires presented the same questions, but the language was adapted to the target group. Gathered information was based on a 7-day recall of the last week or an average week. Detailed questions on exercise and sleep were only asked at the Wassenberg School. At the Alexander group, only 'sports activity in leisure time' was recorded.

The nutrition part of the questionnaire focused on the consumption of carbohydrate rich in-between foods, drinks during the day, and main meals. A variety of drinks and foods were categorized and grouped by their caloric contents per 100g, vegetable spoon, or pieces. Drinks were grouped in: soda, fruit juice, lemonade, fortified milk drinks and milk. Subgroups were created when light or zero kcal options were available. Based on the carbohydrate rich components, main meals were grouped as potato, rice, pasta, brown rice and whole wheat pasta. Data was collected on how often a given starchy food was consumed in an average week and how many vegetable spoons were consumed of that starchy food. Bread meals were grouped as whole grain, white/brown bread and cereals. Candy and cookies were measured loosely and only by type due to very large variety of candy available and the unawareness of children and parents to indicate exact caloric

contents and amounts given/eaten. The food questionnaire provided an estimate of the amount of carbohydrates consumed over 7 days, in total, and from the different drink and food groups.

The exercise part of the questionnaire focused on the child's physical activities in leisure time and general level of activity during the day. A variety of the most common sports activities was presented in the questionnaire, including a blank space for any missed or unusual sports. Based on the average burned kcal per hour, the intensity level of sports activity was classified in 4 classes: 1= no/low activity (no sport or sitting sports), 2 = moderate activity (dancing, horseback riding, and ballet), 3 = active activity (soccer, aerobics, biking) and 4 = very active activity (basketball, hockey, swimming, athletics and self-defence sports). General physical activity was measured subjectively by asking about details on reading, watching TV, playing and intensity of playing, and free time cycling. As mentioned, at the Alexander school only sports in leisure time without any details on intensity or other daily activity was included. A positive answer on the 'sports in leisure' question was sufficient for allocation to the group 'sportsmen'.

The sleep part of the questionnaire provided general insight in sleep habits of the child, by asking how the child felt after waking up and the time it took to become mentally and physically awake. Only the parents were asked about the time it took the child to fall asleep and was used to estimate fatigue and regularity of bedtimes.

**Actiwatch**

Data on sleep quality was assessed in a sub-cohort of children by using an accelerometer (Philips Respironics, Actiwatch-2, and Murrysville, PA, USA). This is a watch-sized solid-state "Piezo-electric" device capable of recording body movements by tracking fluctuations in G-force varying

between 0.5 and 2.0G with a sensitivity of 0.025G. The device is waterproof to IPX7 standards and is modelled for ease of use and durability. Users of the device can not alter the settings or observe results registered by the device without a docking station, thus preventing them from falsifying or altering records. Children had to wear the watches for a full week and data was only processed if at least 3 uninterrupted, consecutive days were registered. When possible or necessary, the children were briefly interviewed regarding the results the day after retrieving the watches. Sleep quality was expressed as sleep efficiency [(total sleep time - time wake after sleep onset/total sleep time) \*100].

**Statistical Evaluation**

Changes in parameters between groups were evaluated using relevant parametric statistical tests, such as independent T-test, One-Way ANOVA. Correlations between different parameters were assessed by using parametric Pearson correlation test. Simple or multivariate linear regression was used to test the relationship between parameters. Relevant confounders used in multivariate linear regression were school, time (year), BMI, carbohydrate intake, gender, and being a sportsman or not.

Data evaluation was performed using SPSS (SPSS 18.0, IBM, New York, USA) and STATA (version 2, Stata Corporation, College Station, Texas, USA).

**Results**

An overview of characteristics, parameters and outcomes per school and per category of sportsmen and non-sportsmen is presented in Table 1. A total of 126 children was evaluated; 65 (59 sportsmen and 6 non-sportsmen) from the Wassenberg school and 61 (26 sportsmen and 35 non-sportsmen) from the Alexander school.

**Table 1:** An overview of the several parameters in sportsmen and non-sportsmen (no leisure time sports activities), based on single measurements in an observational study in Dutch elementary schoolchildren from two different schools (Wassenberg School and Alexander School).

Parameter	Wassenberg School				Alexander School			
	Sportsmen	Non-sportsmen	All children	p-value	Sportsmen	Non-sportsmen	All children	p-value
N=	59	6	65		26	35	61	
Subject age (y)	8.5±1.8c	9.3±2.7	8.6±1.9e	0.331	9.5±1.4c	9.2±1.4	9.3±1.4e	0.372
Total carbohydrates (g/week)	623±168a	678±105d	629±163a	0.387	1140±221a	1140±424d	1140±349a	0.997
Carbohydrates from juice-soft drinks (g/week)	136±75a	93±41d	132±73a	0.259	554±178a	530±246d	540±218a	0.671
AGE-score	1.03±0.13c	1.02±0.12e	1.02±0.13	0.946	0.95±1.4c	1.11±0.10e	1.04±0.13	0
Weight (kg)	31.9±6.9b	34.1±9.3	32.1±7.1b	0.474	37.3±9.5b	35.4±9.8	36.2±9.6b	0.443
Height (cm)	139.1±13.1b	145.8±19.1	139.8±13.7c	0.286	148.1±14.1b	144.0±10.3	145.8±12.1c	0.192
Waist (cm)	59.7±3.4d	60.3±4.0	59.8±3.5d	0.76	63.6±5.9d	62.8±9.5	63.1±8.1d	0.701
Waist to Height (cm/cm)	0.43±0.04	0.42±0.04	0.43±0.04	0.396	0.43±0.04	0.44±0.06	0.43±0.05	0.751

Extremely slim (%)	0	0	0		3.85	5.71	4.92	
Healthy slim (%)	76.27	83.33	76.92		65.38	65.71	65.57	
Healthy (%)	20.34	16.67	20		30.77	20	24.59	
Overweight (%)	3.39	0	3.08		0	8.57	4.92	
Very overweight (%)	0	0	0		0	0	0	
BMI	16.3±2.0	15.8±1.0	16.3±1.9	0.549	16.8±2.1	16.8±2.9	16.8±2.6	0.936
Severe underweight (%)	1.69	0	1.54		0	0	0	
Underweight (%)	3.39	0	3.08		3.85	0	1.64	
Healthy (%)	76.27	100	78.46		84.62	82.86	83.61	
Overweight (%)	16.95	0	15.38		11.54	14.29	13.11	
Obese (%)	1.69	0	1.54		0	2.86	1.64	
Sleep efficiency (%)	96.9±1.5 (n=17)	93.3±5.0 (n=5)	96.1±3.0	0.187				
Sleep hours	9.0±0.6 (n=17)	8.7±0.6 (n=5)	9.0±0.6	0.274				

Data are expressed as Mean ± SD.

Significant differences between sportsmen and non-sportsmen per school are indicated in a separate column in the table. Significant differences between schools, as well as between sportsmen and non-sportsmen sub-categories, are indicated by the same letter: a: p<0.001; b: p<0.01; c: p<0.02; d: p<0.006; e: p<0.05

BMI cut-off values for children 5-12 y are derived from the Dutch national growth diagrams [27].

Waist to height ratio classifications for children (0-15 y) are derived from Schneider et al. [28]: extremely slim (<0.35), healthy slim (0.35-0.40), healthy (0.46-0.51), overweight (0.52-0.63), and very overweight (>0.63).

For the Wassenberg School, activity score for non-sportsmen was zero since this score is based on leisure time sports activities only. The activity score is also not available for the Alexander School since only sports in leisure time and not the intensity was monitored. Sportsmen from the Wassenberg School were younger, lighter and shorter than sportsmen from the Alexander school. The absolute weekly total carbohydrate intake as well as carbohydrate intake from juices and soft drinks was lower (p<0.001) in children from the Wassenberg School as compared to those from the Alexander School (Table 1).

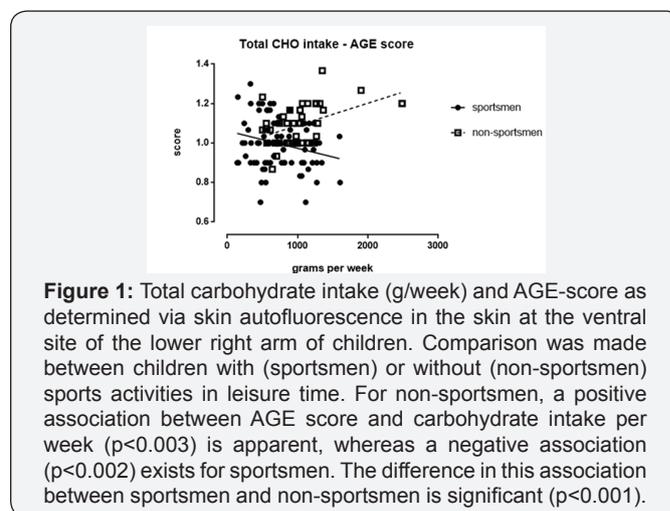
### Age score

The mean AGE-score was significantly higher in non-sportsmen than in sportsmen at the Alexander School. In contrast, this difference did not exist at the Wassenberg School. Between schools, the mean AGE score of sportsmen was higher at the Wassenberg School than at the Alexander School, whereas this score was lower in the non-sportsmen from the Wassenberg School relative to the non-sportsmen from the Alexander school (Table 1).

### Total carbohydrate intake versus AGE score

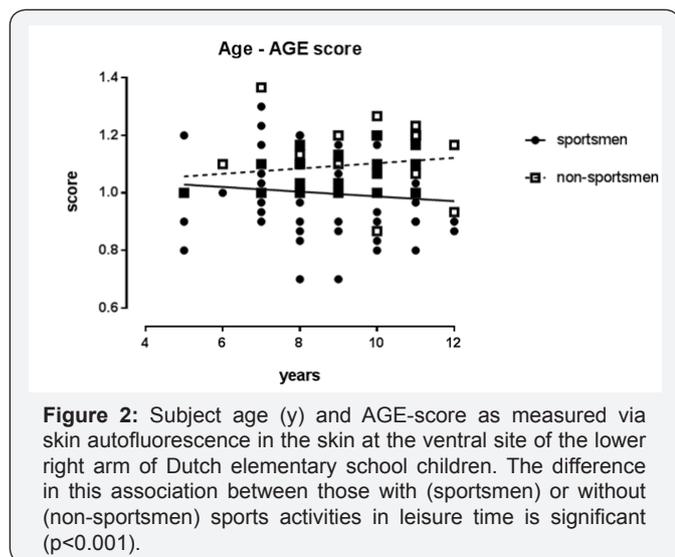
Without distinction between sportsmen and non-sportsmen, no association is observed between the amount of carbohydrates consumed per week and AGE score (p>0.10).

However, when data are split into sportsmen and non-sportsmen (Figure 1), a positive association exists between carbohydrate intake per week and AGE score for the non-sportsmen (p<0.003), in contrast to a negative association for sportsmen (p<0.002). Moreover, there is a significant difference between sportsmen and non-sportsmen regarding the effect of carbohydrate intake on AGE score (p<0.001). This is also reflected in the absolute difference in AGE score between both groups (p<0.008), with higher AGE score levels in non-sportsmen as compared to sportsmen.



### Subject age versus AGE score

Without distinction between sportsmen and non-sportsmen, no association ( $p > 0.99$ ) exists between subject age and AGE score. However, a significant difference was observed when comparing sportsmen and non-sportsmen ( $p < 0.001$ , Figure 2). Thus, in sportsmen, higher age was correlated with lower AGE scores, whereas the opposite was true for non-sportsmen. Separate associations (subject age of sportsmen and non-sportsmen with AGE score) did not yield significance ( $p > 0.30$ ).



### Carbohydrate intake versus waist to height and BMI

Classification based on the waist to height ratio (fat mass indicator) shows that children from the Wassenberg School and Alexander School appear to be healthy, meaning  $>95\%$  had a normal fat mass or were even slim (Table 1). Although carbohydrate intake by sportsmen and non-sportsmen from the Alexander School was significantly higher relative to the Wassenberg School, no correlation was found between the waist to height ratio and carbohydrate intake in any of the groups. Also, no differences existed in mean waist to height ratio between the sportsmen and non-sportsmen of either school (Table 1). Waist to height ratio was positively associated with BMI ( $p < 0.001$ , the higher the BMI the higher the waist-to height ratio), and inversely associated with subject age ( $p < 0.001$ , the higher the age the lower the waist-to height ratio).

With regard to BMI, no associations were found with total carbohydrate intake ( $p > 0.10$ ). The same is true when a distinction is made between sportsmen and non-sportsmen. A significant association was observed between BMI and subject age ( $p < 0.003$ ), the older the subject the higher the BMI.

### Carbohydrate intake and subject age

Overall, a positive association exists between total carbohydrate intake and subject age ( $p < 0.004$ ), but when

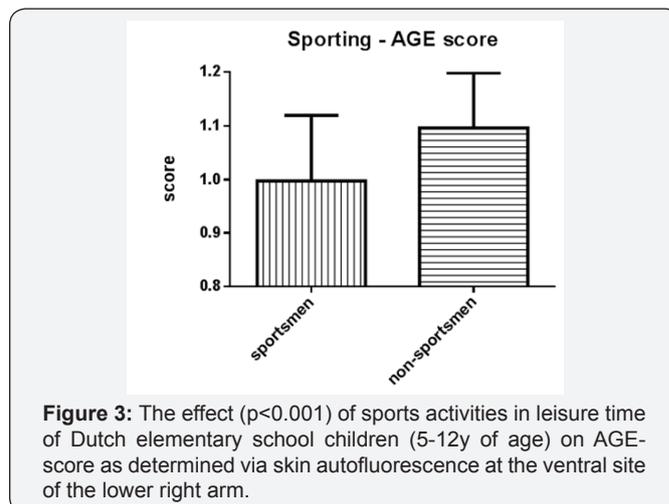
groups were studied separately, this linear association was only observed in sportsmen ( $p < 0.002$ ), not in non-sportsmen ( $p > 0.50$ ). Both groups are significantly different from each other with regard to this association ( $p < 0.01$ ).

### Activity score versus sleep efficiency

In this sub group, limited in number and only derived from the Wassenberg School, as well as for the sportsmen separately, no association was found between the activity score and sleep efficiency ( $p > 0.45$ ). Overall, sleep efficiency was high:  $96.1 \pm 3.0\%$ . Age of children was inversely related to sleep efficiency ( $p < 0.04$ ).

### Parameter with the strongest impact on AGE-score

When evaluating every principal parameter in one model, AGE score in particular relies on being a sportsman or not (Figure 3). In those with sports activities in leisure time the AGE score is significantly ( $p < 0.001$ ) lower than without these sports activities. No additional impact was found for school, total carbohydrate intake, BMI, gender and age.



### Discussion

In the present study, skin autofluorescence (AGE score) was measured in Dutch school children (age 5-12y) from two elementary schools. Children from these schools differed in mean intake of total carbohydrates per week and in the number of gym classes taken at school, 5 versus 2 times a week. Based on leisure time sports activities, the children were classified as 'sportsmen' or 'non-sportsmen'. Despite several differences between schools, the study clearly shows that the impact of daily physical activity on AGE score is significant. Daily physical activity also determines the difference in the relation between waist to height ratio and body weight, and between subject age and AGE score. Surprisingly, total carbohydrate intake as only related with the AGE score for the non-sportsmen.

### AGE score, carbohydrate intake and physical activity

The formation of glycated proteins, lipids and nucleic acids (AGEs) is stimulated by hyperglycaemia and oxidative stress

[29]. Nevertheless, an increase in skin AGEs is considered as a normal feature of ageing and has been translated into a reference line for the ages of 0 to 80y, (n=231) based on subjects from an outpatient clinic for pre-surgery screening. Most participants were adults, with the number of included infants and children being very low [30]. Based on these reference values, the skin AGE score is used to check lifestyle of adult diabetic patients and to identify an increased risk of cardiovascular diseases in vulnerable target groups. Furthermore, the AGE score is associated with metabolic syndrome and its individual components [29]. Since this method is non-invasive and fast, it might be an excellent way to screen populations on lifestyle and related risks or benefits. In the present study, we investigated whether the assumed high intake of carbohydrates by Dutch elementary school children was reflected by an increased AGE score, and whether physical activity could play a protective role.

The difference in total carbohydrate intake (as well as carbohydrates from juices and soft drinks) between the two schools was substantial. At the Wassenberg School mean total carbohydrate intake (90g/day) was below the IOM recommended standard (130g/day). The mean intake of carbohydrates from juices and soft drinks (19g/week) at this school was less than half the intake (48g/day) as found in a Dutch food survey from 2007-2010 [5] amongst children aged 7-11y, and even lower than the recommended limit of the WHO for added sugars (25-50g/day). In contrast, on average, total carbohydrate intake (162.8g/day) at the Alexander School exceeds the IOM recommendation, whereas the intake of carbohydrates from juices and soft drinks (77g/day) was well above the WHO limit and close to four times higher as compared to the Wassenberg School. Despite these significant differences, carbohydrate consumption did not translate into a clear association with the AGE score for the total group, indicating other parameters might be more important.

Physical activity is known to improve insulin sensitivity in diabetic patients by stimulating glucose uptake (and use) in muscles via an insulin receptor independent route [19-22]. In the present study, children were divided in sportsmen and non-sportsmen based on sports activities in leisure time. Of note, children from the Wassenberg School had gym classes daily as compared to 'only' 2 classes per week at the Alexander School; at both schools each class lasted roughly 45minutes. Interestingly, only in non-sportsmen a positive association between AGE score and carbohydrate intake per week was observed. In other words, additional sports activities prevent this positive association. Concomitantly, this implies that 'only' 90 minutes of gym at school (most non-sportsmen were located at the Alexander school) is not sufficient to counteract a higher intake of carbohydrates. Since the AGE score between sportsmen and non-sportsmen at the Wassenberg School was not different, 5x45 minutes of gym seems to be sufficient. Considering all known confounders (school, time [year], BMI,

carbohydrate intake, gender, and being sportsman or non-sportsman), additional sports activities were the strongest predictor for a lower AGE score. With regard to the required duration of an effective physical activity (gym classes + sports in leisure time), the present study indicates that 2.5 hours (90min of gym classes and 1h of leisure time sports for sportsmen at the Alexander School) per week can be considered as minimally desired.

### Body composition

An increase in BMI would be expected when carbohydrate intake is above recommendations. However, in this study no such association was found despite the wide range in carbohydrate consumption. BMI did increase with age, which is in line with the expectations for this target group. It is of interest to see a difference in classification of 'healthy or normal body composition' based on BMI or waist-to-height ratio (Table 1). Based on BMI, about 85% of children at both schools are considered to have healthy weight-length proportions, whereas based on the waist-to-height ratio, >95% of the children are considered to be healthy.

### Sleep

In the small group (n=22) of subjects (all from the Wassenberg School) involved in the Actiwatch sub-study, no association was found between physical activity (activity score) and sleep efficiency. The expectation was that being more active throughout the week would result in a better sleep, which would be an additional benefit of increased physical activity. Noteworthy, typical sleep efficiency in school aged children should be >95% [31], which means that the majority of the participants (sleep efficiency for the whole group being  $96.1 \pm 3.0\%$ ) in this sub study can be qualified as good sleepers, and therefore further improvement might be difficult.

### Weaknesses of this study

The weaknesses of this study were the unexpected low number of non-sportsmen at the Wassenberg School, the daily gym classes at the Wassenberg School as compared to two gym classes weekly at the Alexander School, and the fact that the measurements were performed one year apart from each other, but fortunately in the same season and performed by the same researcher using the same equipment. Although based on these differences school could represent an important confounder, school had no impact on the AGE score.

### Conclusion

To prevent or at least limit a high intake of added carbohydrates is a well-accepted strategy to improve health. However, the present observational study in Dutch elementary school children indicates that increased intake of carbohydrates (>130g/day) can be counteracted by sufficient physical activity (indicated >2.5 hours per week), at least based on skin autofluorescence. Furthermore, our findings reveal

that measuring skin autofluorescence is a fast and non-invasive method to screen populations, including school children, for life style, and that it represents a useful tool to monitor 'health certified schools'. The outcome of this observational study would benefit from confirmation by an intervention study with physical activity as the active parameter.

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