*To AGE or not to age*

# The effect of physical activity and advanced glycation end-products (AGEs) on the vasculature in older individuals

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# The effect of physical activity and advanced glycation end-products (AGEs) on the vasculature in older individuals

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*"Yesterday is history*
*Tomorrow is a mystery*
*Today is a gift*
*That's why it's called the present"*

*Alice Morse Earle (1851-1911)*

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Chapter 1

# General introduction

*"The soul is born old and grows young; that's life's comedy. However, the body is born young and grows old; that's life's tragedy."* Oscar Wilde (1854-1900)

## Aging

Aging is characterized by changes in human appearance, for example turning grey, getting wrinkles, losing elasticity and becoming slower. Aging classically is defined as the progressive loss of function accompanied by decreasing fertility and increasing mortality.[1] The biological process that underlies the progressive loss of function is an accumulation of damage to somatic cells.[1,2] Several intrinsic and extrinsic stressors may lead to injuries of the body. The main theory concerning aging addresses the accumulation of risk factors (e.g. physical inactivity, high fat diet) and accumulation of damage that remains after restoration. Although most of the injury acquired during life is repairable (e.g. scratches or bruises), the repair is often not flawless and little damage remains after injury.[1] As a consequence, risk factors and damage accumulate gradually over time resulting in organ dysfunction (e.g. increased arterial stiffness, diminished endothelial function or atherosclerosis) that may lead to symptomatic diseases (e.g. myocardial infarction) and ultimately to increased vulnerability and death (Figure 1.1).[2]

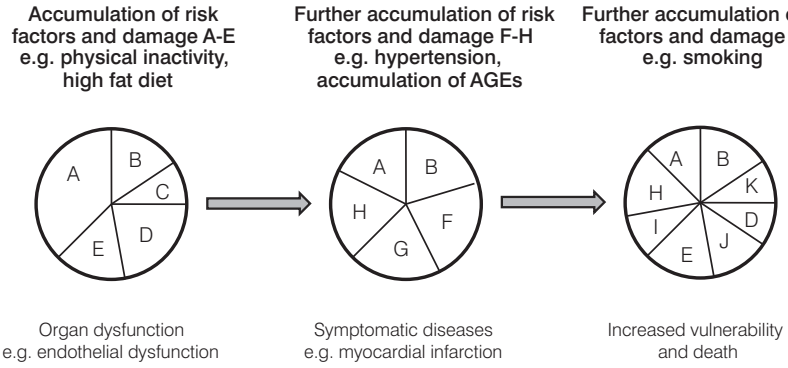


**Figure 1.1** Conceptual presentation of aging with the progressive loss of function and increased vulnerability. Adapted from Rothman, 1976.[2]

Worldwide demographics are changing and people are growing older. It is expected that the next 30 years, the number of individuals older than 65 years will increase from 2.5 million to 4.6 million in the Netherlands.[3] That will be more than 25% of the total

Dutch population (Figure 1.2). Furthermore, the number of frail older individuals also increases from approximately 650.000 individuals nowadays to approximately 1.000.000 individuals by the year 2030.[3] Frail older individuals are an extremely vulnerable group and preventive medicine to encourage and maintain a healthy and independent living is crucial for preventing older individuals to become frail and care dependent.

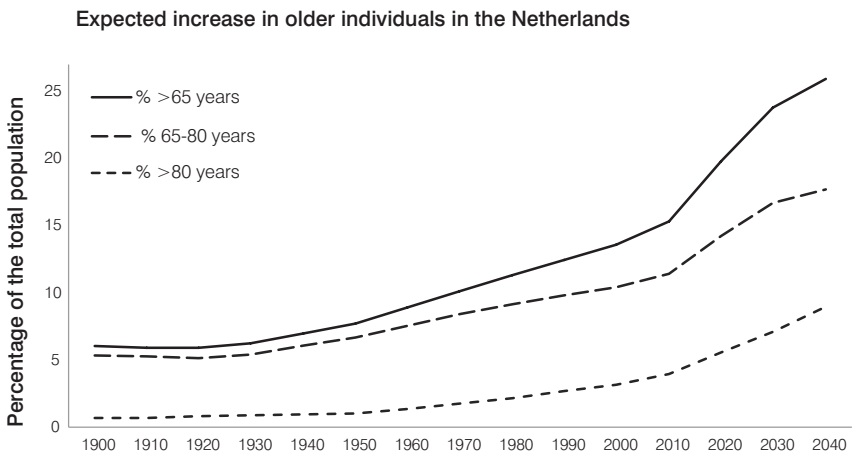


**Figure 1.2** Representation of the expected increase in the older population in the Netherlands, expressed as the percentage individuals (>65 years, 65-80 years and >80 years) of the total population in the Netherlands. Source: Centraal Bureau voor de Statistiek,[3] August 2013

With advancing age the incidence of chronic diseases, such as cardiovascular diseases, increases.[4,5] Despite multiple interventions and reduction in death rates over the last few decades, cardiovascular diseases remain the leading cause of morbidity and mortality in modern societies, and especially in the aging population.[6,7] In this chapter, the physiological changes will be reviewed that occur with aging, especially cardiovascular and cerebrovascular changes, the influence of physical activity thereon, and the role of advanced glycation endproducts (AGEs) in physiological aging. Furthermore, the impact of AGEs on the vascular system, possible interventions to reduce the negative influence of AGEs and the novel agent the AGE-crosslink breaker Alagebrium will be discussed. At the end of this chapter, the aims and outline of this thesis will be addressed.

## Aging and physical (in)activity

Aging is associated with a gradual loss of organ function and increased risk for development of diseases. Large differences exist between individuals in the speed of aging and related morbidity rates due to different coping strategies to minimize the accumulation of risk factors and damage. Besides genetic influences, environmental and lifestyle habits play important roles in disease development. An important lifestyle factor is physical inactivity, which is strongly related to cardiovascular diseases.[8-10] It has been demonstrated that sedentary aging leads to marked cardiovascular dysfunction.[4,11-13] In contrast, lifelong exercise and elevated physical fitness seem to protect against cardiovascular diseases.[5,14-16,5,10,17-19] Improvements in physical fitness, especially among those who are the least fit, are associated with substantial health gains.[9,20] Consequently, physical exercise training is widely recommended as a primary or adjunctive therapy for reducing cardiovascular risk.[21] Nevertheless, most older individuals prefer daily life activities (e.g. walking, cycling and gardening) rather than participation in exercise training programs.[22] Furthermore, a decline in physical fitness occurs with physiological aging. This age-associated decline may be delayed or slowed in those who are engaged in regular vigorous aerobic exercise, but it appears to be inevitable, regardless of (pre-existing) fitness level and exercise status.[23] This reduction in physical fitness results in a decrease in functional capacity that contributes to a loss of independence, increased incidence of disability, and reduced quality of life with age.[23] Therefore, it is crucial to remain physically fit, especially for the older population to maintain a healthy and independent living.

## Aging and cardiovascular changes

It is well known that advancing age is a major and independent risk factor for the development of cardiovascular diseases.[4,5] Even with presumably healthy aging the risk for development of cardiovascular diseases increases. An increased exposure time to risk factors, and thus having a longer time of risk factor and damage accumulation, and occult cardiovascular adaptations explain part of this rise in cardiovascular diseases with aging.[4] A wide array of adverse changes to the arteries contribute to the increased risk of cardiovascular diseases with aging. These changes include structural changes such as thickening of the intima media and stiffening of the arterial walls, and functional changes like the development of vascular endothelial dysfunction.[4,5] These parameters will be discussed separately in the text below.

## Increased intima media thickness

Intima-media thickness (IMT) is the thickness of the tunica intima and tunica media, the innermost two layers of the arterial wall, see Figure 1.3. Intima media thickness correlates well with major cardiovascular risk factors and an increased IMT is an independent predictor of future cardiovascular events.[24-28] Even in healthy older individuals without clinical cardiovascular diseases, the carotid artery IMT increases markedly.[29,30]

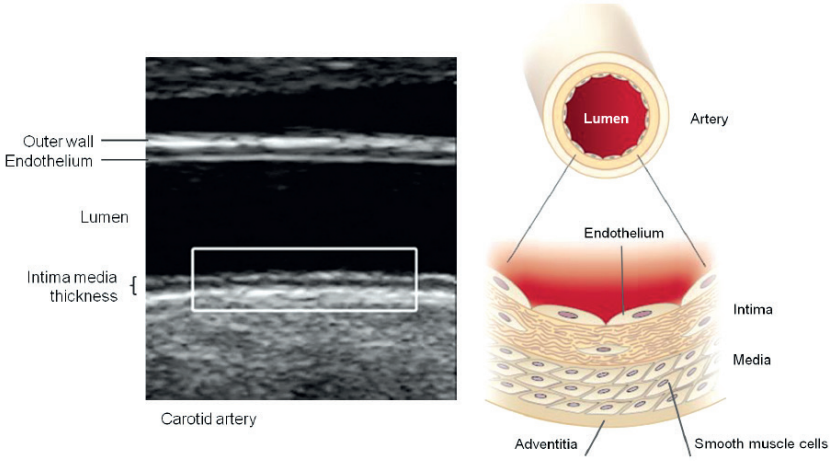


**Figure 1.3** On the left side is a B-mode ultrasound image of the carotid artery seen during the ultrasound measurement. The rectangular box shows the area where the intima media thickness can be measured offline. On the right is a schematic cross-sectional view of an artery with the intima, media and adventitia layers, and endothelial lining (adapted from Libby et al.[31]).

Since the carotid artery is easily accessible due to the anatomical position, and since it is a usual location for the development of atherosclerosis, the carotid artery IMT is used most frequently in clinical practice and scientific studies as a surrogate marker for cardiovascular diseases.[28,32] In addition, the carotid artery is an elastic artery with relatively small muscular media,[33] and thus an increased IMT of the carotid artery is considered to represent mainly intima thickening.[34] Measurements of the IMT are usually performed noninvasively by using B-mode ultrasound (Figure 1.3).[24,35] Numerous studies have shown that non-invasive measures of the carotid artery IMT can be measured with high reproducibility.[24-28]

## Arterial stiffness

Besides intima media thickening, generalized arterial stiffening is another important consequence of aging that significantly contributes to the increased risk for developing cardiovascular diseases in older individuals.[36,37] Arterial compliance represents the capacity of an artery to expand in response to a change in (blood) pressure and is primarily determined by the intrinsic elastic properties of the artery.[38] Vascular aging is strongly associated with elastin depletion and fragmentation, as well as with collagen deposition.[37,39] These changes result in increased arterial stiffness, allowing smaller diameter expansions in response to elevations in blood pressure, that leads to clinical manifestations of a rise in systolic blood pressure and an increase in pulse pressure (systolic – diastolic blood pressure).[40,41] The increase in systolic blood pressure and pulse pressure affects the cardiac function by inducing left ventricular hypertrophy and reduced left ventricular performance.[42] Furthermore, increased arterial stiffness induces altered vascular wall properties such as intima media thickening.[43] Numerous studies have indicated that increased arterial stiffness is an independent predictor for future cardiovascular events.[4,44,45]

Beside (systolic) blood pressure measurements and calculation of pulse pressure, pulse wave velocity (PWV) is a more accurate method to measure arterial stiffness. Pulse wave velocity is measured noninvasively using ultrasound echo Doppler or tonometry. It is calculated as the distance ($\Delta D$) traveled by a pulse wave divided by the time ($\Delta t$) taken to travel this distance: $PWV = \Delta D/\Delta t$ (m/s).[46,47] A higher PWV indicates an increased stiffness of the conduit arteries.[46,47]

Increased arterial stiffness does not solely depend on structural changes of the arterial wall, but is also influenced by the endothelial function to regulate the vascular smooth muscle tone which is also impaired with aging.[4,48,49]

## Endothelial (dys)function

The vascular endothelium is a single layer of cells lining blood vessels that plays a key role in regulating the function and health of arteries.[50,51] The endothelium represents a dynamic interface between the intima of the vasculature and the blood flow (Figure 1.3).[31,52] It is an important regulatory organ that modulates the tone of the underlying vascular smooth muscle cells and maintains cardiovascular homeostasis.[53] Endothelial dysfunction, on the other hand, represents a key step in the early development of atherosclerosis which can ultimately lead to clinical manifestations of cardiovascular diseases.[54] Age-associated endothelial dysfunction is, at least partly, mediated by reduced nitric oxide (NO) bioavailability.[5,55] This is due to an imbalance between the production and removal of nitric oxide. Oxidative stress and inflammation are other mechanisms by which aging leads to reduced nitric oxide bioavailability and thereby reduced endothelium dependent vasodilation.[42,56,57] Vascular oxidative stress develops with aging as a result of increased production of reactive oxygen

species, while antioxidant defense mechanisms remain similar or attenuate.[58] Several factors are known to influence endothelial function, including regular aerobic exercise, dietary factors (e.g. high fat diet), body fatness and fat distribution, and conventional (e.g. smoking, hypertension, diabetes) and non-conventional risk factors for cardiovascular diseases. Examples of non-conventional risk factors are inflammatory markers, such as high sensitive C-reaction protein (hs-CRP) or the accumulation of glycated proteins, also known as advanced glycation end-products or AGEs. These will be discussed later in this chapter.

Endothelial dysfunction can be measured in different vascular beds, e.g. conduit and resistance arteries, in different limbs, e.g. upper and lower limbs, and using different techniques. A frequently used noninvasive technique to measure endothelial function of conduit arteries is the method of flow mediated dilation (FMD). This technique is based on the characteristics that (healthy) arteries dilate in response to an increase in shear stress (increase in blood flow) as a results of an increase in the endothelium derived nitric oxide. The nitric oxide differs to the smooth muscle cells causing a relaxation of the smooth muscle cells and thus a vasodilation and increase in arterial diameter.[59,60] Another technique to examine endothelial function is by using venous occlusion plethysmography to quantify changes in limb volume from changes in limb blood flow using mercury-in-silastic strain gauges.[61] Changes in blood flow can be induced by intra-arterial infusion of vasoactive drugs, e.g. nitric oxide donors like sodium nitroprusside, endothelium dependent agents like acetylcholine or vasoconstricting agents like L-NMMA.[62] Depending on the type and dose of the vasoactive drugs, in combination with the structural and functional properties of the conduit and resistance arteries, the blood flow increases or decreases resulting in a change of limb volume that is registered by the mercury-in-silastic strain gauges. This method to examine endothelial function is considered to be the “gold standard”.[63,64]
The arm-model is used more often to examine the endothelial function than the leg-model. It is known that upper and lower limb vasculature demonstrate different vascular responses to shear [65,66] and pharmacological vasoactive substances.[67,68] Studies have reported that the endothelial function of the brachial artery correlates well with the coronary artery vasomotor function.[69-71] This correlation is less well established for the lower limb endothelial function and coronary arterial function. Nevertheless, since the lower limb demonstrates a higher incidence of clinical vascular diseases and more symptomatic atherosclerosis in older individuals, we will be studying this vascular bed.

**Arterial stiffness and endothelial dysfunction**

Even though structural properties, arterial stiffening, and functional properties, endothelial function, of the vasculature seem to be different, studies have reported interactions

between the two in which arterial stiffening leads to endothelial disturbances and these in turn worsen arterial stiffening.[37,56] Given the increasing number of older individuals and associated health care burden, effective strategies are needed for the prevention and treatment of age-related arterial stiffening and endothelial dysfunction to attenuate the development of cardiovascular diseases and improve healthy, independent aging.

## Aging and cerebrovascular changes

Besides age-related changes in the cardiovascular system, the cerebrovascular system also encounters age-related changes. Most of these changes are inherent to the cardiovascular changes, such as arterial stiffening and endothelial dysfunction.[72] The main goal of the cerebrovascular system is to maintain a stable cerebral blood flow during all circumstances. In order to maintain a stable cerebral blood flow, the cerebral circulation has an autoregulatory system based on myogenic, metabolic, and neurogenic mechanisms that adapt cerebrovascular resistance in response to alterations in blood pressure.[73] The term cerebral autoregulation, first introduced by Lassen in 1959,[74] refers to the ability of the brain to keep cerebral blood flow constant despite changes in blood pressure. Besides cerebral autoregulation, cerebrovascular reactivity to carbon dioxide ($CO_2$) is also involved in optimizing cerebral blood flow. Cerebrovascular $CO_2$ reactivity describes adaptations of cerebral blood flow to changes in arterial carbon dioxide, which is a very potent cerebral vasodilator and constrictor.[75,76] Both cerebral autoregulation and cerebrovascular $CO_2$ reactivity are directly related to the perfusion and oxygenation of the brain tissue and therefore to human brain function.[77,78]

Cerebral autoregulation has been shown to remain stable in participants up to the age of 75 years.[76,79,80] The effects with aging above the age of 75 years have not been examined, and the effects of aging on cerebrovascular $CO_2$ reactivity are less clear. Cerebrovascular pathology, on the other hand, is known to occur in the aging population. Reduced cerebral blood flow in older individuals [79-81] may result in stroke,[82] and it is a major risk factor for the development of neurodegenerative diseases, such as Alzheimer's disease.[83] Furthermore, reduced blood flow and cerebrovascular $CO_2$ reactivity are not only risk factors for the development of neurodegenerative diseases, patients with early stage Alzheimer's disease also have a reduced cerebral blood flow, increased cerebrovascular resistance and reduced cerebrovascular $CO_2$ reactivity.[77,84] Thus, age-related cerebrovascular changes play an important role in age-related disease pathology.

## Impact of physical activity on age-related vascular changes

Arterial stiffness and endothelial dysfunction develop over time and can ultimately manifest in clinical cardiovascular and cerebrovascular diseases. Since arterial stiffness and endothelial dysfunction are precursors in the process of cardiovascular diseases, it would be desirable to intervene in time to delay or improve arterial stiffness and endothelial function in order to postpone the development of clinical vascular diseases.

### Influence on cardiovascular parameters

Physical exercise is said to be the best method to improve healthy independent aging. Prior research has demonstrated an independent, dose-dependent, inverse association between physical fitness and cardiovascular diseases amongst individuals with and without clinical cardiovascular diseases.[85-87] The beneficial effects of physical activity on the cardiovascular system can not only be explained by changes in (traditional) cardiovascular risk factors, such as blood pressure, body composition, glucose metabolism or cholesterol level, but may also be due to arterial remodeling and/or improvement of endothelial function.[88] Even after accounting for traditional risk factors, such as blood pressure, lipids, and diabetes, the inverse relation between physical activity and risk for cardiovascular diseases persists.[86,89] A large study performed by Mora et al.[10] reported that differences in traditional risk factors explain about 59% of the exercise associated reduction in cardiovascular risk, with inflammatory biomarkers making the largest contribution, followed by blood pressure, lipids, and body mass index. Part of this unexplained risk factor gap (41%) could be explained by the beneficial effects of physical activity on vascular structure and/or function, such as improved arterial stiffness and endothelial function (Figure 1.4).[88]

It is known that older individuals who have been exercising most of their lives do not endure the age-related increase in arterial stiffness [38,90] or decrease in endothelial function [15,16] to the same extend as sedentary older individuals. Furthermore, aerobic exercise training improves arterial stiffness [38] and endothelium function, in middle-aged and older men, both with and without clinical presentations of cardiovascular diseases.[15,91]

### Influence on cerebrovascular parameters

The beneficial effects of exercise training on cardiovascular function, also applies to cerebrovascular function by enhancing cerebral blood flow and cerebrovascular $CO_2$ reactivity. A study by Ainslie et al. reported that the cerebral blood flow velocity is higher in endurance trained individuals compared with sedentary peers, independent of age.[81] Murrel et al. showed that a 12-week training intervention induced an increase

in cerebrovascular $CO_2$ reactivity, both in young and older individuals.[92] Even though data concerning the influence of physical activity and exercise training on cerebrovascular function is not overwhelming, the positive influence of physical activity and exercise training seems to be reinforced.

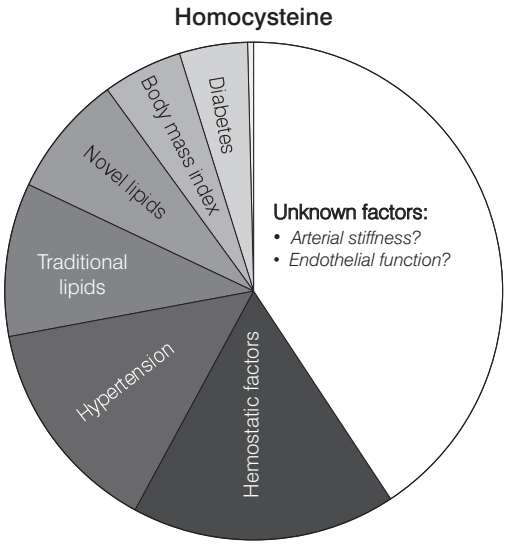


**Figure 1.4** Differences in cardiovascular risk factors explain approximately 59% of the cardiovascular risk reduction associated with physical activity. This suggests that about 40% of the reduction in cardiovascular risk attributable to physical activity remains unexplained. Possibly, physical activity has a direct effect on the structural and functional properties of the vascular system that may explain (part of) this risk factor gap (adapted from Mora et al. 2007 [10] and Thijssen et al. 2010 [88]).

## Aging and advanced glycation end-products

### Formation of advanced glycation end-products (AGEs)

Besides age per se and lifestyle factors, such as physical inactivity, another mechanism involved in the age-related vascular changes is the (lifelong) accumulation of advanced glycation end-products, also known as AGEs. In 1912 the French chemist Louis Maillard described for the first time the non-enzymatic chemical reaction, also known as the Maillard reaction, between reducing sugars, such as glucose, and amino groups on proteins to form protein-protein crosslinks and complex yellow-brown pigment on a crème brûllée or roasted turkey for instance.[93] His thoughts that this process was not only applicable to the food industry, but that

this process might also play an important role in the pathological complications of diabetes mellitus, has taken approximately 80 years before it reached awareness in clinical medicine. Nowadays, evidence has emerged that the pathological effects of advanced glycation end-products (AGEs) are not only relevant in the presence of diabetes mellitus, but also play a role with physiological aging and the development of cardiovascular diseases.[94]

The Maillard reaction begins when a reducing sugar non-enzymatically reacts with a biological amine from a protein, lipid or amino acid to produce a Schiff base (Figure 1.5).[94] This initial reaction usually takes a few hours to occur, is swift, highly reversible and dependent on the concentrations of available glucose.[95,96] A Schiff base can undergo further chemical rearrangements over several weeks to form a more stable glycated protein, an Amadori product. An example of an Amadori product is the glycated hemoglobin HbA1c that clinician use as an indicator of the average blood glucose level over the past 6 to 12 weeks.[97,98] The Amadori product, which is still reversible but far more stable than the Schiff base, can over a period of several months to years undergo structural rearrangements through a series of reactions such as oxidation, dehydration and degradation to form irreversible complex arrangements of crosslinked proteins called Advanced Glycation Endproducts or AGEs.[99,100] Unlike the Schiff base and Amadori product, AGEs are permanently bound and can irreversibly crosslink proteins. Due to the irreversible nature of AGEs, they accumulate gradually with aging.[101,102]

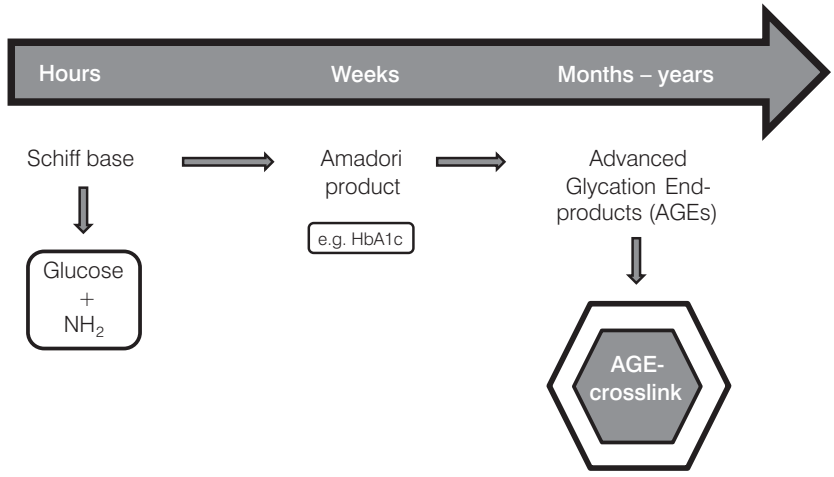


**Figure 1.5** Schematic overview of the Maillard reaction with formation of AGE-crosslinks.

## Impact of AGEs on the vascular system

Due to the nature of glycosylation, this process has long been thought to be related to patients with diabetes mellitus with high levels of glucose. Nowadays, it becomes more apparent that this is a physiological process that occurs in every human being, which consequently contributes to the aging phenotype.[103,104] The physiological consequences of the Maillard reaction have been described in multiple reviews.[98,104-107] The main mechanisms by which AGEs lead to cardiovascular diseases are by increasing arterial stiffness and inducing endothelial dysfunction.

### Arterial stiffness

Crosslinking of extracellular matrix proteins is essentially a physiological phenomenon.[98] It strengthens tissues enduring tissue integrity, without compromising flexibility. Advanced glycation endproducts, however, can covalently bind on long-lived proteins and form additional crosslinks between matrix proteins, like collagen and elastin.[106] The excessive crosslinking caused by AGE accumulation undermines the flexibility of matrix proteins. This results in prominent stiffening of the extracellular matrix and stiffening of the cardiovascular system, e.g. arterial and myocardial stiffening (Figure 1.6).[94,98,101,105] Due to the continuous accumulation of AGEs and irreversible crosslinking to proteins, aging is accompanied with a gradual increase in cardiovascular stiffening.[94,95,99,103]

### Endothelial dysfunction

AGE formation not only changes the physiological properties of (long-lived) proteins, but also induces cellular dysfunction through interaction with cell surface receptors. There are many AGE receptors, but the most important one is the Receptor for AGE (RAGE). RAGE was identified in 1992, and is still considered as a representative AGE receptor on endothelial cells.[108,109] Activation of AGE receptors causes cascades of inflammatory responses and induction of oxidative stress. Furthermore, AGEs can quench nitric oxide inhibiting flow mediated release of nitric oxide, and AGEs induce expression the potent vasoconstrictor endothelin-1.[42,95,106,107,110] These actions lead to endothelial dysfunction, which as mentioned before, precedes the development of atherosclerosis and increases the risk for development of cardiovascular and cerebrovascular diseases.

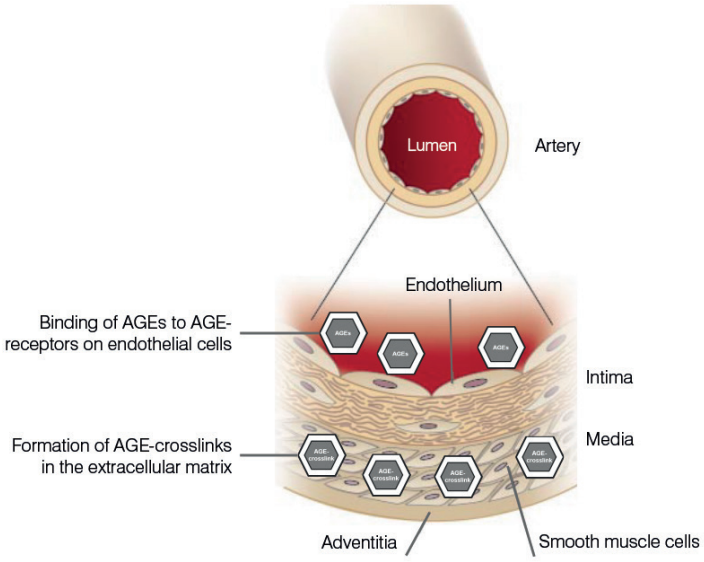


**Figure 1.6** AGE-crosslinks increase the risk for cardiovascular diseases by increasing arterial stiffening and endothelial dysfunction.

## Interventions to reduce AGEs

The recognition that AGEs and AGE-crosslinks play important roles in arterial stiffening and endothelial dysfunction, is driving the search for novel agents that can interact with these AGEs and, consequently, lower the cardiovascular risk. Different approaches may be used to reduce the accumulation of AGEs and its deleterious effects (Table 1.1). The first approach is to reduce the intake of AGEs by limiting the amount of exogenous AGEs in food products [111] or by motivating individuals to quit smoking.[112] A next approach is to diminish the formation of AGEs at one of the first steps in the Maillard reaction, e.g. by interventions to reduce the glucose level, especially in patients with diabetes mellitus, or by inhibiting the transfer from the Shiff base to Amadori products. Another step is to break down the formed AGEs and AGE-crosslinks, for example by using the phase II drug Alagebrium. Another approach is to prevent the deleterious effects of AGEs by blocking the receptors for AGEs. Interventions that have been examined most extensively are aminoguanide, which will be discussed briefly, and Alagebrium, which will be discussed in more detail.

**Table 1.1** Interventions to reduce the deleterious effects of AGEs

| |
|---|
| **Reduce the intake of exogenous AGEs** |
| · Reduce the intake of AGEs in food |
| · Quit cigarette smoking |
| **Inhibition of AGE formation** |
| · Non-pharmaceutical interventions: lower blood glucose level, use of anti-oxidants |
| · Pharmaceutical intervention: aminoguanidine |
| **Break already formed AGE-crosslinks** |
| · Pharmaceutical intervention: phase II drug Alagebrium |
| **Blockers of the Receptor for AGEs to prevent AGE-RAGE interaction** |

### Inhibition of AGE formation using aminoguanide

In 1986 Brownlee et al showed that aminoguanide prevented both the formation of AGEs and the formation of glucose-derived collagen crosslinks in vitro.[113] Even though, aminoguanide seemed promising in the first few trials to reduce microvascular and macrovascular complications in patients with diabetes,[114,115] trials were terminated early due to unfavorable perceived risk-to-benefit ratio's [115] with pro-oxidant activities [116] and inhibition of nitric oxide synthase.[117,118]

## AGE-crosslink breaker Alagebrium

In search of novel agents that would decrease arterial stiffening and improve endothelial dysfunction to lower cardiovascular risk, a new agent was created to specifically break already formed AGE-crosslinks.[119] This drug, ALT-711, also known as Alagebrium, a thiazolium derivative (3-phenyacyl-4,5-dimethylthiazolium chloride), breaks established AGE-crosslinks between proteins.[120] By cleaving AGE-crosslinks, Alagebrium may have the ability to restore compliance of aged and/or diabetic vascular tissue and myocardium.

The pathophysiological mechanism in combination with animal studies and initial phase I and II human studies have shown encouraging results in reducing cardiovascular stiffness [120-127] and improving endothelial function,[128] see Table 1.2.

### (Pre)clinical trials with Alagebrium

The multicenter phase II clinical trials have examined over 1000 patients receiving Alagebrium for up to 9 months. The focus of most clinical trials has been on patients

**Table 1.2** Shows an overview of the animal and patient studies that have been performed with Alagebrium

| Animal studies | Population | Study design |
|---|---|---|
| **Wolffenbuttel *et al.*** [121] 1998, PNAS | Male Wistar rats with 9 weeks streptozotocin-induced diabetes | Four groups:<br>Nondiabetic controls rats<br>Diabetic rats without Alagebrium, n = 13<br>One group 1 week Alagebrium, n= 8-10<br>One group 3 weeks Alagebrium, n = 8-10 |
| **Asif *et al.*** [122] 2000, PNAS | Young and aged dogs | Three groups:<br>Young dogs, n = 7<br>Older dogs without Alagebrium, n = 5<br>Older dogs with Alagebrium, n = 8 |
| **Vaitkevicius *et al.*** [123] 2001, PNAS | Older rhesus monkeys | Single arm study, n = 6 |
| **Candido *et al.*** [124] 2003, Circ Res | Male Sprague Dawley rats with 16 weeks streptozotocin-induced diabetes | Four groups:<br>Control rats, n = 11<br>Control rats with Alagebrium, n = 10<br>Diabetic rats without Alagebrium, n = 12<br>Diabetic rats with Alagebrium, n = 10 |
| **Guo *et al.*** [125] 2009, J Geront Biol Sci | Young and aged Sprague-Dawley rats | Three groups:<br>Young rats, n = 10<br>Older rats without Alagebrium, n = 10<br>Older rats with Alagebrium, n = 10 |
| **Steppan *et al.*** [126] 2012, Exp Geront | Young and aged Male Fisher 344 rats | Five groups:<br>Young rats, n = 8<br>Older rats, n = 30, randomized into 4 groups<br>Alagebrium/Placebo and with/without exercise training |
| **Patient studies** | | |
| **Kass *et al.*** [120] 2001, Circulation | Systolic blood pressure >140mmHg<br>Pulse pressure >60mmHg<br>Male & female; mean age 67 years | Randomized placebo vs. Alagebrium<br>Alagebrium n = 62; Placebo n = 31 |
| **Little *et al.*** [127] 2005, J Cardiac Failure | Diastolic heart failure<br>Left ventricular ejection fraction ≥50%.<br>Male & female; mean age 71 years | Open label, single arm trial, n = 23 |
| **Zieman *et al.*** [128] 2007, J Hypertension | Systolic blood pressure >140mmHg<br>Diastolic blood pressure <90mmHg<br>Pulse pressuer >60mmHg<br>Male & Female; mean age 65 years | Single arm & single blind, n = 13<br>2 weeks placebo & 8 weeks Alagebrium |
| **Hartog *et al.*** [129] 2011, Eur J Heart Failure | Chronic heart failure<br>Left ventricular ejection fraction <45%.<br>Male & Female; mean age 62 years | Randomized, double blind, placebo controlled<br>Alagebrium n = 50; Placebo n = 52 |

| | Dose of Alagebrium | Main outcome |
|---|---|---|
| | 1 mg/kg body weight<br>3 weeks | Reversion of diabetes-induced increased large artery stiffness<br>Decrease in collagen crosslinking measured in tail tendon |
| | 1 mg/kg body weight<br>4 weeks | Reduction in age-related left ventricular stiffness |
| | 1 mg/kg every other day<br>3 weeks | Improvement in compliance of the large arteries and LV function |
| | 10 mg/kg body weight<br>16 weeks | Attenuation of diabetes-associated cardiac abnormalities in rats<br>Improvement in LV weight<br>Decrease in myocardial collagen crosslinking and myocardial AGE accumulation<br>Decreased LV RAGE gene expression |
| | 10 mg/kg body weight<br>16 weeks | Improvement of cardiac mass and diastolic function<br>Decreased AGE accumulation in myocardial collagen<br>Increased anti-oxidant activity in the aging hearts |
| | 1 mg/kg body weight<br>4 weeks | Alagebrium combined with exercise training improved systolic and diastolic cardiac function and arterial stiffness |
| | | |
| | 210 mg per day<br>8 weeks | Lowered pulse pressure<br>Improved large artery compliance and distensibility |
| | 210 mg twice daily<br>16 weeks | Regression of LV hypertrophy<br>Improvements in LV diastolic properties<br>Subjective improvements in quality of life by the patients |
| | 210 mg twice daily<br>8 weeks | Improvement in brachial endothelial function |
| | 200 mg twice daily<br><br>9 months (36 weeks) | No improvement in exercise capacity or cardiac diastolic function |

with diabetes mellitus, systolic hypertension and heart failure. There are no studies examining the effects of Alagebrium in healthy (older) individuals. Even though patient population are more amendable to improvement with Alagebrium, the rational to study *healthy older* individuals in our study is well considered since it is known that healthy older individuals have stiffer arteries and decreased endothelial function compared with healthy young controls.[4,5] Thus, favorable effects of improving arterial stiffness and endothelial function are certainly expected using Alagebrium.

Most patient studies using Alagebrium used short term application (8-16 weeks),[120,127,128] with the exception of one recent study using Alagebrium for 9 months in heart failure patients.[129] However, short term use of AGE-crosslink breakers alone may not be sufficient to alter arterial stiffness formed by decades of accumulation of AGEs in healthy older individuals. The combination of AGE-crosslink breakers with exercise training, the latter having demonstrated to improve cardiovascular compliance in sedentary individuals,[15,16,38] may be particularly effective in improving arterial stiffness and endothelial function in healthy older sedentary individuals. Preliminary work in rats suggested that the combination of exercise training and Alagebrium reverses age-related cardiovascular adaptations in rats.[126] Whether AGE-crosslink breakers enhance the cardiovascular benefits from exercise training in humans is currently unknown. This leads us to the founding interests of this thesis.

## Aims and outline of this thesis

### General aim of this thesis

Advancing age, physical inactivity and accumulation of AGEs are important factors in the development of cardiovascular diseases. Increased arterial stiffness and endothelial function are known to play key roles in this age-related process. However, the effects of exercise training and the AGE-crosslink breaker Alagebrium on arterial stiffness and endothelial function in healthy older individuals have not been clarified yet. Therefore, the general aim of this thesis is to elucidate the individual and combined effects of exercise training and the AGE-crosslink breaker Alagebrium in older individuals on different aspects of the vascular system.

### Outline of this thesis

The general aim has been translated in a series of experiments that are presented in the subsequent chapters of this thesis. First, we examined the impact of physical fitness level on arterial stiffness, endothelial function and cardiovascular risk in a group of older *non-exercising* individuals. It is known that exercise training is beneficial for the reduction of cardiovascular diseases.[10,19,130] However, older individuals, who

are more prone to develop cardiovascular diseases, prefer daily life activities, such as gardening, low intensity cycling or walking, over exercise training to remain physically fit.[22] It is currently unknown whether a lower or higher level of physical fitness, related to daily life activities, influences vascular characteristics and cardiovascular risk profile in healthy non-exercising older individuals. We hypothesized that a higher physical fitness level, related to more daily life activities, is associated with a lower arterial stiffness, better endothelial function and more favorable cardiovascular risk profile in older individuals. These results are discussed in ***Chapter 2***.

Besides influences on cardiovascular function and cardiovascular risk profile, physical activity is also known to improve sleep quality in individuals with sleep disorders.[131,132] Sleep quality deteriorates with age [133,134] and impaired sleep quality is a frequently reported medical complaint.[135] Nevertheless, the influence of physical activity in older individuals without medical sleep disorders is unknown. Therefore, the aim of this study, that is described in ***Chapter 3***, was to (1) investigate the relationship between physical fitness and daily energy expenditure with sleep efficiency in young adults and older participants, and (2) using a randomized controlled trial to examine the effects of a 12-month exercise training program on sleep quality measures, such as sleep onset latency and sleep efficiency, in a group of older participants. We hypothesized that a higher physical fitness level and a higher daily activity level are both associated with a better sleep efficiency and that 12 months of exercise training will enhance sleep efficiency in healthy older participants.
The cerebrovascular function across different age ranges are examined in ***Chapter 4***. Cerebral blood flow is the result of a delicate balance between blood supply, perfusion pressure and tissue oxygenation.[76,77] While the effects of age-related deterioration on the cardiovascular system have been extensively examined, the influence of age on the cerebrovascular system has been examined far less. This chapter examines the effects of age on cerebral blood flow and cortical oxygenation by investigating the relationship between blood pressure, cerebral blood flow and cerebral cortical oxygenation in different age groups. We hypothesized that the cerebrovascular function, and thereby the cortical oxygenation, which is crucial for human brain function, are diminished in healthy older individuals compared with younger individuals.

In ***Chapter 5*** we examine the effects of a 12-month intervention program with the AGE-crosslink breaker Alagebrium, alone and in combination with exercise training in a factorial design trial, on endothelial function, arterial stiffness and cardiovascular risk in healthy older individuals. Age-related changes in vessel characteristics lead to endothelial dysfunction and arterial stiffening.[4,5] Furthermore, the formation of AGEs

decreases endothelial function and the accumulation of AGEs in the arterial wall increases arterial stiffening.[94,95,103] Preliminary work in rats suggested that the combination of exercise training and an AGE-crosslink breaker reverses cardiovascular adaptations to advanced age in rats.[126] Whether AGE-crosslink breakers enhance the cardiovascular benefits from exercise training in humans is currently unknown. The primary hypothesis was that both breaking AGE-crosslinks using Alagebrium and performing aerobic exercise training for one year improves endothelial function and arterial stiffness. Furthermore, we speculate that this effect is enhanced when both interventions are combined.

Finally, in ***Chapter 6*** we combine and discuss the current knowledge and shine new light across the domain of AGEs and the AGE-crosslink breaker Alagebrium. Furthermore, we speculate on future interventions to enhance healthy aging to maintain independent living in the aging society.

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Chapter 2

# Impact of fitness level on cardiovascular risk and vascular function in older non-exercising individuals

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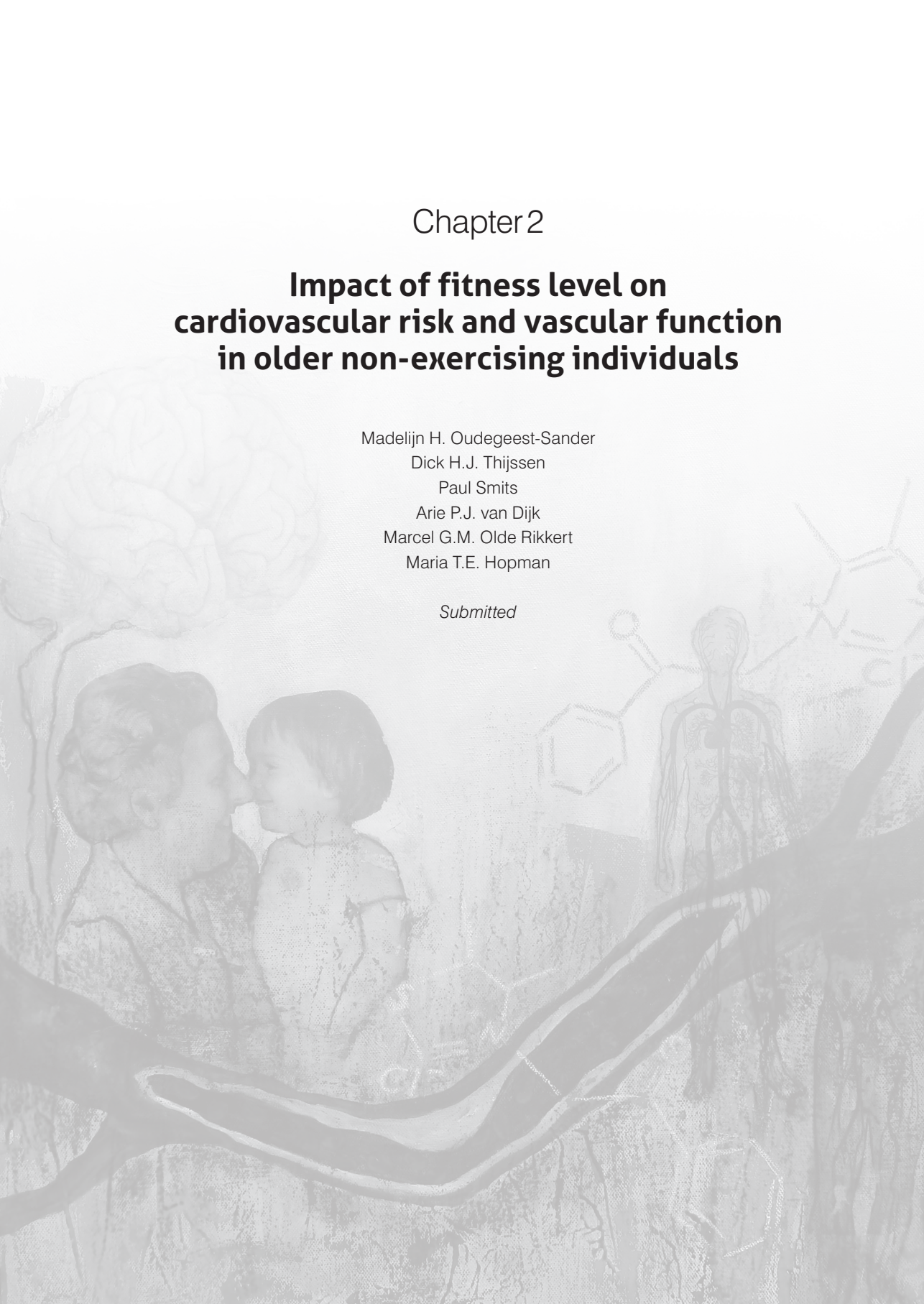

## Abstract

**Background**: The level of physical fitness in exercising versus non-exercising individuals directly correlates with cardiovascular risk. Most older individuals prefer being active in daily life activities rather than performing exercise training. Whether differences in physical fitness in older, non-exercising individuals affect the cardiovascular risk profile and vascular function is currently unknown.

**Methods**: Forty older non-exercising individuals (age 69 ± 4 years), free of any overt disease, were included. Stratified for gender and age, participants were allocated to a lower physical fitness ($VO_2$max 20.7 ± 2.4 $mlO_2$/min/kg) or higher physical fitness group ($VO_2$max 29.1 ± 2.8 $mlO_2$/min/kg, $p<0.001$). Cardiovascular risk profile was calculated by using the Lifetime Risk Score. Vascular function was examined using venous occlusion plethysmography to assess blood flow changes in response to intra-arterial infusion of acetylcholine, sodium nitroprusside and $N^G$-monomethyl-L-arginine (L-NMMA).

**Results**: The higher physical fitness group showed a higher daily life activity level (accelerometry) compared with the lower physical fitness group ($p=0.04$). Lifetime Risk Score was significantly higher and blood flow ratio response to acetylcholine was significantly lower in the lower physical fitness group ($p<0.001$, $p=0.04$, respectively). Blood flow ratio responses to sodium nitroprusside and L-NMMA were similar between both groups.

**Conclusions**: A higher physical fitness in an older, non-exercising population was associated with a better cardiovascular risk profile and vascular function. This study supports a physiological basis that supports the beneficial effects of differences in physical fitness level related to daily life activities on cardiovascular health in older individuals. Therefore, older individuals should be encouraged to enhance their daily life activities.

## Introduction

Physical exercise training has strong protective effects on the cardiovascular system and decreases morbidity and mortality.[1-3] The beneficial effects of exercise training are partly explained via improvements of the cardiovascular risk profile (i.e., blood pressure, lipids, weight balance, insulin/glucose regulation and inflammatory markers), but may also relate to a direct effect of physical exercise on the vasculature.[2,4,5] Elevated physical fitness levels importantly contribute to cardiovascular risk reduction. In subjects with similar cardiovascular risk factors, those with a higher physical fitness level have a lower risk of cardiovascular morbidity and mortality.[6] Therefore, the Lifetime Risk Score (LRS) incorporates physical fitness level and is found to be superior compared with the Framingham Risk Score to predict future cardiovascular events.[7,8]

Differences in physical fitness levels are present, even within those who do not regularly perform exercise training.[9] Whether differences in physical fitness in older, *non-exercising* individuals contribute to differences in cardiovascular risk profile and vascular function is currently unknown. Therefore, the aim of the present study is to assess the effects of a lower or higher physical fitness level in older, non-exercising individuals on cardiovascular risk profile and vascular function. We hypothesize that a higher physical fitness due to more daily life activities is associated with a healthier cardiovascular risk profile and better vascular function in older individuals. Knowing the impact of daily life activities on cardiovascular health may alter and improve the lifestyle advices for older individuals.

## Methods

### Participants

Forty older individuals, who did not perform regular physical exercise training for the past 5-10 years, were recruited. All participants were 65 years or older without cardiovascular diseases or disorders that could compromise physical activity. The study was approved by the local Medical Ethics Committee and all participants gave written informed consent.

### Experimental Design

The participants reported to our laboratory three times. First, participants were screened for general health and cardiovascular risk factors. On a separate day participants performed an incremental maximal bicycle exercise stress test to examine the maximal oxygen uptake ($VO_2$max).[10] Participants were then allocated to a lower ($VO_2$max <25 ml$O_2$/min/kg body weight) or higher ($VO_2$max >25 ml$O_2$/min/kg body weight) physical fitness group, stratifying for gender and age. The division of

both groups below or above a $VO_2$max of 25 $mlO_2$/min/kg was based on the median value combining all 40 participants. On a separate day, which was at least 2 days after the maximal exercise stress test, vascular function was assessed using venous occlusion plethysmography and intra-arterial infusion of vaso-active substances. Furthermore, participants received an activity monitor to examine daily life activity levels across 7 consecutive days.

## Measurements

*General characteristics*

All participants underwent clinical examination with measurements of height, weight, waist and hip circumference. A four-point skinfold thickness measurement was obtained in order to calculate fat percentage and lean body mass.[11] Blood pressure and resting heart rate were measured three times in the supine position using a manual sphygmomanometer around the left arm after a 10 minute rest.

*Incremental maximal bicycle exercise stress test*

An incremental maximal exercise stress test on a bicycle ergometer (Lode, Excalibur Sport, Groningen, the Netherlands) was performed to measure maximal oxygen uptake ($VO_2$max, $mlO_2$/min/kg). After 3 minutes rest, participants started cycling at a workload of 50 Watt, which was increased by 10 Watt/minute. Continuous measurement of oxygen uptake ($VO_2$) was performed using an automatic gas analyzer (Oxycon alpha, Jaeger, Breda, the Netherlands). Peak oxygen uptake was calculated as the average oxygen uptake during the last minute of the test and then scaled for body weight and lean body mass. Heart rate was measured continuously with a 12-lead ECG. Three out of the following 4 criteria had to be met, otherwise the test was repeated; clinical signs of exhaustion of the participant, respiratory quotient ≥1.10, finishing within 10 beats of the maximum predicted heart rate (=220-age), and flattening of $VO_2$ uptake curve (≤110 mL increase during the last minute).[10]

*Daily life activities*

A validated bi-axial accelerometer (SenseWear Pro3, BodyMedia, Pittsburg, PA, USA) was used to quantify daily life activities.[12,13] The accelerometer was continuously worn around the right upper arm during 24 hours for 7 consecutive days. Data from the accelerometers were analyzed offline using the data from 7.00AM to 11.00PM from each day. Data were rejected if <90% of the data on a single day or less than 4 days of data was collected.

Metabolic equivalent units (METs) were used to quantify the intensity of daily life activities. MET is a physiological measure expressing the ratio of intensity of physical activity to that of the reference metabolic rate 1 MET during quiet sitting.[14] Moderate-intensity activities are considered 3.0-5.9 METs and vigorous-intensity activities

are considered ≥6.0 METs.[14] Besides the intensity of daily life activities, we also measured the duration (minutes) of activities with an intensity of ≥3 METs. Both intensity and duration of physical activities were averaged over the 7 days.

*Cardiovascular risk profile*

*Venous blood samples* were taken after an overnight fast to measure lipid levels (total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), and triglycerides), glucose and glycosylated haemoglobin (HbA1c). In addition, high sensitive C-reactive protein (hs-CRP) was examined as a measure of inflammation.

*Arterial wall thickness* of the left common carotid artery (CA) was measured using high-resolution echo ultrasonography with a 7.5 MHz linear array transducer (Picus, Pie Medical Benelux, Maastricht, the Netherlands) under standardized conditions. The left common carotid artery was measured 2 cm proximal to the bulbus.[15] Four separate longitudinal ultrasound images were made with both arterial walls clearly displayed for 6 consecutive heartbeats to analyse off-line. Distance from lumen-intima interface to media-adventitia interface indicated the wall thickness.[16] Values of the intima media thickness (IMT) were averaged from these 4 images after excluding the highest and lowest value of the 6 heartbeats per ultrasound image.

*Framingham Risk Score* (FRS) was calculated to estimate the percentage 10-year risk for general cardiovascular diseases.[17] Predictors taken into account were age, gender, body mass index, diabetes mellitus, smoking, treated and untreated systolic blood pressure, and total and HDL cholesterol. Besides the more traditional FRS, we also calculated the novel *Lifetime Risk Score* (LRS) [7,8] with a 30-year risk prediction for cardiovascular disease mortality. It incorporates factors similar to the FRS, but also adds physical fitness level to the risk calculation.

*Vascular function*

To standardize this measurement, participants were asked to refrain from coffee, tea, alcohol, chocolate, vitamin C supplements or fruit 14 hours prior and fasting overnight prior to the examinations. Room temperature was set at 22 ± 1 °C.

The endothelial function of the lower limb was measured from resistance artery blood flow responses using venous occlusion plethysmography during intra-arterial infusion of vasoactive substances [18,19] in the upper leg, previously described in detail by Kooijman et al..[20] Using a modified Seldinger technique, an intra-arterial cannula (Angiocath 16 gauge, Becton Dickinson, Sandy, Utah, USA) was introduced into the right common femoral artery at the level of the inguinal ligament under local anaesthesia (10 ml lidocaine 1%). This cannula was used for intra-arterial administration of three vaso-active substances by an automatic syringe infusion pump (CardinalHealth, Rolle, Switzerland) and for intra-arterial blood pressure monitoring (Type PX600F, Edwards Lifesciences Services GmbH, Unterschleissheim, Germany).

We infused the endothelium dependent vasodilator acetylcholine (ACh), the endothelium independent vasodilator sodium nitroprusside (SNP), and the nitric oxide synthase inhibitor $N^G$-monomethyl-L-arginine (L-NMMA). Rate of infusion was adjusted to leg volume that was determined by anthropometry as described and validated by Jones et al..[21]

Bilateral blood flow in the upper legs was measured by ECG-triggered venous occlusion plethysmography. Thigh occlusion cuffs (12cm) were connected to a rapid cuff inflator (Hokanson Inc., Bellevue, WA, USA), which simultaneously inflated the cuffs to a pressure of 50mmHg.[22] Cuff inflation was triggered and sustained by the R-waves on the ECG during 8 heart cycles and subsequently deflated during 8 heart cycles. Mercury-in-silastic strain gauges (Hokanson Inc., Bellevue, WA, USA) were placed at mid thigh to quantify changes in leg volume from changes in upper leg blood flow.[23] Calf circulation was occluded during substance infusion by inflating cuffs directly below the knee to suprasystolic values (≥220 mmHg) to avoid the use of high dosages with subsequent possible systemic effects of the vasoactive substances.[20]

After instrumentation and at least 45 minutes after cannulation of the femoral artery, infusion of the vaso-active substances started. Acetylcholine was administered at 1, 4, 16, 32 and 64 $\mu$g/mL/dL leg volume, SNP 0.06, 0.20 and 0.60 $\mu$g/mL/dL leg volume, and L-NMMA 0.05, 0.10, 0.20 and 0.40 mg/mL/dL leg volume. The order of infusion was fixed and each substance was infused for 5 minutes. Two consecutive infusions were performed before deflating the lower leg cuffs to restore normal blood flow for 5 minutes. During these 5 minutes of recovery, 0.9% saline (or 5% glucose during SNP measurements) was infused to maintain a constant flow rate. Between administration of different substances, a 20 minute rest period was inserted with continuous flow of 0.9% saline.

Plethysmography data of both legs were digitalized with a sample frequency of 100 Hz and analyzed offline by a customized computer program (MIDAC, Instrumental Department, Radboud University Medical Center, Nijmegen, the Netherlands). Upper leg blood flow (ml/min/dL leg volume) was calculated as the slope of the volume change curve over a 4-s interval, starting 2.5 seconds after the cuff inflation, to avoid inclusion of induced cuff artefacts.[23] From each infusion dosage, the last 6-8 slopes were averaged to calculate the leg blood flow. To correct for systemic effects, blood flow ratio between the infusion and control leg was calculated.

## Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA). Data are presented as mean ± standard deviation unless otherwise indicated. Differences between the lower and higher physical fitness group were analyzed with an unpaired *t*-test. The Linear Mixed Model

was used (Dose*Group) to compare changes in blood flow response and blood flow ratio to increasing dosages of each vasoactive substance (Dose) between both groups (Group). A Pearson correlation was used to correlate physical fitness in terms of $VO_2$max per lean body mass with the maximal blood flow response. Statistical significance was set at a (2-sided) *p*-value ≤0.05.



## Results

As a consequence of our allocation process, both groups consisted of 20 participants with an equal distribution in gender and age (Table 2.1). Daily minutes of moderate intensity activities and mean METs per day were significantly lower in the lower physical fitness group compared with the higher physical fitness group (Table 2.1).

**Table 2.1** Description of the lower and higher physical fitness ($VO_2$max) group.

| | Lower $VO_2$max | Higher $VO_2$max | *p*-value |
|---|---|---|---|
| Number of participants | 20 | 20 | |
| Male : Female (n) | 12 : 8 | 13 : 7 | 0.75 |
| Age (years) | 70 ± 3 | 69 ± 4 | 0.55 |
| Daily minutes of moderate intensity activities¥ | 79 ± 39 | 108 ± 43 | **0.04** |
| Mean METs per day¥ | 1.43 ± 0.21 | 1.59 ± 0.21 | **0.02** |
| $VO_2$ max ($mlO_2$/min/kg) | 20.7 ± 2.4 | 29.1 ± 2.8 | **<0.001** |
| $VO_2$ max per lean body mass ($mlO_2$/min/kg) | 31.2 ± 3.0 | 41.5 ± 4.6 | **<0.001** |
| Maximal load (Watt) | 133 ± 33 | 175 ± 41 | **<0.01** |

Data are presented as mean ± standard deviation. ¥ Due to technical problems daily life activity data could not be analyzed for two subjects. Thus, n=19 for the lower and n=19 for the higher $VO_2$max group.
**Abbreviations:** METs metabolic equivalent units.

### Cardiovascular risk profile

The lower physical fitness group demonstrated a significantly higher body mass index, fat percentage, waist and hip circumference, waist-to-hip ratio, hs-CRP levels and Lifetime Risk Score compared with the higher physical fitness group (Table 2.2). No significant differences were observed between both groups in lean body mass, blood pressure, heart rate, lipid levels, glucose, HbA1c, carotid artery IMT or Framingham Risk Score (Table 2.2).

**Table 2.2** Cardiovascular risk profile of the lower and higher physical fitness ($VO_2$max) group.

| | Lower $VO_2$max | Higher $VO_2$max | *p*-value |
|---|---|---|---|
| Body Mass Index (kg/m$^2$) | 27.4 ± 3.5 | 24.9 ± 2.2 | **0.01** |
| Lean Body Mass (kg) | 55.8 ± 11.4 | 52.6 ± 9.4 | 0.34 |
| Fat percentage (%) | 33.4 ± 6.8 | 29.6 ± 5.1 | **0.05** |
| Waist circumference (cm) | 99.8 ± 8.4 | 88.7 ± 8.6 | **<0.001** |
| Hip circumference (cm) | 105.1 ± 6.4 | 99.1 ± 3.9 | **<0.01** |
| Waist-to-hip ratio | 0.95 ± 0.05 | 0.90 ± 0.07 | **<0.01** |
| Systolic blood pressure (mmHg) | 132 ± 10 | 132 ± 14 | 1.00 |
| Diastolic blood pressure (mmHg) | 77 ± 7 | 79 ± 6 | 0.20 |
| Heart rate (bpm) | 63 ± 7 | 60 ± 8 | 0.23 |
| Total cholesterol (mmol/l) | 5.4 ± 0.6 | 5.4 ± 1.0 | 0.88 |
| HDL (mmol/l) | 1.3 ± 0.3 | 1.3 ± 0.3 | 0.90 |
| LDL (mmol/l) | 3.6 ± 0.7 | 3.6 ± 0.9 | 0.94 |
| Triglycerides (mmol/l) | 1.1 ± 0.3 | 1.1 ± 0.4 | 0.84 |
| Glucose (mmol/l) | 5.0 ± 0.4 | 5.0 ± 0.4 | 0.91 |
| HbA1c (%) | 5.7 ± 0.3 | 5.6 ± 0.3 | 0.44 |
| Hs-CRP (mg/l) | 3.0 ± 2.5 | 1.6 ± 1.7 | **0.05** |
| IMT CA ($\mu$m) | 714 ± 113 | 680 ± 99 | 0.32 |
| Framingham Risk Score (%) | 26 ± 16 | 24 ± 12 | 0.71 |
| Lifetime Risk Score (%) | 41.5 ± 5.1 | 30.1 ± 9.4 | **<0.001** |

Data are presented as mean ± standard deviation. **Abbreviations:** HDL high density lipoprotein, LDL low density lipoprotein, HbA1c glycolized hemoglobin, hs-CRP high sensitivity C-reactive protein, IMT CA intima media thickness carotid artery.

## Vascular function

Due to technical problems, 4 participants (2 in each group) were excluded from analyses of vascular function. L-NMMA was only given to 24 participants in total.

The increase in blood flow to acetylcholine and sodium nitroprusside was significantly larger in the higher physical fitness group (Dose*Group $p=0.03$ and $p=0.02$, respectively for ACh and SNP) compared with the lower physical fitness group (Figure 2.1). When presented as the blood flow ratio, a significantly larger increase during

infusion of acetylcholine was observed in the higher physical fitness group compared with the lower physical fitness group (Dose*Group $p=0.04$) (Figure 2.2). The increase in blood flow ratio in response to sodium nitroprusside, however, was not significantly different between both groups ($p=0.17$) (Figure 2.2). The magnitude of the decrease in blood flow and blood flow ratio in response to L-NMMA did not differ between both groups (Figures 2.1 and 2.2).



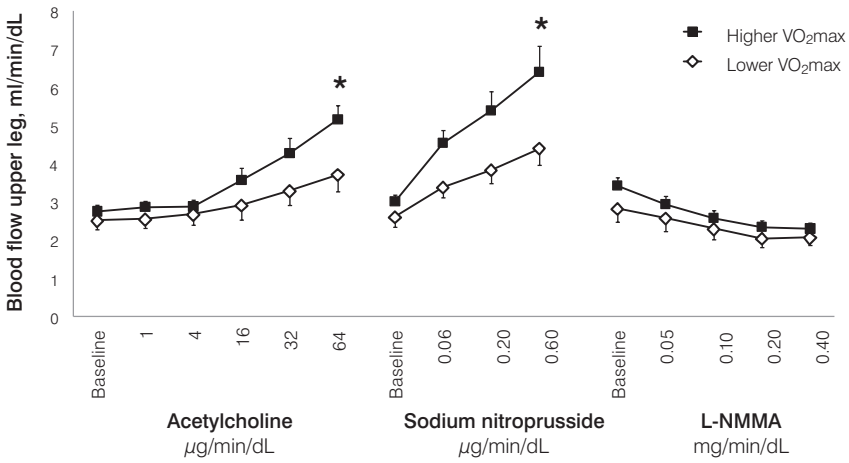


**Figure 2.1** Mean blood flow response (± SEM) of the infusion leg shows a significantly larger endothelium dependent and independent blood flow response to infusion of acetylcholine (* $p=0.03$) and sodium nitroprusside (* $p=0.02$) in the higher physical fitness group (■ n=18) compared with the lower physical fitness group (◇ n=18). No differences were observed in contribution of nitric oxide to baseline vascular tone in response to L-NMMA infusion (n=10 higher physical fitness group and n=14 lower physical fitness group).

## Correlation between physical fitness and vascular function

A significant correlation was observed between $VO_2$max per lean body mass and the maximal blood flow response corrected for baseline blood flow to ACh (r=0.341, $p=0.045$), SNP (r=0.388, $p=0.021$), and L-NMMA (r=-0.450, $p=0.031$) (Figure 2.3).

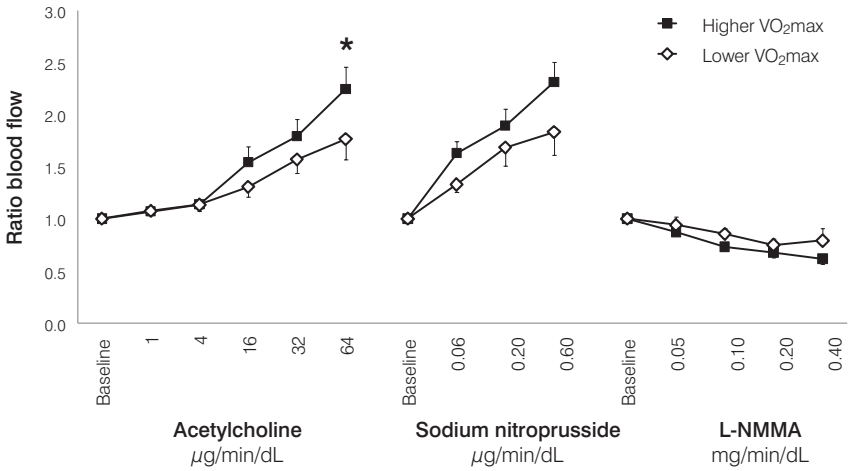


**Figure 2.2** Mean blood flow ratio infusion/control leg (± SEM) shows a significantly larger increase in the higher physical fitness group (■ n=18) compared with the lower physical fitness group (◇ n=18) during infusion of acetylcholine (* $p$=0.04). No statistically significant differences were observed in blood flow ratio between both groups in response to infusion of sodium nitroprusside and L-NMMA (n=10 higher physical fitness group and n=14 lower physical fitness group).

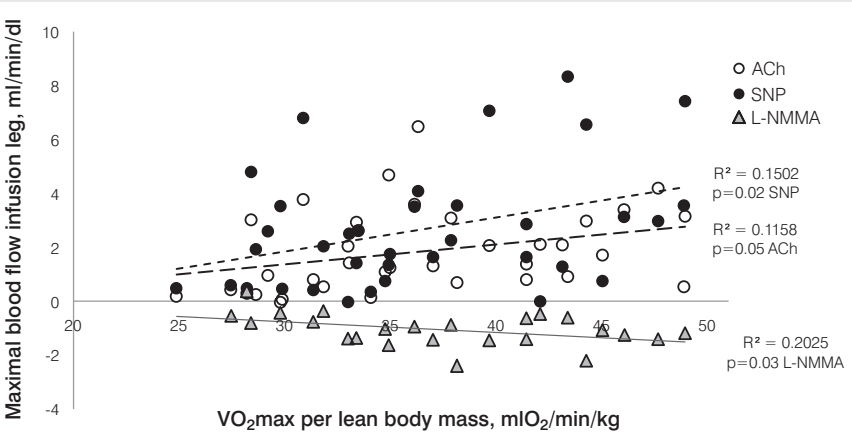


**Figure 2.3** A significant correlation was found between physical fitness ($VO_2$max per lean body mass) and the maximal blood flow response corrected for baseline blood flow in response to acetylcholine (○ n=36, long dashed correlation line), sodium nitroprusside (● n=36, short dashed correlation line) and L-NMMA (grey Δ n=24, grey correlation line).

## Discussion

In this study we examined cardiovascular risk profile and vascular function in a homogenous group of non-exercising older individuals, who were allocated to a lower and higher physical fitness group. This study for the first time shows that a higher level of physical fitness, unrelated to exercise training, is associated with a better cardiovascular risk profile and a better vascular function in older individuals. Results of this study implicate that promotion of daily life activities can be a successful strategy to improve cardiovascular risk in the older population.



The marked differences in physical fitness between our groups cannot be explained by differences in exercise training as all subjects were non-exercisers for at least the past 5-10 years. Accelerometry data suggests that the differences in physical fitness may be related to differences in duration of daily moderate intensity activities (≥3 METs) and in intensity of daily life activities (mean METs/day). This finding is strengthened by the results of McGuire & Ross [9] that both duration and intensity of incidental physical activity are positively associated with physical fitness. Although these correlations were not very strong, on average the higher physical fitness group spend half an hour per day *more* at moderate intensity physical activity level than the lower physical fitness group. In addition, the intensity of daily life activities was significantly higher in the higher physical fitness group.

Our most important finding is the higher blood flow response to acetylcholine in the higher physical fitness group, indicating a better vascular function of the lower limb resistance arteries in this group. The observation that physical fitness is positively related to vascular function, is further supported by the significant and positive correlations between physical fitness and blood flow responses to the vaso-active substances. This novel finding adds to previous studies that demonstrated that exercise training has a beneficial effect on forearm vascular function in healthy young,[1,24-26] as well as in endurance trained older individuals.[24-26] An important difference is that these studies compared either exercise trained subjects with sedentary peers, or included previously sedentary subjects that were examined before and after a period of intensive physical exercise training. In marked contrast, we included non-exercising individuals who differed in physical fitness level primarily based on daily life physical activities. Moreover, we add the interesting finding that a better vascular function is present in non-exercising older individuals who perform half an hour per day more on daily life activities.
Our finding of a better lower limb resistance artery endothelial function in the group with a higher physical fitness questions the underlying mechanism for this finding. Infusion of SNP, an endothelium independent vasodilator, did not result in differences

between both groups when data was presented as blood flow ratio. This indicates that the larger response to acetylcholine in the higher physical fitness group is unlikely explained by differences in smooth muscle cell sensitivity to nitric oxide, a finding which is in agreement with previous findings with sedentary older individuals and between sedentary vs. trained older individuals.[24,25] Another mechanism for the difference in endothelium dependent vasodilation between our groups may relate to an increased NO production and/or release.[1,25] However, the similar blood flow responses to NO synthase inhibitor L-NMMA in our study, suggests that the contribution of NO to the basal vascular tone was similar in both groups. This discrepancy might be due to the fact that previous studies either compared sedentary vs. endurance trained older individuals or performed an exercise training intervention, and therefore the differences in physical fitness were much larger than in our relative homogenous group.

The higher body fat percentage in the lower physical fitness group, may have impacted vascular function. Previous studies found that obesity, independent of other cardiovascular risk factors, relates to an impaired (conduit artery) endothelial function, partly due to higher levels of inflammatory markers.[27] However, we did not include subjects with obesity and the difference in BMI was relatively small (2.5 kg/m$^2$). Moreover, we found no correlation between fat percentage or BMI and vascular function or hs-CRP (data not shown). Therefore, differences in BMI and fat percentage did not alter the major outcomes of our study.
The higher level of inflammatory marker hs-CRP in the lower physical fitness group is believed to reflect inflammation of the arterial wall. Hs-CRP is a strong independent predictor of cardiovascular adverse events in a large multivariate analysis,[28] that even exceeds the predictive capacity of traditional LDL cholesterol level.[29,30] Physical exercise has shown to have a beneficial effect in terms of reducing the concentration of several inflammatory markers,[31,32] and some evidence supports the hypothesis that hs-CRP concentrations positively correlate with vascular dysfunction.[33] In our study, we add the novel observation that a higher physical fitness level related to daily life activities, independent of exercise training, is associated with lower values of hs-CRP.

Another prominent finding was that the Lifetime Risk Score (LRS) was significantly better in the higher physical fitness group than in the lower physical fitness group, while the Framingham Risk Score (FRS) did not differ between groups. The additive of physical fitness, which is an established marker with predictive capacity for future cardiovascular morbidity and mortality,[6,34] in the LRS results in a relevant difference in the 30-year cardiovascular disease mortality risk prediction. Thus, adding physical fitness seems to represent a more reliable risk prediction. This also reinforces our

findings with favourable differences seen in cardiovascular risk profile and vascular function in our higher physical fitness group.

Our result of the favourable effects of differences in physical fitness level, not related to exercise training, is supported by previous findings from a large meta-analysis performed by Löllgen et al in 2009.[35] They included 38 studies involving more than 271,000 participants of both sexes, age ranging from 20-80 years with different activity levels, from sedentary to vigorous activities. The most important risk reduction in cardiovascular and all cause mortality occurred in the change from sedentary behaviour to low or moderate physical activity levels with only a minor additional reduction with further increase of activity level. Our study adds to this by strong direct physiological measurements that even differences in physical fitness level related to daily life activities alone, are beneficial to the cardiovascular risk profile and vascular function.



## Conclusion

In summary, we observed that differences in physical fitness in a representative group of 40 healthy older non-exercising individuals are related to a more favourable cardiovascular risk profile and a better vascular function. This study supports a physiological basis for the beneficial effects of differences in physical fitness level related to daily life activities on cardiovascular health in the older population. Therefore, older individuals should be encouraged to enhance their daily life activities.

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Chapter 3

# The impact of physical fitness and daily energy expenditure on sleep efficiency in young and older humans

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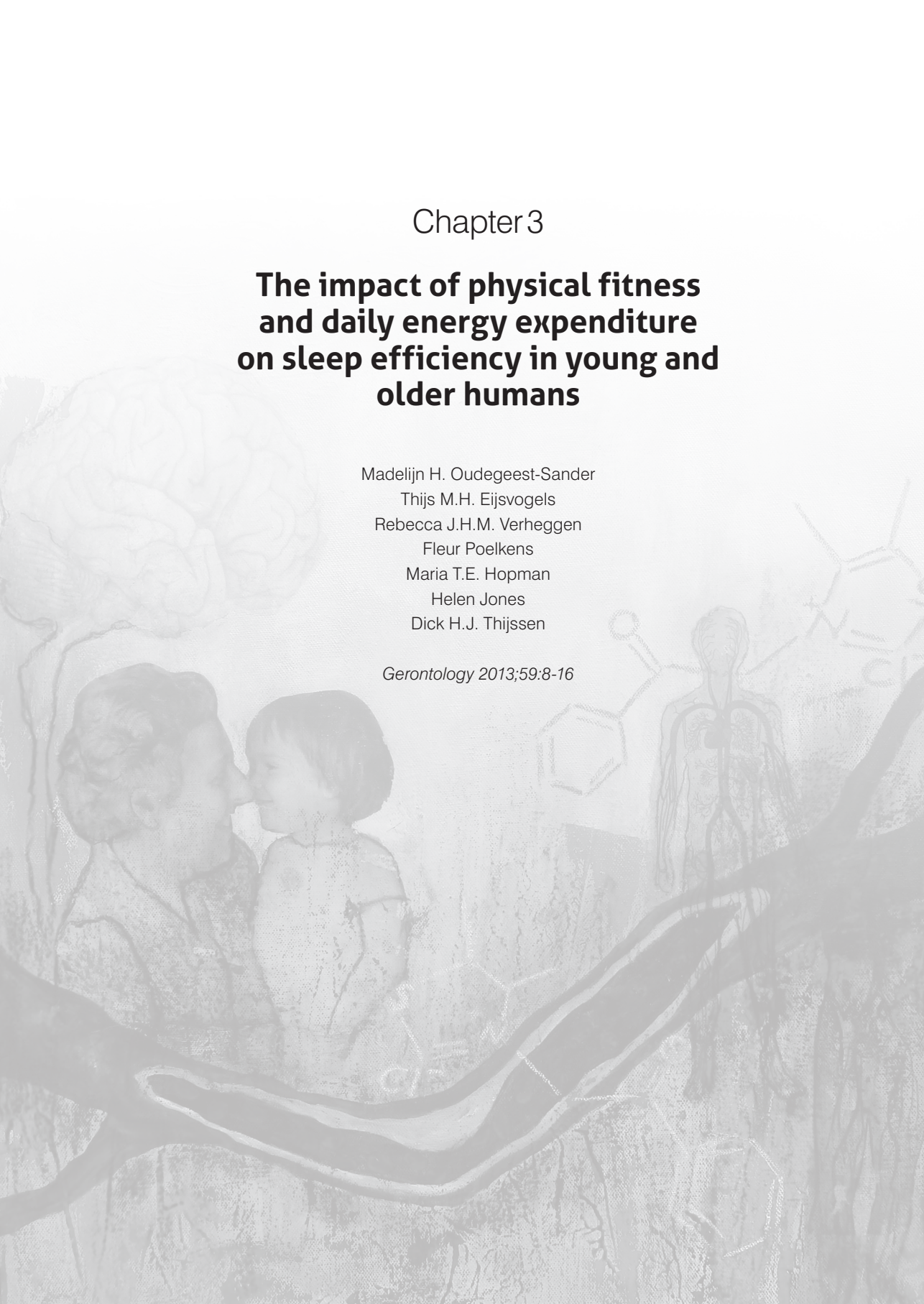

## Abstract

**Background.** Physical activity is known to influence sleep efficiency. Relatively little is known about the relationship between physical activity and sleep efficiency in young and older humans and the impact of exercise training on sleep efficiency in healthy older individuals. Our aim was to determine the relationship between physical fitness and daily energy expenditure with sleep efficiency in young and older subjects, and assess the effect of 12-month exercise training on sleep efficiency in healthy older participants.

**Methods.** The relationship between physical fitness (maximal cycling test) and daily energy expenditure (accelerometry) with sleep efficiency (accelerometry) was examined cross-sectionally in 12 healthy young adults (27 $\pm$ 5 years) and 21 healthy older participants (69 $\pm$ 3 years). Subsequently, the effect of 12-month exercise training (n=11) or control period (n=10) on sleep efficiency in older participants was examined using a randomized controlled trial.

**Results.** Daily energy expenditure and sleep efficiency did not differ between young and older subjects. A significant correlation was found between energy expenditure and sleep efficiency (r=0.627, $p$=0.029) in young adults, but not in older participants (r=-0.158, $p$=0.49). Physical fitness did not correlate with sleep efficiency in either group. Exercise training significantly improved physical fitness (15.0%, $p$<0.001), but failed to alter sleep characteristics such as sleep efficiency, sleep onset latency and awakenings.

**Conclusions.** We found that young adults with higher daily energy expenditure have greater sleep efficiency, whilst this relationship is diminished with advanced age. In contrast, we found no correlation between physical fitness and sleep characteristics in healthy young or older participants, which may explain the lack of improvement in sleep characteristics in older participants with 12-month exercise training. Exercise training may be more successful in subjects with existing sleep disturbances to improve sleep characteristics rather than in healthy older subjects.

## Introduction

Impaired sleep quality is a frequently reported medical complaint,[1] and sleep quality deteriorates with age.[2,3] For example, 74-88% of the older population report sleep disturbances,[3] whilst prevalence rates of insomnia in older subjects range between 12-54%.[3,4] The most common treatment for sleep disturbances is a pharmacological intervention.[5,6] However, frequent use of sleep medication is associated with several adverse side effects (e.g. sedation, drowsiness, risk of falling and dependence),[7,8] which are more common in the older population.[9,10] Therefore alternative strategies to improve sleep quality in this population are required.

Physical activity is known to influence sleep quality. Previous studies have found that higher energy expenditure during the day is associated with energy conservation and tissue restoration during sleep,[11-13] whilst the exercise-induced increase in core body temperature may activate sleep-associated heat-loss mechanisms (see Driver *et al*[13] for an overview). In addition, physical exercise has been shown to have a positive influence on longer sleep duration.[14-16] Indeed, epidemiological studies have demonstrated an association between exercise levels and sleep quality.[17,18] Several studies showed that exercise training can improve subjective and objective measures of sleep in groups with sleep disturbances,[19-21] whilst good sleepers might benefit less from exercise training due to a ceiling effect in sleep quality,[22,23] As most previous studies predominantly focus on groups with (mildly) impaired sleep, relatively little is known about the impact of age *per se* on the relationship between physical fitness or energy expenditure and sleep efficiency; i.e. an objective measure for sleep quality.[24] Moreover, the potential effects of long-term (12 months) exercise training to improve sleep efficiency in healthy, older humans are not frequently examined.

The purpose of the current study was to (i) investigate the relationship between physical fitness and daily energy expenditure with sleep efficiency in young adults and older participants and (ii) using a randomized controlled trial to examine the effect of a 12-month exercise training program on sleep quality measures, such as sleep onset latency and sleep efficiency, in a group of healthy older participants. We hypothesized that a higher physical fitness and a higher daily activity level are both associated with a better sleep efficiency and that 12-months of exercise training will enhance sleep efficiency in healthy older participants.

## Methods

### Participants

Twenty-one healthy older participants (11 males and 10 females, age 69 ± 3 years) and twelve healthy young sex-matched controls (6 males and 6 females, age 27 ± 5 years) were recruited from the local community (see Figure 3.1 for CONSORT

diagram). Older participants, at least 65 years of age, were screened extensively through medical history, physical examination and blood testing. Young adults were screened through medical history and physical examination. Both the older participants and the young adults had a sedentary lifestyle (less than one hour physical activity of at least moderate intensity per week examined by self-report questionnaire). All participants were free of self-reported sleep and mood disturbances and did not use sleep medication, anti-depressants or cardiovascular medication. Individuals with cardiovascular disease and diseases that may interfere with sleep quality such as obesity (BMI >30 kg/m$^2$), diabetes mellitus and hypertension (<160/90 mmHg for older participants, <140/90 mmHg for young controls) were excluded. The local ethics committee approved the study and conformed to the Declaration of Helsinki, all participants gave their written informed consent before participation.

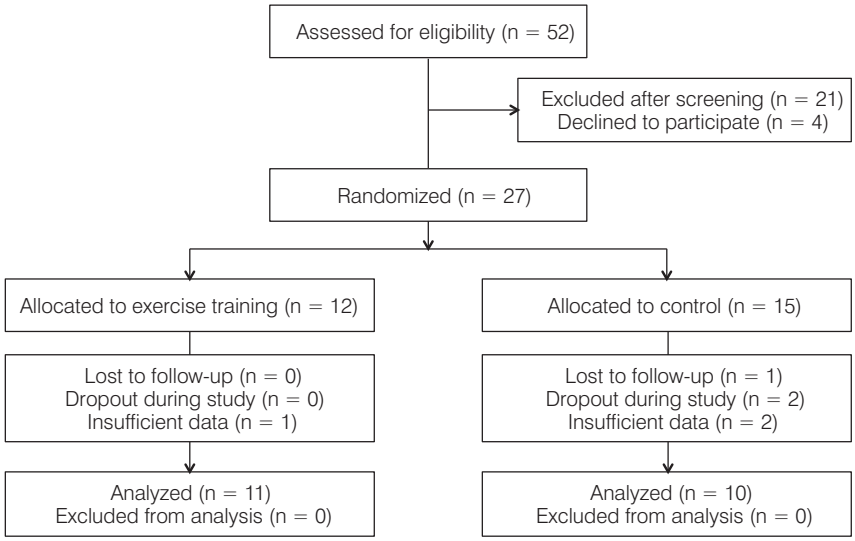


**Figure 3.1** Consort-style flowchart regarding inclusion and drop-out of subjects for our study.

## Experimental Design

Sleep efficiency was examined using accelerometry over a 7-day period. In addition, all participants performed a maximal cycling test to determine physical fitness level. Young participants did not undergo a 12-month intervention, unlike the older participants

who were randomly allocated after the baseline measurements to a 12-month intervention of either cycling exercise training or maintaining their current sedentary lifestyle (control). Participants in the control group were firmly instructed to maintain their normal physical activity level. All measurements were repeated after 6 and 12 months. Efforts were made not to change medication throughout the intervention period, whilst participants who developed health problems that could interfere with sleep quality were excluded from the study.

## Measurements

### *Energy expenditure and physical fitness*

*Energy expenditure.* Daily energy expenditure (EE) was assessed using an activity monitor (SenseWear Pro3 Armband, SWA, Body Media) that was worn around the right upper arm. Sampling frequency was 32 Hz and data from the activity monitor were measured in 60 second epochs. The activity monitor measured 24 hours per day for 7 consecutive days. Each 24 hour interval was analyzed from 12:00 to 12:00 the following day, and was included when the activity monitor recorded at least 1296 minutes per 24-hour cycle (>90% of the total data). Data collected during the time in bed (see later for specific definition) was not included in the analysis for energy expenditure. Energy expenditure was calculated as total number of kilocalories (kcal) used per day. The activity monitor has been validated to examine energy expenditure in humans against indirect calorimetry, i.e. the gold standard to assess energy expenditure in resting and exercise conditions.[25]

*Physical fitness.* Physical fitness was assessed in all participants using a maximal exercise stress test on a bicycle ergometer (Lode, Excalibur Sport, Groningen, the Netherlands) with an incremental protocol (50 Watt and 10 Watt/min for older participants; 0 Watt and 20 Watt/min for young adults). During the test, participants were verbally encouraged to reach their maximum performance. Continuous measurement of oxygen consumption ($VO_2$) was performed using an automatic gas analyzer (Oxycon alpha, Jaeger, Breda, the Netherlands). Peak oxygen uptake, in $mlO_2$/min/kg, was taken as the average oxygen uptake of the last minute of the test and corrected for body weight. Heart rate was measured continuously with a 12-lead ECG. Criteria for the quality of the maximal performance test were: clinical signs of full exhaustion of the participant, respiratory quotient ≥1.10, finishing within 10 beats of the maximum predicted heart rate (=220-age), and flattening of $VO_2$ uptake curve (≤150 $mlO_2$ increase during the last minute).[26] Three out of 4 criteria had to be met for the test to be successful. Furthermore, blood lactate (mmol/l) was measured before and 2 minutes after the test and the 10-point Borg scale was used to rate the perceived exertion. When criteria of maximal performance were not reached, the test was repeated after two weeks.

*Sleep characteristics*

The accelerometer (SenseWear Pro3 Armband, SWA, Body Media) utilizes a combination of sensors (heat flux, galvanic skin response, skin temperature, near body ambient temperature) and a bi-axial accelerometer which allows for assessment of sleep characteristics. The SenseWear data were reduced to binary forms for 'lying' ('0' = no, '1' = yes) and 'sleeping' ('0' = no, '1' = yes). These data were used to determine the sleep onset latency, total sleep time, time in bed and the number of awakenings (see below). Each 24 hour interval was analyzed from 12:00 to 12:00 the following day for a detailed description of one entire sleeping episode. These data were analyzed using a customized analysis software system, which is independent of observer bias. Subsequently, sleep characteristics were averaged across the 7 days and used for further analysis. The activity monitor has been validated to examine sleep against polysomnography;[27,28] i.e. a technique that is considered by many the gold standard to assess sleep physiology in humans.

*Sleep onset latency.* The sleep onset was defined as the first encounter of ten minutes of which at least 90% of the minutes were scored sleeping after positional change from upright to supine position (i.e. change from '0' to '1' for 'lying').[29,30] The sleep onset latency was defined as the duration from the positional change to the start of the sleep onset. This definition is in agreement with various other studies that used accelerometry as an objective measure for sleep quality and efficiency.[24,31,32]

*Total sleep time and sleep efficiency*. The time in bed was defined as the duration from positional change from upright to supine position (i.e. change from '0' to '1' for 'lying') to the first encounter of positional change from supine to upright after awakening (i.e. change from '1' to '0' for 'lying'). The total sleep time was defined as the total sum of the minutes scored sleeping from sleep onset to the end of the sleeping episode. The sleep efficiency was calculated by: ([total sleep time/ time in bed]*100).[24]

*Awakenings*. The number of awakening is the amount of awake periods of at least one minute, excluding the final awakening before arising.[24]

## Exercise Intervention

Exercise training was performed 3 times per week for 12 months on a cycling ergometer (Medgraphics, Corival Cycle Ergometer). Each exercise session was supervised and consisted of 10 minutes warming up at 60% of the individual heart rate reserve (calculated as ([(maximum heart rate – resting heart rate)*0.60] + resting heart rate)). This was followed by 30 minutes cycling exercise at 70-85% of the individual heart rate reserve and ended with 5 minutes cooling down. Heart rate was continuously monitored during exercise using heart rate monitors (Polar RS800; Polar Electro Oy, Kempele, Finland). Workload was individually adjusted throughout the training to correct for improvements in physical fitness.

### Statistical Analysis

Statistical analysis was performed with the use of Statistical Package for the Social Sciences (SPSS, version 16, Chicago, IL, USA). Exploration of distribution indicated the data were normally distributed, therefore baseline differences between the young adults and older participants were analyzed using unpaired *t* tests for continuous variables and Chi square test for categorical data. Correlations between physical fitness, energy expenditure and sleep parameters were determined using Pearson's correlation coefficient. In the older participants, a two-way repeated measures ANOVA was used to analyze changes in physical fitness, energy expenditure and sleep efficiency outcome variables across the 12-month period (Time Points: 0, 6 and 12 months), and to analyze differences between training *versus* control interventions. Data are presented as mean ± SD and the level for significance was set at $\alpha \leq 0.05$.



## Results

All subjects successfully completed our intervention study. Adherence to the exercise training sessions was 91 ± 7% (range 72-97% with a median of 92%). Adherence was defined as completing a training session of at least 30 minutes cycling at an intensity of >70% of the individual's heart rate reserve. Subject characteristics of the two study groups are shown in Table 3.1. Young adults had a lower body mass index and higher physical fitness compared to the older participants. No differences in sleep onset latency, total sleep time and sleep efficiency were observed between young adults and older participants. A significant higher number of awakenings were evident in young adults compared with older participants (Table 3.1).

### Correlation physical fitness/energy expenditure and sleep characteristics

A significant and positive correlation was found between daily energy expenditure and sleep efficiency in young adults (Figure 3.2). This correlation was not present in the older cohort (Table 3.2). Energy expenditure did not correlate with sleep onset latency, total sleep time and awakenings in young adults nor in older participants (Table 3.2). No correlation was found between physical fitness ($VO_2$max) and sleep characteristics in young adults or older participants (Table 3.2).

### Randomized controlled trial in older participants

No differences were found in baseline characteristics between both older groups prior to the 12-month intervention (Table 3.3). Nor were there differences in physical fitness, maximal workload or sleep characteristics between both older groups prior to the 12-month intervention (all $p>0.05$, Table 3.4). A significant increase in physical

**Table 3.1** General and sleep characteristics in healthy young (n=12) and older participants (n=21).

| | Young participants | Older participants | *p*-value |
|---|---|---|---|
| **General characteristics** | | | |
| Age (years) | 27 ± 5 | 69 ± 3 | **<0.001** |
| Height (cm) | 178 ± 8 | 171 ± 8 | **0.02** |
| Weight (kg) | 72.8 ± 9.2 | 74.0 ± 11.2 | 0.84 |
| Body mass index (kg/m2) | 22.9 ± 2.3 | 25.1 ± 2.5 | **0.03** |
| $VO_2$max ($mlO_2$/kg/min) | 40.3 ± 8.2 | 25.1 ± 4.3 | **<0.001** |
| Daily EE (kcal) | 1739 ± 346 | 1773 ± 307 | 0.77 |
| Systolic BP (mmHg) | 122 ± 8 | 131 ± 17 | 0.09 |
| Diastolic BP (mmHg) | 74 ± 8 | 79 ± 11 | 0.20 |
| **Sleep characteristics** | | | |
| Sleep onset latency (min) | 9 ± 6 | 10 ± 6 | 0.88 |
| Total sleeping time (min) | 400 ± 34 | 373 ± 53 | 0.12 |
| Sleep efficiency (%) | 82 ± 5 | 82 ± 9 | 0.83 |
| Number of awakenings (n) | 13 ± 2 | 9 ± 4 | **<0.001** |

Data are presented as mean ± standard deviation. *P*-value refers to an unpaired *t* test between young and older participants. **Abbreviations:** EE energy expenditure, BP blood pressure.

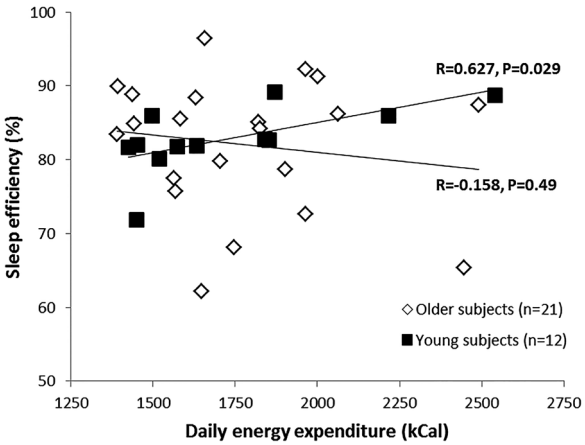


**Figure 3.2** Correlation between energy expenditure (kcal) and sleep efficiency (%) in young adults (solid square) and older participants (open diamonds).

**Table 3.2** Correlation of physical fitness ($VO_2$max) and energy expenditure (EE) with sleep characteristics (sleep onset latency, total sleep time, sleep efficiency and number of awakenings) in young (n=12) and older participants (n=21).

| | Young Participants | *p*-value | Older Participants | *p* -value |
|---|---|---|---|---|
| **Physical fitness** | | | | |
| Sleep onset latency (min) | 0.198 | 0.54 | -0.046 | 0.84 |
| Total sleeping time (min) | -0.162 | 0.62 | -0.014 | 0.95 |
| Sleep efficiency (%) | 0.273 | 0.39 | -0.239 | 0.30 |
| Number of awakenings (n) | 0.299 | 0.35 | 0.422 | 0.06 |
| **Energy expenditure** | | | | |
| Sleep onset latency (min) | -0.167 | 0.60 | 0.138 | 0.55 |
| Total sleeping time (min) | 0.231 | 0.47 | -0.149 | 0.52 |
| Sleep efficiency (%) | **0.627** | **0.029** | -0.158 | 0.49 |
| Number of awakenings (n) | -0.154 | 0.63 | -0.013 | 0.96 |

**Table 3.3** General characteristics of the older population included in the 12-month intervention of either exercise training (n=11) or control group that maintained their physical activity level (n=10).

| | Exercise training | Control | *p*-value |
|---|---|---|---|
| **Baseline characteristics** | | | |
| Gender Male : Female | 5 : 6 | 5 : 5 | |
| Age (years) | 68 ± 2 | 70 ± 2 | 0.08 |
| Height (cm) | 173 ± 8 | 170 ± 7 | 0.49 |
| Weight (kg) | 76.5 ± 12.4 | 71.3 ± 10.4 | 0.31 |
| Daily EE (kcal) | 1863 ± 364 | 1675 ± 207 | 0.17 |
| Systolic BP (mmHg) | 125 ± 12 | 137 ± 19 | 0.11 |
| Diastolic BP (mmHg) | 76 ± 10 | 82 ± 13 | 0.31 |

Data are presented as mean ± standard deviation. *P*-value refers to an unpaired *t* test between both groups. **Abbreviations:** EE energy expenditure, BP blood pressure.

**Table 3.4** Physical fitness and sleep characteristics of the exercise group (n=11) and control group (n=10). Participants were measured at 0, 6 and 12 months.

| | | Time (months) | | | |
|---|---|---|---|---|---|
| | Group | 0 | 6 | 12 | 2-way ANOVA |
| **Characteristics** | | | | | |
| Body mass index | Training | 25.5 ± 2.1 | 24.9 ± 2.1 | 24.7 ± 2.3 | **Intervention: 0.007** |
| ($kg/m^2$) | Control | 24.5 ± 2.8 | 24.6 ± 2.4 | 24.3 ± 2.3 | Time*Group: 0.084 |
| Energy expenditure | Training | 1863 ± 364 | 1954 ± 319* | 1751 ± 309 | **Intervention: 0.01** |
| (kcal) | Control | 1675 ± 207 | 1721 ± 222 | 1647 ± 220 | Time*Group: 0.35 |
| $VO_2$max | Training | 24.7 ± 3.6 | 28.5 ± 3.3 | 28.4 ± 3.7 | **Intervention: 0.013** |
| ($mlO_2/kg/min$) | Control | 26.0 ± 5.2 | 25.5 ± 4.9 | 25.1 ± 4.1 | **Time*Group: <0.001** |
| Maximal workload | Training | 141 ± 31 | 169 ± 33 | 168 ± 32 | **Intervention: 0.005** |
| (Watt) | Control | 144 ± 41 | 140 ± 35 | 141 ± 38 | **Time*Group: <0.001** |
| **Sleep characteristics** | | | | | |
| Sleep onset latency | Training | 11 ± 7 | 10 ± 6 | 11 ± 5 | Intervention: 0.92 |
| (min) | Control | 8 ± 4 | 10 ± 2 | 8 ± 3 | Time*Group: 0.45 |
| Total sleeping time | Training | 371 ± 52 | 366 ± 42 | 367 ± 55 | Intervention: 0.73 |
| (min) | Control | 386 ± 46 | 374 ± 44 | 379 ± 34 | Time*Group: 0.97 |
| Sleep efficiency | Training | 82 ± 11 | 84 ± 10 | 80 ± 12 | Intervention: 0.06 |
| (%) | Control | 84 ± 8 | 81 ± 9 | 82 ± 7 | Time*Group: 0.51 |
| Number of | Training | 9 ± 4 | 8 ± 4 | 10 ± 6 | Intervention: 0.20 |
| awakenings (n) | Control | 10 ± 3 | 9 ± 3 | 10 ± 3 | Time*Group: 0.58 |

Data are presented as mean ± standard deviation. A 2-way ANOVA was used to examine changes in parameters across the 12-month period ('Intervention'; 0 *versus* 6 *versus* 12) and whether the change across the intervention differed between both groups ('Time*Group'). *P*-values for these comparisons are provided in this table. * Post hoc significantly different from 0.

fitness and maximal workload was found in older participants who performed the exercise training (Table 3.4). These findings indicate that our exercise training program was successful to improve physical fitness levels in healthy older individuals. A similar change across time was found in energy expenditure in both groups, with higher levels at 6 months (Table 3.4). However, we found no effect of the 12-month intervention on sleep onset latency, total sleep time, sleep efficiency and the number of awakenings (Table 3.4).

## Discussion



The purpose of this study was to examine the impact of advanced age on the relation between physical fitness and daily energy expenditure with sleep characteristics. We have a number of unique findings in this study. First, a strong relationship between energy expenditure and sleep efficiency was found in young adults and this relationship was absent in the older population. Second, physical fitness and sleep characteristics were not significantly correlated in young adults or older participants. Finally, despite significantly improved physical fitness in healthy older participants, exercise training had no significant effect on sleep characteristics in this population. Collectively, these findings suggest that the relationship between energy expenditure and sleep efficiency is altered in healthy older participants compared with young peers. The absence of a correlation between physical fitness and sleep efficiency could explain the lack of improvement in sleep efficiency after a 12-month exercise training program in healthy older participants.

We found no significant differences in sleep time and sleep efficiency between young adults and older participants in our study. This finding is consistent with observations from Jean-Louis et al,[33] but contrasts with others that reported that older age is associated with lower sleep efficiency.[4,34] A potential explanation for the conflicting results is that some previous studies used sleep questionnaires to assess sleep efficiency,[4] which are found to be less reliable than activity monitors to examine sleep efficiency.[35] Another important difference between studies is that we included healthy participants without the presence or history of sleep problems, whilst others included older participants who were institutionalized [34] and who were likely to demonstrate altered sleep patterns.[36] Finally, one previous study did not control for pharmacological interventions [34] that may have interfered with sleep efficiency. An unexpected finding in our study relates to the higher number of awakenings in young adults than in older people, which contrasts with findings of previous studies.[34,37] However, one previous study also found more awakenings in younger women using an actigraph.[33] Young adults may demonstrate more movement during sleep, which is not necessarily

associated with awake episodes. As a result, accelerometry may (incorrectly) identify these periods of higher activity level during sleep as awake periods.[31,32,38] Collectively, we found no differences in sleep efficiency between young adults and older participants, which indicates that we have included healthy young adults and older participants without sleep disturbances.

We found a strong positive correlation between daily energy expenditure and sleep efficiency in young people. This finding is in agreement with meta-analyses indicating that sleep may improve following physical exercise of longer durations [14,16] and that duration, rather than intensity or time of day, might be more predictive of better sleep.[14] Another study examined physical activity levels and found no significant association between day-to-day physical activity and sleep in healthy young and older subjects.[23] However, they also found a small but significantly better self-reported sleep on the most active days compared to the least active days in young subjects. Although speculative, activation of thermoregulatory responses during periods of increased physical activity may relate to the positive correlation between daily energy expenditure and sleep efficiency in young adults.[13] Physical activity, even when performed at moderate intensity such as walking [39,40] or cycling,[41] causes a mild rise in core body temperature, and is followed by activation of heat-loss processes.[42] When performed in the evening, these responses may play a role during the initiation of sleep.[43,44] However, the exact role of the thermoregulation to explain the link between activity level and sleep should be further examined. Another explanation relates to the ability of sleep for energy conservation and tissue restoration. Increased daily energy expenditure will deplete energy stores, leading to a larger energy restoration during sleep, which consequently is associated with an enhanced sleep efficiency.[12,13] Finally, high catabolic activity during exercise is associated with a higher energy expenditure, which leads to elevated anabolic activity during sleep. The higher anabolic activity is believed to promote energy use for tissue restoration, but also contributes to improved sleep efficiency.[11,13]

An important and novel finding is that the positive relationship between energy expenditure and sleep efficiency is altered with advanced age, as older participants did not demonstrate a significant correlation. This observation raises questions about the potential mechanisms for the altered relationship between energy expenditure and sleep efficiency with advanced age. One potential explanation relates to the age-related degeneration of the suprachiasmatic nucleus in the hypothalamus.[44] The suprachiasmatic nucleus is the major circadian pacemaker that coordinates behavioral and hormonal circadian rhythms, such as core body temperature, and initiates sleep through thermoregulatory responses.[43] Degeneration of the suprachiasmatic nucleus in older individuals may play a role in their diminished rest-activity circadian rhythm.[34] Another explanation relates to the ability of anabolic processes to

regulate sleep. Advanced age is associated with a smaller skeletal muscle mass,[45] but also inadequate protein intake.[45,46] These changes may contribute to impaired anabolic processes in the older group and, therefore, to the impaired relation between energy expenditure and sleep efficiency in older participants. Taken together, our study provides the unique observation that advanced age leads to an attenuated relation between energy expenditure and sleep efficiency.

We found no correlation between physical fitness and sleep characteristics in young adults. This finding is in agreement with some studies,[47,48] which also found that physical fitness does not determine sleep efficiency in young participants. Although others found a correlation between physical fitness and sleep quality,[49] this study used subjective measures to assess sleep. We extend the findings of these previous studies that also healthy older individuals do not demonstrate a relation between physical fitness and sleep characteristics. These findings seem to contrast with the correlation between energy expenditure and sleep characteristics. Nevertheless, higher energy expenditure does not necessarily correlate with a higher physical fitness level.[50] It is demonstrated that higher intensity daily activity levels (METs) rather than energy expenditure, relate to higher physical fitness.[51] Indeed, also in our cohorts of healthy young adults and older participants, we found no significant correlation ($r=0.086$, $p=0.64$) between energy expenditure and physical fitness. Therefore, our results suggest that a higher physical fitness level *per se* does not contribute to an improvement of sleep characteristics in healthy individuals.
The lack of a relationship between physical fitness and sleep characteristics is confirmed by the findings of the 12-month exercise training in older individuals. Whilst a marked improvement in physical fitness and maximal workload was found, no changes in sleep characteristics were evident. Although some previous studies provided evidence that exercise training improves sleep quality in adults with (mild) sleep disturbances, these studies typically employ self-reported, subjective measures of sleep quality.[19,21] When adopting objective measures of sleep, beneficial effects of exercise training on sleep characteristics are rare [52,53] or mild.[20] Another important methodological difference is that previous studies included participants with moderate sleeping complaints, who are therefore more likely to benefit from an (exercise) intervention to alter sleep characteristics than participants with a normal sleep pattern,[13] such as those included in our study. Subjects with normal sleep patterns show a ceiling effect when the effect of an intervention is examined aimed to improve sleep.[13,22] Therefore, our finding that exercise training does not improve sleep characteristics in healthy older subjects, despite the improved physical fitness, further supports our finding that the relationship between energy expenditure and sleep is blunted with age.

### Limitations

A number of limitations should be considered. First, in this study accelerometry was used to measure sleep characteristics. Accelerometry is a valid and accurate method to assess sleep characteristics [27,28,32,38] and is used previously in sleep-activity studies.[31,34] Accelerometry provides the advantage of providing an objective assessment of sleep characteristics in a home-based setting with little disruption to normal sleep pattern, whereas the use of polysomnography is impractical in a home-based setting and typically requires a laboratory visit. Nonetheless, the validity of the SenseWear to examine sleep loses some accuracy when sleep becomes more disturbed.[28] Also, estimation of periods of wakefulness by the SenseWear is less accurate than sleep,[28] which may impact the total sleep time variable, and therefore the calculation of sleep efficiency. Nevertheless, a previous study found minor discrepancy (14 minutes) between the SenseWear and polysomnography for the total sleep time.[28] Whilst the use of the SenseWear is associated with some limitations, we have not included subjective measures of sleep quality in our study. Moreover, participants were not informed about the purpose of this study, which excludes the potential for subject bias. Therefore, based on the purpose and methodology of our study, accelerometry is an appropriate and valid tool to examine sleep efficiency.
Another limitation of this study is that we included healthy older subjects without sleep disturbances. Inclusion of such a homogeneous group makes it difficult to extrapolate our results to different groups, whilst the absence of sleep disturbances resulted in low *a priori* chances to improve sleep characteristics. Also, the relatively small sample size may have contributed to the lack of improvement in sleep characteristics after training. However, it should be taken into consideration that subjects trained for 12 months; i.e. a methodological design that corrected for potential seasonal influences and thereby excluded the potential influence of the daylight cycle on sleep characteristics.[13,54] Taken together, our results indicate that exercise training in healthy older participants, without (a history of) sleep complaints, does not alter objective measures of sleep efficiency.

## Conclusion

In conclusion, we found that young adults with higher daily energy expenditure have greater sleep efficiency, whilst this relationship is attenuated with advanced age. In contrast, we found no correlation between physical fitness and sleep characteristics in healthy young adults or older participants. This may explain the lack of improvement in sleep characteristics in healthy older participants with 12 months of exercise training. Exercise training may be more successful in subjects with existing sleep disturbances to improve sleep characteristics rather than in healthy older subjects.

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Chapter 4

# Assessment of dynamic cerebral autoregulation and cerebrovascular $CO_2$ reactivity in aging by measurements of cerebral blood flow and cortical oxygenation

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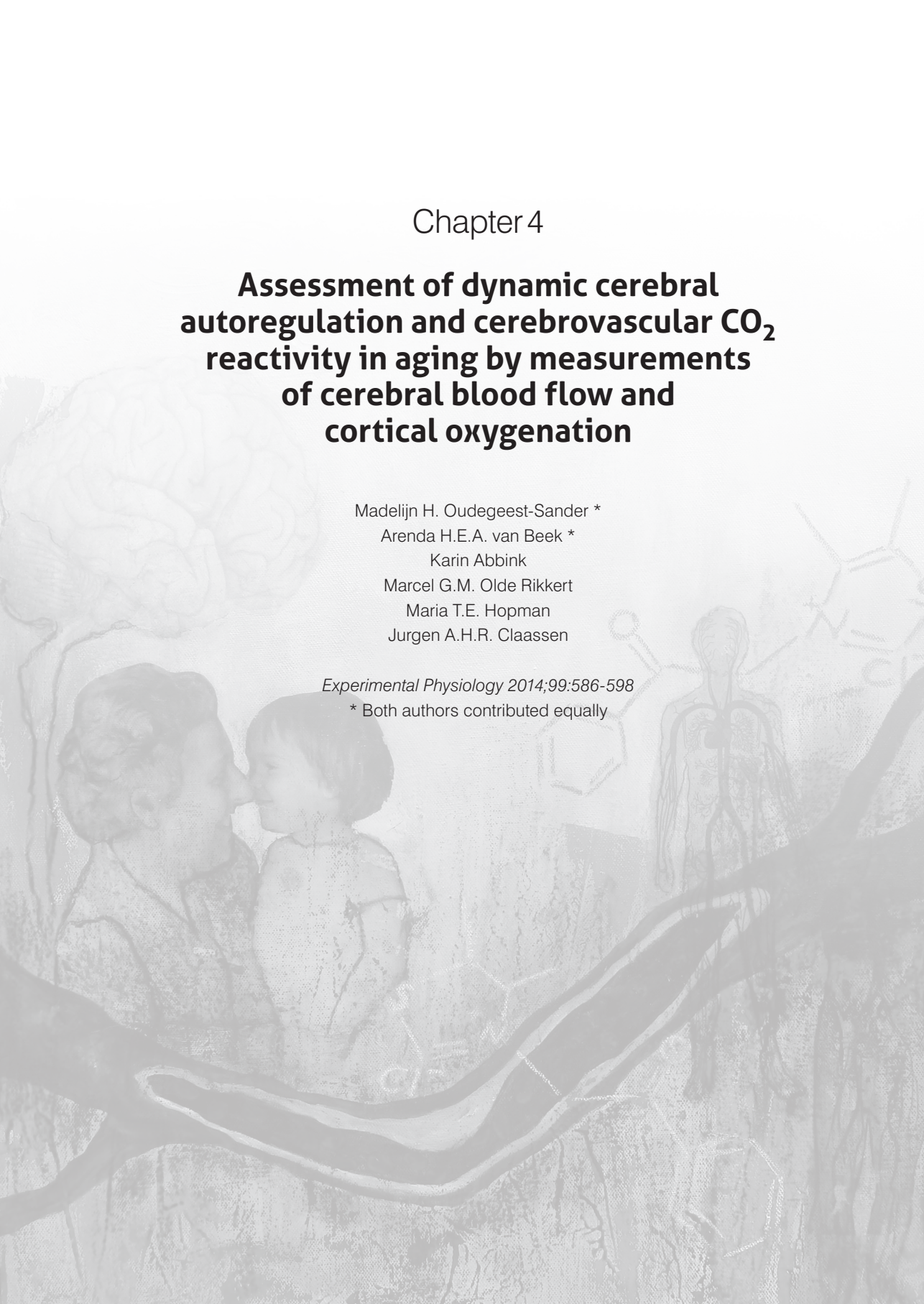

## Abstract

**Background**: With aging cerebral blood flow velocity (CBFV) decreases. However, to what extent dynamic cerebral autoregulation and cerebrovascular carbon dioxide ($CO_2$) reactivity are influenced by aging is unknown. The aim was to examine the dynamic responses of CBFV and cortical oxygenation to changes in blood pressure (BP) and arterial $CO_2$ across different ages.

**Methods**: Fifty-eight participants in three age groups were included: young (n=20, 24 ± 2 years), elderly (n=20, 66 ± 1 years), and older elderly (n=18, 78 ± 3 years). Cerebral blood flow velocity was measured using transcranial Doppler, simultaneously with oxyhemoglobin ($O_2Hb$) using near-infrared spectroscopy and beat-to-beat blood pressure measurements using Finapres. Postural maneuvers were performed to induce blood pressure fluctuations. Cerebrovascular $CO_2$ reactivity was tested with hyperventilation and $CO_2$-inhalation.

**Results**: With age, CBFV decreased (young 59 ± 12 cm/s, elderly 48 ± 7 cm/s, older elderly 42 ± 9 cm/s, $p<0.05$) and cerebrovascular resistance increased (1.46 ± 0.58 mmHg/cm/s, 1.81 ± 0.36 mmHg/cm/s, 1.98 ± 0.52 mmHg/cm/s, $p<0.05$). Normalized gain (autoregulatory damping) increased with age for BP-CBFV (0.88 ± 0.18, 1.31 ± 0.30, 1.06 ± 0.34, $p<0.05$) and CBFV-$O_2Hb$ (0.10 ± 0.09, 0.12 ± 0.04, 0.17 ± 0.08, $p<0.05$) during the repeated sit-stand maneuver at 0.05 Hz. Even though the absolute changes in CBFV and CVRi during the cerebrovascular $CO_2$ reactivity were higher in the young group, the percentage changes in CBFV, CVRi and $O_2Hb$ were similar in all age groups.

**Conclusion**: There was no decline in dynamic cerebral autoregulation and cerebrovascular $CO_2$ reactivity with increasing age up to 86 years. Despite the decrease in cerebral blood flow velocity and increase in cerebrovascular resistance with advancing age, CBFV and cortical oxygenation were not compromised in these elderly during maneuvers that mimic daily life activities.

## Introduction

Physiological aging is associated with well-recognized changes in the systemic and cerebral vasculature.[1-3] Systemic vascular changes lead to an increased risk for cardiovascular events while cerebrovascular adaptations enhance the risk of (ischemic) stroke. Furthermore, resting cerebral blood flow (CBF) is decreased in older individuals.[4,5] Whether this decrease in CBF reflects a decrease in cerebral demand or a true diminution in blood flow, remains unknown. Likewise, whether aging leads to diminished cerebrovascular function remains unclear.

Cerebrovascular function can be studied using two physiological phenomena, namely dynamic cerebral autoregulation and cerebrovascular reactivity to carbon dioxide ($CO_2$). Dynamic cerebral autoregulation reflects the ability of the brain to stabilize CBF despite rapid daily life changes in blood pressure.[6-8] Dynamic cerebral autoregulation remains stable up to the age of 73 years,[4,5,8] but has not been estimated in healthy elderly above this age. Most studies examined the effects of small blood pressure changes [9,10] instead of larger fluctuations in blood pressure during postural changes, which may provide a stronger challenge for dynamic cerebral autoregulation, and therefore may be more sensitive to detect changes in cerebrovascular function.[11]

Cerebrovascular reactivity to changes in carbon dioxide ($CO_2$), the second phenomenon to study cerebrovascular function, declines significantly with increasing age up to 90 year.[12] Unfortunately, most studies investigated either the dynamic cerebral autoregulation [5,11,13,14] or the cerebrovascular $CO_2$ reactivity,[12,15-18] but the studies did not examine both aspects of the cerebrovascular system simultaneously.

Dynamic cerebral autoregulation and cerebrovascular $CO_2$ reactivity are both directly related to the oxygenation of the brain tissue and therefore to human brain function.[19,20] The majority of studies of dynamic cerebral autoregulation and cerebrovascular $CO_2$ reactivity have used transcranial Doppler to obtain continuous measurements of cerebral blood flow velocity, together with continuous blood pressure measurements (Finapres). Transcranial Doppler does not provide information regarding the oxygenation of the brain. The addition of near-infrared spectroscopy can be used to measure concentrations of oxygenated and deoxygenated hemoglobin and provide estimates of cortical brain tissue oxygenation.[21] Thus, by using Finapres and transcranial Doppler sonography (TCD) concomitantly with near-infrared spectroscopy (NIRS), it is possible to determine two different but dependent dynamic relationships, namely the relation between blood pressure and cerebral blood flow on the one hand and the relation between cerebral blood flow and cerebral cortical oxygenation on the other.[19,20]

This study aimed to investigate effects of age on dynamic cerebral autoregulation and cerebrovascular $CO_2$ reactivity in order to examine cerebrovascular function by

investigating the relationship between blood pressure, cerebral blood flow and cerebral cortical oxygenation in a group of young, elderly, and older elderly subjects. The combination of examining dynamic cerebral autoregulation and cerebrovascular $CO_2$ reactivity using both TCD and NIRS in this population is, to our knowledge, unique. We hypothesized that the cerebrovascular function and thereby the cortical oxygenation are diminished in healthy elderly aged 74 years and older compared with young subjects and with elderly below the age of 74 years. To investigate this hypothesis, beat-to-beat blood pressure, cerebral blood flow, and cortical oxygenation were simultaneously measured to investigate cerebrovascular hemodynamics during changes in blood pressure and changes in arterial $CO_2$.

## Methods

### Ethical approval

The study was approved by the Medical Ethics Committee, and was performed according to the Declaration of Helsinki and Good Clinical Practice guidelines. All participants gave written informed consent.

### Participants

Fifty-eight participants in three different age groups were included. A young group (young - mean age 24 ± 2 years, range 21 to 28 years), an older group (elderly - mean age 66 ± 1 years, range 65 to 69 years), and the oldest group (older elderly - mean age 78 ± 3 years, range 74 to 86 years). Participants were recruited at the Departments of Physiology and Geriatric Medicine, Radboud University Medical Center, Nijmegen, the Netherlands. All participants were extensively screened. Participants with hypertension (>160/90 mmHg), diabetes mellitus reported in medical history (fasting glucose >6.9 mmol/l), hypercholesterolemia (total cholesterol >7.5 mmol/l), body mass index >32.5 kg/m$^2$, compromised cognition (Mini Mental State Examination <26) or cardiovascular or cerebrovascular diseases were excluded from participation. Furthermore, auscultation of the carotid arteries was performed and participants with significant atherosclerotic lesions in the carotid arteries indicated by murmurs were also excluded from participation. None of the participants were regularly seeing a general practitioner or medical specialist and were not using medication known to interfere with the cardiovascular system.

### Instrumentation

*Heart rate and blood pressure*. A three-lead electrocardiogram (ECG) was used for heart rate registration and measurement of the R-R interval. Beat-to-beat blood pressures (systolic, diastolic and mean arterial pressure (mean blood pressure)) were

continuously measured on the left index or middle finger using a photoplethysmographic cuff from the Finometer (Finapres Medical Systems, Amsterdam, the Netherlands). The left hand was held at heart level by a supporting sling to eliminate changes in hydrostatic gradient during measurements. During each maneuver data were collected without interruption in beat-to-beat blood pressure measurement. The return to flow calibration that is built-in in this Finometer to calibrate finger pressure to brachial artery pressure was performed between the different maneuvers. This procedure uses a brachial cuff that is inflated above systolic pressure and then gradually released.

*Cerebral blood flow velocity* (CBFV) in the middle cerebral artery was measured bilaterally using TCD with 2 MHz pulsed Doppler probes (Multi-Dop, DWL Medical systems, Singen, Germany). The CBFV signal of the middle cerebral artery was identified according to the criteria of Aaslid et al. using the signal depth, velocity, and wave characteristics.[22] The TCD probes were fixed at a constant angle and positioned with a customized headband (Spencer technologies, Seattle, WA, USA) over the temporal window. Depth, sample volume and signal power of the TCD measurement were noted for each participant. If only one CBFV signal was available, this signal was used for analysis. This was the case in one young subject, five elderly and four older elderly. Unilateral measurements were performed consistently throughout the maneuvers in these individuals. In the majority, both left and right CBFV signal were available and of sufficient quality, and then the average of both CBFV signals was used. Several studies have demonstrated that the diameter of the middle cerebral artery does not change appreciably even during moderate alterations in blood pressure and end-tidal $CO_2$.[23-26] Assuming that the diameter of the insonated artery remains constant within a subject, changes in CBFV represent changes in CBF.

*Frontal cortical oxygenation* was measured using the validated technique of NIRS.[27,28] Changes in the concentration of oxygenated and deoxygenated hemoglobin ($O_2$Hb and HHb, respectively) in the cerebral cortex were measured.[19,29] The basic principle of NIRS is that near-infrared light penetrates the skull and brain, and is absorbed by the chromophores $O_2$Hb and HHb that have different absorption spectra. Assuming constant light scattering, changes in concentrations of these chromophores lead to changes in light absorption, and from the reflected light these $O_2$Hb and HHb concentrations can then be calculated using the modified Lambert-Beer law.[30,31] Two pairs of NIRS optodes were placed and tightly fixed over the left and right frontal cortex in the customized headband that locked the Doppler probes. We applied a continuous wave NIRS device with 3 light bundles with wavelengths 775 nm, 845 nm, and 904 nm (Oxymon, Artinis Medical Systems, Zetten, the Netherlands). An interoptode distance of 5.0 cm was used to minimize contamination from the extracerebral circulation and to maximize signal intensity.[30] The differential pathlength factor, which accounts for the increased distance travelled by light due to scattering,

is age-dependent.[32] At present however, no data are available on the actual variation of differential pathlength factor in adults aged above 50 years.[30,32] The differential pathlength factor was set to 6.6 (corresponding to age 50) in all groups. Moreover, contributions from extracranial tissue, such as skin tissue, to NIRS measurements could not be excluded. However, these contributions are relatively small with an interoptode distance of at least 5.0 cm.[20,21,29,33] Total hemoglobin (THb), the sum of $O_2$Hb and HHb, is associated with local cerebral blood volume, whereas $O_2$Hb is associated with local cerebral blood flow [21] in the frontal cortex.

## Protocol

To standardize the measurements, participants were asked to refrain from heavy exercise 24 hours prior to the examinations, not to take caffeinated drinks or alcohol 14 hours prior, and only have a small breakfast in the morning at least 2 hours before the visit. All participants were measured in the morning in a quiet and temperature controlled room.

The following maneuvers were performed in a fixed order. The measurement started with a ten minute baseline in sitting rest. The different parts of the protocol were separated by two minutes sitting rest. For the repeated sit-stand maneuver, participants were coached to perform a repeated sit-stand maneuver at 0.05 Hz (10 seconds sitting - 10 seconds standing) for five minutes.[11,13] For the single squat-stand maneuver participants performed standing for one minute, then squatting for one minute and then again standing for five minutes. For the cerebrovascular $CO_2$ reactivity measurements, participants were instructed to hyperventilate with deep breaths at a frequency of 0.5 Hz (1 second breath in - 1 second breath out, demonstrated by the researcher during the measurement) for one minute to induce hypocapnia. After a recovery of two minutes, participants inhaled a $CO_2$ gas mixture through a mouthpiece with a Y-valve connected to a five liter rubber bag. This bag was fed by a gas mix system that combines 21% oxygen with variable levels of $CO_2$ and $N_2$, at a flow of 20 l/min. The concentration of $CO_2$ was gradually increased from 0% to 7% in the fourth minute (oxygen was kept constant at 21%, while $N_2$ decreased from 79% to 72%), followed by a recovery period of two minutes. End-tidal $CO_2$ ($EtCO_2$) was monitored via a nasal canule using a capnograph (N1000, Nellcor, Boulder, CO).

## Data processing

All data were simultaneously recorded at 125 Hz for offline analysis (Oxysoft, Artinis Medical Systems, the Netherlands). A running average filter of 1 second was applied to the NIRS measurements to increase the signal to noise ratio and to reduce the heart beat and high frequency noise.[30,31] Baseline heart rate, systolic, diastolic and mean blood pressure, systolic, diastolic and mean CBFV were calculated from the

baseline sitting rest measurement. Cerebrovascular resistance index (CVRi) was calculated as the ratio of mean blood pressure and CBFV. Spectral analysis was performed on beat-to-beat mean blood pressure, mean CBFV and $O_2Hb$ changes.

*Frequency and transfer function analysis*

All transfer function analyses were performed with commercially available software (DADiSP, DSP Development, Cambridge, MA). The signals were aligned with the time of the R wave peaks of the ECG. The time series were then linearly interpolated at 2 Hz to obtain equidistant time intervals and detrended with third-order polynomial fitting. These data then were analyzed in windows containing data segments of 128 points (64 seconds), with 50% overlap. A Hanning-window was applied for spectral estimation. This data segmentation is based on a trade-off between a reduction in spectral variance and keeping sufficient spectral resolution. Each Hanning-windowed segment was fast-Fourier transformed and the periodograms were averaged to calculate the autospectrum of mean blood pressure, CBFV and $O_2Hb$.[19,34] Spectral power of oscillations was calculated as the area under the curve of the power spectrum density (PSD) plots. The HHb signal was excluded from analysis, since the signal demonstrated only very small changes.

*Baseline measurement*

For analysis of the baseline measurement a time period of 400 seconds was used. Transfer function analysis was performed according to 3 predefined frequency domains:[34] very low frequency (VLF: 0.02-0.07 Hz), low frequency (LF: 0.07-0.20 Hz) and high frequency (HF: 0.20-0.35 Hz). The dynamic relationship between blood pressure as input and CBFV as output (mean blood pressure-CBFV) was determined with transfer function analysis. Since the spontaneous oscillations during baseline rest measurement in the $O_2Hb$ signal were very small and therefore coherence was insufficient (see the following paragraph) the relation CBFV-$O_2Hb$ was not assessed. Mean values of coherence, phase and gain of the transfer function were analyzed in the same frequency ranges as described in the previous paragraph. We have previously described these estimates in detail.[8] In short, *coherence* tests the linearity of the relationship between input (mean blood pressure) and output (CBFV). Coherence approaching unity in a specific frequency suggests a linear relationship, whereas coherence approaching zero indicates no relationship between the signals, severe extraneous noise or a non-linear relationship.[35,36] A coherence of 0.4 is considered the lower limit where transfer function estimates can be calculated with confidence.[10,34] The *phase shift* quantifies the displacement in time of a signal relative to another signal. Normal autoregulation causes a positive phase shift between CBFV and blood pressure. *Gain* determines how changes in blood pressure are transmitted into CBFV. A low gain indicates an efficient autoregulation (damping),

whereas an increase in gain represents a diminished efficiency of the dynamic process of cerebral autoregulation. Normalized gain (Gain*CVRi) was used to reduce the influence of individual differences in absolute levels of blood pressure and CBFV on gain. Estimates of coherence, phase and gain were therefore only included if coherence was >0.4 in this study.[11,13,34]

*Repeated sit-stand maneuver at 0.05 Hz*

The repeated sit-stand maneuver causes oscillations that are much larger than the spontaneous oscillations that occur during baseline sitting rest position, therefore the dynamic relationship can be assessed with greater coherence. Transfer function analysis was performed in the very low frequency range 0.02-0.07 Hz. The dynamic relationships between blood pressure as input and CBFV as output (mean blood pressure-CBFV) and CBFV-$O_2$Hb were determined. Data were also only included if coherence was >0.4. We have previously shown that upstream oscillations in CBFV induced by changes in blood pressure contribute importantly to the downstream brain tissue level oscillations in $O_2$Hb.[19] It is our experience that oscillations induced at a frequency of 0.05 Hz result in clear blood pressure and CBFV oscillations with good coherence whereas oscillations induced at a frequency of 0.1 Hz result in more noise and thereby loss of reliable data.[13,19] Furthermore, in accordance with the high pass filter, dynamic cerebral autoregulation is mainly active in the low and very low frequency regions.

*Single squat-stand maneuver*

For the single squat-stand maneuver we analyzed two time periods: 1) squatting and 2) maximum decline after standing up from squatting. For this we used 30 seconds of steady state in squatting position (1) and the period of 5 consecutive seconds in which mean blood pressure and CBFV had decreased the most upon standing (2). Both absolute and relative changes in mean blood pressure and CBFV were calculated.

*Cerebrovascular $CO_2$ reactivity*

Regarding the analysis of the cerebrovascular $CO_2$ reactivity, absolute and relative changes in heart rate, mean blood pressure, CBFV, CVRi and $O_2$Hb were calculated. The full range of cerebrovascular $CO_2$ reactivity was calculated,[16] which represents the percentage change in CBFV from hypocapnia (hyperventilation) to hypercapnia (7% $CO_2$-inhalation) relative to baseline CBFV:

$$=((\text{CBFVmax}[7\%CO_2] - \text{CBFVmin}[\text{hyperventilation}]) / \text{CBFVrest}) * 100\%.$$

We also calculated the change in CBFV between hypocapnia and hypercapnia per mmHg change in $EtCO_2$ between hypocapnia and hypercapnia.[37]

### Statistical analysis

Data are presented as mean ± standard deviation. Comparisons between the different age groups were performed using the one-way ANOVA with Bonferroni correction for multiple comparisons. We checked the data with the Hochberg method because of sample size differences (especially with the single squat-stand maneuver) and with the Games-Howell method because of different group variances. Since these three methods did not show relevant differences, we only mention the results from the one-way ANOVA with Bonferroni correction. Statistical significance was set at a $p$-value $<0.05$. All statistical analysis were executed using statistical software SPSS (SPSS, Inc, Chicago, IL, USA).

## Results

Baseline characteristics for the three age groups are presented in Table 4.1. Body mass index was higher in the elderly compared with the young group ($p<0.001$) and the older elderly ($p=0.05$). There were no differences in baseline blood pressure or heart rate between the three groups. The young group had a higher mean CBFV compared with both elderly groups ($p<0.01$) and a lower CVRi compared with the older elderly ($p<0.01$).



### Cerebral autoregulation for spontaneous oscillations (baseline measurement)

The transfer functions coherence, phase, gain and normalized gain derived from spontaneous oscillations in blood pressure and CBFV during baseline sitting position are presented in Table 4.2. In accordance with the high pass filter model, all participants (except for two individuals, both aged 65 years) showed a pattern with decreasing phase and increasing gain with increasing frequency of oscillations.

Below we will briefly summarize these transfer function parameters (see Table 4.2).
*Power Spectrum Density (PSD)*: The older elderly overall had lower values for PSD for both blood pressure and CBFV in all frequency ranges compared to the younger age groups. However, these differences did not reach statistical significance for all comparisons (Table 4.2).
For transfer function analysis only data with coherence >0.4 were used. *Coherence*: There were no relevant differences in coherence results between age groups. The older elderly had a slightly lower coherence in the LF compared with the young group ($p=0.001$), but mean coherence remained well above 0.4. *Phase*: Values for phase were very similar across age groups, but young subjects had a higher mean phase in the VLF (where cerebral autoregulation is most active), this difference reached statistical significance when compared to younger elderly ($p=0.023$). However, VLF

phase did not differ between younger and older elderly. *Gain:* Table 4.2 lists absolute gain, which is sensitive to individual differences in CVFV or blood pressure, and normalized gain, which is not affected by absolute CBFV or blood pressure. Absolute gain was marginally higher in the LF compared with the elderly ($p=0.05$). Normalized gain revealed no differences between age groups in the VLF and LF range where cerebral autoregulation is active, however, normalized gain was higher in the HF in the older elderly compared with the young group ($p=0.006$).

### Repeated Sit-Stand maneuver 0.05 Hz

The results from the repeated sit-stand maneuver are presented in Table 4.3. Two subjects (one in each elderly group) lost bilateral TCD signal, but the remaining unilateral TCD signal was of sufficient quality to use. CBFV was higher in the young group compared with both elderly groups ($p<0.001$), and the CVRi was lower in the young group compared with the older elderly ($p=0.008$).

**Table 4.1** Baseline characteristics of the three groups.

| | Young | Elderly | Older elderly |
|---|---|---|---|
| **General characteristics** | | | |
| Male : Female (n) | 9 : 11 | 13 : 7 | 15 : 3 |
| Age (years) | 24 ± 2 *1,2 | 66 ± 1 *2 | 78 ± 3 |
| Age ranges (years) | 21 - 28 | 65 - 69 | 74 - 86 |
| Body mass index (kg/m$^2$) | 22.3 ± 2.7 *1 | 26.8 ± 3.4 #2 | 24.2 ± 2.5 |
| **Hemodynamics** | | | |
| Systolic blood pressure (mmHg) | 121 ± 22 | 128 ± 20 | 122 ± 22 |
| Diastolic blood pressure (mmHg) | 62 ± 15 | 64 ± 14 | 56 ± 12 |
| Mean arterial pressure (mmHg) | 82 ± 16 | 86 ± 15 | 78 ± 15 |
| Pulse pressure (mmHg) | 59 ± 12 | 64 ± 11 | 66 ± 13 |
| Heart rate (beats/minute) | 69 ± 10 | 66 ± 10 | 63 ± 7 |
| **Cerebral perfusion** | | | |
| Mean CBFV (cm/s) | 59.2 ± 11.5 *1,2 | 48.2 ± 7.3 | 41.9 ± 8.9 |
| Systolic CBFV (cm/s) | 94.4 ± 18.2 *1,2 | 77.4 ± 14.2 | 69.5 ± 15.0 |
| Diastolic CBFV (cm/s) | 40.5 ± 8.4 *1,2 | 30.0 ± 5.2 | 24.7 ± 5.8 |
| CVRi (mmHg/cm/s) | 1.46 ± 0.58 *2 | 1.81 ± 0.36 | 1.98 ± 0.52 |

Data are presented as mean ± standard deviation. * $p<0.05$ compared with the elderly[1] or older elderly[2]. # $p=0.05$ compared with the older elderly[2]. **Abbreviations:** CBFV cerebral blood flow velocity, CVRi cerebrovascular resistance index.

**Table 4.2** Transfer function analyses mean blood pressure - cerebral blood flow velocity was used to examine the dynamic cerebral autoregulation during spontaneous blood pressure oscillations in sitting rest position.

| | Young | Elderly | Older elderly |
|---|---|---|---|
| **Coherence >0.4** | | | |
| VLF (n) | 15 | 15 | 13 |
| LF (n) | 20 | 17 | 15 |
| HF (n) | 20 | 14 | 17 |
| **Coherence** | | | |
| VLF | 0.53 ± 0.06 | 0.55 ± 0.09 | 0.52 ± 0.08 |
| LF | 0.77 ± 0.11 *2 | 0.68 ± 0.15 | 0.62 ± 0.09 |
| HF | 0.72 ± 0.13 | 0.77 ± 0.13 | 0.70 ± 0.15 |
| **Phase (rad)** | | | |
| VLF | 0.99 ± 0.36 *1 | 0.69 ± 0.28 | 0.74 ± 0.24 |
| LF | 0.49 ± 0.21 | 0.47 ± 0.23 | 0.51 ± 0.20 |
| HF | 0.07 ± 0.15 | 0.11 ± 0.11 | -0.01 ± 0.14 |
| **Gain (cm/s/mmHg)** | | | |
| VLF | 0.60 ± 0.15 | 0.52 ± 0.15 | 0.50 ± 0.15 |
| LF | 0.83 ± 0.18 #1 | 0.68 ± 0.20 | 0.70 ± 0.15 |
| HF | 0.91 ± 0.25 | 0.84 ± 0.19 | 0.85 ± 0.20 |
| **Normalized Gain (%cm/s/%mmHg)** | | | |
| VLF | 0.77 ± 0.29 | 0.93 ± 0.17 | 0.90 ± 0.23 |
| LF | 1.16 ± 0.31 | 1.20 ± 0.31 | 1.43 ± 0.54 |
| HF | 1.24 ± 0.32 *2 | 1.50 ± 0.24 | 1.65 ± 0.50 |
| **PSD BP ($mmHg^2/Hz$)** | | | |
| VLF | 8.16 ± 4.75 | 10.34 ± 6.54 *2 | 4.64 ± 2.24 |
| LF | 4.22 ± 3.26 *1,2 | 2.28 ± 1.81 | 0.80 ± 0.50 |
| HF | 0.62 ± 0.41 | 0.74 ± 0.70 | 0.37 ± 0.39 |
| **PSD CBFV ($(cm/s)^2/Hz$)** | | | |
| VLF | 6.41 ± 3.53 *2 | 6.36 ± 4.32 *2 | 2.79 ± 2.10 |
| LF | 3.54 ± 2.80 *1,2 | 1.66 ± 1.23 | 0.54 ± 0.29 |
| HF | 0.73 ± 0.57 *2 | 0.56 ± 0.47 | 0.28 ± 0.16 |

Data are presented as mean ± standard deviation. * $p<0.05$ compared with the elderly[1] or older elderly[2]. # $p=0.05$ compared with the elderly[1]. **Abbreviations:** PSD power spectrum density, BP blood pressure, CBFV cerebral blood flow velocity, VLF very low frequency, LF low frequency, HF high frequency.

Below we will briefly summarize these transfer function parameters (see Table 4.3). *Power Spectrum Density (PSD):* The induced oscillations led, as expected, to higher PSD between baseline and the repeated sit-stand maneuver ($p<0.001$) for the three age groups (Tables 4.2 and 4.3).

**Table 4.3** Transfer function analyses mean blood pressure - cerebral blood flow velocity (MAP-CBFV) and cerebral blood flow velocity - oxygenated hemoglobin (CBFV-$O_2$Hb) were used to examine the dynamic cerebral autoregulation and cortical oxygenation during induced blood pressure oscillations using the repeated sit-stand maneuver at 0.05 Hz.

| | Young (n=20) | Elderly (n=20) | Older elderly (n=18) |
|---|---|---|---|
| **Hemodynamics** | | | |
| Mean arterial pressure (mmHg) | 89 ± 18 | 91 ± 15 | 88 ± 16 |
| Heart rate (beats/minute) | 79 ± 8 | 75 ± 10 | 76 ± 9 |
| **Cerebral perfusion** | | | |
| CBFV (cm/s) | 59.7 ± 9.9 *[1,2] | 48.1 ± 8.3 | 45.2 ± 9.3 |
| CVRi (mmHg/cm/s) | 1.54 ± 0.47 *[2] | 1.91 ± 0.40 | 2.06 ± 0.63 |
| **Dynamic cerebral autoregulation** | | | |
| *MAP-CBFV* | n=19 | n=19 | n=16 |
| Coherence | 0.63 ± 0.06 | 0.67 ± 0.08 | 0.63 ± 0.11 |
| Phase (rad) | 0.73 ± 0.33 | 0.61 ± 0.21 | 0.80 ± 0.28 |
| Gain (cm/s/mmHg) | 0.62 ± 0.17 | 0.70 ± 0.16 *[2] | 0.55 ± 0.20 |
| Normalized Gain (%cm/s/%mmHg) | 0.88 ± 0.18 *[1] | 1.31 ± 0.30 *[2] | 1.06 ± 0.34 |
| *CBFV–$O_2$Hb* | n=13 | n=16 | n=14 |
| Coherence | 0.54 ± 0.09 | 0.56 ± 0.08 | 0.50 ± 0.07 |
| Phase (rad) | -0.64 ± 0.31 | -0.41 ± 0.49 *[2] | -0.89 ± 0.25 |
| Gain (cm/s/mmHg) | 0.07 ± 0.03 | 0.06 ± 0.02 | 0.08 ± 0.02 |
| Normalized Gain (%cm/s/%mmHg) | 0.10 ± 0.09 #[2] | 0.12 ± 0.04 | 0.17 ± 0.08 |
| **Power Spectrum Density** | | | |
| Blood pressure ($mmHg^2$/Hz) | 50.30 ± 22.27 | 62.77 ± 33.25 | 42.90 ± 18.20 |
| CBFV ($(cm/s)^2$/Hz) | 27.25 ± 14.34 *[2] | 34.67 ± 17.69 *[2] | 15.02 ± 6.03 |
| $O_2$Hz ($(\mu mol/l)^2$/Hz) | 0.23 ± 0.17 | 029 ± 0.25 | 0.17 ± 0.09 |

Data are presented as mean ± standard deviation. * $p<0.05$ compared with the elderly[1] or older elderly[2]. # $p=0.05$ compared with the older elderly[2]. **Abbreviations:** CBFV cerebral blood flow velocity, CVRi cerebrovascular resistance index, MAP mean arterial pressure, $O_2$Hb oxygenated hemoglobin.

As with spontaneous oscillations, PSD for blood pressure and CBFV appeared smaller in the oldest elderly, but this was only statistically significant for CBFV ($p<0.05$).

Due to inclusion of data with coherence >0.4, some participants were excluded from the transfer function analysis *MAP-CBFV* (n=4) and *CBFV-$O_2$Hb* (n=15) as indicated in Table 4.3. *Blood pressure-CBFV relationship*: Similar to the transfer function analysis for spontaneous oscillations, there were no differences between age groups for coherence and phase during induced VLF oscillations at 0.05 Hz. However, gain and normalized gain were higher in the elderly group compared with the older elderly and for normalized gain also compared with the young group (Table 4.3).

*CBFV-$O_2$Hb relationship*: Phase shift between CBFV and $O_2$Hb was lower in the elderly compared with the older elderly ($p=0.003$), and the normalized gain was marginally higher in the older elderly compared with the young group ($p=0.05$). When performing a non-parametric Spearman's rho linear regression using all three groups combined (n=58) with transfer function analysis metrics and age, phase CBFV-$O_2$Hb (r=-0.320, $p=0.034$), normalized gain MAP-CBFV (r=0.276, $p=0.041$) and normalized gain CBFV-$O_2$Hb (r=0.454, $p=0.002$) were statistically significant, indicating a small decrease in phase (CBFV-$O_2$Hb) and increase in gain (MAP-CBFV and CBFV-$O_2$Hb) with advancing age.



## Single squat-stand maneuver

Table 4.4 and figure 4.1 present the results from the single squat-stand maneuver. Nine older elderly were included in this analysis since not all older elderly performed this maneuver due to comorbidity, e.g. osteoarthritis of hip or knee. The squat-stand maneuver induced large changes in blood pressure and CBFV, but overall, there were no differences in relative changes in blood pressure and CBFV between age groups. Heart rate increased significantly more in the young group compared with both elderly groups ($p<0.001$), but the absolute and percentage changes in mean blood pressure were similar in all groups. The CBFV was lower and CVRi was higher with increasing age during both squat and stand position ($p<0.01$). The fall in absolute CBFV upon standing was largest in young subjects ($p=0.013$), however relative changes did not differ between age groups. The absolute decrease in CVRi with standing was larger in the elderly compared with the young group ($p=0.029$).

## Cerebrovascular $CO_2$ reactivity

Table 4.5 presents the results from hyperventilation and $CO_2$-inhalation measurement. In the older elderly group, 17 participants successfully completed this maneuver. Hyperventilation and $CO_2$-inhalation led to significant changes in $EtCO_2$ in all groups ($p<0.01$), resulting in substantial changes in mean blood pressure, CBFV, CVRi and $O_2$Hb. Absolute CBFV was highest and CVRi was lowest in the youngest age group

throughout the measurement. Absolute changes in CBFV and CVRi during hyperventilation and $CO_2$-inhalation were also highest in the young group. However, relative changes expressed as percentage changes from baseline were similar for all ages (Table 4.5).

**Table 4.4** Single squat-stand maneuver was used to examine the cerebral perfusion and cortical oxygenation during an orthostatic hypotension challenge test in healthy subjects, mimicking daily life activities.

| | Young (n=20) | Elderly (n=20) | Older elderly (n=9) |
|---|---|---|---|
| **Squat** | | | |
| Mean arterial pressure (mmHg) | 87 ± 16 | 96 ± 16 | 102 ± 14 |
| Heart rate (beats/minute) | 75 ± 10 | 72 ± 12 *2 | 83 ± 8 |
| CBFV (cm/s) | 64.4 ± 11.0 *1,2 | 50.5 ± 9.4 | 46.0 ± 8.4 |
| CVRi (mmHg/cm/s) | 1.40 ± 0.44 *1,2 | 1.99 ± 0.46 | 2.29 ± 0.60 |
| **Stand** | | | |
| Mean arterial pressure (mmHg) | 58 ± 15 | 61 ± 10 | 69 ± 13 |
| Heart rate (beats/minute) | 104 ± 10 *1,2 | 84 ± 13 | 88 ± 8 |
| CBFV (cm/s) | 47.7 ± 10.1 *1,2 | 39.2 ± 6.7 | 33.3 ± 5.5 |
| CVRi (mmHg/cm/s) | 1.29 ± 0.58 *2 | 1.62 ± 0.28 *2 | 2.11 ± 0.54 |
| **Hemodynamic response** | | | |
| Δ Mean arterial pressure (mmHg) | -29 ± 7 | -35 ± 14 | -33 ± 9 |
| % Δ Mean arterial pressure (mmHg) | -34 ± 8 | -36 ± 10 | -33 ± 7 |
| Δ Heart rate (beats/minute) | 29 ± 10 *1,2 | 11 ± 6 | 5 ± 3 |
| % Δ Heart rate (beats/minute) | 40 ± 18 *1,2 | 16 ± 10 | 6 ± 4 |
| Δ CBFV (cm/s) | -16.7 ± 5.2 *1 | -11.3 ± 6.6 | -12.7 ± 4.2 |
| % Δ CBFV (cm/s) | -26.2 ± 7.5 | -21.5 ± 10.7 | -27.2 ± 6.0 |
| Δ CVRi (mmHg/cm/s) | -0.11 ± 0.20 *1 | -0.37 ± 0.35 | -0.18 ± 0.39 |
| Δ $O_2$Hb ($\mu$mol/l) | -2.22 ± 1.04 | -2.72 ± 1.42 | -2.98 ± 1.06 |

Data are presented as mean ± standard deviation. * $p<0.05$ compared with the elderly[1] or older elderly[2].
**Abbreviations:** CBFV cerebral blood flow velocity, CVRi cerebrovascular resistance index, $O_2$Hb oxygenated hemoglobin, Δ= standing minus squatting, % Δ percentage change between standing and squatting compared to the squat values.

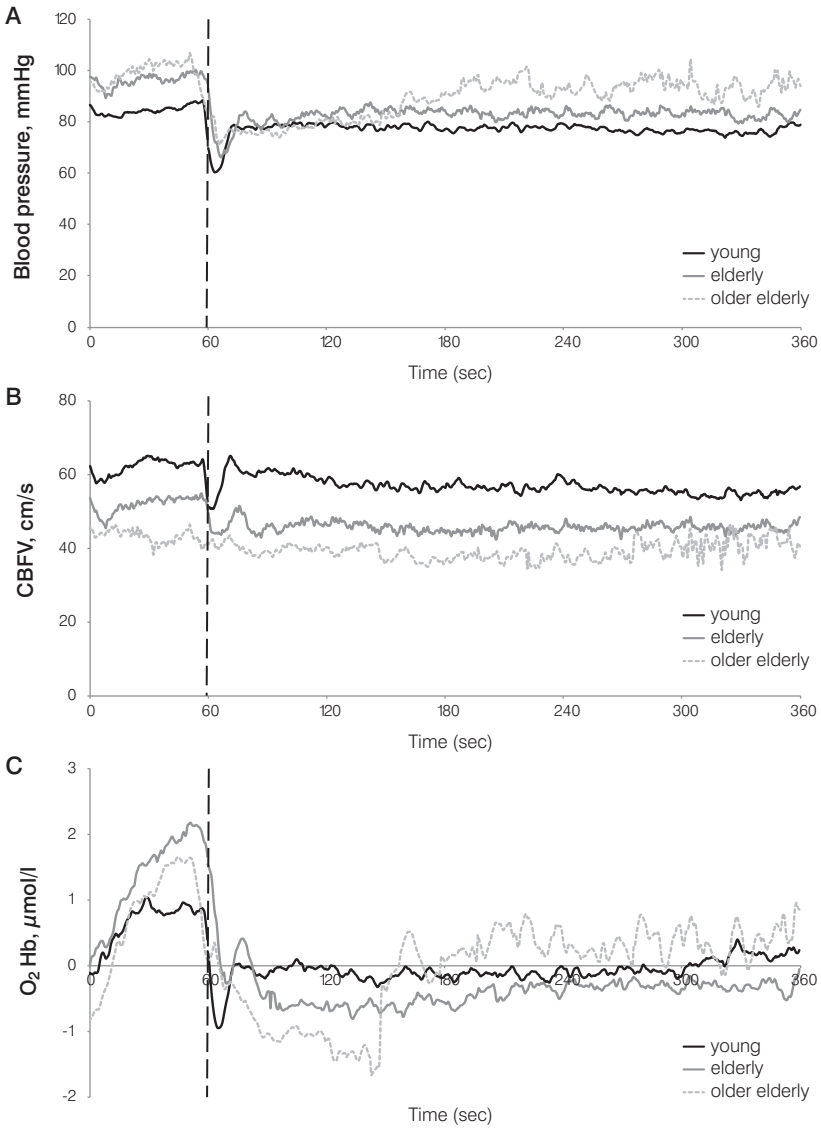


**Figure 4.1** This image shows the blood Pressure (**A**), cerebral blood flow velocity (CBFV; **B**) and cortical oxygenation (oxygenated hemoglobin $O_2Hb$; **C**) responses of the three groups during the single squat-stand maneuver. The vertical dashed line indicates the moment of standing after 1 minute squat. The solid black line represents the young group, the solid grey line the elderly, and the dashed grey line the older elderly.

**Table 4.5** Changes in hemodynamics, cerebral blood flow velocity (CBFV) and cortical oxygenation ($O_2Hb$) in response to changes in carbon dioxide (cerebrovascular $CO_2$ reactivity) with hypocapnia (hyperventilation) and hypercapnia ($CO_2$-inhalation).

| | Young (n=20) | Elderly (n=20) | Older elderly (n=17) |
|---|---|---|---|
| **Baseline** | | | |
| Mean arterial pressure (mmHg) | 81 ± 16 | 85 ± 13 | 84 ± 9 |
| Heart rate (beats/minute) | 78 ± 9 *2 | 70 ± 12 | 66 ± 9 |
| CBFV (cm/s) | 59.8 ± 10.8 *1,2 | 43.6 ± 7.6 | 44.3 ± 10.4 |
| CVRi (mmHg/cm/s) | 1.40 ± 0.41 *1,2 | 2.05 ± 0.42 | 2.02 ± 0.56 |
| $O_2Hb$ ($\mu$mol/l) | -0.18 ± 0.99 | 0.08 ± 0.92 | 0.03 ± 0.84 |
| $EtCO_2$ (mmHg) | 28.8 ± 3.0 | 31.0 ± 2.3 | 31.2 ± 3.8 |
| **Hyperventilation** | | | |
| Mean arterial pressure (mmHg) | 70 ± 20 | 74 ± 10 | 70 ± 11 |
| Heart rate (beats/minute) | 93 ± 14 *1,2 | 75 ± 12 | 75 ± 9 |
| CBFV (cm/s) | 36.0 ± 9.5 *1,2 | 26.6 ± 5.2 | 26.6 ± 5.7 |
| CVRi (mmHg/cm/s) | 1.99 ± 0.69 *1,2 | 2.87 ± 0.69 | 2.74 ± 0.76 |
| $O_2Hb$ ($\mu$mol/l) | -1.61 ± 2.09 | -1.20 ± 0.81 | -1.64 ± 1.74 |
| $EtCO_2$ (mmHg) | 18.3 ± 3.1 | 16.0 ± 5.8 *2 | 20.4 ± 4.4 |
| **$CO_2$-inhalation** | | | |
| Mean arterial pressure (mmHg) | 96 ± 18 | 103 ± 21 | 108 ± 16 |
| Heart rate (beats/minute) | 81 ± 12 *1 | 71 ± 8 | 78 ± 8 |
| CBFV (cm/s) | 97.2 ± 19.5 *1,2 | 76.3 ± 15.5 | 75.9 ± 14.8 |
| CVRi (mmHg/cm/s) | 1.02 ± 0.27 *1,2 | 1.42 ± 0.30 | 1.46 ± 0.32 |
| $O_2Hb$ ($\mu$mol/l) | 3.83 ± 2.11 | 3.55 ± 2.79 | 4.07 ± 2.71 |
| $EtCO_2$ (mmHg) | 50.4 ± 4.5 | 49.0 ± 6.6 | 52.3 ± 4.3 |
| **$CO_2$-inhalation - Hyperventilation** | | | |
| Δ Mean arterial pressure (mmHg) | 26 ± 18 | 26 ± 19 | 38 ± 13 |
| Full range mean arterial pressure (%) | 33 ± 21 | 35 ± 24 | 45 ± 16 |
| Δ Heart rate (beats/minute) | -11 ± 18 *2 | -4 ± 11 | 2 ± 8 |
| Full range heart rate (%) | -15 ± 25 *2 | -5 ± 17 | 4 ± 14 |
| Δ CBFV (cm/s) | 61.2 ± 17.2 #1,2 | 49.7 ± 13.8 | 49.3 ± 12.4 |
| Full range CBFV (%) | 102 ± 20 | 115 ± 27 | 114 ± 30 |
| Δ CBFV / Δ $EtCO_2$ (cm/s/mmHg $CO_2$) | 1.95 ± 0.64 *1,2 | 1.52 ± 0.37 | 1.55 ± 0.34 |

**Table 4.5** Continued.

| | Young (n=20) | Elderly (n=20) | Older elderly (n=17) |
|---|---|---|---|
| **$CO_2$-inhalation - Hyperventilation** | | | |
| Δ CVRi (mmHg/cm/s) | -0.97 ± 0.48 *[1] | -1.45 ± 0.57 | -1.28 ± 0.58 |
| Full range CVRi (%) | -67 ± 18 | -70 ± 21 | -63 ± 23 |
| Δ $EtCO_2$ (mmHg) | 32 ± 6 | 33 ± 7 | 32 ± 5 |
| Full range $EtCO_2$ (%) | 114 ± 31 | 107 ± 24 | 98 ± 36 |
| Δ $O_2Hb$ (μmol/l) | 5.44 ± 2.45 | 4.75 ± 2.76 | 5.71 ± 2.99 |

Data are presented as mean ± standard deviation. * $p<0.05$ compared with the elderly[1] or older elderly[2]. # $p=0.05$ compared with the elderly[1] or older elderly[2]. **Abbreviations:** CBFV cerebral blood flow velocity, CVRi cerebrovascular resistance index, $O_2Hb$ oxygenated hemoglobin, $EtCO_2$ end-tidal $CO_2$, Δ change between $CO_2$ inhalation and hyperventilation.



## Discussion

The aim of the present study was to examine the influence of different ages on dynamic cerebral autoregulation and cerebrovascular $CO_2$ reactivity in combination with the assessment of cerebral cortical oxygenation. The main findings are that in individuals above the age of 70 (age 74 to 86 years in this study) dynamic cerebral autoregulation and cerebrovascular $CO_2$ reactivity retain normal function. Cerebral blood flow and cortical oxygenation were not compromised in these elderly during maneuvers that mimic daily life activities and during changes in arterial $CO_2$.

### Cerebral autoregulation

An intact dynamic cerebral autoregulation is characterized by a high pass filter with decreasing phase and increasing gain with increasing frequency domains.[34,35] For spontaneous oscillations during baseline sitting, the transfer function parameters all followed this pattern in all three age groups, and all older elderly adhered to the high pass filter model. Moreover, the hallmark of dynamic cerebral autoregulation, i.e. the necessary adaptation of CVRi upon a decrease in blood pressure (e.g. when standing after squatting) in order to maintain sufficient CBF, was intact in older individuals. The current study showed that dynamic cerebral autoregulation in this oldest age group was comparable to a group of young subjects and to a group of older adults below the age of 70 years. Moreover, the efficiency of the dynamic cerebral autoregulatory

response, as reflected by the phase of the transfer function analysis during the repeated sit-stand maneuver, was similar in the three age groups indicating that the response time to restore the CBFV after blood pressure changes was comparable between the groups.

There were important differences in baseline cerebral hemodynamics between the three age groups. CBFV decreased and CVRi increased with advancing age. Since there were no differences in blood pressure between the different age groups, the increased CVRi with aging is could be due either to reduced cerebral blood flow or to changes in the characteristics of the blood vessels. It is known that arterial walls become thicker, stiffer, and vascular elasticity diminishes with aging, thereby increasing vascular resistance.[2,3,38] Indeed, Gommer et al. found that higher peripheral vascular resistance correlated with higher CVRi.[39] In this study, we did not measure absolute cerebral blood flow (in ml/min/100 gram of brain tissue) and therefore we are unable to differentiate between these two explanations for the increased CVRi. The transfer function parameters gain and normalized gain, on the other hand, are also thought to be affected by vascular stiffness. Despite the increase in CVRi with age, there was no consistent difference in (normalized) gain with increasing age, as gain was not higher in the older elderly compared with the younger elderly. Nevertheless, linear regression did show a significant increase for normalized gain MAP-CBFV and CBFV-$O_2$Hb with age.

Taken together, we postulate that dynamic cerebral autoregulation is not compromised by the effects of physiological aging up to the age of 86 years.

## Cerebrovascular $CO_2$ reactivity

The relative change in CBFV during changes in $EtCO_2$ was similar in the three age groups. In other words, the ability to vasoconstrict or vasodilatate in response to changes in arterial $CO_2$ is preserved with aging. This finding is in contrast with literature that young subjects have a greater cerebrovascular $CO_2$ reactivity compared with older subjects.[12] Cerebrovascular $CO_2$ reactivity can be affected by structural vascular properties as well as vascular endothelial function.[9,40] Vascular endothelium is an important regulatory organ that modulates the tone of the underlying vascular smooth muscle cells and maintains cardiovascular homeostasis by contributing to vasoconstriction and vasodilation.[41] It is known that the endothelial function in reduced with aging.[40] Despite these potential vascular changes with aging, the percentage change in CBFV and $O_2$Hb in response to changes in $CO_2$ were similar for all groups.

## Cortical oxygenation

The dynamic relation between oscillations in CBFV and cortical $O_2$Hb may reflect a regulatory mechanism to maintain brain tissue oxygenation.[31,42] The relationship between blood pressure, CBFV and $O_2$Hb lends support to the notion that upstream

oscillations in CBFV induced by changes in blood pressure contribute importantly to the downstream brain tissue level oscillations in $O_2Hb$.[19] It must be acknowledged that NIRS also measures $O_2Hb$ in venous blood besides merely the arterial blood supply. However, the NIRS method has been validated to measure changes on cerebral cortical tissue level and the dynamic changes in $O_2Hb$ have been related to changes in the arterial and arteriolar component, with little change in the venous compartment which remains relatively constant.[27,28] NIRS can be influenced by blood in the extracranial tissue (skin), however, the interoptode distance of 5.0 cm that we used has been shown to measure intracranial cerebral hemodynamic changes in $O_2Hb$ and HHb with negligible influence of skin blood flow.[21]

In the current study, repeated sit-stand maneuvers induced strong blood pressure oscillations, which resulted in strong CBFV oscillations leading to $O_2Hb$ oscillations with sufficient coherence to perform transfer function analysis of the relation CBFV-$O_2Hb$. The phase between CBFV and $O_2Hb$ was negative in all three groups, indicating that $O_2Hb$ oscillations follow those of CBFV with a time delay caused by transit time of blood. The gain of the transfer function analysis between CBFV and $O_2Hb$ might reflect either damping of the CBFV fluctuations (perhaps by distensible arterioles or capillaries) resulting in smaller or larger oscillations in cortical oxygenation. Gain may, however, also be influenced by metabolic reserve and its effect on oxygenated hemoglobin, or by a reduced diffusion of oxygen leading to enhanced oscillations in $O_2Hb$ in response to changes in CBFV.[19]

With increasing age, the phase lag between CBFV and $O_2Hb$ became larger, whereas the normalized gain became higher. This is in line with results from patients with Alzheimer's disease, a disease in which cerebral microvasculature is affected. In these Alzheimer patients, the phase lag was larger and gain was higher between CBFV and $O_2Hb$ at 0.05 Hz compared with age-matched controls.[19] Possibly, the microvasculature in older individuals becomes compromised compared with young individuals. We speculate that increased microvascular tortuosity (which leads to increased vessel length and thereby transit time, as has been demonstrated in arterial spin labeling studies) could explain the larger phase shift, and that reduced distensibility and concomitant reduced damping could explain larger gain.

The single squat-stand maneuver elicited a reduction in $O_2Hb$ in the frontal cortex, which was similar in all groups. This challenging maneuver, which is comparable with activities in daily life, such as standing up from a chair, or putting on a sock or shoe, does not seem to compromise oxygenation of the frontal cortex in healthy older individuals. This would confirm our observation of preserved dynamic cerebral autoregulation based on TCD in these healthy subjects. These compensatory mechanisms may however fail in case of more severe stress (for example during septic shock) or with diseases that further compromise cerebral macro- and micro-vasculature like Alzheimer's disease.

### Limitations

In order to induce larger hemodynamic oscillations, a repeated sit-stand maneuver was applied. The induced large blood pressure oscillations during the repeated sit-stand maneuver provide strong and physiological relevant hemodynamic perturbations and lead to an improved estimation of the transfer function parameters and higher power spectrum density and thereby increases reliability of the assessment.[11,13] However, the maneuver could be seen as aerobic exercise and may thereby affect our measurements. However, Ogoh et al.[43] found that mild to moderate intensity exercise did not impair dynamic cerebral autoregulation. It is possible that the older elderly experienced the repeated sit-stand maneuver as mild exercise, however compared with baseline values their heart rate did not increase more than in the other two groups, nor did the $EtCO_2$ change relevantly compared with baseline. Thus, confounding effects of aerobic exercise during the repeated sit-stand maneuver are less likely.

Cerebral blood flow velocity was measured in the middle cerebral artery and NIRS was obtained from the frontal cortex, which receives blood supply from the anterior cerebral artery and the middle cerebral artery.[19] We assumed that relative changes of CBFV would be similar between the middle and anterior cerebral artery in response to blood pressure changes, and thus the supplying anterior cerebral artery most likely reacts similar as the middle cerebral artery.

The cut-off point of a coherence $<0.4$ remains questionable. Theoretically, excluding persons with low coherence might lead to the exclusion of participants with very efficient cerebral autoregulation. However, using the cut-off point warranted optimal data quality and reduced the signal-to-noise ratio. Furthermore, excluding low coherence $<0.4$ was performed in all age groups and considering our findings of sufficient cerebral autoregulation in all age groups, it is highly unlikely that the use of this cut-off value has influenced our results.

## Conclusion

We have shown that a group of healthy older individuals up to the age of 86 years old shows an overall preserved dynamic cerebral autoregulation and cerebrovascular $CO_2$ reactivity leading to a sufficient cerebral cortical oxygenation. The preserved dynamic cerebral autoregulation and cerebrovascular $CO_2$ reactivity were observed despite the decrease in cerebral blood flow velocity and increase in cerebrovascular resistance index with advancing age.

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Chapter 5

# The effect of an advanced glycation end-product crosslink breaker and exercise training on vascular function in older individuals: a randomized factorial design trial

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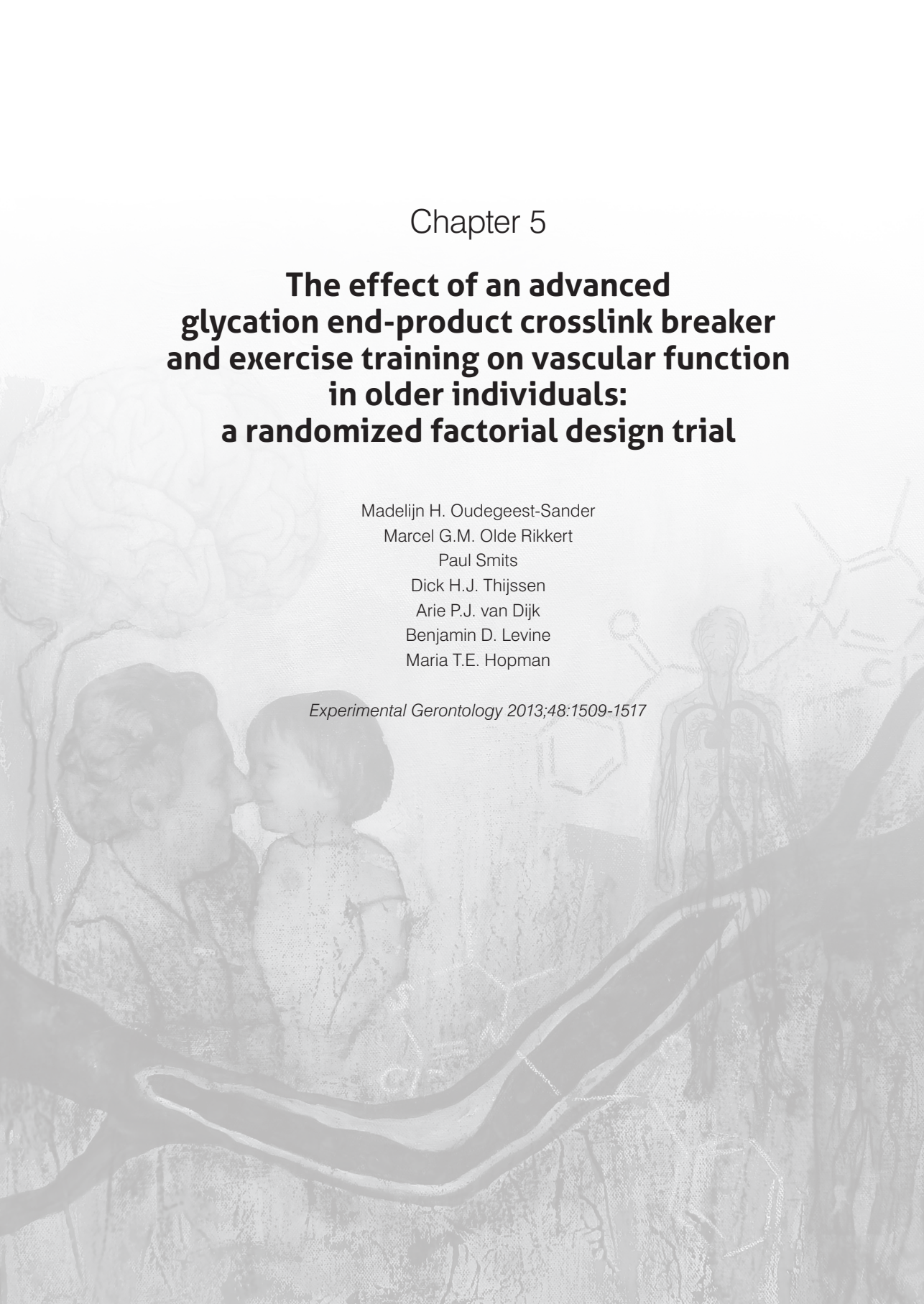

## Abstract

**Background**: Aging leads to accumulation of irreversible advanced glycation end-products (AGEs), contributing to vascular stiffening and endothelial dysfunction. When combined with the AGE-crosslink breaker Alagebrium, exercise training reverses cardiovascular aging in experimental animals. This study is the first to examine the effect of Alagebrium, with and without exercise training, on endothelial function, arterial stiffness and cardiovascular risk in older individuals.

**Methods**: Forty-eight non-exercising individuals (mean age 70 ± 4 years) without manifest diseases or use of medication were allocated into 4 groups for a 1-year intervention: Exercise training & Alagebrium (200 mg/day); Exercise training & placebo; No exercise training & Alagebrium (200 mg/day); and no exercise training & placebo. We performed a maximal exercise test ($VO_2$max) and measured endothelial function using venous occlusion plethysmography and intra-arterial infusion of acetylcholine, sodium nitroprusside and $N^G$-monomethyl-L-arginine. Arterial stiffness was measured using pulse wave velocity, and cardiovascular risk was calculated using the Lifetime Risk Score (LRS).

**Results**: In the exercise training groups, Lifetime Risk Score and $VO_2$max improved significantly (23.9 ± 4.5 to 27.2 ± 4.6 $mlO_2$/min/kg, $p<0.001$). Endothelial response to the vasoactive substances did not change, nor did arterial stiffness in any of the four groups.

**Conclusion**: One year of exercise training significantly improved physical fitness and lifetime risk for cardiovascular disease without affecting endothelial function or arterial stiffness. The use of the AGE-crosslink breaker Alagebrium had no independent effect on vascular function, nor did it potentiate the effect of exercise training. Despite the clinical benefits of exercise training for older individuals, neither exercise training nor Alagebrium (alone or in combination) was able to reverse the vascular effects of decades of sedentary aging within one year of intervention.

## Introduction

Advanced age is associated with an increased risk for cardiovascular diseases, at least partly because of age-related changes in vessel characteristics that lead to arterial stiffening and endothelial dysfunction.[1,2] Another detrimental age-related impact on arterial vessels is the accumulation of Advanced Glycation End-products (AGEs) in the arterial wall.[3-5] AGEs are the end-product of a non-enzymatic reaction with sugar derivatives that leads to irreversible protein-protein crosslinks. This process occurs continuously and ultimately results in an accumulation of complex arrangements of cross-linked proteins and AGEs.[3-5] When AGEs link to long-lived proteins, such as collagen in the arterial wall, they contribute to arterial stiffening.[3-5] Furthermore, AGEs bind to specific AGE-binding receptors on endothelial cells and quench nitric oxide, thereby leading to endothelial dysfunction.[4-6]

Based on the potential role of AGEs in the development of endothelial dysfunction and arterial stiffness, i.e., characteristic vascular adaptations that relate to the increased cardiovascular risk in the older population, therapeutic strategies that reverse the process of AGE formation and accumulation may have beneficial potential. A pharmacologic agent has been created to specifically break already formed AGE-crosslinks.[7] This drug, a thiazolium-derivative known as Alagebrium, breaks established AGE-crosslinks between proteins.[8] Previous animal studies and initial phases I and II patient studies demonstrated a reduced vascular stiffness and improved endothelial function.[3,8,9] Whether older individuals, who typically demonstrate endothelial dysfunction and stiffer arteries, also benefit from an AGE-crosslink breaker is currently unknown.

Physical exercise training is a potent stimulus not only to reduce cardiovascular risk, but also to improve endothelial function and arterial stiffness.[10-12] Preliminary work in rats suggested that the combination of exercise training and an AGE-crosslink breaker reverses cardiovascular adaptations to advanced age in rats.[13] Whether AGE-crosslink breakers enhance the cardiovascular benefits from exercise training in humans is currently unknown. Therefore, the aim of our study was to examine the effects of a 1-year treatment with the AGE-crosslink breaker Alagebrium on endothelial function, arterial stiffness and cardiovascular risk in healthy older individuals, and combine this intervention in a factorial design with exercise training. The primary hypothesis was that both 1-year AGE-crosslink breakers and aerobic exercise training improve endothelial function and arterial stiffness. An additional hypothesis was the presence of a superior effect on endothelial function and arterial stiffness when both interventions were combined.

# Methods

## Ethics Statement

The study was performed according to Good Clinical Practice standards, and approved by the Medical Ethics Committee (Arnhem/Nijmegen, the Netherlands). All participants gave written informed consent. This study is registered at Clinical Trial.gov (NCT01417663).

## Participants

Forty-eight older individuals (age 70 ± 4 years), who did not perform regular (≤1 hour/week) exercise for the last 5-10 years, were recruited from the local community (Figure 5.1). All participants were non-smoking subjects, aged 65 years or older, without diseases or disorders that could compromise physical activity, were not regularly seeing a general practitioner or medical specialist and were not using medication known to interfere with the cardiovascular system or hormone replacement therapy. None of our participants have (a history of) cardiovascular disease. Furthermore, participants with hypertension (>160/90 mmHg), diabetes mellitus, hypercholesterolemia (total cholesterol >7.5 mmol/l) and body mass index >32.5 kg/m$^2$ were excluded from participation. Because of our assessment of endothelial function in the lower limbs, we also excluded participants with the presence of significant atherosclerotic lesions in the lower limbs found with physical examination indicated by murmers over the femoral artery or the absence of peripheral pulsations of the dorsalis pedis artery and/or posterior tibial artery.

## Experimental Design

After an extensive medical screening, participants were allocated to 4 different intervention groups according to a factorial design for a 1-year intervention: 1) Exercise training & Alagebrium; 2) Exercise training & placebo; 3) No exercise training (control) & Alagebrium; and 4) No exercise training (control) & placebo.

Measurements were performed before, during (6 months) and after one year. First, participants were clinically evaluated to be healthy. Then, we performed non-invasive vascular measurements of the common femoral artery diameter and central pulse wave velocity (i.e., arterial stiffness). On a separate day, participants performed an incremental maximal bicycle exercise stress test. Before and after the 1-year intervention, we also assessed lower limb endothelial function using venous occlusion plethysmography and intra-arterial infusion of vasoactive substances. This measurement was performed on a separate day.

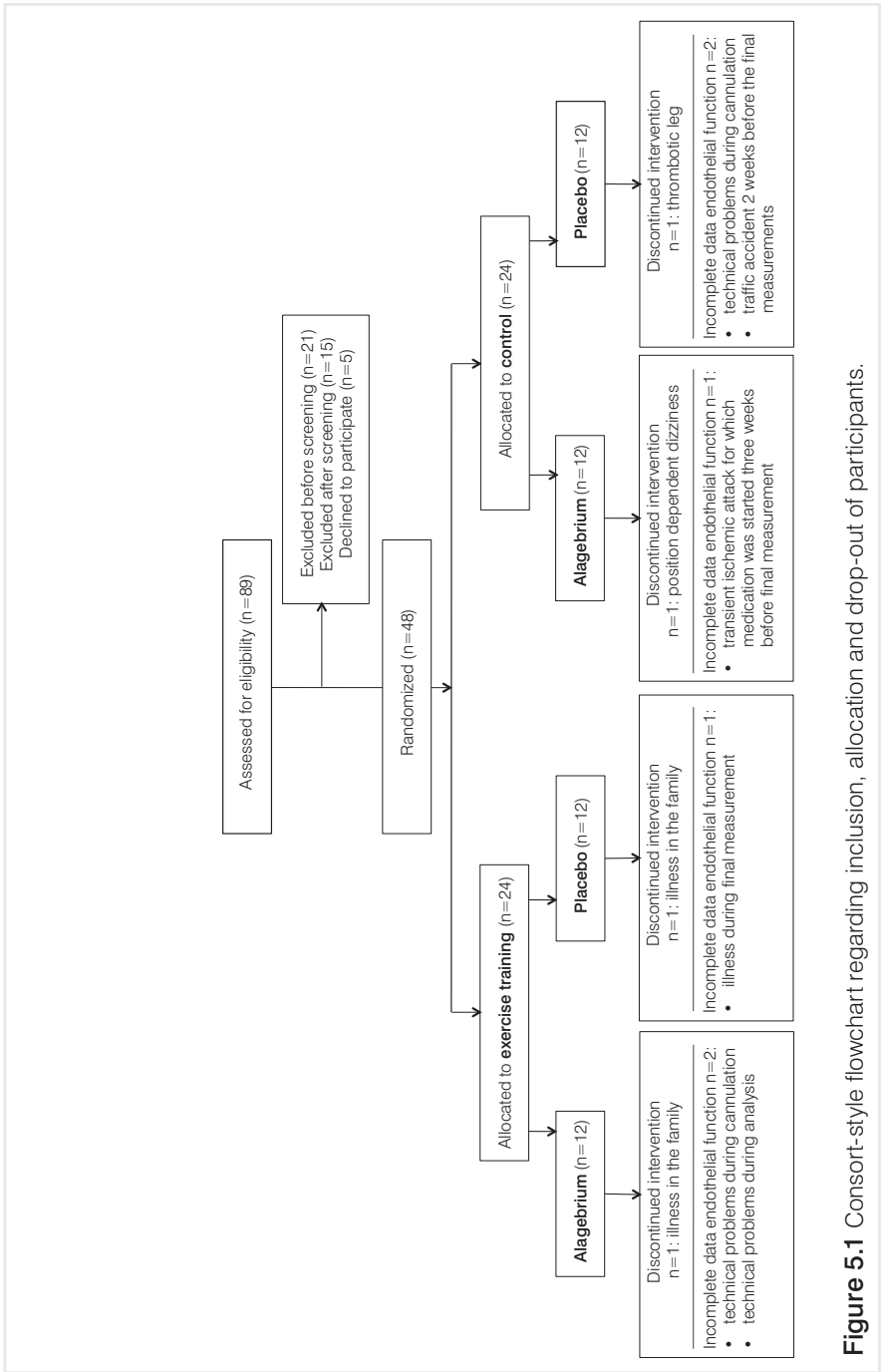


**Figure 5.1** Consort-style flowchart regarding inclusion, allocation and drop-out of participants.

## One year intervention

*Exercise training*

The randomization procedure for exercise training was performed with 48 envelopes. Exercise training was performed 3 times/week for 12 months on a cycle ergometer (Medgraphics, Corival Cycle Ergometer, St Paul, MN, USA). Each exercise session consisted of 10 minutes warm up, followed by 30 minutes of cycling exercise at 70–85% of the individual heart rate reserve and ended with 5 minutes cool down. Heart rate was continuously monitored (Polar RS800; Polar Electro Oy, Kempele, Finland). Workload was individually adjusted throughout the training to enhance physical fitness. The researchers were blinded during data analysis.

*Alagebrium vs. placebo*

Participants were randomized to AGE-crosslink breaker Alagebrium 100 mg twice a day or placebo (twice a day). This dose of Alagebrium, produced by Synvista Therapeutics, was selected as the lowest dose that was effective in both animal [14,15] and human [3,8] studies to maximize efficacy and minimize toxicity. Double-blind randomization was controlled by the university hospital pharmacist and was kept strictly confidential during the study. Compliance with the study drug was controlled by asking participants to keep a journal and sign the time of each ingestion. Every 3 months, this journal was compared with the (empty) strips of tablets.

## Measurements

*General characteristics*

All participants underwent clinical evaluations including measurements of body composition. Blood pressure measurements were performed three times in the supine position using a manual sphygmomanometer around the left arm after a 10 minute rest. Venous blood samples were taken after an overnight fast to measure lipid levels, glucose and glycosylated haemoglobin (HbA1c). In addition, high sensitivity C-reactive protein (hs-CRP) was examined as a measure of inflammation.

*Incremental maximal bicycle exercise stress test*

An incremental maximal exercise stress test on a bicycle ergometer (Lode, Excalibur Sport, Groningen, the Netherlands) was performed to measure maximal oxygen uptake ($VO_2$max).[16] After 3 minutes rest, participants started cycling at a workload of 50Watt, which was increased by 10 Watt/minute. Continuous measurement of oxygen uptake ($VO_2$) was performed using an automatic gas analyzer (Oxycon alpha, Jaeger, Breda, the Netherlands). Peak oxygen uptake (ml$O_2$/min/kg) was calculated as the average oxygen uptake during the last minute of the test and then scaled for body weight and lean body mass.

*Cardiovascular risk*

The Lifetime Risk Score (LRS) is based on an algorithm that incorporates gender, age, systolic blood pressure, diabetes mellitus, total cholesterol, smoking, body mass index, and physical fitness.[17,18] The LRS has a strong predictive capacity for future cardiovascular mortality.[17,18]

*Vascular measurements*

Vascular measurements were performed under standardized conditions. Participants were asked to refrain from coffee, tea, alcohol, chocolate, vitamin C supplements or fruit 14 hours prior and fasting overnight prior to the examinations. Room temperature was set at 22 ± 1 °C.

*Common femoral artery diameter.* Using high-resolution echo ultrasonography with a 7.5 MHz linear array transducer (Picus, Pie Medical Benelux, Maastricht, the Netherlands) we measured the right common femoral artery (CFA) diameter 2 cm proximal to the bifurcation.[19] The percentage change in arterial diameter was calculated and used for analysis.

*Arterial stiffness.* We measured systemic arterial stiffness using central pulse wave velocity (PWV).[20] A three lead electrocardiogram (ECG) was used for R-wave detection. The pulse wave was measured by echo-Doppler ultrasound (WakiLoki Doppler, 4 MHz, Atys) at the left carotid artery and right CFA.

*Lower limb endothelial function.* Endothelial function of the lower limb was measured from resistance artery blood flow responses using venous occlusion plethysmography during intra-arterial infusion of vasoactive substances [21,22] in the upper leg, previously described in detail by Kooijman *et al.*.[23] In short, an intra-arterial cannula was introduced into the right CFA at the level of the inguinal ligament. This cannula was used for intra-arterial administration of vasoactive substances and for intra-arterial blood pressure monitoring. Bilateral blood flow in the upper legs was measured by ECG-triggered venous occlusion plethysmography. Mercury-in-silastic strain gauges were placed at mid thigh to quantify changes in leg volume from changes in upper leg blood flow.

After instrumentation and at least 45 minutes after cannulation of the femoral artery, infusion of the vasoactive substances started. We infused the endothelium dependent vasodilator acetylcholine (ACh), the endothelium independent vasodilator sodium nitroprusside (SNP), and the nitric oxide synthase inhibitor $N^G$-monomethyl-L-arginine (L-NMMA). Acetylcholine was administered at 1, 4, 16, 32 and 64 μg/mL/100mL leg volume, SNP 0.06, 0.20 and 0.60 μg/mL/100mL leg volume, and L-NMMA 0.05, 0.10, 0.20 and 0.40 mg/mL/100mL leg volume. The order of infusion was fixed and each substance was infused for 5 minutes. During each substance infusion, the calf circulation was occluded by inflating cuffs directly below the knee to suprasystolic values (≥220 mmHg) to avoid the use of high dosages with subsequent possible

systemic effects of the vasoactive substances.[23] Two consecutive infusions were performed before deflating the lower leg cuffs to restore normal blood flow for 5 minutes. During these 5 minutes of recovery, 0.9% saline (or 5% glucose during SNP measurements) was infused to maintain a constant flow rate. Between administration of different substances a 20 minute rest period was inserted with continuous flow of 0.9% saline.
Besides changes in blood flow, the blood flow ratio between the infusion and control leg was also calculated to correct for possible systemic effects.

## Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA). Data are presented as mean ± standard deviation (SD), except for the figures in which mean ± standard error of the mean (SEM) are used. At baseline, a one-way ANOVA (*Analysis of variance*) comparison was performed between the 4 groups with Bonferroni correction. Differences between the 4 groups in response to the 1-year intervention were analyzed using the Linear Mixed Model. The interaction between exercise training and Alagebrium was analyzed (Time*Training*Medication), and both individual effects of exercise training vs. no exercise training and Alagebrium vs. placebo were analyzed (Time*Training and Time*Medication, respectively). Changes in blood flow and blood flow ratio responses to increasing dosages of vasoactive substances were also analyzed using the Linear Mixed Model at baseline and after one year. Statistical significance was set at a (2-sided) $p$-value $<0.05$.
Sample size was calculated for an effect of Alagebrium and exercise training on endothelial function. Based on previous studies [11,12,23] that examined the influence of physical activity on endothelial function using intra-arterial infusion of ACh, SNP, and L-NMMA, a group size of 10 subjects is sufficient to detect relevant changes in endothelial function of 3.9 ml/min/dl with exercise training with a power of $>0.95$, given a standard deviation of 4.7 ml/min/dl and an $\Delta$error of 0.05. Moreover, based on previous work from our lab,[19] 8 subjects would allow for a power of $>0.95$ to detect a change in arterial compliance with exercise training of 0.015 $mm^2$/mmHg given a standard deviation of 0.01 $mm^2$/mmHg and an alpha error of 0.05. The magnitude of both of these changes would be considered clinically and physiologically significant. Due to the long intervention period and possibility for drop-outs, group sizes of 12 participants were created.

## Results

Four individuals, 1 per intervention arm, did not finish the intervention due to reasons unrelated to the intervention (Figure 5.1). At baseline, we found no differences between the 4 groups in gender, age, and physical fitness (Table 5.1). Also, no differences were observed in cardiovascular risk factors, e.g. body composition, blood pressure, total cholesterol, triglycerides, glucose, HbA1c, and hs-CRP among the 4 groups at baseline (Table 5.1). Drug compliance was high with >95% of the drug taken.
One year of exercise training significantly improved physical fitness by 15% (Time*Training $p<0.001$, Figure 5.2). Alagebrium did not influence physical fitness (Time*Medication $p=0.969$ and Time*Training*Medication $p=0.757$).

The 1-year intervention did not alter body composition, blood pressure, lipid levels, glucose, and HbA1c in any of the 4 groups (all $p>0.10$, Table 5.1). A small interaction effect was seen in hs-CRP when combining the 4 groups (Time*Training*Medication $p=0.048$). However, neither the individual effect of exercise training, nor the individual effect of medication had a significant influence on hs-CRP levels (Time*Training $p=0.194$ and Time*Medication $p=0.987$, respectively). Furthermore, no overall difference was seen in hs-CRP before and after one year (n=44, pre 1.83 ± 1.38 mg/l vs. post 1.60 ± 1.14 mg/l, paired t-test $p=0.166$).

### Cardiovascular risk prediction

Lifetime Risk Score significantly improved after exercise training (Time*Training $p=0.014$), with an 8% decrease in 30-year risk prediction. Alagebrium did not change LRS or alter the impact of exercise training on Lifetime Risk Score (Time*Medication $p=0.972$ and Time*Training*Medication $p=0.476$, respectively, Figure 5.3).

### Vascular measurements

*Common femoral artery diameter.* Due to technical problems, we had to exclude 7 participants from the echo Doppler measurements. Exercise training was associated with an increase in CFA diameter of 6% (Time*Training $p=0.043$). Use of the AGE-crosslink breaker Alagebrium did not change CFA diameter or alter the magnitude of effect observed with exercise training (Time*Medication $p=0.948$ and Time*Training*Medication $p=0.837$, respectively, Table 5.2).
*Arterial stiffness.* We did not observe changes in arterial stiffness measured with pulse wave velocity after any of the four 1-year interventions (Table 5.2).

**Table 5.1** Characteristics and cardiovascular risk factors of the four intervention groups at baseline and after one year intervention are shown on the left side (first 4 columns *Baseline characteristics*). These data include the participants who completed the 1-year intervention, thus n=11 per group. Differences (*p*-values) between the four groups in response to the 1-year interventions, analyzed using the linear mixed model, are shown on the right side (last 3 columns *Linear Mixed Model: changes due to the 1-year interventions*).

| | Characteristics Baseline & after one year intervention | | | | Linear Mixed Model: changes due to the 1-year interventions | | |
|---|---|---|---|---|---|---|---|
| | Exercise training | | No exercise training | | Time* Training | Time* Medication | Time* Training* Medication |
| | *Alagebrium* | *placebo* | *Alagebrium* | *placebo* | *p-value* | *p-value* | *p-value* |
| **Male : Female (n)** | 6:5 | 8:3 | 8:3 | 3:8 | | | |
| **Age (years)** | 69 ± 3 | 68 ± 3 | 70 ± 3 | 71 ± 5 | | | |
| **$VO_2$max ($mlO_2$/min/kg)** | | | | | **<0.001** | 0.969 | 0.757 |
| Baseline | 23.2 ± 4.2 | 24.5 ± 4.9 | 25.6 ± 4.3 | 24.1 ± 4.2 | | | |
| 12 months | 26.7 ± 4.0 | 27.7 ± 5.2 | 25.0 ± 4.7 | 23.9 ± 3.6 | | | |
| **$VO_2$max per lean body mass ($mlO_2$/min/kg)** | | | | | **<0.001** | 0.740 | 0.864 |
| Baseline | 36.4 ± 6.7 | 35.5 ± 5.7 | 36.0 ± 5.1 | 35.5 ± 5.9 | | | |
| 12 months | 40.2 ± 4.9 | 40.3 ± 5.7 | 34.5 ± 5.5 | 34.9 ± 5.1 | | | |
| **Body Mass Index ($kg/m^2$)** | | | | | 0.812 | 0.209 | 0.706 |
| Baseline | 26.9 ± 3.5 | 27.0 ± 2.6 | 26.6 ± 3.0 | 24.3 ± 3.3 | | | |
| 12 months | 26.2 ± 4.0 | 26.8 ± 2.9 | 26.2 ± 3.1 | 24.3 ± 3.1 | | | |
| **Lean Body Mass (kg)** | | | | | 0.225 | 0.610 | 0.777 |
| Baseline | 53.8 ± 13.3 | 57.1 ± 9.6 | 55.2 ± 8.5 | 46.6 ± 8.7 | | | |
| 12 months | 53.2 ± 11.5 | 56.6 ± 9.5 | 55.4 ± 8.8 | 47.1 ± 8.7 | | | |
| **Waist circumference (cm)** | | | | | 0.790 | 0.366 | 0.763 |
| Baseline | 97.7 ± 11.5 | 96.4 ± 7.5 | 93.3 ± 9.6 | 88.2 ± 11.0 | | | |
| 12 months | 96.8 ± 11.9 | 96.3 ± 7.5 | 92.8 ± 8.5 | 89.0 ± 10.4 | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Hip circumference (cm)** | | | | | 0.491 | 0.735 | 0.772 |
| Baseline | 103.4 ± 5.9 | 103.2 ± 5.8 | 101.9 ± 6.0 | 99.6 ± 7.1 | | | |
| 12 months | 102.5 ± 7.2 | 102.9 ± 6.1 | 100.6 ± 5.9 | 98.6 ± 6.3 | | | |
| **Waist-to-Hip ratio** | | | | | 0.228 | 0.597 | 0.869 |
| Baseline | 0.94 ± 0.06 | 0.94 ± 0.04 | 0.92 ± 0.08 | 0.88 ± 0.07 | | | |
| 12 months | 0.94 ± 0.06 | 0.94 ± 0.03 | 0.92 ± 0.08 | 0.90 ± 0.07 | | | |
| **Systolic blood pressure (mmHg)** | | | | | 0.230 | 0.275 | 0.189 |
| Baseline | 128 ± 10 | 131 ± 9 | 137 ± 15 | 134 ± 15 | | | |
| 12 months | 128 ± 14 | 128 ± 8 | 138 ± 17 | 135 ± 16 | | | |
| **Diastolic blood pressure (mmHg)** | | | | | 0.869 | 0.376 | 0.703 |
| Baseline | 80 ± 9 | 76 ± 6 | 79 ± 8 | 79 ± 12 | | | |
| 12 months | 80 ± 9 | 74 ± 3 | 80 ± 10 | 76 ± 10 | | | |
| **Pulse Pressure (mmHg)** | | | | | 0.155 | 0.629 | 0.150 |
| Baseline | 47 ± 7 | 56 ± 8 | 58 ± 14 | 56 ± 9 | | | |
| 12 months | 49 ± 9 | 53 ± 9 | 58 ± 16 | 59 ± 9 | | | |
| **Total cholesterol (mmol/l)** | | | | | 0.467 | 0.162 | 0.422 |
| Baseline | 5.3 ± 1.0 | 5.7 ± 0.9 | 5.5 ± 0.6 | 5.2 ± 0.9 | | | |
| 12 months | 5.1 ± 0.8 | 5.9 ± 0.9 | 5.2 ± 0.7 | 5.1 ± 0.5 | | | |
| **HDL cholesterol (mmol/l)** | | | | | 0.763 | 0.662 | 0.918 |
| Baseline | 1.4 ± 0.3 | 1.2 ± 0.2 | 1.2 ± 0.3 | 1.6 ± 0.2* | | | |
| 12 months | 1.4 ± 0.4 | 1.3 ± 0.3 | 1.2 ± 0.3 | 1.6 ± 0.3 | | | |
| **LDL cholesterol (mmol/l)** | | | | | 0.789 | 0.204 | 0.437 |
| Baseline | 3.4 ± 1.0 | 4.1 ± 0.7 | 3.7 ± 0.5 | 3.2 ± 0.7* | | | |
| 12 months | 3.1 ± 0.7 | 4.1 ± 0.8 | 3.5 ± 0.5 | 3.1 ± 0.5 | | | |
| **Triglycerides (mmol/l)** | | | | | 0.125 | 0.401 | 0.673 |
| Baseline | 1.2 ± 0.4 | 1.0 ± 0.3 | 1.2 ± 0.4 | 1.0 ± 0.4 | | | |
| 12 months | 1.3 ± 0.5 | 1.2 ± 0.3 | 1.2 ± 0.4 | 1.0 ± 0.3 | | | |

**Table 5.1** Continued.

| | Characteristics Baseline & after one year intervention | | | | Linear Mixed Model: changes due to the 1-year interventions | | |
|---|---|---|---|---|---|---|---|
| | Exercise training | | No exercise training | | Time* Training | Time* Medication | Time* Training* Medication |
| | *Alagebrium* | *placebo* | *Alagebrium* | *placebo* | *p-value* | *p-value* | *p-value* |
| **Glucose (mmol/l)** | | | | | 0.831 | 0.390 | 0.502 |
| Baseline | 5.2 ± 0.6 | 4.9 ± 0.5 | 5.1 ± 0.4 | 4.9 ± 0.3 | | | |
| 12 months | 5.1 ± 0.8 | 5.2 ± 0.6 | 5.1 ± 0.5 | 4.9 ± 0.3 | | | |
| **HbA1c (%)** | | | | | 0.358 | 0.244 | 0.728 |
| Baseline | 5.6 ± 0.4 | 5.8 ± 0.3 | 5.7 ± 0.4 | 5.6 ± 0.2 | | | |
| 12 months | 5.6 ± 0.4 | 5.8 ± 0.4 | 5.5 ± 0.6 | 5.5 ± 0.2 | | | |
| **Hs-CRP (mg/l)** | | | | | 0.194 | 0.987 | **0.048** |
| Baseline | 1.4 ± 1.0 | 2.3 ± 1.3 | 2.1 ± 1.7 | 1.4 ± 1.3 | | | |
| 12 months | 1.9 ± 1.4 | 1.4 ± 1.0 | 2.0 ± 1.3 | 1.2 ± 0.9 | | | |

Data are presented as mean ± standard deviation. * At baseline (one-way ANOVA) HDL cholesterol was higher in the no exercise training & placebo group compared with the exercise training & placebo group ($p$=0.011) and no exercise training & Alagebrium group ($p$=0.012). LDL cholesterol was lower in the no exercise training & placebo group compared with the exercise training & placebo group ($p$=0.049). **Abbreviations:** HDL high density lipoprotein, LDL low density lipoprotein, HbA1c glycosylated hemoglobin, hs-CRP high sensitivity C-reactive protein.

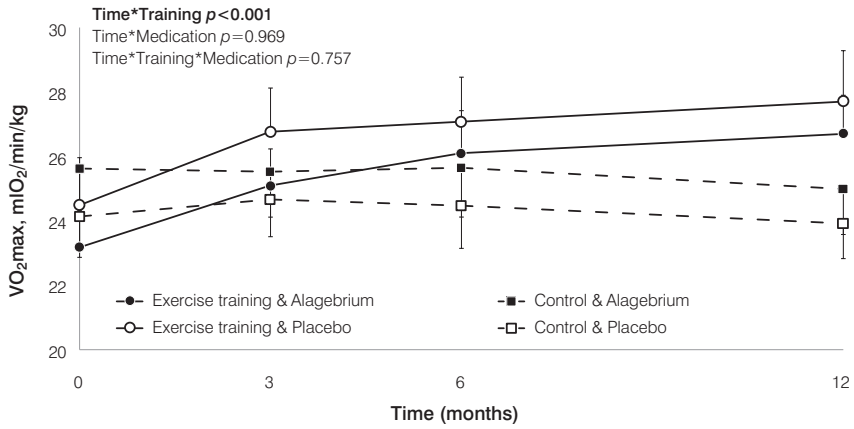


**Figure 5.2** Exercise training groups (solid lines: with Alagebrium ●; with placebo ○) significantly improved their physical fitness, while the control groups (dashed lines: with Alagebrium ■; with placebo □) showed a small, non-significant, decline in $VO_2$max.

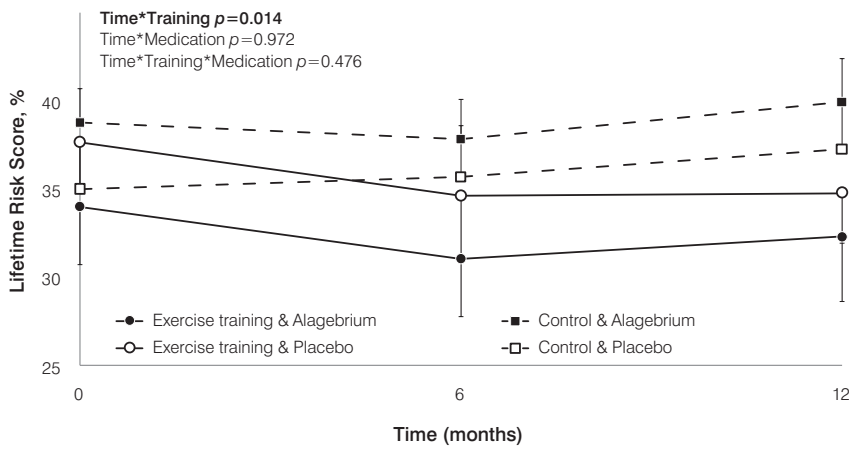


**Figure 5.3** Lifetime Risk Score improved significantly in the exercise training groups (solid lines: with Alagebrium ●; with placebo ○) while the control groups (dashed lines: with Alagebrium ■; with placebo □) did not change significantly. Alagebrium had no influence on the Lifetime Risk Score.

**Table 5.2** Results of the common femoral artery diameter and arterial stiffness of the four groups are shown here. Data during the year intervention are shown on the left side (first 4 columns). These data include the participants who completed the 1-year intervention, thus n=11 per group. Differences (*p*-values) between the four groups in response to the 1-year interventions, analyzed using the linear mixed model, are shown on the right side (last 3 columns *Linear Mixed Model: changes due to the 1-year interventions*).

| | Vascular measurements | | | | Linear Mixed Model: changes due to the 1-year interventions | | |
|---|---|---|---|---|---|---|---|
| | Exercise training | | No exercise training | | Time*Training | Time* Medication | Time*Training* Medication |
| | *Alagebrium* | *placebo* | *Alagebrium* | *placebo* | *p-value* | *p-value* | *p-value* |
| **Common femoral artery diameter (mm)** | | | | | **0.043** | 0.948 | 0.837 |
| Baseline | 9.5 ± 0.9 | 10.1 ± 1.3 | 10.7 ± 1.8 | 9.8 ± 1.6 | | | |
| 6 months | 10.0 ± 1.0 | 10.5 ± 1.4 | 10.9 ± 1.5 | 9.9 ± 1.6 | | | |
| 12 months | 10.1 ± 1.0 | 10.6 ± 1.3 | 10.9 ± 1.5 | 10.0 ± 1.6 | | | |
| **Arterial stiffness, pulse wave velocity (m/s)** | | | | | 0.425 | 0.106 | 0.886 |
| Baseline | 13.3 ± 5.4 | 11.0 ± 3.2 | 13.2 ± 4.3 | 11.5 ± 3.0 | | | |
| 6 months | 11.7 ± 3.7 | 10.8 ± 2.4 | 12.7 ± 2.8 | 12.4 ± 3.4 | | | |
| 12 months | 12.3 ± 4.1 | 11.4 ± 2.6 | 12.2 ± 1.7 | 12.7 ± 3.9 | | | |

Data are presented as mean ± standard deviation.

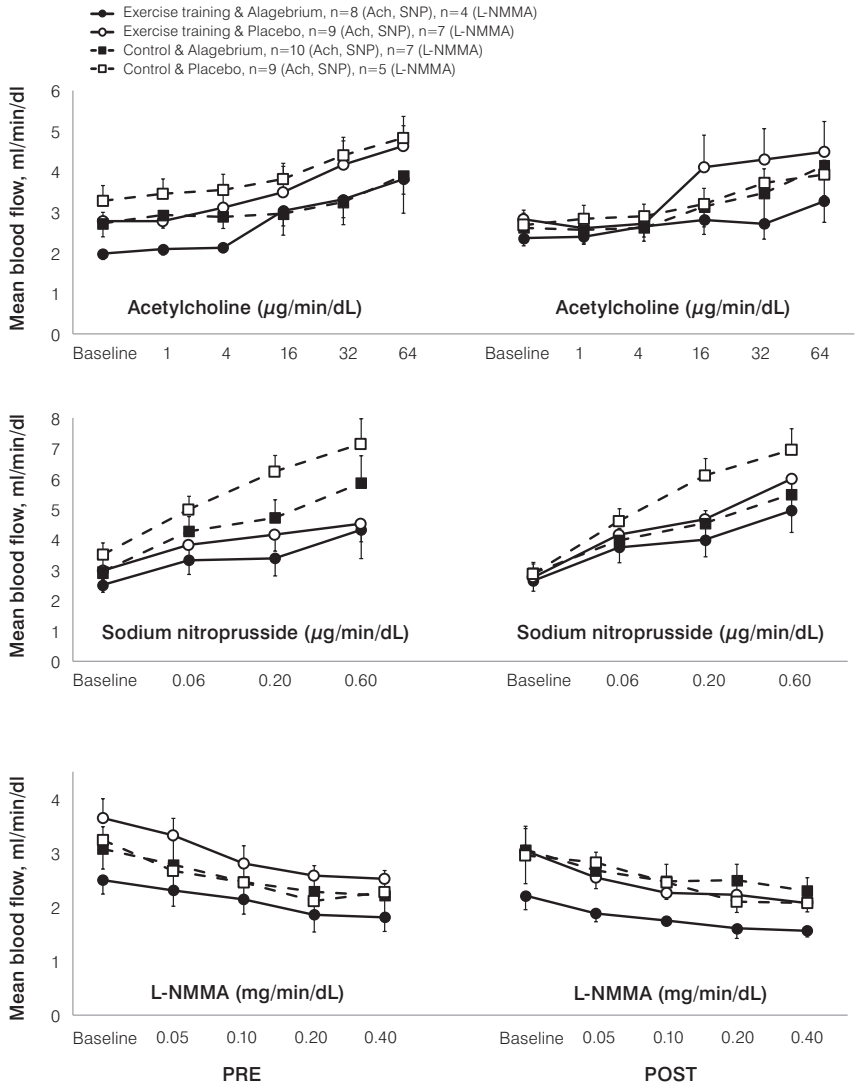


**Figure 5.4** Mean blood flow response to intra-arterial infusion of vasoactive substances. No changes were observed in mean blood flow response to increasing dosage of vasoactive substances between any of the four groups during the year of intervention: exercise training & Alagebrium (solid line + •), exercise training & placebo (solid line + ○), control & Alagebrium (dashed line + ■), and control & placebo (dashed line + □). Pre = baseline measurements, post = after 1-year interventions.

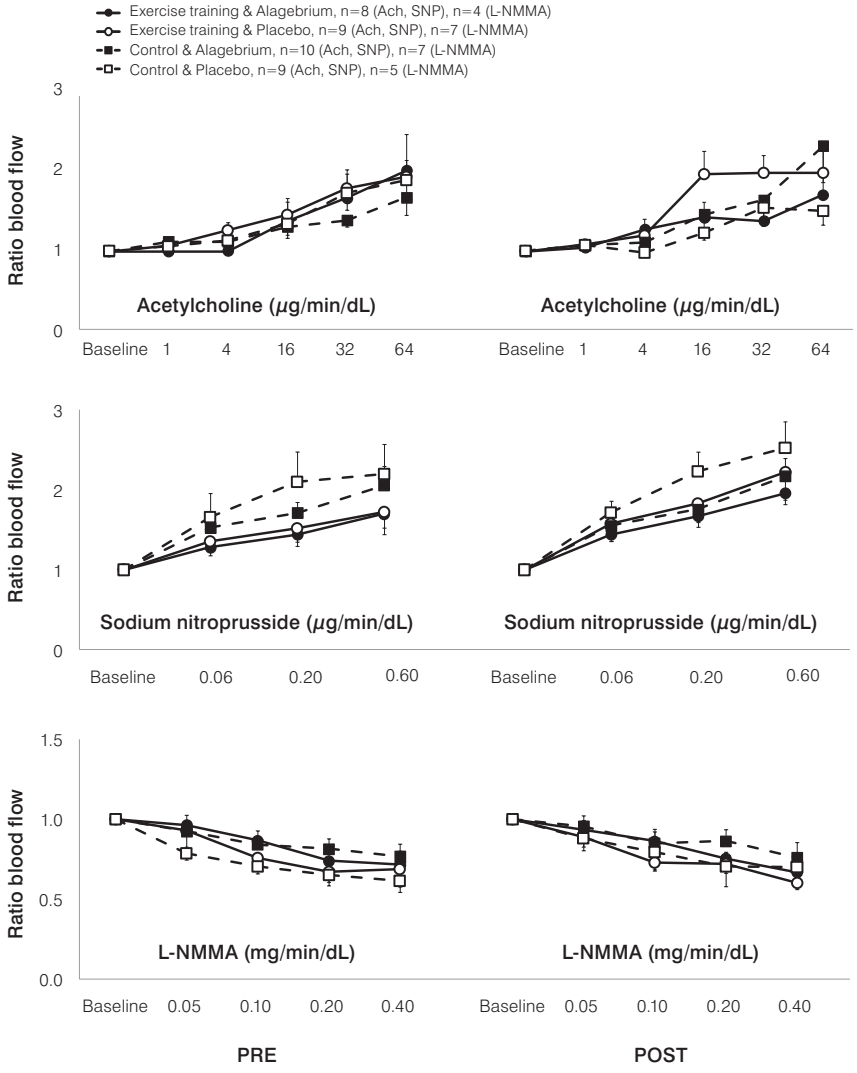


**Figure 5.5** Blood flow ratio response to intra-arterial infusion of vasoactive substances. No changes were observed in blood flow ratio (infusion/control leg) response to increasing dosage of vasoactive substances between any of the four groups during the year of intervention: exercise training & Alagebrium (solid line + ●), exercise training & placebo (solid line + ○), control & Alagebrium (dashed line + ■), and control & placebo (dashed line + □). Pre = baseline measurements, post = after 1-year interventions.

*Endothelial function.* All participants received ACh and SNP, and by design 24 participants were given L-NMMA. Due to technical problems, 8 participants were excluded from analyses (Figure 5.1). We found no significant effect of the interventions (exercise training, Alagebrium, or both) on blood flow or blood flow ratio responses to the incremental doses of ACh, SNP or L-NMMA (all $p>0.10$, Figures 5.4 and 5.5). In addition, no differences were observed among the four groups in blood flow or blood flow ratio responses to the pharmacological stimuli at baseline as well as after one year of intervention (Figures 5.4 and 5.5).

## Discussion

We examined the effects of the AGE-crosslink breaker Alagebrium, alone and in combination with aerobic exercise training on endothelial function, arterial stiffness and cardiovascular risk in sedentary older individuals. Although our interventions were performed successfully (i.e., improvement in physical fitness of 15% and drug intake compliance of >95%), none of the interventions improved endothelial function or arterial stiffness. Nonetheless, the Lifetime Risk Score improved significantly in the exercise training groups, while the AGE-crosslink breaker was not associated with any further improvement. Therefore, the cardioprotective effects of 1-year exercise training in previously sedentary older subjects cannot be potentiated by the AGE-crosslink breaker Alagebrium, nor be explained by improvements in endothelial function or arterial stiffness.



Our primary outcome measure, the endothelial function, was measured using intra-arterial infusion of (incremental doses of) vasoactive drugs, which is widely considered to be the "gold standard".[21,22] Despite a 15% improvement in physical fitness and >95% compliance in drug intake, neither endothelium-dependent or -independent vasodilation, nor the contribution of nitric oxide to basal vascular tone, changed in any group over the intervention period. While most studies investigating Alagebrium have focused on arterial and ventricular stiffness, only one study examined the influence of short term (8 weeks) Alagebrium on endothelial function.[9] They reported an improvement in brachial artery endothelial function. However, this outcome was measured in 10 patients with isolated systolic hypertension (*versus* our healthy older individuals), in a different vascular bed (i.e., a conduit artery in the upper arm *versus* resistance vessels in the lower limbs in the present study), and using the widely used method of flow mediated dilation; i.e., an indirect method to measure endothelial function.
Even though there is consistency in the literature about the positive effects of exercise training on improving endothelial function in patient groups in whom endothelial

function is initially depressed, the effect of exercise training on endothelial function of subjects with marginal endothelial dysfunction is less obvious.[19,24,25] In contrast to several exercise training studies that reported improved endothelial function in the brachial artery after training,[2,11,12] the endothelial function in the lower limbs did not improve in our study groups. A first explanation may relate to differences in vascular responses between the arms and legs, with lower limbs being less responsive to exercise training as the lower limbs already demonstrate a higher activity level than the upper limbs during daily living (e.g., during walking, cycling, climbing the stairs).[26,27] Another explanation for this discrepancy may be the long duration of exercise training in our study. Indeed, previous studies found that exercise training initially leads to functional adaptations (improved endothelium), followed by structural adaptations (increase in vascular diameter) when exercise training continues, allowing endothelial function to return towards baseline levels.[24] Interestingly, we observed an increase in CFA diameter in the exercise training groups, most likely as a result of the repeated exposure of elevation in shear stress on the arterial wall during exercise.[24] Subsequently, functional endothelial responses might have returned to baseline near the end of our study. However, this remains speculative since we did not perform repeated measures to assess the time course of endothelial function.

We found no change in arterial stiffness after our interventions. The observation of unaltered stiffness after training is in line with a recent study that found that intrinsic structural characteristics of the arterial wall remained unaffected in previously sedentary elderly after one year of exercise training.[28] However, both animal and human studies found that Alagebrium decreased arterial stiffness.[8,9,15,29] In our study, we found a small, albeit non-significant, trend of Alagebrium to improve central PWV. More recently, the combination of Alagebrium with exercise training, in a design similar to the present study but performed in rats, reversed the effects of cardiovascular aging in older sedentary rats.[13] Differences between studies not only in doses of Alagebrium and exercise, but also in species examined may explain these results.

An important difference between our study and previous human studies that examined AGE-crosslink breakers is the inclusion of patient populations *versus* non-diseased participants in our study. Cardiovascular patients demonstrate *a priori* endothelial dysfunction and arterial stiffening that likely exceeds that of non-diseased, sedentary older individuals. Therefore, interventions in such populations are more amenable to an improvement. Nonetheless, the rationale of our study to examine older individuals is strong, since it is known that this population is associated with an increased cardiovascular risk, has decreased endothelial function and reports stiffer arteries compared with young individuals.[1,2,10-12]

This study represents the longest duration of therapy with an AGE-crosslink breaker in humans reported in the literature, while we are the first to combine AGE-crosslink breakers with exercise training in humans. Some short duration (open-label) AGE-crosslink breaker studies with patient groups found an improvement in endothelial function [9] and a decrease in arterial and possibly myocardial stiffness,[8,9,29] while a longer 9-month study with stable heart failure patients showed no improvements in cardiac function with Alagebrium.[30]

Whilst the lack of improvement in endothelial function and arterial stiffness after a 1-year intervention is somewhat disappointing, our results strongly reinforce previous suggestions that vascular changes induced by biological aging and physical inactivity cannot be easily reversed. According to Byberg *et al.*,[31] it takes several years for middle-aged men to achieve a decrease in (all cause) mortality after becoming physically active after years of physical inactivity. Also in our study, we included older subjects who have not performed (regular) exercise for the last several years. Thus, despite the strong rationale for direct effects of AGE-crosslink breaker Alagebrium and exercise training on the arterial wall, our 1-year intervention may be insufficient to undo the negative effects of decades of sedentary aging.

Despite the absence of a *direct* effect on the vasculature, significant improvement in physical fitness has important health benefits.[31,32] Physical fitness has recently been demonstrated to have the strongest predictive capacity for future cardiovascular diseases and all-cause mortality.[33,34] Indeed, the improvement in physical fitness in the exercise training groups importantly contributed to the significant improvement in Lifetime Risk Score, while Alagebrium did not have any (additional) effects. Therefore, it must be emphasized that, despite the absence of a direct vascular effects, the performance of a 1-year exercise training program in previously sedentary elderly resulted in a significant and clinically meaningful reduction in risk for future cardiovascular disease.



## Limitations and strengths

The use of invasive and highly valid measures of endothelial function, the long intervention period with a unique combination of novel interventions, and the high compliance with drug-intake and exercise training represent unique aspects of our study. Nonetheless, our study has a number of potential limitations. For example, power analysis supported a sample size of 8-10 participants per group to detect relevant differences with a small chance of type II error. Even though not all participants could be included in the analysis of the endothelial function, even the Control & Alagebrium group (n=10) failed to show differences after the one year intervention. Nor were there trends in any of the other groups. Thus, even if all data from the

venous occlusion plethysmography could have been used, it is unlikely that a physiologically meaningful improvement in endothelial function was missed. Also, arterial stiffness, with presumably sufficient participants per group, did not improve with these interventions. This suggests that the beneficial effects of exercise training on cardiovascular risk are not explained by improvements in endothelial function or arterial stiffness. Another potential limitation is that we did not measure blood levels and pharmacokinetics of Alagebrium. However, we were meticulous at ensuring compliance with the study medication. Moreover, the doses used in this study were comparable to the doses used in other pre-clinical and clinical trials where an effect of Alagebrium to break AGE-crosslinks was evident.[3,8,14,15]

## Conclusion

One year of exercise training in older individuals significantly improved physical fitness and lifetime risk for cardiovascular disease without affecting endothelial function or arterial stiffness. The use of the AGE-crosslink breaker Alagebrium had no independent effect on vascular function, nor did it potentiate the effect of endurance training. Despite the benefits and cardioprotective effects of exercise training for older individuals, neither exercise nor Alagebrium (either alone or in combination) was able to reverse the effects of decades of sedentary aging on the vasculature.

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Chapter 6

# General discussion

*To AGE or not to age*

*"We cannot solve our problems with the same thinking we used when we created them." Albert Einstein (1879-1955)*

When we started the research described in this thesis several years ago, the studies concerning the effects of advanced glycation end-products (AGEs) on the cardiovascular system and the influence of the AGE-crosslink breaker Alagebrium thereon were very promising. The first animal [1-4] and human studies showed beneficial effects of Alagebrium in improving cardiovascular stiffness [5,6] and endothelial function.[7] Unexpectedly, a large randomized clinical trial with patients with chronic heart failure showed no improvements in physical fitness level or diastolic heart function.[8] Together with our colleagues from the Institute of Exercise and Environmental Medicine, Dallas, Texas, USA, we found no changes in arterial stiffness, endothelial function or in cardiac left ventricular hemodynamics and geometry in a group of non-exercising older individuals without clinical or symptomatic cardiovascular diseases.[9,10] The hypothesis that Alagebrium could reduce cardiovascular complications in patients with diabetes, hypertension, heart failure and aging was based on the beneficial animal and human studies. Surprisingly, in our studies Alagebrium did not show these beneficial effects and the question remains what the overall conclusion on the potential effects of Alagebrium in humans should be.
In this chapter I will discuss the rationale of AGEs in aging physiology, followed by an overview of literature with studies involving Alagebrium and possible explanations for the different outcomes. Next, the knowledge concerning plasma AGEs and tissue AGEs will be discussed, and new data will be presented on the influence of exercise training and Alagebrium on plasma AGEs. I will conclude this general discussion with a reconsideration of advanced glycation end-products in human aging and options for future research will be addressed on how to improve healthy aging by addressing the AGE challenges.



## AGEs in aging physiology

### AGEs (patho)physiology

AGEs are formed when a reducing sugar non-enzymatically reacts with a biological amine from a protein, lipid or amino acid to produce a Schiff base. This initial reaction is swift, highly reversible and can undergo further chemical rearrangements to form a more stable glycated protein, an Amadori product. This is still reversible, but it can undergo structural rearrangements through a series of reactions such as oxidation, dehydration and degradation to form irreversible complex arrangements of crosslinked proteins, the Advanced Glycation End-products or AGEs.[11-14] Due to the

irreversible nature of AGEs, they accumulate with aging leading to unfavorable situations and age-related complications.[15-17]

Multiple mechanisms have been proposed by which AGEs are detrimental in aging physiology. First, AGEs damage cells and tissues by forming cross-links with proteins in the extracellular matrix.[18] These crosslinks result in a decrease in elasticity and increase of stiffness of tissues.[19,20] Second, circulating AGEs bind to cellular receptors (RAGEs) and activate cell signaling pathways with subsequent modulation of gene expression, cascades of inflammatory responses and induction of oxidative stress.[21-24] Oxidative stress, on the other hand, increases the formation of AGEs resulting in a vicious cycle.[25] Third, intracellular glycation of proteins may lead to impaired cell function and reduction of the expression of endothelial nitric oxide synthase (eNOS) leading to endothelial dysfunction.[26] Furthermore, AGEs quench nitric oxide (NO) leading directly to inactivation of NO and induce the expression of the potent vasoconstrictor endothelin-1 leading to a further deterioration of endothelial function.[12,27,28] Since AGEs accumulate slowly over the years, older individuals encounter more effects of AGEs and this may contribute to the development of cardiovascular diseases.

## AGEs and cardiovascular diseases

AGEs affect virtually every tissue in the body,[19] but the most examined and elaborate influence of AGEs is that on the cardiovascular system. The best evidenced consequence of AGEs is the accumulation in the extracellular matrix that contributes to stiffening of the arterial walls and myocardium.[11,15,20,29] Arterial stiffening induces arterial systolic hypertension and an increase in pulse pressure, which is a strong predictor for the development of cardiovascular morbidity and mortality.[30-32] Besides the accumulation of AGEs in the arterial walls, AGEs also accumulate in the myocardium which leads to stiffening of the myocardium resulting in heart failure. Both conditions, arterial and myocardial stiffening, are associated with age-related diseases and mortality.

Besides the influence of AGEs on structural vascular changes, AGEs also influence the endothelial function. Healthy vascular endothelium plays a crucial role in the protection of cardiovascular diseases by protecting against the development of atherosclerosis and maintaining vascular tone and homeostasis.[33,34] As mentioned above, AGEs activate RAGEs causing cascades of inflammatory responses, induction of oxidative stress, reduced availability of NO, and induction of endothelin-1. This all contributes to endothelial dysfunction which precedes the development of atherosclerosis and thereby increases the risk for cardiovascular and cerebrovascular diseases.[35] Endothelial function is known to decrease with aging.[36]

The exact interaction between the development of age-related diseases and the influence of accumulation of AGEs thereon remains unclear. Probably AGEs are part

of the accumulation of molecular and cellular damage that is accompanied with aging, and thus AGEs may contribute to age associated morbidity and mortality.

## The rationale for the AGE-crosslink breaker Alagebrium as a cardiovascular drug

The recognition that AGEs play a role in the development of cardiovascular diseases was driving the search for novel agents that could interact with the AGE-pathophysiology and thereby lower cardiovascular risk. In 1996 a new agent was created, phenylthiazolium bromide, to specifically break already formed AGE-crosslinks.[37] Even though this drug was not further developed because of the instability in aqueous solutions,[38] it provided proof of concept that AGE-crosslink breakers are potential pharmacological drugs. Based on this experience, ALT-711, also known as Alagebrium, a thiazolium derivative (3-phenacyl-4,5-dimethylthiazolium chloride) was developed in 1998 as the highly potent breaker of AGE-crosslink between proteins.[5] Multiple animal [1-4,39,40] and human studies [5-7] suggested that by cleaving AGE-crosslinks, Alagebrium could restore compliance of the aged and/or diabetic vasculature and the myocardium. Thus, the development of Alagebrium was a unique opportunity to reduce the cardiovascular risk and thereby improve the quality of life in populations at risk for cardiovascular diseases.
The next paragraphs will provide an overview of current literature concerning both animal and human studies (Table 6.1), and possible explanations for the different outcomes will be presented.

### Animal studies

Primarily two animal models were used to examine the effects of Alagebrium: rats with streptozotocin-induced diabetes [1,4] and aged animals (dogs,[2] monkeys [3] or rats [39,40]). The first in vivo study published was in 1998 by Wolffenbuttel *et al.*.[1] They found that three weeks treatment with Alagebrium (daily 1.0 mg/kg body weight) in diabetes induced rats reversed the large artery stiffness and reduced collagen crosslinking in the tail tendon. In 2003, Candido *et al.*[4] confirmed that Alagebrium reduced the AGE deposition and collagen crosslinking in the myocardium of diabetic rats with a higher daily dose of 10.0 mg/kg body weight during sixteen weeks. Furthermore, the reduction in left ventricular mass and brain natriuretic peptide (BNP) expression suggested an improved cardiac function.
Besides studies with diabetic rats, also animal models of aging have been examined with similar findings. Asif *et al.*[2] found a significant reduction of approximately 40% in age-related left ventricular stiffness in aged dogs after four weeks of treatment of Alagebrium (daily 1.0 mg/kg body weight). These results were confirmed in older

**Table 6.1** Overview of literature.

| *Animal studies* | Population | Trial design |
|---|---|---|
| **Wolffenbuttel *et al.*** [1] 1998, PNAS | Diabetes induced rats | Open label study |
| **Asif *et al.*** [2] 2000, PNAS | Young and aged dogs | Open label study |
| **Vaitkevicius *et al.*** [3] 2001, PNAS | Older rhesus monkeys | Open label study<br>Double crossover |
| **Candido *et al.*** [4] 2003, Circ Res | Diabetes induced rats | Open label study |
| **Guo *et al.*** [39] 2009, J Geront Biol Sci | Young and aged rats | Open label study |
| **Steppan *et al.*** [40] 2012, Exp Gerontol | Young and aged rats | Randomized, placebo controlled trial<br>Not blinded |
| *Patient studies* | | |
| **Kass *et al.*** [5] 2001, Circulation | Systolic hypertension | Randomized, placebo controlled trial<br>Double blind |
| **Little *et al.*** [6] 2005, J Cardiac Failure | Diastolic heart failure<br>LV ejection fraction ≥50%. | Open label study |
| **Zieman *et al.*** [7] 2007, J Hypertension | Isolated systolic hypertension | Open label study<br>Single blind |
| **Hartog *et al.*** [8] 2011, Eur J Heart Failure | Chronic heart failure<br>LV ejection fraction <45%. | Randomized, placebo controlled trial<br>Double blind |
| *Healthy older individuals* | | |
| **Fujimoto *et al.*** [9] 2013 | Healthy older non-exercising individuals | Randomized, placebo controlled trial<br>Double blind |
| **Oudegeest-Sander *et al.*** [10] 2013 | Healthy older non-exercising individuals | Randomized, placebo controlled trial<br>Double blind |

**Abbreviation:** LV left ventricle

rhesus monkeys with improvements of arterial compliance and left ventricular function after three weeks treatment with Alagebrium (1.0 mg/kg body weight every other day).[3] Similar to the study by Candido *et al.*[4] in diabetic rats, Guo *et al.*[39] found the reduction in AGE deposition and collagen crosslinking in the myocardium of aged rats and a reduction in left ventricular mass and improved diastolic function with a daily dose of 10.0 mg/kg body weight during sixteen weeks. Recently, an interesting study in which the individual and combined effects of four weeks of exercise training

| Dose of Alagebrium | Positive/Negative outcome |
|---|---|
| 1 mg/kg body weight<br>1-3 weeks | Positive |
| 1 mg/kg body weight<br>4 weeks | Positive |
| 1 mg/kg every other day<br>3 weeks | Positive |
| 10 mg/kg body weight<br>16 weeks | Positive |
| 10 mg/kg body weight<br>16 weeks | Positive |
| 1 mg/kg body weight<br>4 weeks | Positive |
| | |
| 210 mg per day<br>8 weeks | Positive |
| 210 mg twice daily<br>16 weeks | Positive |
| 210 mg twice daily<br>8 weeks | Positive |
| 200 mg twice daily<br>9 months | Negative |
| | |
| 100 mg twice daily<br>12 months | Negative, except for a minor improvement in<br>LV stiffness when combined with exercise training |
| 100 mg twice daily<br>12 months | Negative |

and Alagebrium 1.0 mg/kg body weight were examined on cardiovascular properties in sedentary older rats, showed that the combination of exercise training and Alagebrium had synergistic effects, and significantly improved systolic and diastolic cardiac function, and decreased vascular stiffness in a rat model of aging.[40]

In summary, the animal studies showed beneficial effects of Alagebrium on cardiovascular compliance, left ventricular function, AGE accumulation, and collagen crosslinking.

## Humans studies with cardiovascular patients

Human studies have primarily examined patient populations with either increased arterial stiffening (systolic hypertension and increased pulse pressure) [5,7] or myocardial stiffening (heart failure).[6,8] After phase I clinical studies with Alagebrium, Kass *et al.*[5] performed the first human study, a randomized, double blind, placebo controlled trial in patients with arterial stiffening. Alagebrium 210 mg per day or placebo (2-to-1 design) was given for eight weeks. Pulse pressure lowered and large artery compliance improved with Alagebrium compared with the placebo group. Another study that examined patients with isolated systolic hypertension used Alagebrium 210 mg twice daily for eight weeks in a single arm, open label trial.[7] On group average Alagebrium resulted in a marginal enhancement in brachial artery endothelial function in ten patients in this study.

Furthermore, the effect of Alagebrium was studied in patients with diastolic heart failure and preserved left ventricular ejection fraction.[6] This was again a single arm, open label trial in which Alagebrium 210 mg twice daily was used for sixteen weeks. Treatment resulted in a regression of left ventricular mass, improvement in left ventricular diastolic properties and subjectively perceived quality of life by the patients. The most recent randomized, double blind, placebo controlled, clinical trial examined heart failure patients with a reduced left ventricular ejection fraction and the influence of Alagebrium 200 mg twice daily or placebo during nine months.[8] This study found no changes in exercise tolerance or in systolic or diastolic cardiac function.

## Humans studies with healthy older individuals

Up to recently, only patients with arterial stiffness or heart failure have been examined with the AGE-crosslink breaker Alagebrium. However, even though researchers acknowledge the role of AGE accumulation with aging and induced age-related cardiovascular diseases, the older population had not yet been the subject of AGE-intervening investigations while this is a particularly interesting population to study due to the growing aging population. Therefore, we started an Alagebrium study in healthy older sedentary individuals together with our colleagues from the Institute of Exercise and Environmental Medicine, Dallas, Texas, USA (Table 6.1).[9,10] Even though it is known that older individuals have stiffer arteries, stiffer myocardium and endothelial dysfunction,[36,41,42] we did not find the results as were expected based on previous literature. Besides a modest improvement on age-related cardiac left ventricular stiffness when Alagebrium was combined with exercise training for a year, we did not find a significant improvement in arterial stiffness, endothelial function, hemodynamics or cardiac geometry.

In summary, most patient oriented studies showed a positive effect of Alagebrium on cardiovascular structure and function, however, the more recent human studies with a long duration of intervention did not show beneficial effects of Alagebrium.
Importantly, in all human studies Alagebrium was well tolerated independent of dosage or duration. In the randomized trials, side effects were reported equally between the Alagebrium and placebo groups.[5,6,8-10] Most common side effects were gastrointestinal symptoms and infections.

## Understanding the different study outcomes

What could explain the differences between the convincing beneficial results from animal studies, the first human studies and the negative findings in the more recent human studies? Differences in study population, study design, dosage and duration, and measurement techniques may, at least partly, explain the different results.

### Study population

Four clinical patient studies have been published: two with arterial stiffening [5,7] and two with heart failure patients.[6,8] Three of these studies have shown beneficial results. The randomized clinical trial including heart failure patients (n=102, mean age 62 years) with reduced ejection fraction (≤45%) showed no improvement in cardiac function or in exercise tolerance after nine months Alagebrium 400 mg per day,[8] while the other (open label) study including heart failure patients (n=23, mean age 71 years) with preserved ejection fraction (≥50%) did find an improvement in cardiac function after sixteen weeks 420 mg per day.[6] In both studies an equal ratio of patients were in New York Heart Association (NYHA) class II or III (ratio 3:2). Even though we cannot completely exclude that the difference between the involvement of systolic dysfunction could influence these results, patient selection does not seem to explain the main differences in results between these two studies. There is, however, an important difference in risk of bias due to the study design (see below).[43]
Cardiovascular patients are more likely to show improvements when using Alagebrium compared with healthy older individuals. Since AGEs accumulate with aging, healthy older individuals are likely to improve cardiovascular compliance with the use of Alagebrium.[12,44,45] The aged animal models showed improvements in cardiovascular stiffness and function with Alagebrium.[2,3,39,40] Steppan *et al.*[40] showed in aged rats that Alagebrium combined with exercise training had a synergistic effect and significantly improved cardiac function and decreased vascular stiffness. It was therefore unexpected that our trial [10] and the one from our colleagues in Dallas, Texas, USA,[9] did not show relevant improvements in vascular and myocardial structure and function in older individuals after one year Alagebrium intervention nor with the combined intervention with exercise training.

Apparently, the effect of Alagebrium is different in animals versus humans. In addition, it seems that Alagebrium has a more pronounced effect in cardiovascular patients than in healthy older individuals.

## Study design

The animal studies were all open label studies. Two of the patient studies [6,7] were open label and two were randomized clinical trials [5,8] of which Kass *et al.*[5] increased power by using a 2-to-1 design favoring Alagebrium. Thus, only two of the ten published studies examining the effects of Alagebrium before 2013 were randomized, placebo controlled, double blinded trials, of which only one with an equal distribution between Alagebrium and placebo.[8] Open label trials are more likely to be (unintentionally) biased, especially when analyzing interpretable data.[43] Therefore, results and conclusion from these studies should be interpreted with caution. The two high-quality clinical trials showed conflicting results with one study showing a positive effect of Alagebrium on arterial stiffening [5] and one study finding no effect on cardiac stiffening.[8] Our recent studies in healthy older individuals add new high quality clinical trials without profound beneficial effects of Alagebrium on cardiovascular structure and function.[9,10]

## Dosage and duration

Dosage and duration of Alagebrium were different in most animal and human studies. The animal models used daily dosages ranging from 0.5 mg/kg body weight to 10.0 mg/kg body weight per day for one to sixteen weeks. The human studies used dosages between 200 mg and 420 mg per day with durations from eight to fifty-two weeks. Dosage did not seem to influence the outcomes, since both higher and lower dosages resulted in positive or negative results. For example, 210 mg per day (eight weeks) resulted in improved arterial compliance,[5] while 200 mg per day (twelve months) did not.[10] Also, 420 mg per day (sixteen weeks) improved left ventricular diastolic function,[6] while 400 mg per day (nine months) did not.[8]

On the other hand, it seems odd that the long duration interventions (nine and twelve months) [8-10] did not show the beneficial effects to the same degree as the short-term (eight and sixteen weeks) studies.[5-7] In animals, the duration did not influence the beneficial results since all studies (one to sixteen weeks) showed improvements in cardiovascular structure and function, even though three weeks Alagebrium resulted in a larger improvement in carotid artery compliance in diabetic rats than one week Alagebrium.[1] In older rhesus monkeys three weeks was sufficient to improve the carotid artery compliance and left ventricular function. The improved vascular compliance persisted over time with a gradual return to baseline 39 weeks after treatment was stopped.[3]

It is difficult to explain why long-term intervention clinical studies with Alagebrium did not show beneficial effects whereas short-term intervention studies did. One could speculate that Alagebrium works primarily in the initial phase and that in the long-term intervention studies the effect has already faded. If this is the case, this would mean that Alagebrium has no use as a long-term intervention to improve the cardiovascular system.

## Measurement techniques

Differences in measurement techniques are likely to explain part of the different outcomes. For example, cardiac function was measured invasively with catheterization and pressure-volume curves [2,9,40] and non-invasively using echocardiography.[2,3,6,8] The invasive measurement is more accurate, especially when used in decapitated or anesthetized animals due to minimization of the influence of stress, breathing, blood pressure, and depression of the autonomic nerves system. Magnetic resonance imaging (MRI) [6] and echocardiography [3,39] were used to measure cardiac mass. It is known that MRI is more sensitive to measure cardiac mass than echocardiography, whereas decapitating animals [4,39] to measure cardiac mass is more sensitive than MRI. Possibly, the use of echocardiography in the randomized, double blind, placebo controlled, clinical trial that examined heart failure patients with a reduced left ventricular ejection fraction [8] was not sensitive enough to measure small changes induced with Alagebrium. On the other hand, the study performed in Dallas, Texas, USA, showed only a modest improvement in left ventricular stiffening and not on other invasively or sensitively measured cardiac parameters.[9] However, these were healthy older individuals and may have been less amenable to benefit from Alagebrium compared with heart failure patients.

Arterial stiffness was measured using different techniques, for example using MRI to calculate thoracic aorta distensibility,[6] echo Doppler to calculate pulse wave velocity,[5,40] and arterial pressure waveforms and carotid augmentation index were measured using tonometry.[3,5,7] In addition, repeated measurements improve statistical findings.[3] Furthermore, one of the advantages of animal studies is the ability to perform both in vivo and in vitro measurements of arterial stiffness and carotid artery compliance [1] and to take myocardial biopsies to measure the actual AGE-crosslinks and tissue AGE accumulation using sophisticated techniques, such as electron microscopy and mitochondrial DNA measures.[4,39]

Endothelial function was measured with different methods. It was measured invasively in the resistance vessels in the lower limb in our study (Chapter 5) [10] with intra-arterial infusion of vasoactive substances and venous occlusion plethysmography, which is considered to be the gold standard.[46,47] It was also measured non-invasively in the conduit artery of the arm using the frequently used method of flow mediated dilation.[7] This is, however, an indirect method to measure endothelial function and is more

susceptible to personal interpretation during analysis, especially when used in an open label study.[7]
Recapitulating, the differences in measurement techniques do not easily explain the different outcomes. There are studies with positive outcomes using sophisticated and excellent measurement techniques, and also studies with weaker techniques. The contrary is also seen that studies without beneficial findings used sophisticated and invasive measures, but also non-invasive measures.

In conclusion, neither the differences between the animal and human studies, nor the differences between the multiple human studies can be easily explained. Perhaps a mismatch exists between the translation from animal to human studies. This has been described before and may be due to failure of the animal models to mimic clinical diseases or functions adequately.[48] However, this does not explain the discordance between the human studies concerning Alagebrium. This discordance is most likely due to the inclusion of different populations (patients versus healthy older individuals) as well as study design and study duration.

## Does Alagebrium break the established AGE-crosslink in human tissues?

One of the most important questions remains unanswered: does Alagebrium in fact break the established AGE-crosslink in human (cardiovascular) tissues? In tail tendons and in myocardial biopsies of rats, Alagebrium has been shown to reduce crosslinking of matrix proteins.[1,4,39] On the contrary, the accumulation of AGEs in the skin of heart failure patients did not change with 9 months use of Alagebrium.[8] None of the human studies has, due to eligible reasons, examined the AGE deposition before and after the use of Alagebrium in different cardiovascular tissues, e.g. myocardial biopsies or samples of the arterial walls. In addition to the inconclusive results, we cannot be certain that Alagebrium actually works as an AGE-crosslink breaker in cardiovascular tissues in humans. Contemplating the current data from this thesis and literature, it is not likely that Alagebrium will have a relevant impact on cardiovascular morbidity and mortality in the (healthy) aging population.

## Plasma AGEs and tissue AGEs

In addition to the remaining uncertainties concerning Alagebrium, the interaction and clinical relevance between the level of plasma AGEs and tissue AGEs are still unclear. Accumulation of AGEs occurs throughout all organs, including in skin, neural, renal,

vascular and cardiac tissue.[49,50] Numerous AGEs have been described. The most well known and extensively examined are $N^{\varepsilon}$-(carboxyethyl)lysine (CEL), which is a putative marker of intracellular protein glycation, $N^{\varepsilon}$-(carboxymethyl)lysine (CML), which is the major non-crosslinking AGE and the potent ligand of RAGE (AGE-RAGE axis), and pentosidine, which is one of the cross-linking AGEs.[13,18,51,52] Even though multiple studies have confirmed positive associations between elevated levels of circulating AGEs and a variety of cardiovascular diseases in individuals with or without type 2 diabetes mellitus and with or without pre-existing cardiovascular diseases,[52-56] lack of associations have also been found.[57-59] Furthermore, it is uncertain whether elevated levels of plasma AGEs adequately reflect the burden of the glycation pathway in tissues,[60] because intracellular glycation is thought to be the major local source of AGEs and not all AGEs may end up in the circulation.[57,61]
Since many questions concerning plasma and tissue AGEs are still unanswered, we tried to gain more insight by examining the levels of plasma AGEs and linking these to cardiovascular measurements and interventions such as exercise training and the use of Alagebrium. In this section we analyzed two sets of data using our sedentary older individuals and using unique data with healthy aging athletes.

## Plasma AGEs and vascular stiffening

It is difficult to obtain human cardiovascular biopsies to examine the relation between plasma and tissue AGEs. Therefore, a derivative parameter of tissue AGEs could be used, such as arterial stiffness. To obtain some insight in this complex interplay between plasma and tissue AGEs, we examined the correlations between protein-bound plasma AGEs and pulse wave velocity (PWV) as an indicator of arterial stiffness in healthy older individuals (population described in Chapter 5). We found a negative correlation between CEL and both the central and peripheral pulse wave velocity in healthy older individuals (Table 6.2), and no significant correlation for the other AGEs. Thus, with increasing levels of pulse wave velocity (increased arterial stiffness), the level of plasma CEL declined. This is contradictory to what we expected and reinforces the gap in current understanding of the relation and interaction between plasma AGEs and tissue AGEs. They almost appear to be two separate identities. This is in line with the variable results of multiple investigations that either found or did not find an association between the level of plasma AGEs and the presence of cardiovascular diseases. [52-61]

## Influence of exercise training on plasma AGEs

Since it is known that AGEs are formed under the influence of oxidative stress, strategies to reduce the production of oxidative stress may reduce the amount of AGEs. Exercise training is renowned for its beneficial cardiovascular effects,[62-64] and lifelong exercise training prevents age-related endothelial dysfunction by preserving

**Table 6.2** Correlation between levels of protein-bound plasma AGEs and central and peripheral pulse wave velocity (PWV) in 44 healthy older individuals.

| | CML | CEL | Pentosidine |
|---|---|---|---|
| **Central PWV** | r = -0.130<br>$p$ = 0.417 | r = -0.340<br>**$p$ = 0.030** | r = -0.061<br>$p$ = 0.706 |
| **Peripheral PWV** | r = -0.284<br>$p$ = 0.076 | r = -0.344<br>**$p$ = 0.030** | r = -0.017<br>$p$ = 0.915 |

Statistical analysis: Pearson correlation using SPSS 20.0.

nitric oxide availability due to reducing oxidative stress.[65] Thus, one could hypothesize that exercise training, and especially lifelong exercise training, reduces oxidative stress and increases the anti-oxidant capacity and nitric oxide availability, and thereby reduces the formation of AGEs. Furthermore, lifelong endurance training protects from the development of age-related cardiovascular stiffening,[66] possibly by reducing the accumulation of AGEs. With these theories in mind, we compared the effects of lifelong exercise training with a long-term sedentary lifestyle on the level of plasma AGEs in healthy older individuals without clinical cardiovascular diseases. For this purpose, we combined two study populations of healthy individuals that were either lifelong athletes or sedentary individuals (Table 6.3). The lifelong athletes exercised between three to seven sessions a week at moderate to high intensity level for at least one hour per session for at least the past twenty years. The sedentary individuals did not perform regular exercise (≤1 hour per week) during the last 5-10 years (described in Chapter 5).

*Lifelong exercise training*

Unlike we hypothesized the lifelong athletes had significantly higher levels of protein bound CML and CEL, and a substantially higher level of pentosidine compared with the sedentary individuals (Figure 6.1). We also found a positive correlation between physical fitness level (expressed as $VO_2$max (ml$O_2$/min/kg body weight)) and CML (Figure 6.2). It seems that (lifelong) exercise training might induce the production of AGEs. One can speculate on the mechanisms. Perhaps exercise training induces (temporarily) more oxidative stress, especially during high intensity training,[67] as performed by the lifelong athletes, and thereby stimulates the formation of AGEs. Nevertheless, this does not explain the discrepancy between the increased levels of plasma AGEs and the tissue induced beneficial effects of lifelong exercise training on endothelial function and cardiovascular stiffening.[66]

**Table 6.3** Characteristics of the lifelong athletes versus sedentary individuals.

| | Lifelong athletes | Sedentary individuals | *p*-value |
|---|---|---|---|
| **General characteristics** | N = 12 | N = 44 | |
| Gender (M : F) | 12 : 0 | 25 : 21 | |
| Age (years) | 64 ± 8 | 70 ± 4 | **0.001** |
| $VO_2$max ($mlO_2$/min/kg) | 44.7 ± 10.3 | 24.4 ± 4.3 | **<0.001** |
| **Hemodynamics** | | | |
| Systolic blood pressure (mmHg) | 140 ± 12 | 133 ± 13 | 0.061 |
| Diastolic blood pressure (mmHg) | 85 ± 11 | 78 ± 9 | **0.022** |
| Pulse Pressure (mmHg) | 55 ± 10 | 53 ± 9 | 0.620 |
| **Blood samples** | | | |
| Glucose (mmol/l) | 4.6 ± 0.3 | 5.0 ± 0.5 | **0.002** |
| HbA1c (%) | 5.5 ± 0.3 | 5.7 ± 0.3 | 0.174 |
| Total cholesterol (mmol/l) | 5.5 ± 0.8 | 5.4 ± 0.9 | 0.812 |

Data are presented as mean ± standard deviation. Statistical analysis: unpaired t-test.

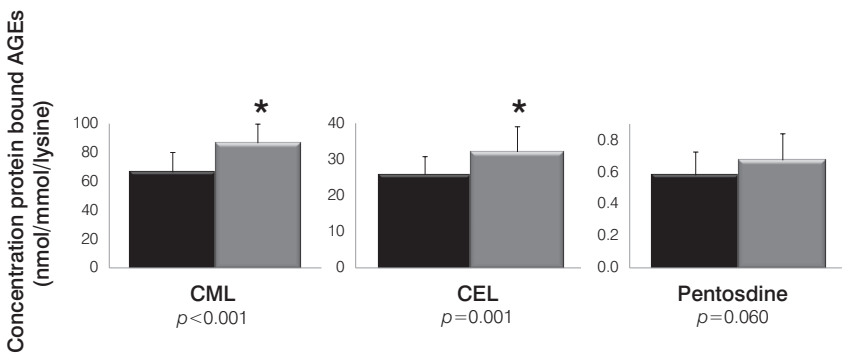


**Figure 6.1** The lifelong athletes (light bars) have significantly higher levels of protein bound $N^{\varepsilon}$-(carboxymethyl)lysine (CML) and $N^{\varepsilon}$-(carboxyethyl)lysine (CEL) and a substantially higher level of pentosidine compared with the sedentary individuals (dark bars).

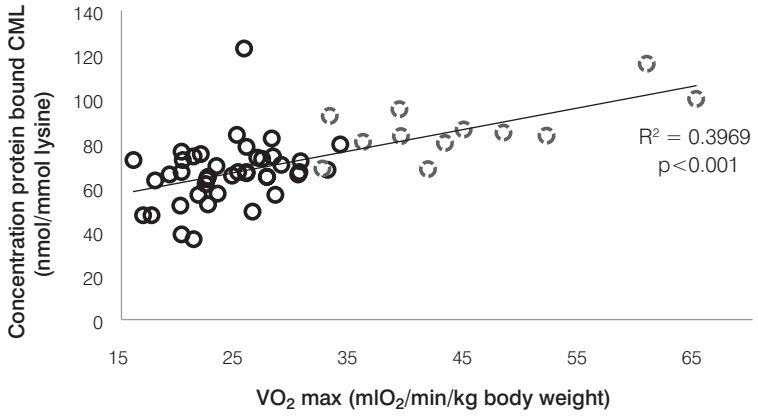


**Figure 6.2** A significant correlation was found between physical fitness level (expressed as $VO_2$max) and protein bound $N^{\varepsilon}$-(carboxymethyl)lysine (CML). This figure shows the correlation with the combined populations of the lifelong athletes (n=12, half open grey circles) and the sedentary individuals (n=44, closed circles). However, the correlation remains statistically significant when using the more homogenous group of only the sedentary individuals (n=44, r=0.327, $p$=0.037).

*Short-term exercise training*

One year of exercise training did not change the level of plasma AGEs (Table 6.4). It is known that one year of exercise training does not induce major improvements in cardiovascular stiffening after decades of physical inactivity.[9,10,68] In the same line, tissue AGEs may not change sufficiently with one year exercise training after decades of physical inactivity. On the other hand, based on the possible formation of oxidative stress and/or anti-oxidants with exercise training, a change in plasma AGEs was expected. Goto *et al.*[67] showed that moderate intensity exercise training augments endothelium function through an increased production of nitric oxide (NO), whereas high-intensity exercise training increases plasma concentrations of oxidative stress. Our training intervention was moderate to high intensity, thus a noteworthy increase in oxidative stress was not anticipated. On the contrary, a slight decrease in plasma AGEs was expected due to an increase in anti-oxidant capacity with moderate intensity training.

## Influence of Alagebrium on plasma AGEs

In search of understanding the results in this thesis, we also analyzed the plasma AGEs in relation to the one year Alagebrium intervention. The level of plasma AGEs did not change with the use of Alagebrium or placebo (Table 6.4). Alagebrium, which

should break tissue AGE-crosslinks, would not directly influence the plasma AGEs. However, it has been shown that Alagebrium reduces oxidative stress in aged rats that were treated with Alagebrium (high dose 10 mg/kg body weight) during 16 weeks.[39] Therefore, the anti-oxidant function of Alagebrium, which has also been described in laboratory settings,[69,70] was thought to lead to lower levels of plasma AGEs with the one year treatment, especially in combination with the exercise training. The lack of change in plasma AGEs with the use of Alagebrium, could be due to the differences in AGEs. There are AGEs that are more specific to intracellular damage, like CEL. CML, on the other hand, is more prominent in plasma and is known to be a potent ligand of the AGE-receptor (RAGE). Pentosidine is known to form crosslinks and was perhaps more susceptible to Alagebrium.[18,52] Nevertheless, the common denominator in the formation of AGEs is oxidative stress [25] and Alagebrium has been shown to have anti-oxidant properties [39,69,70] and was, therefore, expected to lower the level of AGEs, especially in combination with exercise training. It can be argued that it takes several months to years for irreversible AGEs to form,[13,14] but then the one year intervention should have been sufficient to show some changes. Furthermore, the blood samples were taken at multiple time-points (baseline and after 3, 6 and 12 months), in fasting state under standardized conditions and kidney function was good in all participants. However, we did not take the diet into account. Thus, it is possible that dietary intake of AGEs could have influenced our results. Nevertheless, the variation between participants was similar and there were no outlier values within participants during the year intervention, thus the influence of diet is unlikely to have influenced our results relevantly.

## The issue of plasma and tissue AGEs

The unexpected results concerning the plasma AGEs and the influence of lifelong and short-term exercise training, with and without the combination with Alagebrium, do not directly result in more insight into the relation between plasma AGEs and tissue AGEs. The cardiovascular effects that are likely to be induced by the tissue AGEs, e.g. cardiovascular stiffening, are contrary to the levels of plasma AGEs in lifelong athletes, e.g. higher levels of plasma AGEs with less cardiovascular stiffening. Also, the anti-oxidant effect of exercise training and of Alagebrium cannot be confirmed by changes in the levels of plasma AGEs. This reinforces the necessity to further investigate the relation between plasma and tissue AGEs in humans. Especially, if new treatment options for cardiovascular and/or age-related diseases aiming at AGE interventions will be examined.

**Table 6.4** Characteristics of the 4 intervention groups as described in Chapter 5, data at baseline and after one year intervention.

| | Exercise training | | No exercise training | | Linear Mixed Model: changes due to the 1-year interventions | | |
|---|---|---|---|---|---|---|---|
| | | | | | Time*Training | Time* Medication | Time*Training* Medication |
| | *Alagebrium* | *placebo* | *Alagebrium* | *placebo* | *p*-value | *p*-value | *p*-value |
| Male : Female (n) | 6 : 5 | 8 : 3 | 8 : 3 | 3 : 8 | | | |
| Age (years) | 69 ± 3 | 68 ± 3 | 70 ± 3 | 71 ± 5 | | | |
| **$VO_2$max ($mlO_2$/min/kg)** | | | | | **<0.001** | 0.969 | 0.757 |
| Baseline | 23.2 ± 4.2 | 24.5 ± 4.9 | 25.6 ± 4.3 | 24.1 ± 4.2 | | | |
| 12 months | 26.7 ± 4.0 | 27.7 ± 5.2 | 25.0 ± 4.7 | 23.9 ± 3.6 | | | |
| **Pulse Pressure (mmHg)** | | | | | 0.155 | 0.629 | 0.150 |
| Baseline | 47 ± 7 | 56 ± 8 | 57 ± 14 | 56 ± 9 | | | |
| 12 months | 49 ± 9 | 53 ± 9 | 58 ± 16 | 59 ± 9 | | | |
| **Glucose (mmol/l)** | | | | | 0.831 | 0.390 | 0.502 |
| Baseline | 5.2 ± 0.6 | 4.9 ± 0.5 | 5.1 ± 0.4 | 4.9 ± 0.3 | | | |
| 12 months | 5.1 ± 0.8 | 5.2 ± 0.6 | 5.1 ± 0.5 | 4.9 ± 0.3 | | | |
| **HbA1c (%)** | | | | | 0.358 | 0.244 | 0.728 |
| Baseline | 5.6 ± 0.4 | 5.8 ± 0.3 | 5.7 ± 0.4 | 5.6 ± 0.2 | | | |
| 12 months | 5.6 ± 0.4 | 5.8 ± 0.4 | 5.5 ± 0.6 | 5.5 ± 0.2 | | | |
| **CML (nmol/mmol lysine)** | | | | | 0.494 | 0.735 | 0.515 |
| Baseline | 56.7 ± 12.1 | 63.4 ± 8.3 | 75.0 ± 16.8 * | 67.8 ± 7.2 | | | |
| 12 months | 56.9 ± 13.9 | 66.3 ± 13.9 | 82.6 ± 22.8 | 71.7 ± 9.5 | | | |
| **CEL (nmol/mmol lysine)** | | | | | 0.052 | 0.989 | 0.625 |
| Baseline | 24.2 ± 5.1 | 26.8 ± 6.4 | 28.0 ± 8.0 | 26.6 ± 4.7 | | | |
| 12 months | 23.5 ± 3.8 | 27.5 ± 5.2 | 30.4 ± 7.9 | 29.0 ± 6.1 | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Pentosidine (nmol/mmol lysine)** | | | | | 0.200 | 0.580 | 1.000 |
| Baseline | 0.55 ± 0.16 | 0.55 ± 0.12 | 0.63 ± 0.17 | 0.56 ± 0.14 | | | |
| 12 months | 0.53 ± 0.16 | 0.57 ± 0.26 | 0.60 ± 0.12 | 0.55 ± 0.13 | | | |

Data are presented as mean ± standard deviation. Statistical analysis: At baseline a one-way ANOVA was performed with Bonferroni correction to check for group differences. The only difference at baseline was found in CML ***** between the exercise & Alagebrium vs. no exercise & Alagebrium group $p=0.002$. Differences between the 4 groups in response to the 1-year intervention were analyzed using the Linear Mixed Model using SPSS 20.0. The interaction between exercise training and Alagebrium was analyzed (Time*Training*Medication), and both individual effects of exercise training vs. no exercise training and Alagebrium vs. placebo were analyzed (Time*Training and Time*Medication, respectively). **Abbreviations:** HbA1c glycosylated hemoglobin, CML $N^{\varepsilon}$-(carboxymethyl)lysine, CEL $N^{\varepsilon}$-(carboxyethyl)lysine. These data represent the study that is described in Chapter 5.

## Future implications for research on AGEs

Considering the data on Alagebrium and its influence on the cardiovascular system in relation with age-related changes, it must be noticed that the results in literature and this thesis are not supporting a clinically relevant effect of long-term use in healthy older individuals. At this moment, Alagebrium is not an evidence based method to reduce cardiovascular morbidity and mortality in cardiovascular patient populations or healthy older individuals. Even though accumulation of AGEs plays a role in (patho)physiology of cardiovascular and age-related diseases, the exact mechanism between plasma AGEs and tissue AGEs and, more importantly, ways to influence the detrimental effects of AGEs remain unknown. The most interesting topics for future research should first consider some basic questions. How are plasma AGEs and tissues AGEs related? What favors AGE accumulation in different tissues, besides the level of oxidative stress, and what are modifiable routes? Only after answering these questions, we may study how AGEs can be influenced to reduce the cardiovascular risk and burden in cardiovascular patients and aging individuals. Could lifestyle changes, such as reducing dietary intake of AGEs, positively influence plasma and/or tissue AGEs? Or could weight reduction and slowing down the epidemic of type 2 diabetes mellitus help to lower the AGEs burden and could this be a pathway towards improved cardiovascular outcomes?

Physical activity and exercise training have been shown to improve physical fitness and cardiovascular health in older individuals.[62-64] However, these benefits could not be extrapolated to differences in plasma AGEs, since higher levels of plasma AGEs were found in lifelong athletes and no changes were found in plasma AGEs with one year of exercise training. Major direct effects of AGEs on cardiovascular parameters should, therefore, be hypothesized with caution.

Having translated preliminary work from bench to bedside, research should return to the bench again. The multiple metabolic pathways involved in plasma and tissue AGEs and the cardiovascular and age-related consequences should be disentangled first. In the meantime, exercise interventions should be used much more at the bedside, also in older individuals. We added some evidence to these well proven beneficial effects of exercise on cardiovascular aging. Remaining physically fit, also by maintaining regular daily life activities, prolongs healthy and independent living in older individuals.[71] Therefore, encouraging individuals of all ages, but especially children and older individuals, to engage in physical activities will improve health worldwide, and especially in the sedentary and aging western societies. As such, the rhetorical question '*to exercise or not to exercise´* will translate itself in the physiological consequence of aging slowly or aging more rapidly. However, far more complex is the question *´to AGE or not to age*'.

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Chapter 7

# Summary
# Nederlandse samenvatting

# Summary

On average, aging is characterized by a progressive decline in function across multiple organ systems and a reduced ability to maintain homeostasis. With advancing age, the incidence of chronic diseases, e.g. cardiovascular diseases, increases. Despite multiple interventions, such as stimulation of physical activity, cardiovascular diseases remain the leading cause of morbidity and mortality in modern societies, and especially in the aging population. Besides advancing age, risk factors such as physical inactivity and the accumulation of advanced glycation end-products (AGEs) are important contributing factors in the development of cardiovascular diseases. Increased arterial stiffness and endothelial dysfunction are known to play key roles in this age-related process. Even though exercise training has been shown to be beneficial and accumulation of AGEs is detrimental, the (combined) effect of exercise training and the developmental AGE-crosslink breaker Alagebrium in older individuals has not yet been examined. Therefore, the general aim of this thesis was to elucidate the individual and combined effects of exercise training and the AGE-crosslink breaker Alagebrium on different aspects of the vascular system in older individuals without pre-existing symptomatic cardiovascular diseases.

***Chapter 1*** presents an outline of the studies included in this thesis with extensive background information on aging, the effects of aging on the cardiovascular and cerebrovascular system, the influence of exercise training on vasculature in older individuals, the role of AGEs in the pathophysiology of cardiovascular diseases, and the influence of the experimental drug the AGE-crosslink breaker Alagebrium on the cardiovascular system. At the end of this first chapter, the aims and hypotheses of the individual chapters are presented.

In ***Chapter 2*** we examined the influence of a lower or higher physical fitness level, due to daily life activities, on vascular function in a homogenous group of older non-exercising individuals. Vascular function was measured invasively using the upper leg model with intra-arterial infusion of vasoactive substances. We used the endothelium dependent vasodilator acetylcholine (ACh), the endothelium independent vasodilator sodium nitroprusside (SNP), and the nitric oxide synthase inhibitor $N^G$-monomethyl-L-arginine (L-NMMA). Responses in blood flow changes were measured using venous occlusion plethysmography. Cardiovascular lifetime risk score was calculated using predictors such as age, gender, body mass index, diabetes mellitus, smoking, blood pressure, lipid levels and physical fitness level. The group with the higher physical fitness level had a better cardiovascular lifetime risk score and vascular function compared with the group with a lower physical fitness level. This study provides a physiological basis that supports the beneficial effects of

differences in physical fitness level related to daily life activities on cardiovascular health. Therefore, older individuals should be encouraged to enhance their daily life activities.

***Chapter 3*** describes the influence of physical activity on sleep characteristics in a group of young adults and older individuals. A one year exercise training program was performed with the older individuals to examine the influence of exercise training on sleep characteristics. We found that young adults with higher daily energy expenditure have a greater sleep efficiency, whilst this relationship was attenuated with advanced age. In contrast, we found no correlation between physical fitness and sleep characteristics in healthy young adults or older participants. This may explain the lack of improvement in sleep characteristics in healthy older participants with 12 months of exercise training. Exercise training may be more successful in subjects with existing sleep disturbances to improve sleep characteristics rather than in healthy older individuals without sleep problems.

The effect of age on cerebrovascular function is described in ***Chapter 4***. Significant differences were found in cerebral blood flow between the young and older individuals with a decline in cerebral blood flow velocity and an increase in cerebrovascular resistance with age. Nevertheless, the group of healthy older individuals, up to the age of 86 years, that we examined were able to maintain sufficient cerebral perfusion and cortical oxygenation by preservation of the dynamic cerebral autoregulation and cerebrovascular $CO_2$ reactivity during maneuvers that mimic daily life activities.

The main results of this thesis are presented in ***Chapter 5*** where the effects of a one year intervention with either exercise training and/or the use of the AGE-crosslink breaker Alagebrium 100 mg twice daily on cardiovascular health in older individuals are described. Even though one year of exercise training in older individuals significantly improved physical fitness and cardiovascular lifetime risk score, endothelial function and arterial stiffness did not improve. The use of the AGE-crosslink breaker Alagebrium had no independent effect on endothelial function, nor did it potentiate the effect of exercise training. Despite the clinical benefits and cardioprotective effects of exercise training for older individuals, neither exercise training nor Alagebrium (either alone or in combination) was able to reverse the effects of decades of sedentary aging on the vasculature.

***Chapter 6*** reviews the knowledge that has been gained in the field of AGEs and the AGE-crosslink breaker Alagebrium throughout the past decade. The first animal and human studies showed promising results of Alagebrium in improving cardiovascular stiffness and endothelial function. Unexpectedly, recent clinical trials showed no or

only minor improvements in cardiovascular structure and function. Possible explanations are provided for the different study outcomes between the animal and human studies. For instance, a mismatch between animal to human studies is possible which may be due to failure of the animal models to mimic clinical diseases or functions adequately. Furthermore, discordances between the human studies concerning Alagebrium are discussed, such as inclusion of different populations (patient versus healthy older individuals), study design and study duration, and measurement techniques.

In addition, we tried to gain more insight in how plasma and tissue AGEs may be linked by examining the levels of plasma AGEs and linking this to cardiovascular measurements. Furthermore, we examined the influence of lifelong and short-term (one year) exercise training and the use of Alagebrium on plasma AGEs. These results did not directly lead to more insight into the relation between plasma AGEs and tissue AGEs. This reinforces the necessity to further investigate the relation between plasma and tissue AGEs in humans.

Finally, future perspectives and new research topics are presented and the importance of remaining physically fit, also by maintaining regular daily life activities, to prolong healthy and independent living in older individuals, is accentuated.

# Nederlandse samenvatting

In dit hoofdstuk geef ik een overzicht van de bevindingen die in dit proefschrift worden beschreven. Daarbij worden medisch-wetenschappelijke termen zoveel mogelijk achterwege gelaten en beschrijf ik de onderzoeksresultaten in begrijpelijke alledaagse taal.

## Veroudering

Men zegt vaak "*oud worden is een zegen, oud zijn valt tegen*". Bekende tekenen van veroudering zijn onder andere het krijgen van grijze haren, rimpels en achteruitgang in beweeglijkheid en zelfstandigheid. Daarnaast komen ook vaker chronische ziekten voor, zoals hart- en vaatziekten. Aangezien de bevolking steeds ouder wordt en mensen dus steeds meer te maken krijgen met ongemakken en ziekten die gepaard gaan met veroudering, wordt er veel onderzoek gedaan hoe we de negatieve gevolgen van veroudering kunnen verminderen.

Bij veroudering treedt er onder andere een verstijving van de wand van de bloedvaten op (vaatstijfheid). De toegenomen vaatstijfheid zorgt ondermeer voor hoge bloeddruk en verhoogde kans op een hartaanval of beroerte. Daarnaast vermindert de functie van de binnenbekleding van de bloedvaten (vaatfunctie). De vaatfunctie zorgt voor een verwijding of vernauwing van de bloedvaten ten behoeve van de juiste doorbloeding van de spieren en organen. Tevens beschermt een goede vaatfunctie tegen het ontstaan van opstoppingen en afsluitingen in bloedvaten. Een belangrijke oorzaak voor het ontstaan van vaatstijfheid en verminderde vaatfunctie zijn versuikerde eiwitten. Deze versuikerde eiwitten worden in het Engels "*Advanced Glycation End-products*", ofwel AGEs, genoemd. De stapeling van AGEs vindt bij iedereen plaats maar omdat oudere personen meer tijd hebben gehad om deze AGEs te stapelen, ondervinden zij er meer hinder van. Nu is een belangrijke vraag hoe we de vaatstijfheid en verminderde vaatfunctie bij ouderen kunnen verbeteren.

Onlangs is er een nieuw medicijn ontwikkeld dat in dieronderzoeken heeft aangetoond dat het de versuikerde eiwitten kan afbreken en daarmee de vaatstijfheid en vaatfunctie kan verbeteren. Dit medicijn heet de "*advanced glycation end-product (AGE-) crosslink breaker*", ofwel Alagebrium. In gezonde oudere personen is dit geneesmiddel nog niet onderzocht, maar het is de verwachting dat Alagebrium de vaatstijfheid en de vaatfunctie zal verbeteren. Het is al wel bekend dat lichamelijke beweging en sporten erg gezond zijn voor het hart en de bloedvaten, ook bij oudere personen. Dit brengt ons tot het belangrijkste doel van dit proefschrift: meer inzicht krijgen in de (gecombineerde) effecten van Alagebrium en van sporten op het functioneren en op de structuur van bloedvaten bij gezonde oudere personen.

## Hoofdstuk 1 Introductie van het proefschrift

In dit hoofdstuk wordt uitgebreide achtergrond informatie gegeven over veroudering, de invloed van veroudering op de bloedvaten in het menselijk lichaam, de effecten van sporten op de vaatfunctie bij ouderen, de rol die AGEs spelen in het ontstaan van hart- en vaatziekten, en potentiële effecten van het nieuwe geneesmiddel Alagebrium. Aan het einde van dit hoofdstuk worden de doelstellingen en verwachtingen beschreven van de individuele hoofdstukken van dit proefschrift.

## Hoofdstuk 2 De invloed van lichamelijke conditie op de vaatfunctie bij niet-sportende ouderen

In dit hoofdstuk onderzoeken we de invloed van een lagere of hogere lichamelijke conditie (fysieke fitheid) ten gevolge van dagelijkse activiteiten op de vaatfunctie in een groep niet-sportende ouderen. De vaatfunctie werd gemeten door via een infuus in de slagader van het bovenbeen drie verschillende middelen in te brengen. Deze middelen zorgden voor een verwijding of vernauwing van de bloedvaten waardoor de doorbloeding in het bovenbeen veranderde. Deze verandering in doorbloeding werd gemeten door zogenaamde kwiktouwtjes die oprekken of inkrimpen. Daarnaast werd het risicoprofiel voor het ontstaan van hart- en vaatziekten berekend. Dit werd berekend met een formule waarbij onder andere leeftijd, geslacht, verhouding lengte en gewicht, aanwezigheid van suikerziekte, rookgedrag, bloeddruk, cholesterolwaarden en lichamelijke conditie werden meegenomen. De groep met de hogere lichamelijke conditie had een betere vaatfunctie. Zij reageerden beter op de verschillende middelen om de doorbloeding te veranderen. Tevens hadden zij een gunstiger risicoprofiel voor hart- en vaatziekten vergeleken met de groep met een lagere lichamelijke conditie. Dit onderzoek ondersteunt de theorie dat de hoeveelheid dagelijkse lichamelijke activiteiten een gunstig effect hebben op de gezondheid van hart- en bloedvaten. Op grond hiervan moeten oudere personen meer gestimuleerd worden om hun dagelijkse lichamelijke activiteiten uit te breiden en meer te gaan bewegen.

## Hoofdstuk 3 De invloed van lichamelijk conditie en dagelijks energieverbruik op de slaapeigenschappen bij jongeren en ouderen

Dit hoofdstuk beschrijft de relatie tussen lichamelijke conditie en dagelijks energieverbruik op de slaapeigenschappen in een groep jongeren en een groep ouderen. Daarnaast werd er gedurende een jaar een sporttrainingsprogramma uitgevoerd door een groep oudere personen om de invloed van sporttraining op de slaapeigenschappen te onderzoeken. Onze resultaten tonen aan dat de jongeren met een hoger dagelijks energieverbruik een betere slaapkwaliteit hebben, terwijl dit verband bij oudere personen is verzwakt. Desondanks vonden we geen verband tussen lichamelijke conditie en slaapeigenschappen bij jongeren en bij oudere personen. Dit

kan een verklaring zijn waarom de slaapeigenschappen van ouderen niet verbeterden na een jaar sporttraining. Sporttraining is waarschijnlijk alleen effectief bij personen met aanwezige slaapproblematiek om de slaapeigenschappen te verbeteren en niet bij gezonde oudere personen zonder slaapklachten.

### Hoofdstuk 4 Evaluatie van hersendoorbloeding en zuurstofvoorziening in verschillende leeftijdsgroepen

De invloed van leeftijd op de hersendoorbloeding en zuurstofvoorziening van de hersenen wordt in dit hoofdstuk beschreven. Hierbij hebben we in drie leeftijdsgroepen (jongeren van 21-28 jaar, jongere ouderen van 65-69 jaar en oudere ouderen van 74-86 jaar) de hersendoorbloeding, de zuurstofvoorziening van de hersenen en de bloeddruk gemeten tijdens verschillende lichaamshoudingen en ademhalingsoefeningen. Onze resultaten tonen dat met het toenemen van de leeftijd de hersendoorbloeding daalt en de weerstand in de hersenbloedvaten stijgt. Desalniettemin zijn gezonde oudere personen (tot 86 jaar) in staat om onder dagelijkse omstandigheden de hersendoorbloeding en zuurstofvoorziening van de hersenen voldoende in stand te houden.

### Hoofdstuk 5 De (gecombineerde) effecten van Alagebrium en sporten op de vaatfunctie bij gezonde ouderen

De belangrijkste resultaten van dit proefschrift zijn gepresenteerd in dit hoofdstuk. Hierin zijn de (gecombineerde) effecten van het nieuwe medicijn de AGE-crosslink breaker Alagebrium en van sporten onderzocht op de gezondheid van de bloedvaten (vaatfunctie en vaatstijfheid) bij gezonde oudere personen. In dit onderzoek werden de deelnemers verdeeld over 4 groepen: In groep 1 gingen de deelnemers een jaar sporten en namen zij dagelijks de Alagebrium in. Groep 2 ging ook een jaar sporten maar zij kregen dagelijks een placebo tablet (een neppil). De deelnemers in Groep 3 gingen niet sporten maar zij namen wel dagelijkse de Alagebrium in. Tot slot Groep 4, deze deelnemers hoefden niet te sporten en zij kregen een placebo tablet. Dit was de controle groep om de effecten van een jaar veroudering op de bloedvaten te onderzoeken.

Ondanks dat een jaar sporttraining de lichamelijke conditie aanzienlijk verbeterde, evenals het risicoprofiel op het ontstaan van hart- en vaatziekten, verbeterde de vaatfunctie en vaatstijfheid niet. De Alagebrium had geen invloed op de vaatfunctie en het versterkte het effect van de sporttraining niet. Van sporten is reeds aangetoond dat het een gunstig en beschermend effect heeft op het ontstaan van hart- en vaatziekten, ook bij oudere personen. Desondanks waren de sporttraining en Alagebrium in onze studie niet in staat om gedurende een jaar de negatieve gevolgen van jarenlange lichamelijke inactiviteit bij oudere personen ongedaan te maken.

## Hoofdstuk 6 Discussie van het proefschrift

Dit hoofdstuk geeft een overzicht van de kennis die de afgelopen jaren is opgedaan over de versuikering van eiwitten en de effecten van Alagebrium. De eerste dieronderzoeken gaven aanwijzingen dat Alagebrium de stijfheid van hart en bloedvaten en de vaatfunctie kan verbeteren. Onverwachts, toonden de studies van de afgelopen paar jaar met mensen weinig of geen effect op de stijfheid van hart en bloedvaten en de vaatfunctie. Mogelijke verklaringen hiervoor kunnen zijn dat de vertaling van dieronderzoek naar mensgebonden onderzoek niet goed is verlopen. Daarnaast worden de verschillen tussen de diverse mensgebonden onderzoeken besproken om de uiteenlopende resultaten te begrijpen. De mensgebonden onderzoeken verschillen onder andere in onderzoekspopulatie, studie opzet, studie duur en meetmethoden.

Vervolgens hebben we gekeken naar een mogelijk verband tussen de AGEs die in het bloed zitten en de AGEs die in de organen zitten. Daarbij hebben we het effect van levenslang versus kortdurend (een jaar) sporten op de AGEs in het bloed onderzocht. Dit was om te onderzoeken of sporten invloed heeft op de AGEs, en of dat een mogelijke verklaring zou kunnen zijn voor de gunstige effecten van sporten op de gezondheid van hart en bloedvaten. Deze resultaten gaven niet direct meer duidelijkheid in het werkingsmechanisme van de AGEs. Het leverde juist meer vragen op. Dit benadrukt dat het verband tussen de AGEs in het bloed en de AGEs in de weefsels nader moet worden onderzocht.

Tot slot, worden er aanbevelingen en suggesties gedaan voor toekomstig onderzoek en wordt het belang van lichamelijke beweging nogmaals benadrukt. Met name voor de ouder wordende samenleving zijn lichamelijke beweging en dagelijkse lichamelijke activiteiten van groot belang om de gezondheid en het zelfstandig functioneren van ouderen te bevorderen.

## Eindconclusie

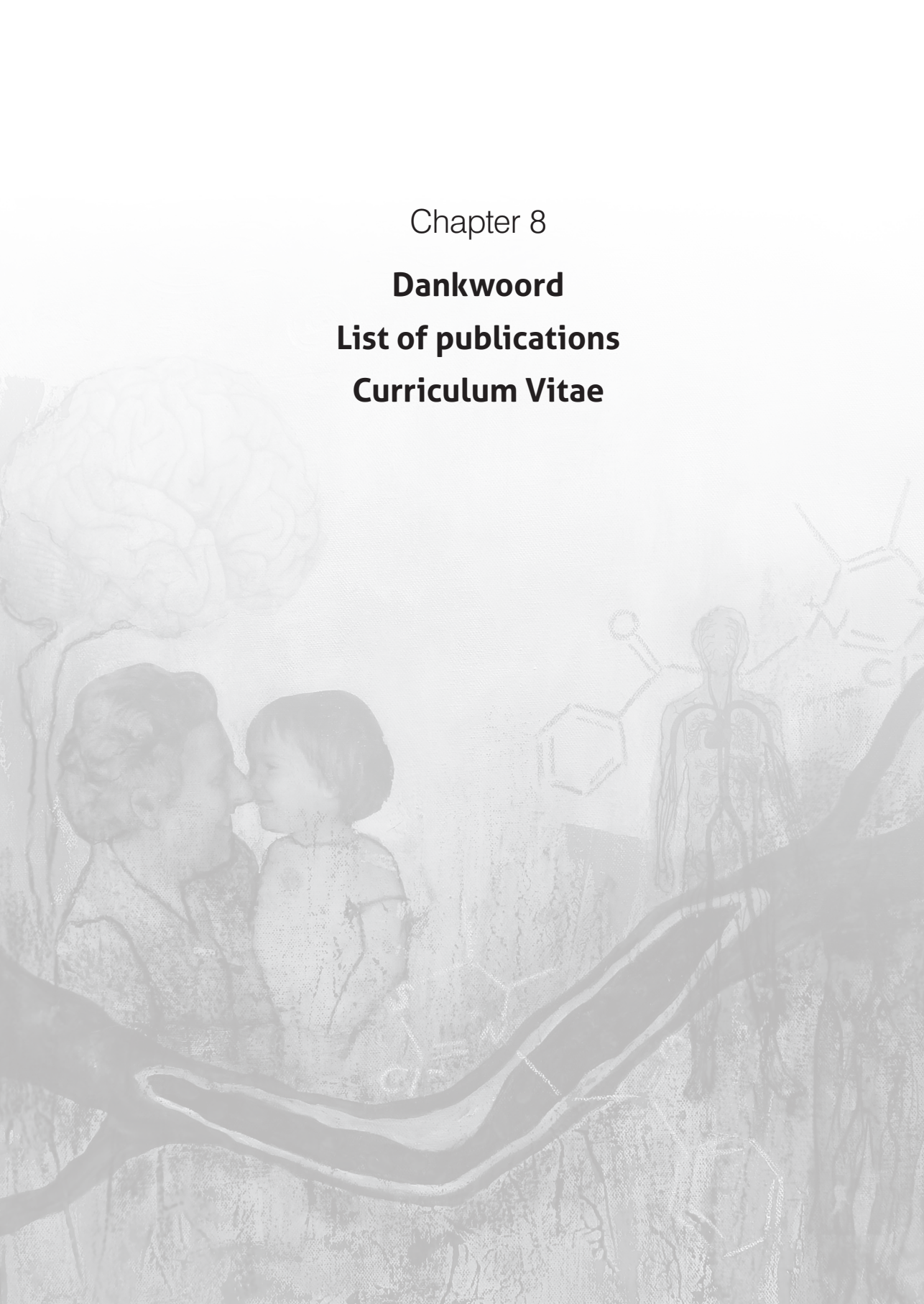
De belangrijkste conclusies van mijn onderzoek zijn dat de gunstige effecten van Alagebrium bij mensen nader onderzocht moeten worden. Momenteel is het onvoldoende bewezen dat Alagebrium de gezondheid van hart en bloedvaten bij gezonde ouderen of bij patiënten verbetert. Daarnaast moet het verband tussen de AGEs in het bloed en de AGEs in de weefsels nader onderzocht worden in de hoop dat dit kan leiden tot een geschikt geneesmiddel om hart- en vaatziekten te verminderen.

Van sporten zijn de gunstige effecten voor hart en bloedvaten reeds aangetoond in verschillende patiëntengroepen en bij gezonde personen. Ondanks dat 1 jaar sporten geen effect had op de vaatfunctie en vaatstijfheid, lieten de deelnemers in ons onderzoek een verbetering zien van hun lichamelijke conditie en van het risicoprofiel op het ontstaan van hart- en vaatziekten. Lichamelijke beweging en sporten zijn voor

*alle leeftijden* van groot belang ter bevordering van de gehele gezondheid. Het is dus nooit te laat om te beginnen met sporten.

Chapter 8

# Dankwoord
# List of publications
# Curriculum Vitae



*"Education is an admirable thing, but it is well to remember from time to time that nothing that is worth knowing can be taught."* Oscar Wilde (1854-1900)

# Dankwoord

Waar moet ik beginnen… Er zijn zoveel mensen die de afgelopen jaren een bijdrage hebben geleverd aan dit onderzoek. Ik zal beginnen met de belangrijkste personen: *de deelnemers aan dit onderzoek*. Zonder jullie hulp, medewerking en toewijding was er nooit een letter in dit proefschrift verschenen. Niet alleen hebben jullie een positieve bijdrage geleverd aan de medische wetenschap, maar ook aan mij als persoon en als arts. Ik heb veel van jullie geleerd. Dank jullie wel!

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*Alles is liefde* (BlØf)

# List of publications

**Oudegeest-Sander MH**, van Beek AH, Abbink K, Olde Rikkert MG, Hopman MT, Claassen JC. Assessment of dynamic cerebral autoregulation and cerebrovascular $CO_2$ reactivity in aging by measurements of cerebral blood flow and cortical oxygenation. *Experimental Physiology 2014;99:586-598*

Eijsvogels TH, Hoogerwerf MD, **Oudegeest-Sander MH**, Hopman MT, Thijssen DH. The impact of exercise intensity on cardiac troponin I release. *International Journal of Cardiology 2014;171:e3-e4*

**Oudegeest-Sander MH**, Olde Rikkert MG, Smits P, Thijssen DH, van Dijk AP, Levine BD, Hopman MT. The effect of an advanced glycation end-product crosslink breaker and exercise training on vascular function in older individuals: a randomized factorial design trial. *Experimental Gerontology 2013;48:1509-1517*

**Oudegeest-Sander MH**, Eijsvogels TH, Verheggen RJ, Poelkens F, Hopman MT, Jones H, Thijssen DH. Impact of physical fitness and daily energy expenditure on sleep efficiency in young and older humans. *Gerontology 2013;59:8-16*

Vonk MC, **Sander MH**, van den Hoogen FH, van Riel PL, Verheugt FW, van Dijk AP. Right ventricle tei-index: A tool to increase the accuracy of non-invasive detection of pulmonary arterial hypertension in connective tissue diseases. *European Journal of Echocardiography 2007;8:317-321*

## Submitted for publication

**Oudegeest-Sander MH**, Thijssen DH, Smits P, van Dijk AP, Olde Rikkert MG, Hopman MT. Impact of fitness level on cardiovascular risk and vascular function in older non-exercising individuals.

# Curriculum Vitae

Madelijn Oudegeest-Sander werd op 16 september 1981 geboren in Eindhoven. Na het behalen van het VWO diploma aan het Eckart College in Eindhoven, heeft zij een jaar gestudeerd aan het Peace College in Raleigh, North Carolina, USA, alvorens te starten met de opleiding Geneeskunde aan de Radboud Universiteit Nijmegen.
Tijdens haar studie Geneeskunde deed zij onderzoek op de afdeling congenitale cardiologie naar de echocardiografische kenmerken van patiënten met een Fontan circulatie. Daar werden de eerste stappen gezet voor het verrichten van wetenschappelijk onderzoek. De ervaring bij de cardiologie was mede een reden om te starten met het promotieonderzoek dat heeft geleid tot dit proefschrift waarin de interesses voor zowel cardiovasculair onderzoek als de geriatrie samenkomen.
Januari 2007 behaalde zij haar arts-examen, waarna zij vier maanden op de afdeling klinische geriatrie heeft gewerkt in het Jeroen Bosch Ziekenhuis te 's-Hertogenbosch. Juli 2007 is zij gestart met het promotieonderzoek bij de afdeling fysiologie van het Radboud Universitair Medisch Centrum te Nijmegen in samenwerking met de afdelingen klinische geriatrie en cardiologie en met het Institute for Exercise and Environmental Medicine in Dallas, Texas, USA. In 2012 is Madelijn gestart met de opleiding tot klinisch geriater op de afdeling klinische geriatrie van het Radboud Universitair Medisch Centrum. Momenteel werkt zij, als onderdeel van de opleiding, op de afdeling interne geneeskunde in het Jeroen Bosch Ziekenhuis te 's-Hertogenbosch. De specialisatie tot klinisch geriater hoopt ze eind 2018 succesvol af te ronden.
Madelijn is getrouwd met Michiel Oudegeest en samen hebben zij twee geweldige zonen: Timon (2010) en Diebe (2012).

Madelijn Oudegeest-Sander was born on September 16th 1981 in Eindhoven, the Netherlands. After graduating from high school, she studied at Peace College in Raleigh, North Carolina, USA, for one year before starting medical school at the Radboud University Nijmegen.
During medical school she performed research as a student assistant at the department of congenital cardiology examining the echocardiographic features of patients with a Fontan Circulation. The first steps for performing research were founded here. The experience at the department of cardiology has lead to further exploring the world of research and combining the passion for cardiovascular research and geriatric medicine. This ultimately led to this thesis.
January 2007 she graduated from medical school, after which she worked at the department of geriatric medicine at the Jeroen Bosch Hospital in 's-Hertogenbosch

for four months. In July 2007 Madelijn became a PhD-student at the department of physiology at the Radboud University Medical Center in Nijmegen in collaboration with the departments of geriatric medicine and cardiology and the Institute for Exercise and Environmental Medicine in Dallas, Texas, USA. In 2012 she started the training in geriatric medicine at the department of geriatric medicine at the Radboud University Medical Center. She is currently working, as part of this traineeship, at the department of internal medicine at the Jeroen Bosch Hospital in 's-Hertogenbosch. At the end of 2018, she hopes to successfully finish this traineeship.
Madelijn is married to Michiel Oudegeest and together they have two wonderful sons: Timon (2010) and Diebe (2012).

*You make it easier when life gets hard*
*I'm lucky I'm in love with my best friend*

*Jason Mraz 2009*