# Lifestyle and large arterial properties – insights from a Finnish and a Swiss Cohort Study

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## List of abbreviations

| | |
|---|---|
| AHA | American Heart Association |
| ANCOVA | Analysis of covariance |
| ANOVA | Analysis of variance |
| ARIC | Atherosclerosis Risk in Communities |
| baPWV | Brachial-ankle pulse wave velocity |
| BMI | Body-mass index |
| CCA | Common carotid artery |
| CI | Confidence Intervals |
| cIMT | Carotid intima-media thickness |
| cLD | Carotid lumen diameter |
| COmPLETE | Cardiopulmonary Exercise Testing |
| CVD | Cardiovascular disease |
| DAT | Dietary assessment tool |
| DC | Distensibility coefficient |
| DNASCO | DNA Polymorphism and Carotid Atherosclerosis |
| DR's EXTRA | Dose-Responses to Exercise Training |
| DYARA | Dynamic Artery Analysis |
| ECG | Electrocardiogram |
| HbA1c | Hemoglobin A1c |
| HDL | High-density lipoprotein |
| Kcal | kilocalories |
| Kg | Kilograms |
| LBM | Lean body mass |
| LoA | Limits of agreement |
| LDL | Low-density lipoprotein |
| FDR | False discovery rate |
| FFQ | Food Frequency Questionnaire |
| FMD | Flow-mediation dilation |
| FNR | Finnish Nutrition Recommendations |

| | |
|---|---|
| FR | Food Record |
| FWER | Familywise error rate |
| mm | Millimeter |
| mmHg | Millimeter mercury |
| mmol/L | Millimoles per liter |
| NCD | Non-communicable disease |
| NIH | National Institute of Health |
| PA | Physical activity |
| PPS3 | Paris Prospective Study III |
| PWV | Pulse wave velocity |
| RCT | Randomized-controlled trial |
| SCORE2 | Systematic COronary Risk Evaluation 2 |
| SD | Standard deviation |
| SGE | Schweizerische Gesellschaft für Ernährung (Swiss Society for Nutrition) |
| $\dot{V}O_{2max}$ | Maximal oxygen uptake |
| WHO | World Health Organization |

## Summary

### Background

In the last centuries, life expectancy in industrialized nations has doubled. Many people reach an advanced age, but the period of life in which a manifest cardiovascular disease has to be treated, is continuously lengthening. Vascular biomarkers and risk scores are used to determine cardiovascular risk. In recent years, however, there has been a shift toward measures of healthy lifestyle, which are more focused on determining health potential. However, the relationship between lifestyle, especially nutrition and physical activity, and the traditionally used biomarkers is still poorly understood.

### Aims

This PhD project had several aims. The first one was to validate a visually aided dietary assessment tool (DAT). Second, this project aimed at comparing various lifestyle metrics, calculated as a cardiovascular health score, and their influence on the structure and function of large human arteries in both a healthy Swiss (aged 50+ years old) and Finnish (aged 55-74 years old) population. Third, the effects of a 4-year lifestyle-based intervention on carotid structure in the same Finnish population were analyzed, to determine if atherosclerotic progression could be slowed.

### Methods

The DAT was validated against a weighed seven-day food record (7d-FR). Young (20-40 years old) and older (50-70 years old) adults were recruited. The DAT was compared to the 7d-FR for total energy intake, macronutrients, sugar, water, and portions of fruits and vegetables. The Finnish Dose-Responses to Exercise Training (DR's EXTRA) study was a randomized-controlled trial lasting four years that consisted of six groups: aerobic exercise, resistance exercise, diet, aerobic exercise + diet, resistance exercise + diet, and reference. Carotid intima-media thickness (cIMT), and lumen diameter (cLD) were used as vascular biomarkers to quantify atherosclerotic progression during the intervention and were measured by transcutaneous ultrasound using state-of-the-art wall contour detection techniques.

In a cross-sectional approach of the DR's EXTRA study, adherence to the American Heart Association's "Life's Simple 7" cardiovascular health score, consisting solely of modifiable lifestyle metrics, was compared with cIMT and cLD, as well as carotid distensibility. The Life's Simple 7 score was divided into poor, intermediate, and ideal cardiovascular health.

The Swiss cross-sectional Cardiopulmonary Exercise Testing (COmPLETE) study included a healthy sample of the Swiss population. For this PhD project, only a sub-sample aged 50-91 years old was considered. The Life's Simple 7 were also used to determine cardiovascular health and were compared to arterial properties, namely cIMT, cLD, carotid distensibility coefficient (DC), flow-mediated dilation (FMD), and brachial-ankle pulse wave velocity (baPWV).

**Results**

**DAT:** 51 participants were included. Correlations between the DAT and the 7d-FR ranged from 0.288 (sugar, $p<0.05$) to 0.729 (water, $p<0.01$). The older group had higher correlations for all macronutrients, the highest correlation being for total energy intake at 0.799 ($p<0.01$). Both groups overestimated total calories in kcal (+14.0%), protein (+44.6%), fats (+36.3%), and portions of fruits and vegetables (+16.0%) but strongly underestimated sugar intake (-50.9%).

**DR's EXTRA intervention:** 1132 participants had valid measurements at baseline and follow-up. Over all groups, we found no significant slowing of atherosclerotic progression ($p<0.01$). A sub-group analysis showed that only men who were given dietary goals did not show a significant cIMT increase during the intervention ($p=0.23$). Men in the diet group had significantly smaller cIMT progression than in the reference group (-0.078 mm, 95% CI: -0.146 to -0.009, $p=0.02$). No other group showed a slowed cIMT progression. None of the intervention groups significantly slowed cLD progression when compared to the reference group.

**DR's EXTRA cross-sectional:** 1400 participants were included in the analyses. Those with an ideal cardiovascular health score had lower cLD than those with intermediate (-0.21 mm, 95% CI: -0.37 to -0.05 mm, $p<0.01$) and a poor score (-0.39 mm, 95% CI: -0.65 to -0.12 mm, $p<0.01$). Those with an ideal score also had higher carotid distensibility than those with an intermediate (0.0032 $kPa^{-1}$, 95% CI: 0.009 to 0.0055 $kPa^{-1}$, $p<0.01$) and a poor score (0.0018 1/kPa, 95% CI: 0.0005 to 0.0032 $kPa^{-1}$, $p<0.01$). No differences were found for cIMT.

**COmPLETE:** The analyses comprised 280 healthy participants. None had a low Life's Simple 7 cardiovascular health score. Those with an ideal score had lower cIMT (-0.038 mm, 95% CI: -0.069 mm to -0.007 mm, $p<0.05$, lower cLD (-0.28 mm, 95% CI: -0.46 mm to -0.11 mm, $p<0.01$), and lower baPWV (-0.05 m/s, 95% CI: -0.08 m/s to -0.02 m/s, $p<0.01$) than those with an intermediate health score.

**Conclusions**

The DAT is an adequate method for screening selected dietary habits and may be particularly useful in older populations.

In middle-aged to older Finns, the Life's Simple 7 was negatively associated with cLD and positively associated with carotid artery distensibility but not with cIMT. A healthy lifestyle, probably pursued decades before the study, seems to protect carotid artery structure and function in the general Finnish population.

A four-year lifestyle intervention did not slow atherosclerosis progression, except in a subgroup of men who were given dietary goals. Physical activity interventions based on international guidelines that were in effect during the study must therefore be questioned.

In a healthy sample of the Swiss population, those with a higher Life's Simple 7 score had better arterial properties. This shows that even in the healthiest individuals, a more favorable lifestyle is associated with better vascular health.

The Life's Simple 7 health score appears to be a practical method for assessing cardiovascular health, for which we were able to show that it is associated with traditional vascular biomarkers. In the middle-aged to older Finnish population studied, lifestyle-based interventions are not sufficient to slow the progression of atherosclerosis; other or more intensified approaches may be needed.

## Zusammenfassung

### Hintergrund

In den letzten Jahrhunderten hat sich die Lebenserwartung in den Industrieländern verdoppelt. Viele Menschen erreichen zwar ein hohes Alter, der Lebensabschnitt in denen eine manifeste Herzkreislauf-Erkrankung therapiert werden muss, verlängert sich jedoch kontinuierlich. Zur Bestimmung des Herzkreislaufrisikos wurden bisher vor allem vaskuläre Biomarker und Risikoscores verwendet. In den letzten Jahren jedoch, hat eine Verlagerung hin zu den Kenngrössen stattgefunden, die einen gesunden Lebensstil ausmachen und dadurch eher das Gesundheitspotential bewerten. Der Zusammenhang zwischen Lebensstil, allen voran Ernährung und körperliche Aktivität, und den traditionell verwendeten Biomarkern ist aber nach wie vor kaum bekannt.

### Ziele

Dieses PhD-Projekt verfolgte mehrere Ziele. Das erste Ziel war die Validierung eines visuell unterstützten Ernährungsfragebogens (DAT). Zweitens sollten verschiedene Lebensstilfaktoren, die als Herzkreislauf-Gesundheitsscore ausgedrückt wurden, und deren Zusammenhang auf die Struktur und Funktion der grossen Arterien in einer gesunden Schweizer (über 50 Jahre alt) und einer finnischen (55-74 Jahre alt) Bevölkerung verglichen werden. Drittens wurden die Effekte einer vierjährigen lebensstilbasierten Intervention auf die Struktur der Halsschlagader in derselben finnischen Population analysiert, um festzustellen, ob die Progression der Atherosklerose durch eine Lebensstilintervention verlangsamt werden kann.

### Methoden

Der DAT wurde gegenüber einem siebentägigen Ernährungs-protokoll (7d-FR), validiert. Es wurden junge (20-40 Jahre alt) und ältere (50-70 Jahre alt) Erwachsene rekrutiert. Gesamtkalorien, Makronährstoffe, Zucker, Wasser und Obst- und Gemüseportionen wurden zwischen den beiden Instrumenten verglichen.

Die finnische Dose-Responses to Exercise Training (DR's EXTRA)-Studie war eine randomisierte, kontrollierte Studie mit einer Dauer von vier Jahren, die sechs Gruppen umfasste: aerobes Training, Krafttraining, Diät, aerobes Training + Diät, Krafttraining + Diät und Kontrollgruppe. Die Intima-Media-Dicke der Arteria carotis (cIMT) und der Lumendurchmesser (cLD) wurden als vaskuläre Biomarker verwendet, die das Fortschreiten der Atherosklerose während der Intervention quantifizieren. Sie wurden mittels transkutanem Ultraschall unter Verwendung modernster Wandkontur-Erkennungstechniken gemessen.

Basierend auf die Querschnittsdaten der DR's EXTRA-Studie wurde die Einhaltung der «Life's Simple 7», ein Herzkreislauf-Gesundheitsscore der American Heart Association, der ausschliesslich aus modifizierbaren Lebensstilfaktoren besteht, mit cIMT und cLD sowie der Distensibilität der A. carotis verglichen. Der Life's Simple 7 Score wurde in niedrige, mittlere

und ideale Herzreislaufgesundheit unterteilt.
Die Schweizer Querschnittsstudie Cardiopulmonary Exercise Testing (COmPLETE) umfasste eine gesunde Schweizer Stichprobe. Für dieses PhD-Projekt wurde nur eine Teilstichprobe von Probanden im Alter von 50-91 Jahren berücksichtigt. Die Life's Simple 7 wurden ebenfalls verwendet und mit den arteriellen Eigenschaften verglichen, namentlich cIMT, cLD, Distensibilitätskoeffizient der A. carotis (DC), flussvermittelte Dilatation der A. brachialis (FMD) und Pulswellengeschwindigkeit von der A. brachialis bis zur Knöchelregion (baPWV).

**Ergebnisse**

**DAT:** 51 Studieninteressierte wurden eingeschlossen. Die Korrelationen zwischen DAT und 7d-FR reichten von 0,288 (Zucker, $p<0,05$) bis 0,729 (Wasser, $p<0,01$). Probanden der älteren Gruppe wiesen für alle Makronährstoffe höhere Korrelationen auf, wobei die höchste Korrelation bei den Gesamtkalorien bei 0,799 lag ($p<0,01$). Beide Gruppen überschätzten die Menge an Gesamtkalorien (+14,0%), Eiweiss (+44,6%), Fetten (+36,3%) die Obst- und Gemüseportionen (+16,0%) leicht bis moderat, unterschätzten dagegen die Zuckeraufnahme stark (-50,9%). **DR's EXTRA Querschnittstudie:** 1400 Probanden wurden in die Analysen einbezogen. Diejenigen mit einem idealen Herzreislauf-Gesundheitsscore hatten eine niedrigere cLD als diejenigen mit einem mittleren (-0,21 mm, 95% KI: -0,37 bis -0,05 mm, $p<0,01$) und einem niedrigen Score (-0,39 mm, 95% KI: -0,65 bis -0,12 mm, $p<0.01$). Probanden mit idealem Score hatten auch eine höhere Distensibilität als jene mit mittlerem (0,0032 $kPa^{-1}$, 95% KI: 0.009 bis 0.0055 $kPa^{-1}$, $p<0.01$) und niedrigem Score (0,0018 $kPa^{-1}$, 95% KI: 0,0005 bis 0,0032 $kPa^{-1}$, $p<0.01$). Hinsichtlich der cIMT wurden keine Unterschiede zwischen den Interventionsgruppen und der Kontrollgruppe festgestellt.
**DR's EXTRA-Interventionsstudie**: 1132 Probanden mit gültigen Messungen zu Beginn der Studie und bei der Nachuntersuchung wurden eingeschlossen. Über alle Gruppen hinweg wurde keine signifikante Verlangsamung des cIMT-Anstiegs festgestellt ($p<0,01$). Eine Untergruppenanalyse zeigte, dass Männer in der Diätgruppe einen signifikant geringeren cIMT-Anstieg aufwiesen als die in der Kontrollgruppe (-0,078 mm, 95% KI: -0,146 bis -0,009 mm, $p<0.01$). Keine andere Untergruppe zeigte einen verlangsamten cIMT-Anstieg. Keine der Interventionsgruppen verlangsamte die cLD-Progression im Vergleich zur Kontrollgruppe signifikant.
**COmPLETE:** Die Analysen umfassten 280 gesunde Teilnehmende. Keiner hatte einen niedrigen Life's Simple 7 Score. Jene mit idealem Score hatten niedrigere cIMT (-0,038 mm, 95% KI: -0,069 mm bis -0,007 mm, $p<0,05$), niedrigeren cLD (-0,28 mm, 95% KI: -0,46 mm bis -0,11 mm, $p<0,01$) und niedrigere baPWV (-0,05 m/s, 95% KI: -0,08 m/s bis -0,02 m/s, $p<0,01$) als diejenigen, mit einem mittleren Gesundheitsscore.

**Schlussfolgerungen**

Das DAT ist eine geeignete Methode zum Screening ausgewählter Ernährungsparameter und kann insbesondere bei älteren Bevölkerungsgruppen nützlich sein.

Bei Finnen mittleren bis höheren Alters waren die Life's Simple 7 negativ mit cLD und positiv mit der Distensibilität der A. carotis assoziiert, nicht jedoch mit der cIMT. Ein gesunder Lebensstil – vermutlich Jahrzehnte vor der Untersuchung bestehend – scheint die Struktur und Funktion der A. carotis in der allgemeinen finnischen Bevölkerungsgruppe zu schützen.

Eine vierjährige Lebensstilintervention führte nicht zu einer Verlangsamung der Atheroskleroseprogression, ausser bei einer Untergruppe von Männern, denen Ernährungsziele vorgegeben wurden. Bewegungsinterventionen basierend auf internationalen Richtlinien, die während der Studie in Kraft waren, müssen daher in Frage gestellt werden.

In einer gesunden Stichprobe der Schweizer Bevölkerung wiesen jene mit einem höheren Life’s Simple 7 Score bessere Arterieneigenschaften auf. Dies zeigt, dass selbst bei den gesündesten Personen ein günstigerer Lebensstil mit einer besseren Gefässgesundheit verbunden ist.

Der Life's Simple 7-Gesundheitsscore erscheint den hier vorliegenden Analysen eine praktische Methode zur Bewertung der Herzkreislauf-Gesundheit zu sein, für die wir zeigen konnten, dass er mit traditionellen vaskulären Biomarkern assoziiert ist. In der untersuchten finnischen Bevölkerungsgruppe mittleren bis höheren Alters reichen lebensstilbasierte Interventionen nicht aus, um das Fortschreiten der Atherosklerose zu verlangsamen; möglicherweise sind andere oder intensivierte Ansätze erforderlich.

# Chapter 1 – Introduction to lifestyle and arterial properties in ageing populations

## 1.1 Importance of a healthy lifestyle

For thousands of years, the main causes of deaths worldwide were communicable diseases, which limited life expectancy to less than 40 years [1]. Since the 1950's, medical breakthroughs have enabled us to increase worldwide life expectancy, up until very recently [2]. 2021 marked the first year since 1950 that life expectancy decreased due to the emergence of a novel coronavirus disease. Omitting the current pandemic, life expectancy has more than doubled since the beginning of the 19$^{th}$ century due to the major decrease of deaths related to communicable diseases in most developed countries [3]. Instead, non-communicable diseases (NCD) such as heart disease, cancer, or stroke, have taken over as the main cause of death. People suffering from NCD tend to be affected for years, reducing quality of life, while extending time with morbidities, therefore increasing the proportion of life spent being ill [4]. It is thought that by the end of this decade, there will be more than 1.4 billion people over the age of 60, and more than 2.1 billion by the year 2050, many of which suffering from NCD [5]. To counter this trend of living longer but with multiple morbidities, the World Health Organization (WHO) pronounced the decade from 2020 to 2030 the "Decade of Healthy Ageing" [5]. To achieve healthy ageing, one of the targets is to reduce NCD-related mortality, which can be achieved primarily by improving lifestyle.

Over 20 years ago, the WHO already issued a statement on what is considered a healthy lifestyle [6]. It defined five factors that make up a healthy lifestyle, i.e., no tobacco smoking, no excessive alcohol consumption, regular physical activity, a healthy diet, and not being obese. The benefits of adhering to a healthy lifestyle are well documented, as are the risks of not following it. For example, a study with US-American adults showed, in a follow-up of 34 years, that those who never smoked, were not obese, were physically active, had high quality diet, and did not consume alcohol excessively, had significantly higher life expectancy than those who did not adhere to any of those five lifestyle factors [7]. The difference in life expectancy was quantified as 14 years for women, and over 12 years for men. Sadly, a study including 20 European countries reported that only 5.8% of Europeans followed a healthy lifestyle (i.e., adhering to all five healthy lifestyle habits) [8]. The countries with the lowest adherence rates had a prevalence of healthy lifestyle in less than 2% of the population, while the countries with the highest rates did not even exceed 10%.

Somewhat more encouraging results have come from Germany. A study found that while only 5.2% of the adult population adhered to all five healthy lifestyle behaviors, 18.3% followed four, and 31.9% followed three healthy lifestyle behaviors [9]. This means that more than half of the adult population adhered to at least three out of five healthy lifestyle behaviors. The question therefore arises whether adherence to some healthy lifestyle metrics might be

sufficient to reduce cardiovascular risk. Studies have examined the interplay between mortality and healthy lifestyle metrics and found evidence supporting this negative correlation. For example, a meta-analysis that included 15 studies with over 500,000 participants with a mean follow-up period of 13 years found that adhering to at least four of the five healthy lifestyle factors was associated with a reduction of all-cause mortality by up to 66% [10]. Further, as opposed to not adhering to any healthy lifestyle factor, following one, two, or three lifestyle factors was associated with lower risk of cardiovascular mortality by 28%, 42%, and 54%, respectively [10].

As clearly demonstrated with these studies, there is a strong association between lifestyle and the development of NCD. The current issue of modern societies is that the prevalence of NCD is at record levels. In Switzerland, for example, it has been reported that more than a quarter of the total population suffers from at least one NCD [11] and that NCD are responsible for half of all premature deaths (i.e., deaths before the age of 70 years old) in men, and 60% of all premature deaths in women [12]. While the Swiss Federal Office of Public Health recognizes that genetic predisposition plays a role in the pathogenesis of NCD, it also emphasizes that maintaining a healthy lifestyle is essential to reduce the risk of developing one.

In Switzerland, the most recent cost analysis of NCD dates from 2011 [13]. The total costs of NCD cumulated to 51.2 billion Swiss Francs annually, which equated to about 80% of the total health expenditure. The costs of cardiovascular diseases and musculoskeletal diseases hereby constituted the greatest share. In response to the number of people affected by NCD and their generated costs, the Swiss Federal Office for Public Health has created the "*Nationale Strategie Prävention nichtübertragbarer Krankheiten (NCD-Strategie) 2017–2024*" (Engl. National Strategy for the prevention of non-communicable diseases (NCD-strategy 2017-2024). In that report, the strategies to lower the incidence of NCD are divided into three pillars. The first pillar includes population-based health promotion and prevention. In this pillar, the main goals are to improve lifestyle by reducing certain risk factors such as smoking and alcohol consumption and increasing dietary quality and physical activity. The second pillar targets health care, and the third pillar aims at health promotion and prevention in the economy and at the workplace.

The literature as well as the efforts of national and global health institutions clearly prove how living a healthy lifestyle contributes not only to a longer, but also to a disease-free life. Strategies to reduce the prevalence of NCD in the general population are therefore in great demand. Also, strategies to determine which individuals are at risk of developing lifestyle-related diseases are necessary. For instance, it is important to find diagnostic tools that allow to monitor cardiovascular risk over time. The applied methods should be precise, inexpensive, ideally non-invasive and, of course, reproducible. The following chapters will discuss these methodological challenges by presenting currently applied vascular biomarkers as well as a cardiovascular health score.

## 1.2 Vascular biomarkers

In 2001, the Biomarkers Definitions Working Group, as part of the National Institutes of Health (NIH), defined a biomarker as follows: **"**A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." [14]. Essentially, a vascular biomarker therefore serves to detect structural or functional changes in the vascular system before a disease is diagnosed. Vascular biomarkers have since been widely applied to detect cardiovascular risk. A position paper from the European Society of Cardiology Working Group in 2015 defined the most important non-invasive vascular biomarkers for primary and secondary cardiovascular disease (CVD) prevention [15]. The following chapters will describe the biomarkers that I applied for my PhD project.

### 1.2.1 Carotid arterial properties

The left and right carotid arteries are a pair of vessels that supply blood to the brain, neck, and face. Since flawless blood supply to those areas of the human body are crucial, the importance to assess the carotid arteries as a tool for cardiovascular risk prediction, has long been recognized [16, 17]. The carotid arteries are located superficially, making them easily accessible for ultrasound examination using B-mode sonography. This imaging technique has several advantages: it is safe, inexpensive, painless, radiation-free, and offers the ability to assess arterial wall thickness, stenosis or plaque formation [18].

The arterial walls are made up of three layers. The outermost layer, the tunica adventitia, is made of collagen fibers and elastin. Its function is to supply and innervate the vessels, and to separate the vessels from other structures [19]. The middle layer, the tunica media, is made of smooth muscle cells, elastin, and collagen fibers. With its high proportion of muscle cells, it controls the toning of the vessels in response to nervous or hormonal stimuli. The innermost layer, the tunica intima, is mainly composed of endothelial cells and elastin-rich collagen [20]. The endothelium is mainly responsible for the exchange of substances between tissue and blood as well as for the regulation of blood pressure. The endothelium also regulates the flowability of the blood by inhibiting and activating coagulation processes [21]. While histologically the differences between the various tunicae are clear, the delimitation between the intima and media are difficult to see using B-mode sonography. As such, to determine carotid wall structure, the intima-media complex is taken together and is referred to as carotid intima-media thickness (cIMT) [16]. The adventitia is not used for arterial wall assessment, as its outer border is difficult to discern in B-mode [22].

The carotid wall is known to change with age [23]. Atherosclerosis is a pathological process which describes the build-up of cholesterol, fat, blood cells and other substances in the tunica intima of main arteries. Over time, the accumulation of these substances in the arterial wall, called plaques, result in arterial narrowing which, in turn, reduces blood supply to the periphery and may cause complications, such as ischemia.

Diseases related to atherosclerosis, such as stroke or myocardial infarction, are the number one

and two leading causes of death in the world [24]. Over three quarter of all CVD-related deaths globally are suffered in low- and middle-income countries, which have limited access to diagnostic tools and adequate healthcare [25]. The slow progression of atherosclerosis over decades means that even high-risk individuals remain asymptomatic during a time in which it could still be possible to make adjustments which would alter the course of the disease [26]. The assessment of cIMT may therefore be a valid tool to predict cardiovascular events, especially in symptom-free subjects.

As such, since the 1990's, an extensive amount of literature can be found on cIMT as an independent predictor of cardiovascular events [23, 27, 28]. Of all vascular biomarkers, cIMT is the one that has been studied the most extensively [29]. A meta-analysis that included over 36'000 subjects found that the risk for cardiovascular events was elevated by 16% per 0.1mm increase in cIMT [30]. The evidence further suggests that there are several modifiable and non-modifiable risk factors that influence atherosclerotic progression. The most important non-modifiable risk factors are age [23], sex [31], and race [32]. There is also evidence showing that geographical latitude plays a non-negligible role [33]. Subjects living in Northern European countries (e.g., Sweden, Finland) are known to have significantly higher cIMT than Southern European countries (e.g., France, Italy). Longitudinal data on that matter, however, is still scarce. As for modifiable risk factors, it is well known that those with higher physical fitness, measured as maximal oxygen uptake ($\dot{V}O_{2max}$) [34] and higher levels of physical activity [35], have lower cIMT than less physically active persons. In addition, a recent study found that in adults, systolic blood pressure was the strongest predictor for cIMT [36]. In the same study, the authors found that some dietary components were also associated with cIMT, namely total dietary fiber and fish intake. Dietary fiber is known to be associated with slower atherosclerotic progression [37] and a reduced cardiovascular risk [38]. Similarly, a recent meta-analysis found that fish consumption was associated with lower incidence of coronary heart disease and mortality [39].

More recently however, the benefit of assessing cIMT to measure cardiovascular risk has been questioned, as its additive value appears to be low [15]. For example, a meta-analysis including over 45'000 adults found that there was little, clinically insignificant additive value in cIMT to predict first-time myocardial infarction or stroke [40]. The authors of the meta-analysis concluded that cIMT should not be part of routine CVD risk assessment due to its limited benefits. There may, however, still be a case for sonographic measurement of the carotid arteries, as the longitudinal study by Fritze et al. (2020) showed that carotid lumen diameter (cLD) provided more information than cIMT for all-cause mortality [41]. The authors argue that cLD is easier to measure than cIMT, but that more evidence is needed to investigate whether cLD progression can be slowed via pharmacological and/or non-pharmacological interventions.

For both cIMT and cLD, however, it must be noted that changes are slow. Interventions using carotid properties as an endpoint are inconclusive at best [42]. Other vascular biomarkers may therefore be superior to determine the effects of interventions.

### 1.2.2 Arterial stiffness

Arterial stiffness indicates how rigid an arterial wall is, i.e., how much it dilates with each heartbeat. Arterial stiffness is commonly measured by pulse wave velocity (PWV), which is defined as the speed at which the blood ejected from the left ventricle of the heart during systole produces arterial pulsation [43]. PWV stands in an inverse relationship with arterial distensibility, meaning that PWV is higher in stiffer arteries. As arteries naturally become stiffer from proximal to distal, PWV increases in the periphery and is lowest in the ascending part of the aorta. With age and with other cardiovascular risk factors, arteries become stiffer, especially large elastic-type arteries [15, 44]. This process is mainly a consequence of remodeling of the tunica media [45], and it is not linear. As such, longitudinal studies have found that in the third life decade, aortal PWV increased by 0.4 m/s, whereas it increased by 1.8 m/s in the seventh decade [46].

There are multiple ways to calculate PWV invasively or non-invasively, such as carotid-femoral PWV, which largely measures elastic arteries, or brachial-ankle PWV (baPWV), which measures the large elastic arteries, but also smaller arteries [15, 47]. As opposed to the gold standard carotid-femoral PWV, baPWV has the advantage of not measuring in the inguinal region, which may be an area of discomfort for study participants. In addition, by having the blood pressure cuffs placed around the extremities, baPWV it thought to provide information on a longer arterial segment. Disadvantages include the lack of reference values and height-based formulas to calculate baPWV [15]. In this manuscript, I will focus on baPWV, as, due to its ease of use in clinical practice, it was the method used to measure arterial stiffness in my PhD project [47]. The use of baPWV has mostly been documented in Japan, where they showed an association with cardiovascular risk [48, 49]. A meta-analysis found that an increase of baPWV of 1 m/s equated to an increase in total cardiovascular events, cardiovascular mortality, and all-cause mortality by 12%, 13%, and 6%, respectively [50]. In that meta-analysis, however, more than 50% of all participants were affected by a chronic disease with a poor prognosis. More recently, a meta-analysis that included studies which showed results from representative samples of the Japanese population found similar evidence [49]. Per 1 standard deviation increase in baPWV, the cardiovascular risk was increased by 19%. The authors concluded that baPWV was therefore a valid tool to enhance prediction of cardiovascular events. To the best of my knowledge, to date, there is no such evidence for European populations.

### 1.2.3 Endothelial function

The endothelium constitutes the most inner layer of arterial walls, which is in direct contact with blood [51]. As such, the endothelium plays an essential role in blood flow control and vascular tone. Cardiovascular risk factors such as tobacco smoking, hypertension, or aging are known to be associated with reduced endothelial function [52, 53]. As discussed above, atherosclerotic changes in the arterial walls progress slowly over decades and remain difficult to discover for years. A few decades ago, the non-invasive measurement of endothelial function has been suggested, as functional changes occur long before any structural adaptation of the arterial walls begin [54, 55]. This phenomenon, known as endothelial dysfunction, occurs when the bioavailability of endogenous vasodilators, mainly nitric oxide, is reduced [56]. To assess endothelial dysfunction, it is possible to either measure coronary or peripheral circulation [57]. In this thesis, I will only discuss the measurement in peripheral arteries, more specifically, the brachial artery, as this was the only artery assessed for endothelial function in my PhD thesis. Ultrasound-based flow mediated dilation (FMD) of the brachial artery is a validated measurement of endothelial function and a predictor for cardiovascular events [58]. FMD is used to measure the ability of the endothelium to dilate after an acute increase in shear stress. Therefore, a high FMD (i.e., high ability of the brachial artery to distend) is considered being cardio protective. As such, as a meta-analysis in 2014 found that a 1% increase in FMD is associated with 10% risk reduction for cardiovascular events [59]. Non-modifiable risk factors for lower FMD include male sex and older age, which are consistently associated with lower FMD [60-62]. The advantage of using FMD in clinical studies is that it is reactive to physiological interventions, such as exercise or diet [63]. The use of FMD to monitor the effects of lifestyle-based interventions may therefore be superior to the other vascular biomarkers presented above [15]. However, until very recently, normal and reference values were not available for FMD, limiting the ability to compare between different populations [64, 65]. As such, FMD is currently not recommended in routine care but the (novel) availability of reference values may warrant its clinical application in the future.

## 1.3 The Life's Simple 7

As an alternative to vascular biomarkers, which are not used in routine care [15], cardiovascular risk scores have emerged as an accepted method to assess cardiovascular risk in at-risk populations. Nevertheless, their accuracy is questioned [66]. For example, cardiovascular risk in low-risk populations tends to be overestimated, whereas in high-risk populations, cardiovascular risk is underestimated. This issue leads to people being unnecessarily treated with medication on one hand. On the other hand, individuals with high risk are inadequately treated in respect of the severity of their disease.

The most commonly used risk scores, such as the Framingham Risk Score [67], the European SCORE 2 [68], and SCORE 2 old people [69], are all dependent on non-modifiable variables: age, sex, and ethnicity, all of which are indeed non-negligible risk factors for cardiovascular events [70], and are heavily factored in those scores. The calculated risk scores also consider

several lifestyle-related metrics, such as smoking status, or body mass index (BMI). One of the weaknesses of these scores in clinical settings is that, when following a patient over multiple years, the risk score may worsen due to the natural biological ageing process, even if their lifestyle improved. As such, this may lead to loss of motivation for the patient, which, in turn, would negatively affect healthy lifestyle behavior and could ultimately result in a higher cardiovascular risk.

The American Heart Association (AHA) recognized this issue and introduced the "Life's Simple 7" in 2010 as an attempt to reduce cardiovascular events in the USA by 20% over the 10 years following their implementation [71]. The rationale was to move the focus away from disease treatment and more towards health and wellness promotion, and to therefore emphasize modifiable lifestyle habits which can be implemented to improve cardiovascular health [72]. As such, four behavioral (not smoking, regular physical activity, healthy diet, and normal BMI) and three biological (systolic blood pressure, blood lipids and blood glucose) health metrics were included in the Simple 7. Predefined cutoff values and the presence/absence of medication were used to determine cardiovascular health via a scoring system (Table 1) [71]. For each of the metrics, being in the range of an ideal score is awarded two points, intermediate is one point, while poor is zero points. An ideal cardiovascular health score is typically referred to as 10-14 points, an intermediate score ranges from 4-9, and a poor score is considered scoring ≤3 points.

Since the creation the Life's Simple 7, a great amount of data have been published on that matter. For example, an inverse relationship between ideal health metrics and the incidence of cardiovascular events [73-75], and mortality [76, 77] was found. As Sanchez (2018) wrote, the Life's Simple 7 are vital, but not easy to achieve [78]. Only very few adults in the USA achieve ideal cardiovascular health scores, none achieve a perfect score of 14 points, and only 0.5% score 13/14 points [79]. The meta-analysis by Ramírez-Vélez et al. (2018) comprised 12 studies, which in total included over 200'000 participants [80]. The authors confirmed that only few adults had an ideal cardiovascular health score and further found that an ideal health score was associated with risk reduction for CVD by 72%, when compared to those with a poor score. Encouragingly, they found that those with an intermediate score already benefited massively, as they had a 47% lower risk for CVD than those with a poor score. This proves that adhering to at least some of the Life's Simple 7 metrics may already be very beneficial to reduce cardiovascular risk.

Recently, Enserro et al. (2018) demonstrated in a 20-year follow-up study, that subjects who started the study with ideal cardiovascular health and maintained it (ideal-ideal), had the lowest odds of developing a subclinical disease [81]. In contrast, those with poor cardiovascular health metrics who stayed in the same category (poor-poor), showed the highest rates of subclinical cardiovascular disease, incidence of CVD, and mortality. While those results are hardly surprising, the findings from the individuals who changed categories over time (ideal-poor, or poor-ideal) are worth discussing. It is commonly suggested and recommended that it is never too late to improve lifestyle, with studies showing convincing results in elderlies that stopped

smoking [82], became more physically active [83], or improved their diets [84]. These recommendations were confirmed by the findings by Enserro et al. (2018), who showed that those who improved their cardiovascular health metrics (poor-ideal) had lower incidence of CVD than those who stayed in the poor cardiovascular health category (poor-poor) [81].

*Table 1: Life's Simple 7 definitions of ideal, intermediate, and poor cardiovascular health. Data derived from Lloyd-Jones et al., (2010), copyright ©2010, American Heart Association, Inc.*

| | **Ideal** | **Intermediate** | **Poor** |
|---|---|---|---|
| Smoking | Never smoker | Former smoker | Current smoker |
| Body mass index | <25 kg/m$^2$ | 25-29.9 kg/m$^2$ | ≥30 kg/m$^2$ |
| Physical activity | ≥150 min/week moderate or ≥75 min/week vigorous or ≥150 min/week moderate + vigorous activity | 1-149 min/week moderate or 1-74 min/week vigorous or 1-149 min/week moderate + vigorous activity | None |
| Diet score* | 4-5 components | 2-3 components | 0-1 components |
| Total cholesterol | <5.2 mmol/L and not treated | 5.2-6.1 or treated to <5.6 mmol/L | ≥6.2 mmol/L |
| Blood pressure | SBP <120 and DBP <80 mmHg and not treated | SBP 120-139 or DBP 80-88 or treated SBP SBP <120 and DBP <80 mmHg | SBP ≥140 or DBP ≥90 mmHg |
| Fasting glucose | <5.6 mmol/L and not treated | 5.6-6.9 or treated to <5.6 mmol/L | ≥7.0 mmol/L |

SBP indicates systolic blood pressure; DBP diastolic blood pressure.
* Meets criteria: Fruits and vegetables ≥4.5 servings/day, Fish ≥two 3-5 oz/week, Fiber-rich whole grains ≥3 servings/day, Sodium <1500 mg/day, and sugar-sweetened beverages ≤4 glasses/week.

It is, however, more interesting to look at those who started the study in the ideal cardiovascular health category and moved to the poor cardiovascular health category (ideal-poor). In this cohort, the presence of subclinical disease, the incidence of CVD, and mortality, was lower than in the poor-ideal group. This shows that while the Simple 7 metrics are modifiable, starting with poor cardiovascular health is associated with higher cardiovascular risk, regardless of whether lifestyle was improved or not. This further cements the case for early adoption of a healthy lifestyle, reaching ideal cardiovascular health metrics early and, ideally, maintaining them for as long as possible. These results were confirmed by Corlin et al. (2020), who showed in a 25-year longitudinal study that a higher Life's Simple 7 score in midlife was associated with lower risk of hypertension, diabetes, CVD, and all-cause death [85]. Morbidity and mortality can therefore be reduced by improving cardiovascular health.

Starting with a poor cardiovascular health score and improving it may not be as protective as

having an ideal score early on, but it is still proven to be beneficial in secondary prevention. Mok et al. (2018) found that those with an ideal health score had up to 84% lower incidence of myocardial infarction than those with a poor score [86]. In that study, those with a health score of ≥7 at baseline had a 40-60% lower risk of mortality after myocardial infarction, when compared to those with a score of ≤3. These findings are of invaluable importance, as the indicate that the Life's Simple 7 play a key role not only in primary, but also in secondary prevention, and should be implemented in cardiac rehabilitation. Further, the above-mentioned results show that adherence to a healthy lifestyle early in life is superior to changing lifestyle habits in adulthood, but it is never too late to implement healthy lifestyle habits.

### 1.4 Life's Simple 7 and arterial properties

As demonstrated in the previous chapters, both the Life's Simple 7 cardiovascular health score and arterial properties are used to make clinical decisions based on a patient's risk of developing a CVD. To date, only limited evidence is available on the Life's Simple 7 and arterial properties. There is some evidence suggesting that adherence to the Life's Simple 7 is associated with lower PWV in children [87]. In adults, however, current evidence in unclear and controversial. The largest study on that matter, the Paris Prospective Study 3 (PPS3), included over 9'000 participants from primary health care centers and showed that independently from age, sex, and education, a higher Life's Simple 7 score was associated with lower cIMT, lower number of carotid plaques, and higher carotid distensibility [88]. To date, this is the only study that investigated carotid distensibility and the Life's Simple 7. A cross-sectional twin study showed that adherence to the Life's Simple 7 was negatively associated with cIMT, independent of genetic and familial factors [89]. Lastly, the 5-year follow-up AGES-Reykjavik study found that men with a lower Life's Simple 7 score had greater carotid plaque areas [90]. However, this association was not confirmed in women, and cIMT was not associated with the Life's Simple 7 for either sex.

To conclude, there is currently insufficient evidence on the topic of the Life's Simple 7 and arterial properties. Cross-sectional and longitudinal data are scarce and inconclusive and data on interventions are inexistent. The question that has yet to be fully answered is whether adherence to the Life's Simple 7 is associated with better arterial properties. It is also unclear whether lifestyle improvements can slow atherosclerotic progression. The aim of my PhD thesis is to provide new insights on this topic and to demonstrate the importance of the assessment of both modifiable health metrics and arterial properties to adequately determine cardiovascular health.

# Chapter 2 - Aims of the thesis

My PhD project aimed at determining which lifestyle factors influence the structure and the function of large arteries in the ageing human body.

## 2.1 Main objective of this PhD project

| | |
|---|---|
| *Main objective* | To compare various lifestyle metrics and their influence on the function of large human arteries in a healthy Swiss **(**aged 50+ years old**)** and Finnish (aged 55-74 years old) population. |

## 2.2 Further aims of this PhD project

| | |
|---|---|
| *Aim 1* | To validate a visually aided dietary assessment tool (DAT) |
| *Aim 2* | To compare how adherence to certain lifestyle metrics is associated with arterial properties in Swiss and Finnish subjects |
| *Aim 3* | To quantify the effects of a 4-year lifestyle-based intervention on atherosclerotic progression of the carotid arteries in Finns |

## 2.3 Hypotheses

1. The visually aided DAT is a valid tool to assess dietary habits in healthy subjects.
2. Better lifestyle, quantified via the Life's Simple 7 health score, is associated with superior arterial properties in a representative sample of the Finnish population, as well as in a healthy Swiss population.
3. A lifestyle-based intervention including diet, aerobic, or resistance training slows atherosclerotic progression. Combined aerobic or resistance exercise and diet is superior to diet or exercise alone.

## 2.4 Publications

| | |
|---|---|
| *Publication 1* | Validation of a visually aided dietary assessment tool to estimate dietary intake in an adult Swiss population |
| *Publication 2* | Effect of lifestyle interventions on carotid arterial structure – the DR's EXTRA study |
| *Publication 3* | Adherence to Life's Simple 7 is associated with better carotid properties |
| *Publication 4* | Ideal Life's Simple 7 score relates to macrovascular structure and function in the healthy population |

# Chapter 3 – Participants and methods

My PhD project is based on data from one randomized-controlled trial (RCT), one cross-sectional study, and one validation study. Out of the three studies, a total of four manuscripts were published.

The RCT was conducted in Kuopio, Finland, from 2002 (first recruitment) to 2011 (last follow-up measurements). Both the cross-sectional and the validation study were undertaken in Basel, Switzerland. The cross-sectional study lasted from 2017 to 2019 while the validation study took place in 2021. The following chapter will give an overview on the three studies that constitute my PhD project, in order of submission to a peer-reviewed journal.

## 3.1 Study design and population DAT (publication 1)

The goal of the validation study was to validate a visually aided dietary assessment tool (DAT). To validate the DAT, we aimed to include a total of 50 healthy adults (Figure 1). Sample size was determined according to a similarly designed validation study by Steinemann et al. (2017) [91]. To discriminate between young and older adults, we divided the study population in one group with subjects aged 20-40 years old, and one group with subjects aged 50-70 years old. In addition, all subjects were to be physically and mentally able to follow the study protocol. The rationale for the validation of the DAT was that it had been used in the COmPLETE study. The COmPLETE study is a cross-sectional study aimed at investigating cardiovascular health in a disease-free sample the Swiss population of Basel. Its study design is described in chapter 3.3. During the data collection phase of the COmPLETE study, the DAT had not been validated yet. To use information on dietary habits in the COmPLETE population and to complete the Life's Simple 7 score for this population, the DAT had to be validated first. As such, the validation of the DAT served as a one of the pillars for the 4$^{th}$ publication.

All measurements were performed at the Department for Sports, Exercise, and Health, of the University of Basel, Switzerland. The subjects were invited twice within eight days. On the first visit, anthropometric measurements were taken, followed by the health-related questionnaires, the DAT, and instructions on how to fill out the 7-day food-record (7d-FR). The second visit was identical to the first, and the results of the 7d-FR were discussed. The 7d-FR is widely used and known to be an accurate form of dietary assessment [92], which is why the visually aided DAT was validated against the 7d-FR.

For my project, I was involved in every step of the manuscript publication. The initial proposal to include the validation study in the PhD project came from my first supervisor, who was also involved in the entire procedure. Together, we designed the study protocol, formulated the research question and hypotheses. I was responsible for writing the proposal for the ethics commission (swissethics). Also, I was responsible for recruiting two students who would write their Bachelor's theses (under my supervision) as part of this validation project. The students were familiarized with the study procedures and tasked to help me recruit the study participants and measure them. After data acquisition, I cleaned the data and analyzed them with the support

of our in-house statistician. I wrote the initial publication (manuscript 1) and implemented the comments made by the co-authors. I was also responsible for submitting the final manuscript and the exchange with reviewers and editors. The data used for manuscript 1 was used for two Bachelor's theses, that were both written under my supervision.

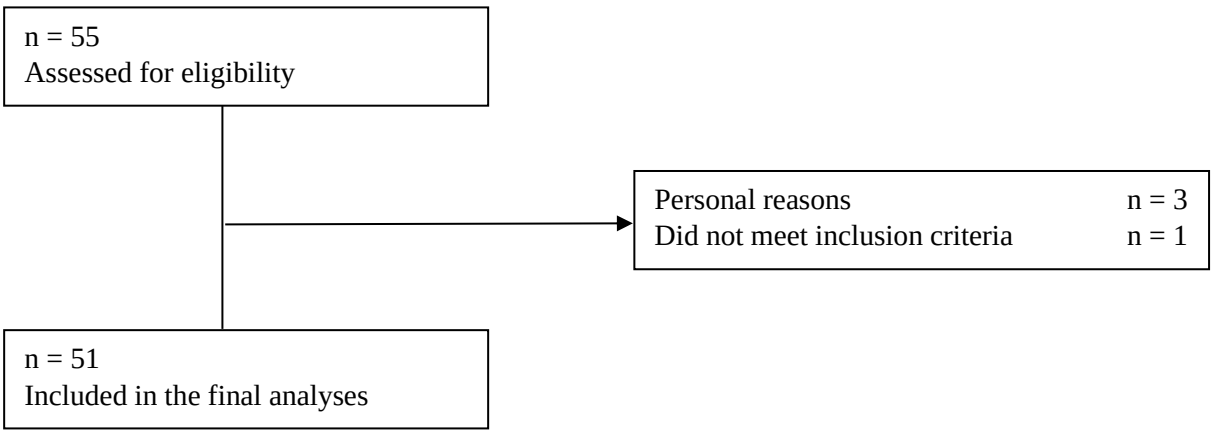


Figure 1: Flowchart of the validation study (publication 1).

### 3.2 Study design and population DR's EXTRA (publications 2 and 3)

The Dose-Responses to Exercise Training (DR's EXTRA) study was designed following the previously performed DNASCO (DNA Polymorphism and Carotid Atherosclerosis) study [42]. Briefly, the DNASCO study was a randomized controlled trial that was undertaken over a period of six years with 140 middle-aged Finnish men. The aim was to identify whether regular aerobic exercise could slow atherosclerotic progress in previously inactive individuals. Although cardiorespiratory fitness, assessed by $\dot{V}O_{2max}$ was improved in the intervention group, the authors concluded that regular aerobic exercise did not slow atherosclerotic progression, measured as cIMT. However, a subgroup analysis detected that men not taking statins had 40% less progression of cIMT than the control group, after adjusting for cardiovascular risk factors such as smoking, blood pressure, low-density lipoprotein cholesterol levels, and obesity. The men in the aerobic exercise group who were not taking lipid-lowering medications started to show reduction of progression of cIMT after three years of intervention. A possible explanation for this phenomenon was that middle-aged men might be slow to respond to exercise intervention. However, the exact reason for the results of the DNASCO study remained speculative.

Consequently, the DR's EXTRA study was designed and set up to be the largest RCT to be undertaken to assess the effects of various lifestyle-related interventions on atherosclerotic CVDs, type 2 diabetes, metabolic syndrome, and dementia in the general population. International recommendations for physical activity were determined by results from observational studies [93]. Therefore, the DR's EXTRA study was designed to address the question of whether increased aerobic or resistance training, improved diet, or a combination

of both exercise training and healthy diet would slow atherosclerotic progression, measured as cIMT (Figure 2). Initially, the study was meant to include additional sub-groups in the aerobic exercise, the resistance exercise, and the dietary intervention group, hence the "Dose-Responses" part of the DR's EXTRA acronym. Both the aerobic and the resistance exercise groups were supposed to have the respective participants exercising at a rate of 1000 – 1500 kcal/week, and after 6 months, some would have increased energy expenditure to >1500 kcal/week by increasing exercise duration. Also after 6 months, the dietary intervention group was supposed to be split into one group that would have followed the Finnish Nutrition Recommendations [94], and another group that would have been given a Special Nutrition treatment, which was stricter than the Finnish Nutrition Recommendations. However, the group split was never implemented for reasons of feasibility, resulting in the final group allocation as shown in Figure 2. The detailed flowchart of the DR's EXTRA study is shown in Figure 3. My PhD project started years after completion of DR's EXTRA. I was given the task to analyze the sonography data of the carotid arteries, which would serve as a fundament for manuscripts 2 and 3. The expert sonographer of the DR's EXTRA study had recorded all measurements of the left and right carotid arteries of each participant at baseline and follow-up. Consequently, for each participant, two screenshots at end-diastole and peak-systole were taken of both the left and right carotid artery. This resulted in eight images per participant, per measuring point, totaling approximately 20'000 images to analyze. We analyzed the images with the help of the semi-automated program Dynamic Artery Analysis (DYARA), which was developed to analyze B-mode ultrasound images [95]. The boundaries of the intima-media complex of the far wall and the adventitial border of the near wall of the ultrasound image were detected over a 10 mm segment proximal to the carotid bifurcation. The analysis of the four images at peak-systole (two on the right, two on the left side) and at end-diastole, were averaged [96]. To gather all the images, I was supported by a Master student involved in the project. In addition, one Bachelor student was involved to merge the data files from each measurement into one document. Two publications resulted from the DR's EXTRA study for my PhD project, namely publication 2 and 3. Publication 2 focused on the intervention effects on carotid structure (cIMT and cLD). Due to a potential systematic error for blood pressure measurements between baseline and follow-up, we were not able to include any functional parameters of the carotid arteries, as they require the use of systolic blood pressure, or pulse pressure [97]. We therefore decided to opt for a cross-sectional approach for publication 3, to include the functional parameters of the carotid arteries. This further allowed the inclusion of a higher number of participants than in publication 2, as only baseline values were considered. In addition to the two publications, this dataset served as a basis for one Master's, and one Bachelor's thesis, which were both written under my supervision.

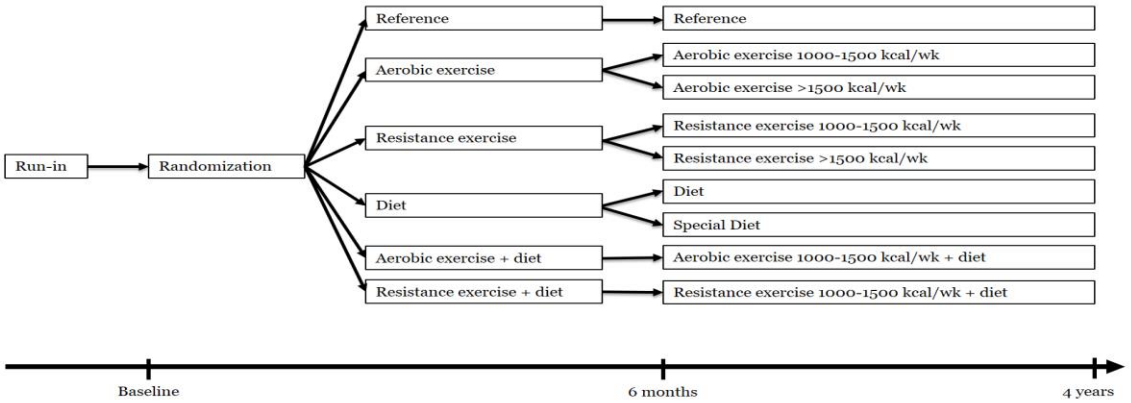


Figure 2: Intervention arms of the DR's EXTRA study.

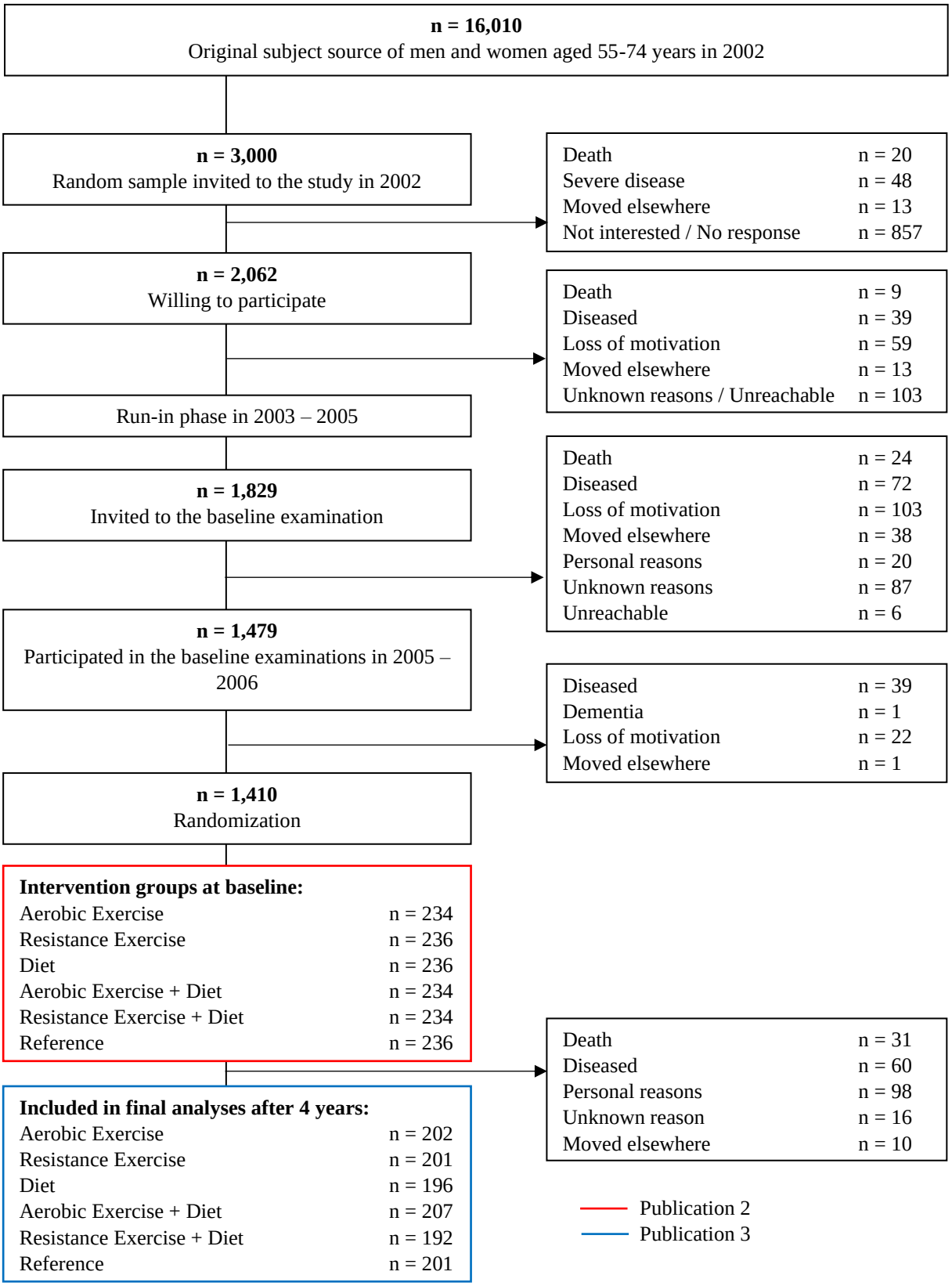


Figure 3: Flowchart of the DR's EXTRA study (publications 2 and 3).

### 3.3 Study design and population COmPLETE (publication 4)

The Basel, Switzerland, based COmPLETE study was designed to investigate cardiovascular health, physical fitness and endurance capacity in subjects free of any chronic diseases, aged 20 – 100 years old, as well as in patients with heart failure [98]. The COmPLETE study therefore comprised two arms, COmPLETE Health and COmPLETE Heart. For my PhD project, only the data from COmPLETE Health was included, therefore COmPLETE Heart will not be further discussed. One of the primary aims of COmPLETE Health was to create new norm values for healthy subjects for physical fitness markers, such as $\dot{V}O_{2max}$. A further goal was to progress maximal cardiopulmonary exercise testing as a diagnostic and prognostic tool for CVD [99]. To achieve that goal, the aim was to recruit 70 participants per age decade, namely 20-29, 30-39, 40-49, 50-59, 60-69, 70-79, and 80+ years old. Anyone interested in participating in the COmPLETE Health study was to be free of any manifested CVD, be non-smoker, not obese (BMI <30 kg/m$^2$) and have a blood pressure of <160/100 mmHg. These criteria were set in place to ensure that only those ageing in an apparently healthy manner were included in the study. The following flowchart (Figure 4) shows the detailed recruitment and exclusion process.

My PhD project started during the data collection phase of COmPLETE Health and COmPLETE Heart, with the goal to finish data collection in both arms of the study before year-end of 2019. After completion of data collection, the validation study (manuscript 1) was set up, to allow for statements on dietary components of the COmPLETE population in manuscript 4.

For manuscript 4, only data from the participants aged 50+ years old were considered, as one of the vascular biomarkers, namely the carotid properties, were only measured in those who were aged 50 years or older. In addition to the carotid properties, manuscript 4 included FMD and baPWV as vascular biomarkers. As in the DR's EXTRA study, the Life's Simple 7 score was calculated for all study subjects. We used the DAT from manuscript 1 to assess dietary habits, and hemoglobin A1c (HbA1c) as a proxy for fasting blood glucose. The other variables we assessed were identical to the ones in DR's EXTRA. The COmPLETE study served as a base for countless Bachelor's and Master's theses, which have been completed during and after the data collection period. As part of the COmPLETE team, I supervised one Master's, and four Bachelor's theses.

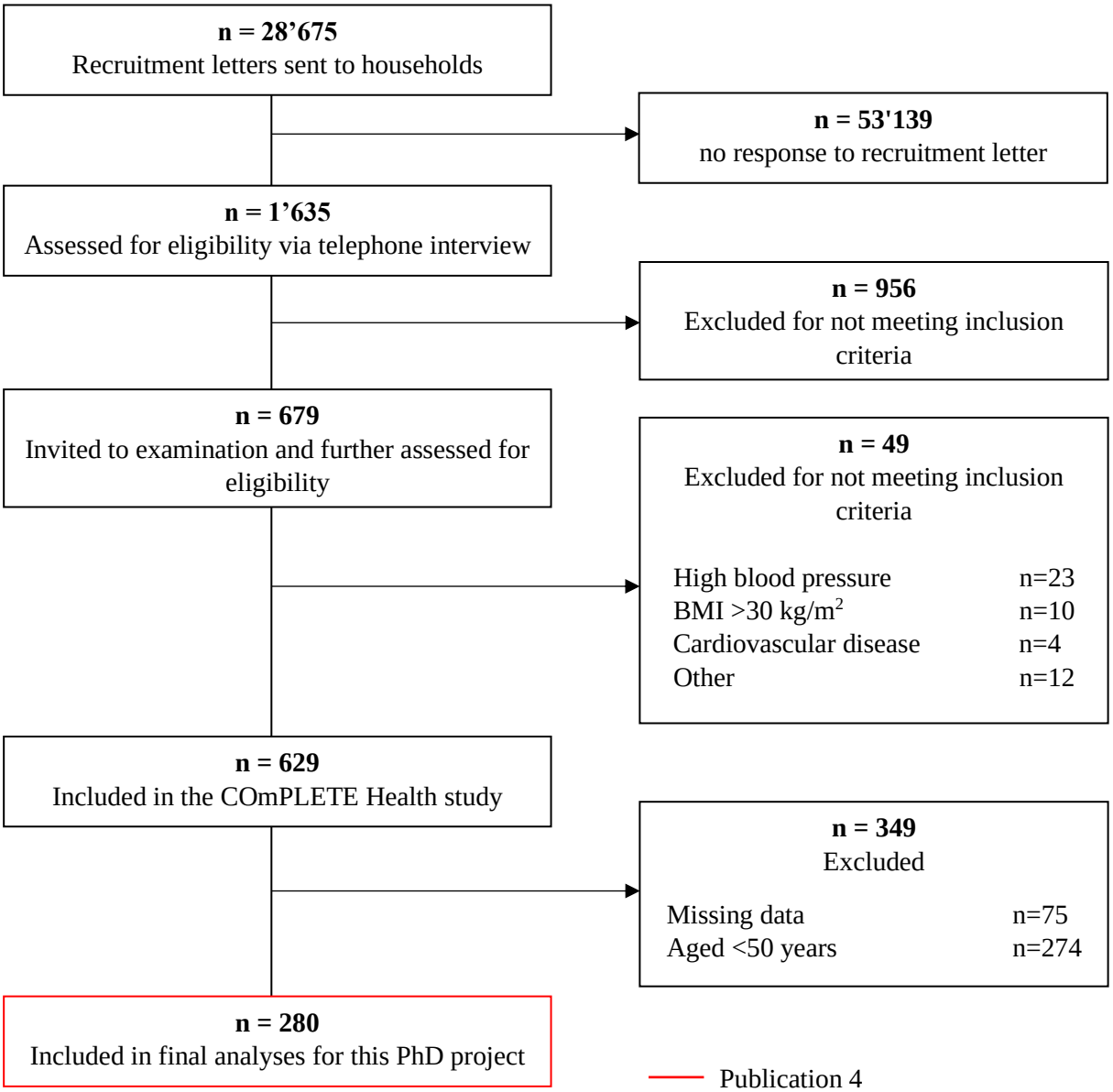


Figure 4: Flowchart of the COmPLETE Health study.

# Chapter 4 – Publication 1

## Validation of a visually aided dietary assessment tool to estimate dietary intake in an adult Swiss population

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**Abstract**

**Background:** Accurately assessing dietary intake is crucial for understanding how diet affects a person's health. In large cohorts, paper-based dietary assessment tools (DAT) such as food recalls or food frequency questionnaires have emerged as valid tools with a low burden for participants.
**Objective:** To validate a visually aided DAT for use in studies with Swiss adults against the gold standard of a weighed 7-day food record (7d-FR).
Design: Fifty-one adults (n=24 women, n=27 males) participated in the study and were recruited within two age groups (20-40y and 50-70y). Each participant filled out the visually aided DAT, then the 7d-FR. The DAT was compared to the 7d-FR for total energy intake, macronutrients, sugar, water, and portions of fruits and vegetables. Pearson correlation and Bland-Altman analyses were used for statistical analyses.
**Results:** Total correlations ranged from 0.288 (sugar, $p<0.05$) to 0.729 (water, $p<0.01$). The older age group showed higher correlations for total energy intake, protein, fats, carbohydrates, and sugar, but not for water ($p<0.05$). Correlations were moderate at $r > 0.5$, whereas only water and protein reached those values in the young group. Both groups overestimated total calories in kcal (+14.0%), grams of protein (+44.6%), fats (+36.3%), and portions of fruits and vegetables (+16.0%) but strongly underestimated sugar intake (-50.9%).
**Conclusions:** This DAT showed that all macronutrients and total energy intake were estimated more accurately by the older age group and therefore might be adequate to capture dietary habits in older Swiss adults.

**KEYWORDS:** Diet assessment tool, weighed food record, dietary intake, validation study, food frequency questionnaire

## 1. Introduction

The assessment of dietary intake in adults is one of the key elements in risk stratification when assessing chronic diseases such as diabetes, cardiovascular diseases, or other lifestyle-related non-communicable diseases [1]. Until now, there has been a trade-off between the accuracy of a dietary assessment method, its practicality, and its manageability in clinical trials [2]. As such, in scientific settings, 24h recalls and weighed food records (FR) as retrospective methods are most widely used [3]. 24h recalls aim to assess the food intake consumed in the 24 hours before the assessment day. The main advantages and disadvantages of 24h recalls have been well described [4]. FR are most used during a 4- or 7-day period (4d-FR / 7d-FR), where participants are asked to weigh and (or) report any food/drink item consumed during that time frame. This yields a precise overview of a person's food consumption, that may give information on dietary patterns during the week and (or) on weekend days. This prospective method, if properly conducted, counteracts a memory bias, which might occur in retrospective methods. However, FRs involve high effort by participants and evaluation of data by the study personnel can be burdensome because of high data volumes [5]. Although the FR is considered the gold standard for dietary assessment, several disadvantages should be considered that have been described previously [6, 7]. As a possible solution, food frequency questionnaires (FFQ) have emerged as more suitable options for large studies, making up for their lack of precision with their ease of use, the low burden for participants and study personnel, as well as the reduced costs when compared to other methods [3]. Further, FFQs assess dietary habits retrospectively, meaning that these habits are not altered during the assessment period [8]. Still, retrospectively assessing food intake may affect accuracy, and problems of underreporting or false reporting are recognized. In addition, each FFQ needs to be validated against the gold standard to ensure quality.

It has been extensively studied that study participants tend to answer questionnaires to fit social desirability [9-12]. This means that behavioral patterns that are commonly seen as "good" or "healthy" (e.g., daily physical activity, being non-smoker) are overreported, whereas patterns and behaviors that are seen as "unhealthy" tend to be underreported (e.g., high consumption of sugary drinks).

In epidemiological studies, it is important to accurately capture nutritional habits such as daily sugar intake or fruit and vegetable consumption. The number of fruits and vegetables consumed is important since an inverse association between fruit and especially vegetable consumption and the prevalence of metabolic syndrome has been reported in a meta-analysis [13-15]. The World Health Organization (WHO) recommends a daily intake of five portions of fruits and vegetables or roughly 400 grams for adults [16]. Conversely, the WHO recommends that sugar intake should be reduced to a maximum of 50 grams per day, as an increased intake of sugar is directly associated with the risk of obesity [13, 17]. To date, one Swiss study has investigated the amount of total sugar intake (in adults) and found that participants consumed 107 grams of sugar per day on average [18]. Although that study showed that sugar intake in Switzerland, as assessed via 24h-recall, is lower than in other countries (e.g., the Netherlands), it remains more than twice as high as the daily recommendations and Switzerland is listed as one of the European nations with the highest sugar consumption per capita [19, 20].

It was demonstrated that in many international studies, dietary habits (e.g., meal frequency, portion size, number of meals per day) differ greatly between younger and older adults [21-23]. At the time of the present study, most validation studies of dietary assessment methods had been conducted in younger adults while the assessment tools are widely used in studies of elderly people. Such tools generally have moderate correlations (r-value 0.40-0.59) between the gold standard and the validated assessment tool. Other tools that have been developed specifically for older populations might not be suitable for younger participants [23-25].
The present validation study was part of the Cardiopulmonary Exercise Testing (COmPLETE) study, which tested over 600 healthy adults and 80 patients with heart failure [26]. In the present study, we validated a visually aided dietary assessment tool (DAT) against the gold standard, the 7d-FR [27]. Total energy intake (kilocalories, kcal), macronutrients (grams), as well as water (liters), fruits and vegetables (portions), and sugar intake (grams) were examined. We aimed to assess whether this tool is useful to provide a valid estimate of all macronutrients, as well as for fruits and vegetables, and daily sugar intake. Finally, the present study aims at validating the assessment tool for younger adults, as well as older adults equally.

## 2. Methods

### *Study population and design*

Study participants were recruited between March and May 2021 through advertisements via email, online flyers, and word of mouth in northwestern Switzerland. Eligible participants were 20-40 or 50-70 years of age (sampling stratified by age), and mentally and physically able to follow the study protocol. Exclusion criteria were as follow: illness during the study period that affected diet, substantial lifestyle changes during the study (e.g., smoking cessation, diets), and a cardiac pacemaker since the conducted study included bio-impedance measures for body composition between the first and second visit. Information about present chronic diseases (e.g., heart failure, cancer, diabetes) and the use of medication were collected via a telephone interview before the first visit. Additionally, smoking status was assessed before the start of the study. Participants received written information detailing the procedures of the study and they gave written informed consent before participation.
On the first visit, anthropometric measurements were taken, including body composition using the bio-impedance (InBody 720, InBody Co. Ltd., Seoul, South Korea). Then, blood pressure was measured twice after 10 minutes of rest with an automatic blood pressure monitor system (Omron Healthcare, Germany). Participants were asked to fill out the paper form DAT before they were instructed on how to complete the 7d-FR. In addition, participants were asked not to change their dietary or physical activity habits during the monitoring period. The second visit occurred 7 or 8 days after the first visit and was identical to it. During the second visit, study personnel verified that the study protocol was followed and discussed the results of the 7d-FR with the participants. The sample size for the present study was determined according to a similar validation study from Switzerland [28]. The present study was approved by the Ethics Committee of Northwestern and Central Switzerland (EKNZ 2021-00406) and complied with the declaration of Helsinki.

***Dietary assessment***

*Dietary assessment tool:* During the first and second visit, to assess habitual food consumption, participants reported which food items they consumed on a "typical day" using the DAT. A "typical day" was defined by the study personnel as a day, on which participants followed a routine they would follow on most weekdays (e.g. normal workday, illness-free). The DAT as used in the present study is provided in the supplement. The DAT shows the food pyramid of the Swiss Society for Nutrition (SGE) (Version 2005 – 2011) on the left third of the page, a portion size equivalent for various food items of the respective category in the middle, as well as five mealtimes (breakfast, snack #1, lunch, snack #2, dinner) and a column for the sum of the five mealtimes. The food pyramid is divided into six levels, with several sub-levels, which are as follows:

1st section (top of the pyramid): Sweets (e.g., chocolate, cake, sweet beverages)

2nd section: part 1: vegetable oils, butter, nuts.

part 2: fatty meals (e.g., sausages, fried food, cream sauces)

3rd section: part 1: Meats and meat-like products (e.g., chicken, fish, tofu, eggs)

part 2: Dairy products (e.g., milk, yogurt, cheese)

4th section: Grains and legumes (e.g., bread, corn flakes, potatoes, pasta, lentils)

5th section: Vegetables and fruits, including fruit juices

6th section (base of the pyramid): Unsweetened drinks (e.g., water, tea, coffee)

Underneath the pyramid: alcoholic beverages (e.g., beer, wine)

*Seven-day food record:* Between the two visits (7-8 days apart), all participants were instructed to record their dietary intake over seven consecutive days. We used a modified version of the previously validated Freiburg Diet Protocol [29], which was developed by the German Federal Research Institute for Nutrition and Food. The FR was handed out in paper form. Participants were instructed to always keep the FR with them and to fill it out after each food or beverage consumption, irrespective of whether it was a meal or snack, to avoid lack of reporting. All participants received verbal and written instruction on how to keep track of their dietary intake and on how to use the DAT. Each page of the FR included additional written instructions. The FR had pre-defined food categories (e.g., bread, dairy products, legumes), with examples of foods for each category. The categories of the DAT and FR were similar, but the FR had more subcategories and food items. Additional space was provided on the paper forms to allow recording of consumed foods not listed. All items were listed with the standard portion sizes, and participants were asked to report the number and size of portions consumed throughout the day or the amount (in grams or milliliters). For best precision, participants were asked to weigh all consumed food items using their own kitchen scale. Because of the COVID-19 pandemic restrictions during the study, restaurants, canteens, and bars were closed, and private gatherings were limited to five people, meaning that most – if not all – meals were consumed at home. This potentially positively affected the precision of the measurements since all participants were

asked to weigh the food items with their own kitchen scale. Participants returned the completed 7d-FR at the time of the second visit, and they were able to discuss any issues they had encountered with the FR with the study personnel at that time.
All food items were entered into NutriGuide® Swiss (Version 4.9), an online software solution that calculates nutritional values of single food items, as well as for meals.

**3. Statistical analysis**

After completion of data collection, all 7d-FR were checked for plausibility and completeness by the study personnel.
Based on the food groups illustrated in the food pyramid of the DAT, all food items were categorized into sweets, fatty meals, fats, meat/meat-like products, dairy, grains, legumes, drinks, and alcohol. Each food group of the 7d-FR was matched with the above-listed food group of the DAT. For the DAT, nutritional values in kcal of the portion size equivalents were calculated with NutriGuide® Swiss and multiplied with the number of portions consumed by the participant for each of the above-mentioned food groups. The total was calculated by summing all food groups. For the 7d-FR, study personnel entered all food items into the NutriGuide® Swiss software, and nutritional values were calculated by averaging the caloric intake of the 7 days recorded.
Prior to data analysis, we tested for normal distribution of the data using the Shapiro Wilk test and found that the data of both the DAT and 7dFR was positively skewed ($p=0.01$ and $p=0.04$, respectively) [30]. The logarithmic transformation of the data showed no proportional bias (unstandardized β-coefficient = 0.106, $p=0.515$). Therefore, all data are presented as median and interquartile range (table 2). To calculate the differences between the medians reported in table 2, we performed a quantile regression for unpaired samples.
Because the data was not normally distributed, all macronutrients, as well as total calorie intake, were logarithmically transformed (log10) for the analyses. Bland-Altman plots (figures 1-4) were created for the log-transformed variables and transformed back to the original scale, as suggested by Euser et al. [31]. The 95% limits of agreement for the Bland-Altman plots were calculated as the average difference ± 1.96 standard deviations of the difference [32]. In accordance with Gerke (2020), we created QQ-plots, histograms of the differences, and histograms of the results of the Preiss-Fisher procedure, which were all non-problematic (not reported) [33].The Bland-Altman plots were created for the entire population and not by age group, as the p-values of the log-transformed data of DAT – 7d-FR were significant for both age groups. The correlation between macronutrients and water intake between the DAT and the 7d-FR were calculated using Pearson's r (Table 3). To check for any abnormal weight changes, a paired-samples t-Test was run between groups for pre and post measurements. Statistical analyses were performed using SPSS (IBM SPSS version 27.0. Armonk, NY). All tests were performed two-sided and p-values $<0.05$ were considered significant.

## 4. Results

One subject did not follow the study protocol correctly and was excluded from the analyses; hence, 51 subjects were included. Participant characteristics are depicted in Table 1. The age range was 21-67 years, with an average age of 24.3 years in the young group and 57.4 years in the old group. There was a significant difference between the groups regarding height, body mass index (BMI), waist-to-hip ratio (WHR), and diastolic blood pressure. No statistically significant difference was observed for systolic blood pressure; however, it has to be noted that three participants of the older group were taking blood pressure-lowering medication. In addition, there was no significant weight change between the first and second visits in either group.

We found significant differences between the 20-40 and 50-70 groups with regard to total energy intake ($p<0.01$) and grams of carbohydrates ($p=0.03$) for the DAT and 7d-FR (table 2). Further, we found that subjects aged 50-70 estimated significantly lower sugar consumption using the 7d-FR than subjects aged 20-40. The results of the present study (Table 3) display that this DAT shows a high correlation with the reference method in the older group. The highest correlation was found for total energy intake in the older group at 0.799, which was much higher than the young group (0.277, $p<0.01$). However, although the correlation for total energy intake in the old group was higher than the young group, the mean difference between total energy intake in the young group was lower (13.5%) than the old group (14.7%). In addition to total energy intake, correlations between the DAT and the 7d-FR were significantly higher in the old versus the young group for carbohydrates (0.776, vs. 0.228, $p<0.01$), fats (0.494, $p<0.05$ vs. 0.136, $p<0.01$), and sugar (0.479, $p<0.05$ vs. 0.184, $p<0.01$). No significant differences were observed for the other variables.

Regarding weight changes, we found that both groups were lighter at the second visit (0.3 kilograms in the young group, $p=0.06$ and 0.2 kilograms in the old group, $p=0.04$), with weight changes ranging from +1.7 to -2.1 kilograms. Only the weight change in the old group was significant ($p=0.04$). No significant changes in lean body mass were seen in either group during the study (all $p\geq0.2$). We found changes in fat mass in the young group ($p<0.01$) but not the old group ($p=0.375$). Table 4 shows the mean bias, as well as upper and lower limits of agreement for the Bland-Altman plots. Due to the data being back transformed on the original scale, table 4 further shows the mean slopes, as well as the slopes for the upper and lower 95% limits of agreement for total energy intake and macronutrients.

The Bland-Altman plots are shown in figures 1-4. Mean and 95% limits of agreement are displayed as solid lines, with the respective 95% confidence intervals displayed as dotted lines. As visible in table 2 and figures 1-4, the DAT appeared to overestimate total calorie intake, protein, and fat intake, whereas carbohydrates intake was underestimated. Bland-Altman analyses were not possible for fruit and vegetables intake, as they did not fulfill the necessary statistical criteria. In addition, all subjects in the old group were within the limits of agreement for all parameters. The difference between DAT and 7d-FR was 237 kcal for total energy intake, 35.1 grams (144 kcal) for protein, 76.7 grams (314 kcal) for carbohydrates, 32.8 grams (295 kcal) for fats, and 47.2 grams (194 kcal) for sugar (table 2).

**Table 1:** Characteristics of participants by age group.

| | Total | 20-40 years | 50-70 years |
|---|---|---|---|
| **Female, n (%)** | 51 (47.1) | 27 (40.7) | 24 (54.2) |
| **Age *(years)*** | 39.9 (17.1) | 24.3 (2.7) | 57.4 (4.5)** |
| **Height *(cm)*** | 174.6 (9.3) | 177.4 (10.2) | 171.5 (7.2)* |
| **Weight pre *(kg)*** | 72.0 (13.8) | 71.2 (14.8) | 73.0 (13.0) |
| **Weight post *(kg)*** | 71.8 (13.7) | 70.9 (14.7) | 72.8 (12.8) |
| **LBM pre *(kg)*** | 30.9 (1.0) | 32.8 (1.5) | 28.9 (5.7)** |
| **LBM post *(kg)*** | 30.9 (1.0) | 32.6 (1.5) | 28.9 (5.8)** |
| **Fat mass pre *(kg)*** | 17.0 (9.2) | 12.8 (8.1) | 21.7 (8.0)** |
| **Fat mass post *(kg)*** | 17.0 (9.3) | 13.2 (8.2) | 21.2 (8.8)** |
| **BMI *($kg/m^2$)*** | 23.5 (3.4) | 22.4 (3.1) | 24.7 (3.3)* |

Data are mean (SD) unless stated otherwise.
* Different from age group 20-40 years ($p<0.05$).
** Different from age group 20-40 years ($p<0.01$).
BMI, Body Mass Index; LBM, Lean Body Mass.

**Table 2:** Energy intake by macronutrients and group.

| Category | | Total | 20-40 years | 50-70 years |
|---|---|---|---|---|
| | | | n=27 | n=24 |
| **Energy (kcal)** | DAT | 2171 (1813-2514) | 2253 (1955-3011) | 1966 (1567-2431)** |
| | 7d-FR | 1934 (1554-2241) | 2122 (1702-2769) | 1609 (1420-2158)** |
| | Difference (%) | 12.3 | 6.2 | 22.2** |
| **Protein (g)** | DAT | 111.5 (87.5-133.0) | 118.2 (88.4-135.3) | 109.0 (79.4-123.7) |
| | 7d-FR | 76.4 (59.9-90.3) | 80.2 (63.7-97.5) | 70.6 (58.6-83.7) |
| | Difference (%) | 45.9 | 47.4 | 54.4 |
| **Carbohydrates (g)** | DAT | 183.8 (136.8-248.2) | 221.9 (179.3-278.8) | 169.7 (111.3-207.6)** |
| | 7d-FR | 206.5 (162.2-265.9) | 241.2 (184.0-292.7) | 184.6 (137.1-241.9)** |
| | Difference (%) | -11.0 | -8.0 | -8.1 |
| **Fats (g)** | DAT | 103.4 (89.2-126.3) | 104.9 (90.4-134.8) | 91.9 (87.0-122.9) |
| | 7d-FR | 70.6 (58.0-96.1) | 76.6 (59.6-115.3) | 65.1 (53.1-79.7) |
| | Difference (%) | 46.5 | 36.9 | 41.2 |
| **Sugar (g)** | DAT | 39.2 (31.1-54.2) | 39.2 (34.1-56.0) | 37.5 (27.1-50.7) |
| | 7d-FR | 86.4 (59.4-124.4) | 93.7 (66.3-133.4) | 74.9 (52.2-116.3)* |
| | Difference (%) | -54.6 | -58.2 | -49.9 |
| **Fruits and Vegetables (P)** | DAT | 3.0 (2.0-4.0) | 3.0 (2.0-4.0) | 2.5 (2.0-3.0) |
| | 7d-FR | 2.0 (1.3-3.3) | 2.0 (1.1-3.3) | 2.1 (1.4-3.5) |
| | Difference (%) | 50.0 | 50.0 | 19.0 |

All values are displayed as Median (Interquartile Range) in kilocalories (kcal), grams (g), or portions (P) per day. Difference in percent is calculated as follows: (DAT / 7d-FR)*100.
DAT, Dietary Assessment Tool; 7d-FR, Seven-Day Food Record.
* Different from age group 20-40 years ($p<0.05$).
** Different from age group 20-40 years ($p<0.01$).

**Table 3:** Correlations between the DAT and 7d-FR, by group.

| Group | Kcal | Protein | Carbohydrates | Fats | Sugar | Water |
|---|---|---|---|---|---|---|
| **Total** | 0.468 | 0.596** | 0.483** | 0.292** | 0.288* | 0.729** |
| **20-40 years** | 0.277 | 0.584** | 0.228 | 0.136 | 0.184 | 0.728** |
| **50-70 years** | 0.799** | 0.606** | 0.776** | 0.494* | 0.479* | 0.650** |

*Significant correlations ($p<0.05$).
** Significant correlations ($p<0.01$).
DAT, Dietary Assessment Tool; 7d-FR, Seven-Day Food Record.

**Table 4:** Mean bias and Limits of Agreement (LoA) of the Bland-Altman plots for total energy intake and macronutrients.

| variable | Mean bias | LoA upper CI | LoA lower CI | Mean bias slope | LoA upper CI slope | LoA lower CI slope |
|---|---|---|---|---|---|---|
| **Kcal** | 0.054 | 0.303 | -0.195 | -0.124 | 0.442 | -0.671 |
| **Carbohydrates** | -0.033 | 0.303 | -0.369 | 0.077 | 0.803 | -0.671 |
| **Protein** | 0.164 | 0.408 | -0.080 | -0.373 | 0.183 | -0.875 |
| **Fats** | 0.159 | 0.496 | -0.178 | -0.362 | 0.404 | -1.032 |

*Legend: CI, 95% confidence intervals.*

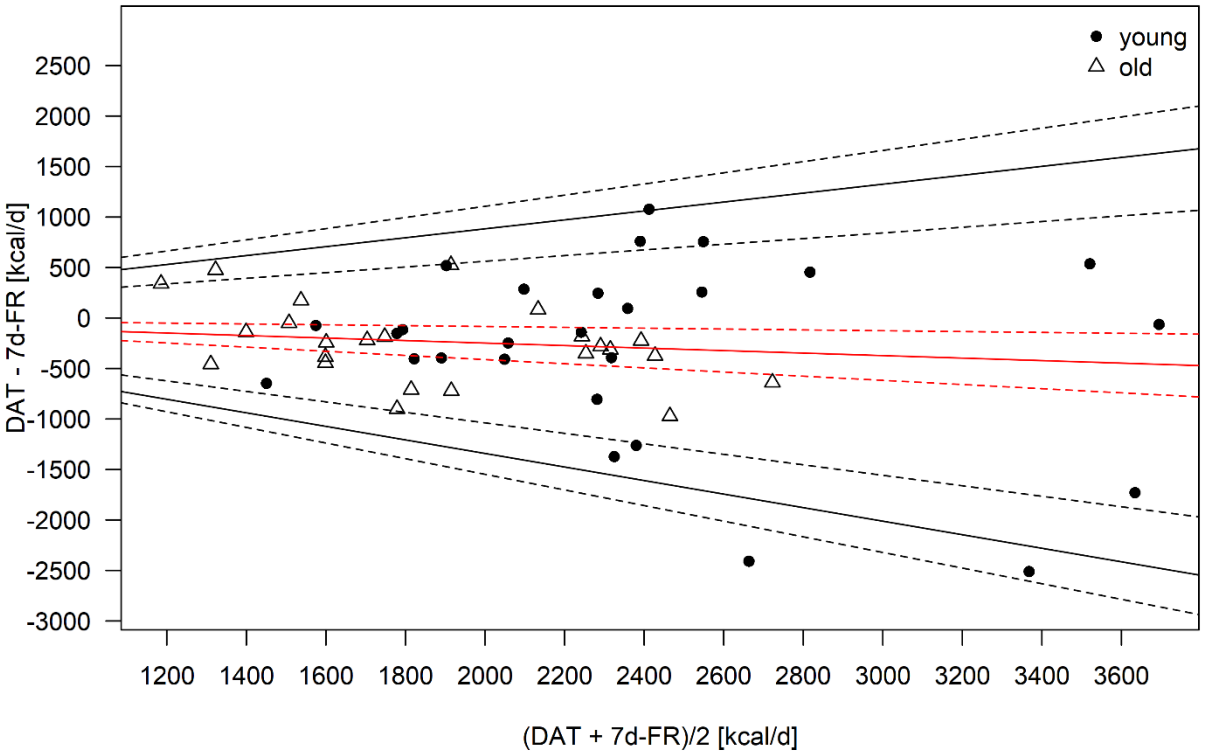


**Figure 1:** Bland-Altman Plot of the total calorie intake as calculated from the 7-day food record (7d-FR) and the visually aided dietary assessment tool (DAT). *Legend: Red solid line, mean; black solid lines, 95% limits of agreement; dotted lines, 95% of the respective solid lines.*

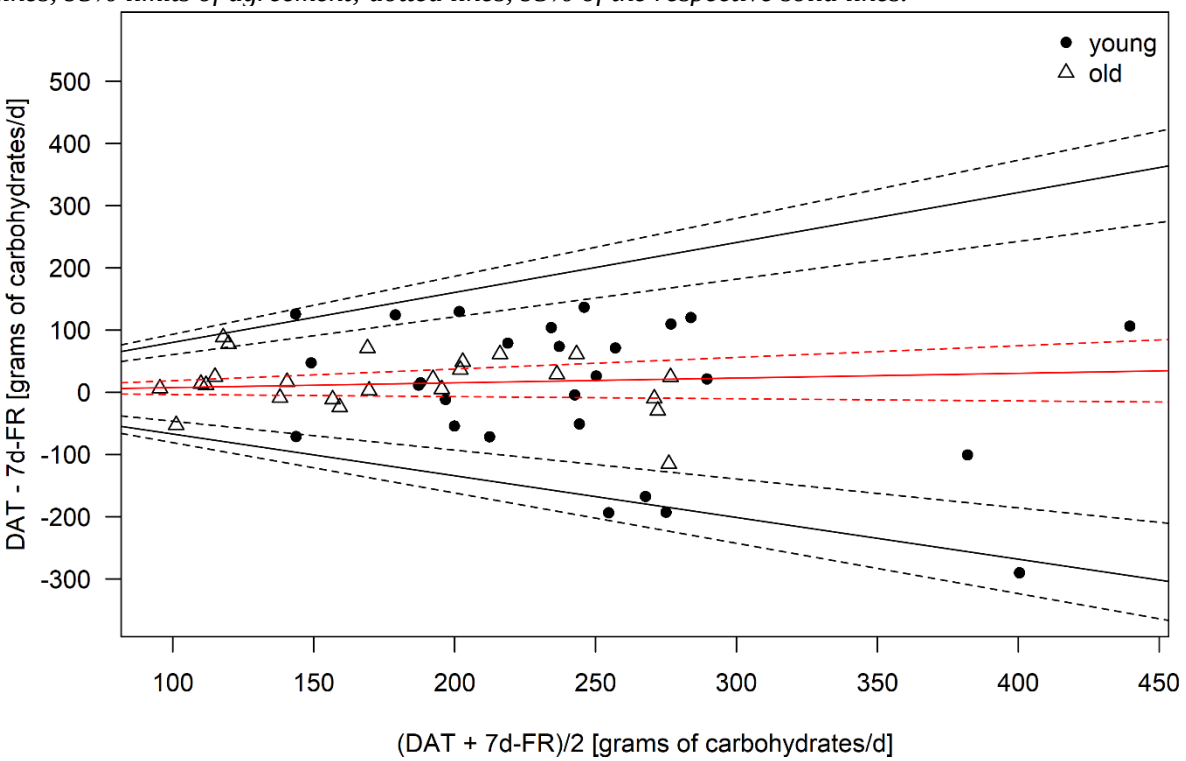


**Figure 2:** Bland-Altman Plot of the carbohydrate intake as calculated from the 7-day food record (7d-FR) and the visually aided dietary assessment tool (DAT). *Legend: Red solid line, mean; black solid lines, 95% limits of agreement; dotted lines, 95% of the respective solid lines.*

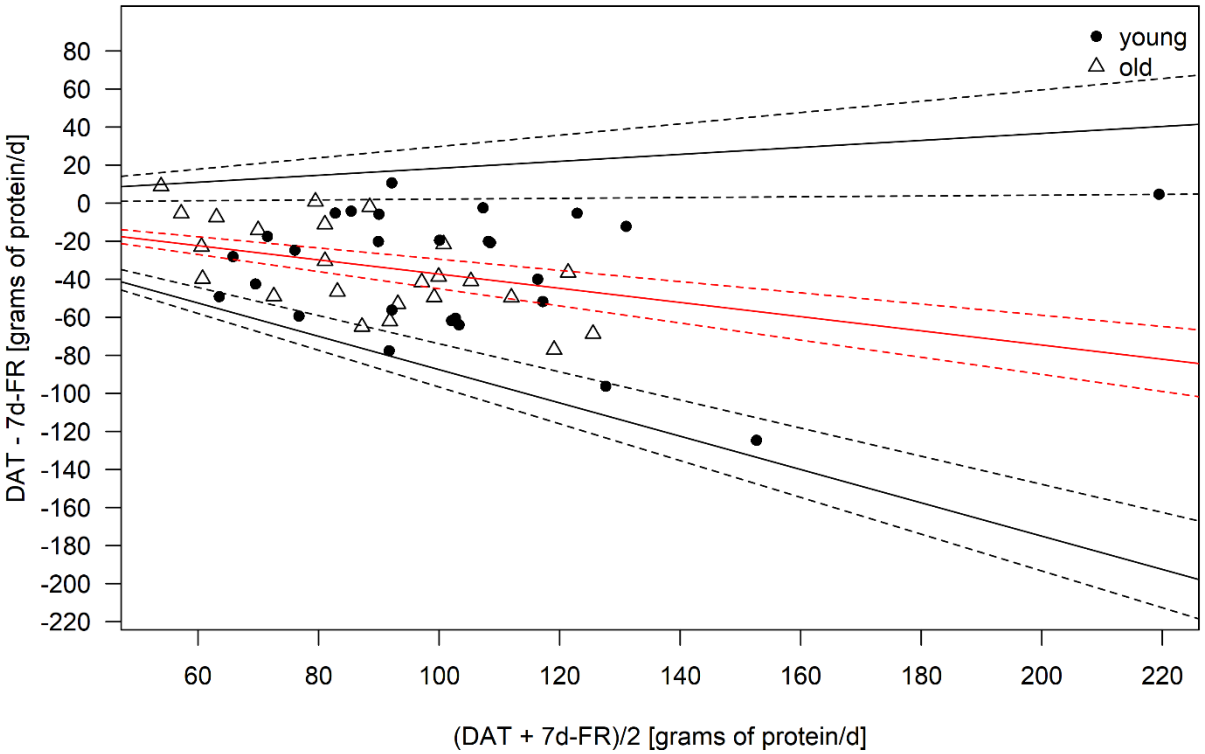


**Figure 3:** Bland-Altman Plot of the protein intake as calculated from the 7-day food record (7d-FR) and the visually aided dietary assessment tool (DAT). *Legend: Red solid line, mean; black solid lines, 95% limits of agreement; dotted lines, 95% of the respective solid lines.*

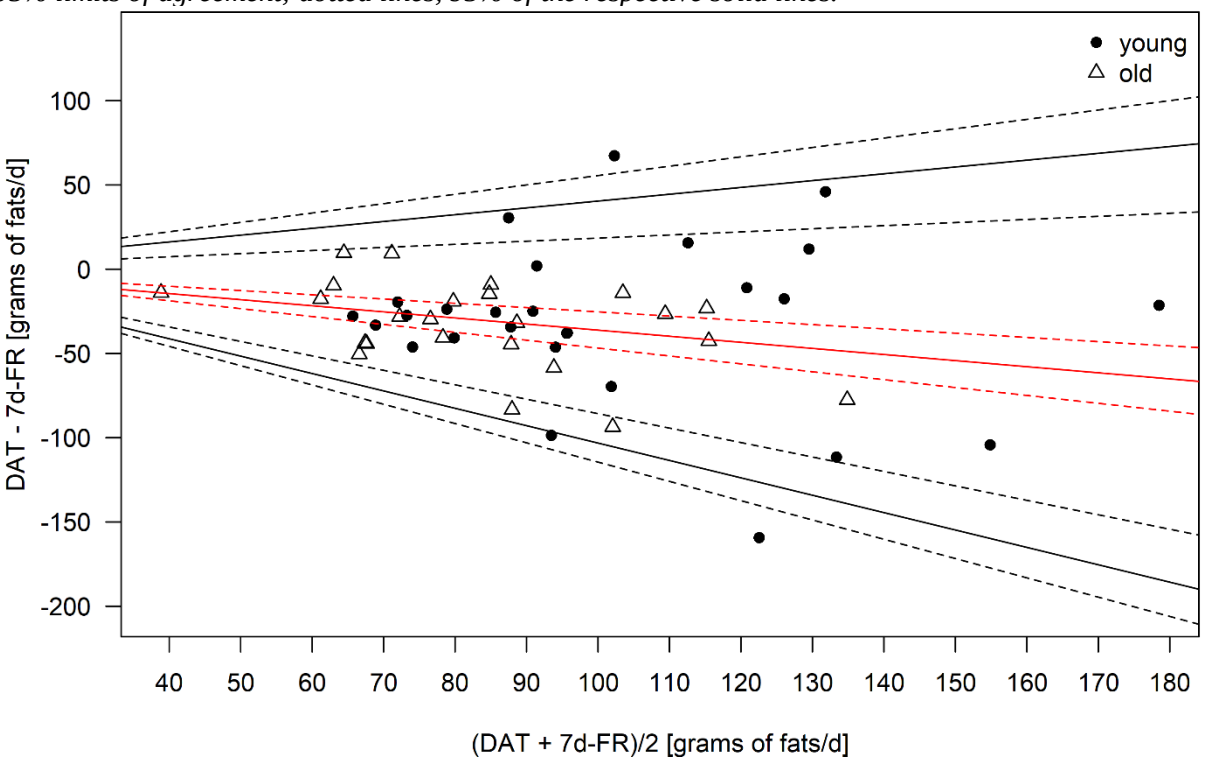


**Figure 4:** Bland-Altman Plot of the fat intake as calculated from the 7-day food record (7d-FR) and the visually aided dietary assessment tool (DAT). *Legend: Red solid line, mean; black solid lines, 95% limits of agreement; dotted lines, 95% of the respective solid lines.*

## 5. Discussion

The present study aimed to assess whether the visually aided dietary assessment tool (DAT) is a valid instrument to estimate food intake in Swiss adults, aged 20-40 and 50-70 years. The gold standard 7d-FR was used as a reference method and validity was investigated for all macronutrients (in grams), as well as total calorie consumption (in kilocalories), number of portions of fruits and vegetables, and sugar intake (in grams). The age groups were defined in a manner to discriminate between young adults, who tend to have less regulated daily routines than older adults [23, 34]. As demonstrated by Willet (1998), FFQs are commonly used to assess long-term dietary habits, which was the key area of interest in the present study [3]. We showed that the young group was able to estimate energy intake and all macronutrients more precisely than the old group. Across both groups, total energy intake, carbohydrates, and portions of fruits and vegetables were estimated more precisely than protein, fats, and sugar. However, correlations between the DAT and 7d-FR were stronger in the old group, with significant correlations for total energy, protein, carbohydrates, fats, sugar, and water. The only significant correlations that were observed in the young group were for protein and water. Notably, the correlation of 0.799 for total energy intake in our old group was much higher than in comparable, recent studies [35, 36].

Our study reveals that the DAT we used overestimated total energy intake. This is in line with other European studies, that reported differences between the DAT and FR [37, 38]. However, the recent systematic review by Sierra-Ruelas et al. (2021) revealed that most DATs tend to underestimate total energy consumption [39]. While the overestimation of energy in the overall population is acceptable (+12.3%), the Bland-Altman plots revealed that the DAT mostly overestimated fat and protein intakes, and that carbohydrates were underestimated. Since fats have an energy density of 9 kcal/g, an overestimation of fat consumption will inevitably affect the estimation of total energy intake. A potential explanation for the overestimation of fat intake in the DAT might be, that fats are highly represented in the DAT, making up a fast part of the upper section of the DAT. Further, the portion size of high-fat food items such as cheese or nuts is relatively high in the DAT, creating an overestimation of the consumption of high-fat foods. To our knowledge, we are the first to report back-transformed data in Bland-Altman plots for the validation of a DAT or FFQ. Therefore, we cannot precisely compare our results with other data. However, when comparing our findings with a similar validation study undertaken in Switzerland [28], we hereby show that the DAT we used appears to be more precise, especially in the older population which consumed less calories. However, no comparison can be made for the macronutrients.

Regarding the assessment of water consumption, there is no consensus on how to precisely assess intake [40]. However, as stated in an overview by Mons et al. (2007), food records should be preferred over retrospective methods because of higher precision [41]. In our study, we were able to demonstrate that contrary to the current consensus, our method assessed water intake with high precision. In contrast, our study showed that sugar intake was poorly estimated with a correlation between the DAT and 7d-FR of 0.184 ($p=0.40$) and 0.479 ($p<0.05$) in the young and old groups, respectively. Our population in the young group estimated a daily sugar intake of 50.6 (± 30.8) grams, which would be in accordance with the WHO recommendations. The old group estimated their sugar intake at 39.3 (± 17.1) grams per day. However, the 7d-FR

showed that daily sugar intake was approximately twice as high (101.7 grams in the young and 81.6 grams in the old group) in both groups, indicating that sugar intake estimation was a challenge regardless of age. The intake measured in the 7d-FR is in line with results published in 2019 on sugar consumption in Swiss adults [18]. The authors found that in a population aged 18-75 years of age total daily sugar intake equaled 107 grams on average, which is comparable to our results of 92.2 grams (101.7 grams for the young group, 81.6 for the young group). We found that both groups lost some weight during the study, on average 0.2 kilograms. It has been well documented that study subjects may change their dietary patterns to a healthier approach when being monitored to fit social desirability [7]. However, as recently reported by Turicchi et al. (2020), within-week weight fluctuations of up to 0.35% in body weight can be observed [42]. This placed both of our groups within acceptable weight change ranges.

The present study has several limitations. Firstly, the assessment method using a visual aid with a colored pyramid is suggestive. Study subjects see which food items are more socially accepted, as they belong to the food category which has a "healthy" image. These categories are shown in green or blue colors, in contrast to yellow, brown, or red for food items that are regarded as less desirable. However, the food pyramid is well known in Switzerland and therefore all participants were familiar with it.

The portion size equivalents shown in the food pyramid and estimated by the SGE were relatively broad. For example, a portion of bread ranged from 75-125 grams, and the type of bread is not specified. Since older people generally consume smaller portions than younger adults, this may have led to an overestimation of the portion size in the old group, and an underestimation in the young group [34]. Portion size estimation is considered one of the main reasons for inaccurate reporting in food questionnaires, and different portion sizes for various aged groups may be a valid solution [43].

Further, study personnel was present when participants filled out the DAT. Although study personnel was not actively watching the participants but rather performing other tasks, the presence of the personnel may have affected the estimations of the participants. In addition, the keyword "typical day" when assessing dietary habits was not defined using a specific period (e.g., last seven days, last month, or last year). While dietary intake may vary because of seasonality in some populations, an analysis using data from Swiss studies determined that seasonality decreased in the last decades and may not play a significant role today [44]. A limitation of our analyses is that we did not define limits of agreement a priori based on biologically and analytically relevant criteria, as has been suggested for the Bland & Altman plot system by Giavarina (2015) [45]. However, we are not aware of any validation studies with comparable dietary assessment methods (e.g., food frequency questionnaires), where the suggested approach has been applied and reported. Lastly, we determined our sample size according to previous studies but did not calculate the power for our analyses, therefore potentially not recruiting enough participants.

All participants used their private household scales since a standardized kitchen scale was not provided. No calibration of scales was therefore available. This may have led to discrepancies when weighing the food items and to systematic over-or underestimation of portion size. Therefore, energy intake as measured with the 7d-FR can only be estimated.

We also did not monitor physical activity. Weight loss, as observed in our study, is the result

of negative caloric balance. This is achieved either by lowering the total energy consumption or increasing physical activity and therefore energy requirements. It is possible that the old group did not achieve the weight loss through nutritional changes but rather by increasing physical activity. Lastly, the food pyramid that was used for the COmPLETE study and the present validation study was in use between 2005 and 2011. While there were no major changes in the pyramid since then, some trendy food (e.g., tofu, nuts, beans, lentils) may be underrepresented. The main strength of the present study was the use of the 7d-FR, which is regarded as superior to retrospective assessment methods for this kind of validation study. In addition, most, if not all meals were consumed at home due to the restrictions of the COVID-19 pandemic. This may have led to more precise dietary monitoring during the study.

## 6. Conclusions

The present study showed that a simple dietary assessment tool can be used effectively in an adult Swiss population. The highest correlation between the DAT and the gold standard 7d-FRwas achieved in people aged 50-70 years old but younger, as well as older adults overestimated total energy, protein, fats, and fruits/vegetables portions. Sugar intake was strongly underestimated. To conclude, this DAT appears to be a valid alternative to the more complex weighed food records in epidemiological studies to estimate dietary habits but not to calculate precise macronutrients intake.

**Author Contributions:** AST and GN were responsible for conceptualization and methodology. GN conducted the research, performed the statistical analyses, and wrote the manuscript. CB and NS provided expert advice for statistical analysis and manuscript review. AST was responsible for funding acquisition. All authors read and approved the final manuscript.

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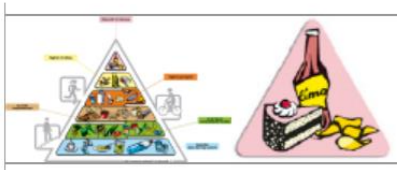
Visite 1 / 2

# FFQ 1 _____

**Supplement**

| profil | 1 Portion entspricht entweder/oder | Portionen/Tag Frühstück | Zwischen-mahlzeit | Mittag-essen | Zwischen-mahlzeit | Abend-essen | Portionen |
|---|---|---|---|---|---|---|---|
|  | 25 g Schokolade (1 Reihe/Riegel)<br>3 dl Süssgetränk<br>25–30 g salzige Snacks<br>3–4 Kekse (Guetzli)<br>30 g Kuchen (1 kleines Stück) | | | | | | Portionen/Tag |
|  | 10 g (1 EL) hochwertige Pflanzenöle (wie Raps-, Baumnuss-, Olivenöl) für die Zubereitung<br>10 g Butter oder Margarine<br>20–30 g (1–2 EL) ungesalzene Nüsse | | | | | | Portionen/Tag |
| | fettreiche Speisen:<br>Frittiertes, Paniertes, Rahmsaucen, ölhaltige Saucen, Wurstwaren (50–100 g) | | | | | | Portionen/Woche |
|  | 100–120 g Fleisch/Geflügel/Fisch<br>100–120 g Tofu, Quorn<br>2 Eier | | | | | | Portionen/Tag |
| | 2 dl Milch<br>180 g Joghurt/Sauermilch<br>150–200 g Quark/Hüttenkäse<br>40 g Hartkäse<br>60 g Weichkäse | | | | | | Portionen/Tag |
|  | 75–125 g Brot<br>45–75 g Getreideflocken, Teigwaren, Reis, Mais oder anderes Getreide (Rohgewicht)<br>180–300 g Kartoffeln<br>30–40 g Frühstücksflocken<br>60–100 g Hülsenfrüchte (z. B. Linsen) | | | | | | Portionen/Tag |
|  | Mind. 120 g Gemüse/Früchte (1 Handvoll)<br>2 dl Gemüse-/Fruchtsaft<br>50 g Blattsalat | | | | | | Portionen/Tag |
|  | Ungesüsste Getränke (Wasser, Mineralwasser, Tee, Kaffee)<br>1 Glas/1 Tasse (1.8–2 dl) | | | | | | Portionen/Tag |
| | Alkoholische Getränke<br>Standarddrinks (nicht für Jugendliche)<br>1 dl Wein oder 3 dl Bier | | | | | | Portionen/Tag |

*Quelle: Schweizerische Gesellschaft für Ernährung SGE*

## Chapter 5 – Publication 2

# Adherence to Life's Simple 7 is associated with better carotid properties

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**Abstract**

**Background and aims**

Cardiovascular health scores have emerged as a simple way to assess the risk to suffer from a cardiovascular disease. The American Heart Association's Life's Simple 7 constitutes of modifiable lifestyle factors to reduce cardiovascular risk. Its association with carotid properties, is yet inconclusive. The aim is to determine the association between the adherence to Life's Simple 7 and carotid properties in middle-aged to elderly Finns.

**Methods**

A representative sample of Finnish men and women aged 55-74 years old was included in the present study. Carotid intima-media thickness (cIMT), lumen diameter (cLD), and carotid distensibility were measured by transcutaneous ultrasound using state-of-the-art wall contour detection techniques. The Life's Simple 7 cardiovascular health score was calculated using seven categories (body-mass index, cholesterol, systolic blood pressure, fasting plasma glucose, smoking status, physical activity, and diet). In accordance with the American Heart Association, for each category, an ideal score was given 2 points, intermediate scores 1 point, and poor scores 0 points.

**Results**

In total, 1400 (49.4% male) subjects were included in the analyes. After adjusting for age and sex, we found that subjects with an ideal cardiovascular health score had lower cLD than those with an intermediate score (-0.21 mm, 95% CI: -0.37 – -0.05 mm, p=0.005) and a poor score (-0.39 mm, 95% CI: -0.65 – -0.12 mm, p=0.001). Similarly, subjects with an ideal health score had higher carotid distensibility than those with an intermediate score (0.0032 1/kPa, 95% CI: 0.009 – 0.0055 1/kPa, p=0.002) and a poor score (0.0018 1/kPa, 95% CI: 0.0005 – 0.0032 1/kPa, p=0.004). We found no differences regarding cIMT.

**Conclusions**

In middle-aged to elderly Finns, higher adherence to the Life's Simple 7 is associated with lower cLD and higher distensibility, but not with cIMT. Adherence to healthy lifestyle habits is therefore associated with better carotid structure and carotid function in middle-aged to elderly Finns.

**KEYWORDS:** cardiovascular health score, carotid lumen diameter, carotid intima-media thickness, carotid distensibility, lifestyle

## Visual Abstract

## Introduction

Trends from 2000-2019 report that cardiovascular diseases are the main cause of death in the world, with 7 out of the top 10 leading causes of death worldwide being noncommunicable diseases [1]. The risk to suffer from a noncommunicable disease is normally the product of multiple risk factors such as high systolic blood pressure, obesity, or physical inactivity [2]. To assess that risk, several cardiovascular risk scores, such as the Framingham Risk Score, or the European SCORE2, have been introduced [3, 4]. These risk scores consist of modifiable and non-modifiable components. In contrast to the risk scores, the American Heart Association created a cardiovascular health score, The Life's Simple 7, in an attempt to reduce cardiovascular death in the USA by 20%. [5]. The Life's Simple 7 define the most important lifestyle habits that should be implemented to achieve optimal cardiovascular health. Ideal cardiovascular health is defined as the absence of any cardiovascular disease (CVD), plus fully implementing the Life's Simple 7 [6]. Previously, adherence to the Life's Simple 7 has been shown to be associated with lower CVD risk in elderly North American [7], and European populations [8].

While CVD risk can be calculated using various formulas, it can also be measured with the assessment of carotid wall structure and function, which are vascular biomarkers for atherosclerosis [9, 10]. Atherosclerosis describes the structural (thickening) and functional (stiffening) of the arterial walls, plus the widening of the arterial diameter. The structural changes of the carotid artery are commonly measured as carotid intima-media thickness (cIMT), whereas arterial stiffening can be measured using carotid distensibility [10]. Carotid lumen diameter (cLD) shows structural and functional changes in the carotid artery [11]. All three parameters are proven to be reliable to assess cardiovascular risk and are predictors for cardiovascular events, such as stroke and myocardial infarction [12-15].

In Finland, the risk of suffering a cardiovascular event is amongst the highest in Europe [16]. While Life's Simple 7 and the assessment of carotid properties have been used separately, their association has yet to be investigated in the general Finnish population.

Therefore, the aim of the present study is to determine the association between the adherence to Life's Simple 7 and carotid properties in middle-aged to elderly Finns.

## Methods

### Design and participants

The Dose-Responses to Exercise Training (DR's EXTRA) study was designed to assess the effects of a lifestyle intervention (regular aerobic exercise, strength training, healthy diet, or a combination of these) on carotid properties, cognition, and endothelial function, over the course of a four-year randomized control trial (ISRCTN45977199, http://isrctn.org). In the present study, we focused on analyzing baseline data. Recruitment for the DR's EXTRA study was undertaken between 2002-2004, when a random sample (stratified by sex) of 3,000 community-dwelling Finnish men and women aged 55-74 years old, was obtained from the people's registry of the city of Kuopio, Finland. Exclusion criteria consisted of any medical or other conditions

that do not allow to participate in an exercise intervention, as judged by a study physician. The full study overview, including the recruitment process, has already been published [17, 18]. Participants provided written informed consent. The study complied with the Declaration of Helsinki and was approved by the Research Ethics Committee of the Hospital District of Northern Savo, Finland.

**Assessment of outcomes: carotid intima-media thickness and carotid lumen diameter**
Four expert sonographers assessed carotid properties using B-mode ultrasonographical imaging. The measurement was performed on the right and left common carotid artery (CCA) and the carotid bulb after the subjects had at least a 15-min rest in a supine position following the Mannheim Consensus [19]. All measurements were recorded in full length and expert ultrasound readers selected the frames where cIMT on the far wall could be best detected for further analyses. To ensure the validity of the cIMT calculations, 4 frames were selected for each subject (2 frames at end-diastole (directly before ascent of R-wave of the ECG), on the left and right CCA, respectively). The frames were analyzed via the fully automatic and validated B-mode image analysis program DYARA (DYnamic ARtery Analysis) [10, 20]. The reader marked a bounding box of 1 cm length in x-axis including far and near wall within the distal 2 cm of the CCA, and the program measured cIMT at the far wall as average over 1 cm, as well as average outer cLD (from adventitia-media border at far wall to media-adventitia border at near wall). If ultrasound quality was partially insufficient for the program to detect the wall or the intima-media complex, the reader manually corrected the respective segment of the carotid wall. The results of each frame were then exported and the mean of 4 end-diastole values, were used for all analyses. Carotid distensibility was calculated as $\frac{(2 \times delta\ cLD \times diastolic\ cLD)+(delta\ cLD)^2}{(PP \times diastolic\ cLD)^2}$, whereas delta cLD equated to systolic cLD minus diastolic cLD, and pulse pressure (PP) equated to systolic blood pressure minus diastolic blood pressure [10].

**Assessment of physical activity and diet**
Physical activity was measured at baseline via a 12-month retrospective questionnaire, which included the most commonly performed activities in Finland (e.g., cross-country skiing, walking, cycling) [21]. Only at-least moderate physical activity had to be considered and participants were asked to report the frequency of each type of physical activity, the duration, and intensity on a 1-4 scale, whereby 1 was considered light, and 4 very heavy physical activity. Dietary intake was assessed using a 4-day food record (4d-FR), which included three weekdays and one weekend day. All instructions on how to fill out the 4d-FR were given verbally and in written form during the study visit. Food intake was estimated using a food use questionnaire that included individual food items, as well as mixed dishes, and a portion size picture booklet [22]. The 4d-FR were then analyzed using the MicroNutricaw nutrient calculation software (version 2.5; Finnish Social Insurance Institution), based on Finnish and international food

consumption tables [23]. To determine dietary quality, a score from 0-4 was used [24]. The four dietary goals were: ≥400 g of vegetables per day, ≥2 servings of fish per week (=30 g of fish per day), ≥14 g of fiber per 1000 kcal, and <10 energy percentage of daily intake of saturated fatty acids.

**Other measurements**

Height and weight were measured by the nearest 0.1 cm and 0.1 kg. Body-mass index (BMI) was calculated as weight in kg divided by the squared value of height in meters. Blood samples were collected after a 12-hour fasting period by a trained physician to determine high-density lipoprotein (HDL), low-density lipoprotein (LDL), fasting blood glucose, and cholesterol, by standard laboratory analysis. Blood pressure was measured in a seated position, after subjects had rested for 5 minutes. Use of medication, smoking status, alcohol consumption, and medication were assessed via questionnaire.

**Cardiovascular health score**

We used the Life's Simple 7, a surrogate for ideal cardiovascular health introduced by the American Heart Association in 2010 [5]. The health score consists of 7 modifiable metrics, 4 of which are considered behavioral (physical activity, diet, smoking status, BMI), and 3 of which are considered biological (systolic blood pressure, fasting plasma glucose, and cholesterol). For each variable used for the health score, a study participant could either score 0 (poor), 1 (intermediate), or 2 (ideal) points. Therefore, the health score ranged from 0 (poor) to 14 (ideal). The limits of each variable used in the health score were the same as the one's presented by the American Heart Association [5]. Scoring 0-4 points was considered poor, 5-9 intermediate, and 10-14 ideal.

**Statistical analyses**

The primary outcome of the present study was to determine the relationship between the cardiovascular health score and carotid artery properties. Continuous variables are shown as means ± SD and dichotomous variables as frequencies and percentages. To determine the differences between men and women, we performed an independent samples t-test.

An analysis of variance (ANOVA) with a Tukey's post-hoc test was performed to determine the difference between the subjects achieving a poor, intermediate, and ideal cardiovascular health score. All ANOVA were adjusted for age, sex, and current medication. We used violin plots to visualize the carotid properties for each cardiovascular health score category (i.e., poor, intermediate, and ideal).

To test whether the behavioral or biological metrics were more predictive for carotid properties, we used a linear regression model adjusted for age, sex, and medication [25]. In a first step, R2 was calculated for the full model (i.e., all 7 health metrics). In a second and third step, the regression was calculated with the behavioral (BMI, physical activity, diet, smoking status) and the biological (fasting plasma glucose, cholesterol, systolic blood pressure) metrics. To determine the fraction of new predictive information provided by either the behavioral or the

biological metrics, we calculated 1 – R2(biological)/R2(full) and 1 – R2(behavior)/R2(full), respectively.

All statistical analyses were 2-sided, and p-values of <0.05 were considered statistically significant. We used the IBM SPSS Statistics for Windows, version 26.0 (SPSS Inc., Chicago, Illinois, USA), and R version 4.1.3 for Windows (R Foundation for Statistical Computing, Vienna, Austria).

**Results**

Of the 1410 participants in the study (47% response rate), 10 (0.7%) did not have valid ultrasound data, therefore 1400 (49.4% male) participants were included in the analyses. In total, 79 (40.5% male) subjects had a poor health score, 1133 (48.8% male) had an intermediate score, and 170 (56.5% male) had an ideal health score. Of note, none of the subjects had 14 points, only one subject had 13 points, while none had 0 points and one subject had 1 point.

Characteristics for the entire population, divided into men and women, are depicted in table 1. Women had a significantly higher health score than men (3.1 ± 1.3 vs 2.6 ± 1.3, p<0.001). Women had significantly higher systolic blood pressure (149 ± 21 mmHg vs. 144 ± 19 mmHg, p<0.001), higher total cholesterol (5.25 ± 0.92 mmol/L vs. 4.89 ± 0.93 mmol/L, p<0.001), lower fasting plasma glucose (5.66 ± 0.84 mmol/L vs. 6.02 ± 1.07 mmol/L), and higher diet score (1.8 ± 1.2 vs. 1.7 ± 1.1, p=0.011) than men. Less women than men were current, former, and non-smokers (p<0.001), while no differences were found for BMI (p=0.910), as well as moderate and vigorous physical activity (p=0.322).

The results of the ANOVA showed a small, but significant effect for an ideal health score being associated with lower cLD: $F(1,1375) = 139.3$, $p<0.001$, $\eta 2 = 0.091$. A posthoc test using Tukey's method showed that subjects with an ideal cardiovascular health score had lower cLD than those with an intermediate (7.03 ± 0.71 mm vs. 7.24 ± 0.85 mm, p=0.005) or poor (7.03 ± 0.71 mm vs 7.42 ± 0.96 mm, p=0.001) cardiovascular health score. There were no significant differences between a poor and an intermediate health score for cLD.

For carotid distensibility, the ANOVA also showed a small, but significant effect for an ideal health score being associated with higher carotid distensibility: $F(2,1375) = 7.1$, $p<0.001$, $\eta 2 = 0.007$.

Subjects with an ideal cardiovascular health score had higher carotid distensibility than those with an intermediate (0.0134 ± 0.0075 1/kPa vs. 0.0116 ± 0.0069 l/kPa, p=0.002) and poor (0.0134 ± 0.0075 1/kPa vs. 0.0102 ± 0.0070 l/kPa, p=0.004) cardiovascular health score. There was a non-significant effect for a higher health score being associated with lower cIMT: $F(2,1375) = 2.48$, $p=0.084$, $\eta 2 = 0.002$ (table 2 and figure 1).

We found that for cIMT, the biological and behavioral health metrics explained very little of cIMT ($R^2$ change = 0.011, p=0.008, and $R^2$ change = 0.005, p=0.455), respectively. Although the $R^2$ change for the biological health metrics was twice as high as the behavioral health metrics, the strongest predictor for cIMT was age ($R^2$ change = 0.098, p <0.001). Sex added

little predictive value ($R^2$ change = 0.005, p=0.008), and for medication, only antihypertensives added low, but significant predictive value ($R^2$ change = 0.003, p=0.036). In contrast, in regards to cLD, behavioral health metrics explained more than the biological health metrics (R2 change = 0.045, p <0.001, and $R^2$ change = 0.017, p <0.001), respectively. The strongest predictor for cLD was sex ($R^2$ change = 0.091, p <0.001), whereas age was less predictive ($R^2$ change = 0.032, p <0.001) and as for cIMT, only antihypertensives added predictive value ($R^2$ change = 0.017, p<0.001). For carotid distensibility, the behavioral health metrics were also a better predictor than the biological metrics ($R^2$ change = 0.042, p <0.001, and $R^2$ change = 0.017, p=0.054), respectively. Age and sex were less predictive than the behavioral health metrics (R2 change = 0.024, p <0.001 for age, and $R^2$ change = 0.006, p=0.004 for sex). For medication, only antihypertensives added predictive value for carotid distensibility ($R^2$ change = 0.017, p <0.001). The results of the logistic regression are depicted in supplement table 1.

Supplement table 2 shows the mean and the differences of the variables used for the health score, as well as the variables for the carotid properties between the poor, average, and ideal cardiovascular health score, by sex.

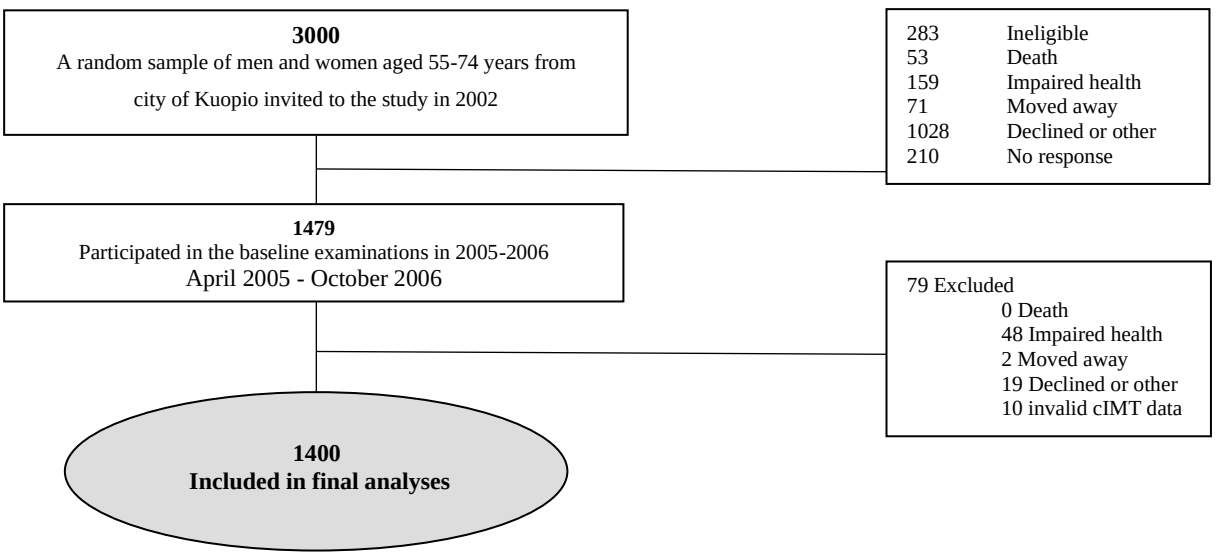


Figure 1: Study flowchart of the DR's EXTRA study.

Table 1: Characteristics of the participants, by sex.

| | All (n=1400) | Men (n=692) | Women (n=708) | p-value |
|---|---|---|---|---|
| **Age (years)** | 66.4 (5.4) | 66.3 (5.4) | 66.6 (5.3) | 0.252 |
| **cIMT (mm)** | 0.824 (0.142) | 0.834 (0.149) | 0.814 (0.134) | 0.009 |
| **cLD (mm)** | 7.23 (0.90) | 7.58 (0.89) | 6.88 (0.76) | <0.001 |
| **Carotid distensibility (1/kPa)** | 0.0117 (0.0070) | 0.0124 (0.0071) | 0.0112 (0.0069) | 0.001 |
| **Systolic blood pressure (mmHg)** | 147 (20) | 144 (19) | 149 (21) | <0.001 |
| **Diastolic blood pressure (mmHg)** | 83 (9) | 84 (9) | 82 (9) | <0.001 |
| **Heart rate (bpm)** | 62 (9) | 61.5 (9.5) | 62.9 (8.8) | <0.001 |
| **Body-mass index (kg/m$^2$)** | 27.6 (4.5) | 27.7 (3.9) | 27.6 (5.0) | 0.910 |
| **Cholesterol (mmol/L)** | 5.07 (0.94) | 4.89 (0.93) | 5.25 (0.92) | <0.001 |
| **Triglycerides (mmol/L)** | 1.35 (0.70) | 1.39 (0.75) | 1.31 (0.64) | 0.031 |
| **HDL (mmol/L)** | 1.69 (0.48) | 1.52 (0.42) | 1.86 (0.48) | <0.001 |
| **LDL (mmol/L)** | 3.21 (0.84) | 3.18 (0.84) | 3.23 (0.84) | 0.256 |
| **Fasting plasma glucose (mmol/L)** | 5.84 (0.97) | 6.02 (1.07) | 5.66 (0.84) | <0.001 |
| **Light PA (min/week)** | 133 (216) | 131 (220) | 135 (213) | 0.781 |
| **Moderate PA (min/week)** | 289 (314) | 282 (331) | 295 (297) | 0.428 |
| **Vigorous PA (min/week)** | 112 (165) | 125 (175) | 99 (154) | 0.003 |
| **Moderate + Vigorous PA (min/week)** | 411 (354) | 421 (376) | 402 (330) | 0.322 |
| **Diet Score** | 1.7 (1.2) | 1.7 (1.1) | 1.8 (1.2) | 0.011 |
| **Diabetes medication, n(%)** | 99 (7.1) | 64 (9.2) | 35 (4.9) | 0.002 |
| **Cholesterol-lowering medication, n(%)** | 490 (35.0) | 244 (35.3) | 246 (34.7) | 0.840 |
| **Antihypertensive medication, n(%)** | 587 (41.9) | 287 (41.5) | 300 (42.4) | 0.733 |
| **Hormone-replacement therapy n(%)** | 371 (26.5) | 24 (3.5) | 347 (49.0) | <0.001 |
| **Smoking status n(%) Never** | 762 (54.4) | 243 (35.1) | 519 (73.3) | <0.001 |
| **Former** | 499 (35.6) | 360 (52.0) | 139 (19.6) | <0.001 |
| **Current** | 137 (9.8) | 88 (12.7) | 49 (6.9) | <0.001 |

Values are means ± SD or n (%). *Legend: BMI, Body-Mass Index; cIMT, carotid intima-media thickness; cLD, carotid lumen diameter; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PA, physical activity.*

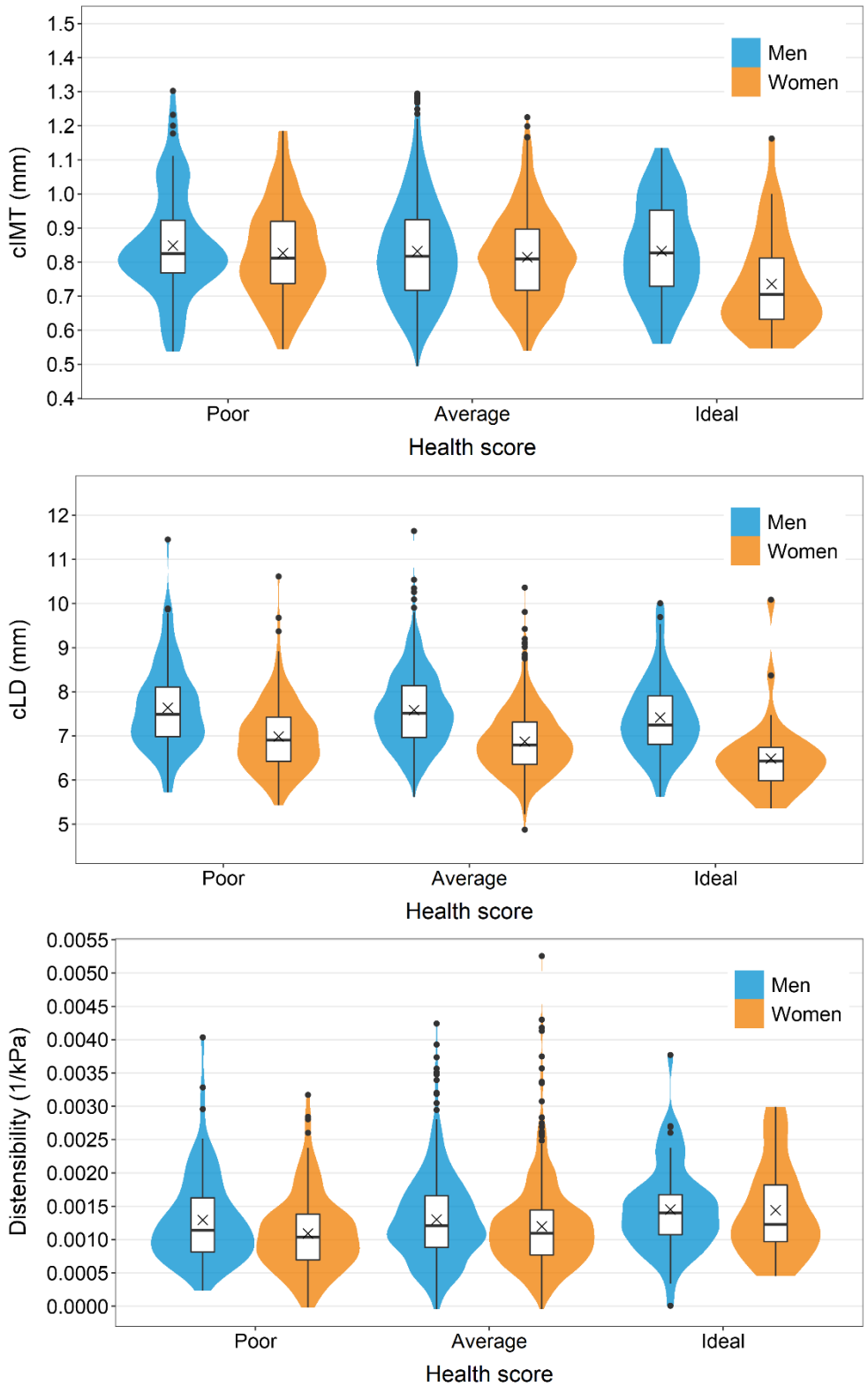


Figure 2: Violin plots of the carotid properties, by sex. *Legend:* cIMT, carotid intima-media thickness; cLD, carotid lumen diameter.

Table 2: Carotid properties, by health score category. Adjusted for age and sex.

| | Simple 7 | N | Mean (SD) | Mean difference | | 95% CI | | p-value |
|---|---|---|---|---|---|---|---|---|
| **cIMT (mm)** | Poor | 79 | 0.824 (0.141) | Poor – Int. | -0.003 | -0.040 | 0.036 | 0.999 |
| | Int. | 1133 | 0.827 (0.145) | Poor – Ideal | 0.022 | -0.067 | 0.022 | 0.532 |
| | Ideal | 170 | 0.802 (0.127) | Int. – Ideal | 0.025 | -0.052 | 0.002 | 0.076 |
| **cLD (mm)** | Poor | 79 | 7.42 (0.94) | Poor – Int. | 0.18 | -0.05 | 0.40 | 0.177 |
| | Int. | 1133 | 7.24 (0.85) | Poor – Ideal | 0.39 | 0.12 | 0.65 | 0.001 |
| | Ideal | 170 | 7.03 (0.72) | Int. – Ideal | 0.21 | 0.05 | 0.37 | 0.005 |
| **Distensibility (1/kPa)** | Poor | 79 | 0.0102 (0.0009) | Poor – Int. | -0.0014 | -0.0033 | 0.0057 | 0.251 |
| | Int. | 1133 | 0.0116 (0.0008) | Poor – Ideal | -0.0032 | -0.0055 | -0.0009 | 0.002 |
| | Ideal | 170 | 0.0134 (0.0007) | Int. – Ideal | -0.0018 | -0.0320 | -0.0046 | 0.004 |

*Legend:* cIMT, carotid intima-media thickness; cLD, carotid lumen diameter; Int., Intermediate cardiovascular health score.

## Discussion

This study aimed at determining the association between poor, intermediate, ideal cardiovascular health, and carotid properties in an elderly Finnish population. We demonstrated that in men and women aged 55-74 years old, a higher cardiovascular health score adjusted for sex and age was associated with lower cLD and distensibility, but not cIMT.

To date, data on the AHA's Life's Simple 7 and carotid structure is scarce and inconclusive. In regards to cLD, to the best of our knowledge, we are the first to show an association between cLD and the Simple 7. The measurement of cLD as a biomarker for cardiovascular risk has gained traction in recent years, as cLD is known to be influenced by age [26], but also by several modifiable risk factors such as physical activity, systolic blood pressure, or BMI [27-29]. In healthy arteries, elastin, a protein in the extracellular matrix which allows the arteries to expand and relax with each cardiac cycle, and collagen, which stiffens the arteries, work in tandem to ensure ideal blood flow to the periphery. With age or because of poor lifestyle, changes in cLD occur through the fragmentation of elastin, which causes the arteries to stiffen [30]. This phenomenon stimulates arterial dilation as a compensatory mechanism, making cLD a sensitive biomarker for cardiovascular health. With our results, we provide further evidence to the case for cLD measurement in middle-aged adults to assess cardiovascular risk, confirming the results from a recent study which found that including cLD improved a model predicting all-cause mortality, as opposed to including cIMT [13].

Regarding carotid function and cardiovascular health scores, only one study that included middle-aged to elderly men and women has been reported. The French Paris Prospective Study III (PPS3) study found that subjects with ideal cardiovascular health had significantly higher carotid distensibility when compared with subjects with a poor cardiovascular health score [31]. Our results are in agreement with PPS3, as we showed that only ideal cardiovascular health was associated with higher carotid distensibility. We hereby strengthen the evidence that maintaining high cardiovascular health is imperative to maintain high carotid function and that average cardiovascular health may not be sufficient.

In contrast to cLD, more data is available on cIMT and the Simple 7. A Vietnam-based study

showed that cardiovascular health was associated with lower cIMT in twins and was not confounded by genetic factors [32]. Our results contradict these findings. Although speculative, the different population and the difference in methodologies of cIMT measurements (radiofrequency vs. image-based) may provide a partial explanation. The Vietnamese results were however confirmed by PPS3, and a Chinese study, with higher effect sizes for men than women in PPS3, but higher effect sizes for women in China [31, 33]. In contrast, a study with Icelandic subjects found no differences in cIMT, regardless of cardiovascular health score and sex [34]. This is in line with our results from a similar region of the world, where we showed no cIMT differences between subjects with a low, average, or ideal cardiovascular health score in a middle-aged to elderly Nordic population. As opposed to PPS3, our study population was recruited using the people's registry and not via a primary health care center, which may better reflect the status of the general population.

In addition, there is a clear north-to-south gradient for cIMT across Europe, with Finnish cIMT measurements being approximately 10% higher than French cIMT values [35]. In elderly subjects, latitude has been found to be the strongest independent determinant for cIMT, followed by age, and sex [35]. On the one hand, this phenomenon explains the higher cIMT values in our study compared with other European-based studies. On the other hand, it shows that latitude is a more important predictor for cIMT than lifestyle-related health metrics, at least in our population.

While cIMT reflects the carotid wall structure, cLD and carotid distensibility offer more information on carotid function. Our study provides evidence that in middle-aged to elderly Finns, adherence to the Simple 7 does not overcome the latitude determinant of cIMT but that nevertheless, carotid function benefits from a healthy lifestyle, further underlining the importance of adherence to a healthy lifestyle, and a holistic approach when analyzing the carotid arteries.

In line with the results from the PPS3 study, we showed that biological health metrics (i.e., systolic blood pressure, cholesterol, and fasting glucose) were a stronger predictor for cIMT than the behavioral health metrics (i.e., diet, physical activity, BMI, and smoking status) [31]. However, in our study, the behavioral health metrics explained cLD and carotid distensibility better than the biological metrics. The health metrics however, explained only little of the carotid properties, whereas age and sex were better predictors. Nevertheless at least in this Finnish population, behavioral health should not be neglected as an important, albeit small, pillar of cardiovascular health.

In this cross-sectional study, we showed that only 9.1% of this representative sample of the Finnish population had ideal cardiovascular health. While this percentage is higher than a comparable study from the United States [36] and China [33], they are lower than in European studies from Spain [37] and France [31]. This underlines the importance of assessing cardiovascular health in Finland, as the prevalence of CVD is among the highest in Europe [16]. In contrast, compared with other European regions, northern European countries have low CVD

mortality. This points towards better diagnostic possibilities and treatment in Nordic countries, which result in higher costs to treat CVD due to long periods of disease.

With this study, we strengthen the case for the use of the Life's Simple 7 as a tool for cardiovascular health assessment. Although not widely used in European studies, the Simple 7 have the advantage of being of solely modifiable metrics and therefore potentially being more communicative and comprehensive than cardiovascular risk scores.

This study has several limitations. First, as with all cross-sectional approaches, causation cannot be determined. Also, the Life's Simple 7 show current adherence to a healthy lifestyle. While some factors are difficult to modify over a short period of time (e.g., cholesterol, systolic blood pressure), others can be modified quickly, such as physical activity or diet. As such, it is this cross-sectional study setting, short-term lifestyle changes cannot be ruled out. Second, the age range of our studied population does not allow to generalize our results to other age groups. Third, we assessed physical activity using a questionnaire. It has been shown that participants may overestimate the amount of physical activity in questionnaires [38]. Strengths of this study include the use state-of-the-art ultrasound measurement techniques, and we included cLD in our models, which to the best of our knowledge, we are the first ones to do so. In addition, our general population-based approach may allow for better comparisons with future studies. Lastly, the use of the 4d-FR to assess dietary habits allowed for precise estimation of the dietary score, which other studies could not do [31, 32].

**Conclusions**

In conclusion, this study demonstrated that higher adherence to Life's Simple 7 was associated with lower cLD and higher carotid distensibility, but not with lower cIMT in middle-aged to elderly Finns. We hereby provide more evidence towards the importance of adhering to healthy lifestyle habits to reduce age-related structural and functional changes in the carotid artery and reflecting the atherosclerotic process as an important vascular imaging biomarker and finally reduce the risk of suffering a cardiovascular event.

**Author Contributions:** PK, KS, RM, MH, RR and AST were responsible for conceptualization and methodology. PK, KS, RM, MH, GN, AST and RR conducted the research. GN performed the statistical analyses. GN and AST wrote the manuscript. PK, KS, RM, MH, RR and AST provided expert advice for statistical analysis and critically reviewed the manuscript. RR was responsible for funding acquisition. All authors read and approved the final manuscript.

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**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** Data described in the manuscript, code book, and analytic code will not be made available because ethical permissions do not allow it.

**Conflicts of Interest:** The authors declare no conflict of interest.

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## Supplement

*Supplement 1:* Predictive values of age, sex, the behavioral, and biological health metrics for the carotid parameters.

| | | | Sum of Squares | df | Mean Square | F | $R^2$ Change | p-value |
|---|---|---|---|---|---|---|---|---|
| cIMT (mm) | Subset Tests | Age | 2.728 | 1 | 2.728 | 152.53 | 0.098 | <0.001 |
| | | Sex (ref.: male) | 0.127 | 1 | 0.127 | 7.083 | 0.005 | 0.008 |
| | | Biological Metrics | 0.312 | 6 | 0.052 | 2.91 | 0.011 | 0.008 |
| | | Behavioral Metrics | 0.139 | 8 | 0.017 | 0.974 | 0.005 | 0.455 |
| | Regression | | 3.378 | 16 | 0.211 | 11.807 | | <0.001 |
| | Residual | | 24.41 | 136 | 0.018 | | | |
| | Total | | 27.788 | 138 | | | | |
| cLD (mm) | Subset Tests | Age | 35.356 | 1 | 35.356 | 59.137 | 0.032 | <0.001 |
| | | Sex (ref.: male) | 99.197 | 1 | 99.197 | 165.91 | 0.091 | <0.001 |
| | | Biological Metrics | 18.101 | 6 | 3.017 | 5.046 | 0.017 | <0.001 |
| | | Behavioral Metrics | 49.287 | 8 | 6.161 | 10.305 | 0.045 | <0.001 |
| | Regression | | 275.803 | 16 | 17.238 | 28.831 | | <0.001 |
| | Residual | | 816.104 | 136 | 0.598 | | | |
| | Total | | 1091.907 | 138 | | | | |
| Carotid distensibility (1/kPa) | Subset Tests | Age | 0.0017 | 1 | 0.0017 | 36.647 | 0.024 | <0.001 |
| | | Sex (ref.: male) | 0.0004 | 1 | 0.0004 | 8.411 | 0.006 | 0.004 |
| | | Biological Metrics | 0.0028 | 6 | 0.0005 | 10.34 | 0.041 | <0.001 |
| | | Behavioral Metrics | 0.0007 | 8 | 0.0001 | 1.914 | 0.01 | 0.054 |
| | Regression | | 0.0061 | 16 | 0.0004 | 8.29 | | <0.001 |
| | Residual | | 0.0624 | 136 | 0.0005 | | | |
| | Total | | 0.0685 | 138 | | | | |

*Supplement 2: Difference between poor, intermediate, and ideal cardiovascular health score for the health score variables and carotid properties. By sex.*

| Sex | | HS | Mean (SD) | Health Score | | Difference | 95% CI | | p-value |
|---|---|---|---|---|---|---|---|---|---|
| **Men** | Age (years) | Poor | 64.3 (5.4) | Poor | Int. | -2.1 | -4.5 | 0.1 | 0.070 |
| | | Int. | 66.5 (5.5) | Poor | Ideal | -1.4 | -4.0 | 1.2 | 0.419 |
| | | Ideal | 65.7 (4.9) | Int. | Ideal | 0.8 | -0.6 | 2.2 | 0.390 |
| | Systolic blood pressure (mmHg) | Poor | 150 (11) | Poor | Int. | 5 | -4 | 12 | 0.421 |
| | | Int. | 145 (18) | Poor | Ideal | 15 | 6 | 24 | <0.001 |
| | | Ideal | 135 (18) | Int. | Ideal | 10 | 6 | 15 | <0.001 |
| | Fasting glucose (mmol/L) | Poor | 7.32 (2.88) | Poor | Int. | 1.31 | 0.87 | 1.75 | <0.001 |
| | | Int. | 6.01 (0.86) | Poor | Ideal | 1.50 | 1.21 | 2.19 | <0.001 |
| | | Ideal | 5.62 (0.72) | Int. | Ideal | 0.39 | 0.13 | 0.66 | 0.002 |
| | Cholesterol (mmol/L) | Poor | 5.39 (1.29) | Poor | Int. | 0.49 | 0.01 | 0.10 | 0.009 |
| | | Int. | 4.90 (0.92) | Poor | Ideal | 0.71 | 0.27 | 1.15 | <0.001 |
| | | Ideal | 4.68 (0.73) | Int. | Ideal | 0.22 | -0.02 | 0.46 | 0.080 |
| | BMI (kg/m$^2$) | Poor | 33.3 (5.4) | Poor | Int. | 5.5 | 4.0 | 7.1 | <0.001 |
| | | Int. | 27.8 (3.6) | Poor | Ideal | 8.4 | 6.7 | 10.1 | <0.001 |
| | | Ideal | 24.9 (2.3) | Int. | Ideal | 2.9 | 2.0 | 3.8 | <0.001 |
| | Pack years (years) | Poor | 9.6 (18.5) | Poor | Int. | -1.5 | -8.2 | 5.2 | 0.860 |
| | | Int. | 11.1 (15.7) | Poor | Ideal | -2.8 | -10.3 | 4.8 | 0.663 |
| | | Ideal | 12.4 (15.0) | Int. | Ideal | -1.3 | -2.8 | 2.8 | 0.743 |
| | At least moderate physical activity (min/week) | Poor | 525 (425) | Poor | Int. | 117 | -40 | 274 | 0.189 |
| | | Int. | 408 (353) | Poor | Ideal | -25 | -83 | 268 | 0.432 |
| | | Ideal | 432 (354) | Int. | Ideal | -93 | -119 | 71 | 0.821 |
| | Diet score | Poor | 0.6 (0.6) | Poor | Int. | -0.9 | -1.36 | -0.48 | <0.001 |
| | | Int. | 1.5 (1.1) | Poor | Ideal | -2.2 | -2.67 | -1.69 | <0.001 |
| | | Ideal | 2.8 (0.9) | Int. | Ideal | -1.3 | -1.52 | -0.99 | <0.001 |
| | cIMT (mm) | Poor | 0.808 (0.131) | Poor | Int. | -0.031 | -0.095 | 0.033 | 0.490 |
| | | Int. | 0.839 (0.153) | Poor | Ideal | -0.005 | -0.078 | 0.066 | 0.981 |
| | | Ideal | 0.814 (0.133) | Int. | Ideal | -0.025 | -0.014 | 0.064 | 0.279 |
| | cLD (mm) | Poor | 7.64 (1.06) | Poor | Int. | 0.03 | -0.35 | 0.40 | 0.987 |
| | | Int. | 7.61 (0.87) | Poor | Ideal | 0.29 | -0.14 | 0.70 | 0.252 |
| | | Ideal | 7.35 (0.80) | Int. | Ideal | 0.26 | 0.03 | 0.48 | 0.021 |
| | Carotid distensibilty (1/kPa) | Poor | 0.0108 (0.0063) | Poor | Int. | -0.0015 | -0.0045 | -0.0015 | 0.477 |
| | | Int. | 0.0123 (0.0072) | Poor | Ideal | -0.0028 | -0.0061 | 0.0006 | 0.123 |
| | | Ideal | 0.0136 (0.0069) | Int. | Ideal | -0.0013 | -0.0032 | 0.0005 | 0.203 |
| **Women** | Age (years) | Poor | 67.9 (5.7) | Poor | Int. | 1.2 | -0.7 | 3.0 | 0.308 |
| | | Int. | 66.7 (5.2) | Poor | Ideal | 3.5 | 1.2 | 5.8 | 0.001 |
| | | Ideal | 64.4 (5.3) | Int. | Ideal | 2.3 | 0.8 | 3.8 | 0.001 |
| | Systolic blood pressure (mmHg) | Poor | 155 (20) | Poor | Int. | 5 | -3 | 12 | 0.283 |
| | | Int. | 150 (21) | Poor | Ideal | 17 | 8 | 26 | <0.001 |
| | | Ideal | 138 (21) | Int. | Ideal | 12 | 6 | 18 | <0.001 |
| | Fasting glucose (mmol/L) | Poor | 6.76 (1.45) | Poor | Int. | 1.14 | 0.86 | 1.41 | <0.001 |
| | | Int. | 5.62 (0.74) | Poor | Ideal | 1.53 | 1.19 | 1.88 | <0.001 |
| | | Ideal | 5.23 (0.39) | Int. | Ideal | 0.39 | 0.17 | 0.62 | <0.001 |
| | Cholesterol (mmol/L) | Poor | 5.52 (1.01) | Poor | Int. | 0.26 | -0.59 | 0.06 | 0.139 |
| | | Int. | 5.26 (0.93) | Poor | Ideal | 0.29 | 0.11 | 0.91 | 0.008 |
| | | Ideal | 5.23 (0.67) | Int. | Ideal | 0.03 | -0.02 | 0.51 | 0.075 |
| | BMI (kg/m$^2$) | Poor | 33.9 (4.9) | Poor | Int. | 6.3 | 4.6 | 7.8 | <0.001 |
| | | Int. | 27.6 (4.6) | Poor | Ideal | 10.4 | 8.4 | 12.3 | <0.001 |
| | | Ideal | 23.5 (2.9) | Int. | Ideal | 4.1 | 2.8 | 5.4 | <0.001 |
| | Pack years (years) | Poor | 0.1 (0.4) | Poor | Int. | -1.9 | -4.3 | 0.4 | 0.136 |
| | | Int. | 2.0 (6.3) | Poor | Ideal | -4.3 | -7.3 | -1.5 | 0.001 |
| | | Ideal | 4.4 (10.2) | Int. | Ideal | -2.4 | -4.4 | -0.5 | 0.008 |
| | At least moderate physical activity (min/week) | Poor | 361 (322) | Poor | Int. | -45 | -162 | 74 | 0.652 |
| | | Int. | 406 (332) | Poor | Ideal | -47 | -192 | 99 | 0.735 |
| | | Ideal | 408 (334) | Int. | Ideal | -2 | -99 | 99 | 0.999 |
| | Diet score | Poor | 0.6 (0.6) | Poor | Int. | -1.2 | -1.6 | -0.8 | <0.001 |
| | | Int. | 1.8 (1.1) | Poor | Ideal | -2.2 | -2.7 | -1.7 | <0.001 |
| | | Ideal | 2.8 (1.2) | Int. | Ideal | -1.0 | -1.3 | -0.7 | <0.001 |
| | cIMT (mm) | Poor | 0.836 (0.141) | Poor | Int. | 0.019 | -0.029 | 0.065 | 0.633 |
| | | Int. | 0.817 (0.133) | Poor | Ideal | 0.068 | 0.011 | 0.088 | 0.017 |
| | | Ideal | 0.768 (0.124) | Int. | Ideal | 0.049 | 0.100 | 0.126 | 0.007 |
| | cLD (mm) | Poor | 7.19 (0.86) | Poor | Int. | 0.30 | 0.03 | 0.57 | 0.023 |
| | | Int. | 6.89 (0.75) | Poor | Ideal | 0.55 | 0.22 | 0.88 | <0.001 |
| | | Ideal | 6.64 (0.76) | Int. | Ideal | 0.25 | 0.03 | 0.47 | 0.022 |
| | Carotid distensibilty (1/kPa) | Poor | 0.0098 (0.0071) | Poor | Int. | -0.0009 | -0.0035 | 0.0014 | 0.576 |
| | | Int. | 0.0109 (0.0068) | Poor | Ideal | -0.0041 | -0.0070 | -0.0010 | 0.005 |
| | | Ideal | 0.0139 (0.0069) | Int. | Ideal | -0.0030 | -0.0050 | -0.0010 | 0.001 |

*Legend:* BMI, Body-mass index; cIMT, carotid intima-media thickness; cLD, carotid lumen diameter; HS, Cardiovascular health score; Int, Intermediate Health Score.

## Chapter 6 – Publication 3

# Effect of lifestyle interventions on carotid arterial structure – the DR's EXTRA study

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## Abstract

### Background

No lifestyle-based interventions with medium-term duration on carotid atherosclerotic have been performed so far. We aimed to investigate whether guideline-based dietary and physical activity interventions slow the progression of atherosclerotic changes in the general elderly population.

### Methods and results

A total of 1410 Finnish men and women from a representative population sample were randomly assigned to one of six groups in the four-year intervention study: 1) reference, 2) aerobic training, 3) resistance training, 4) diet, 5) aerobic training + diet, 6) resistance training + diet. The primary outcome was mean common carotid artery intima-media thickness (cIMT). The lumen diameter of the common carotid artery (cLD) was also analyzed.

A total of 567 men and 565 women aged 57 to 78 years had valid measurements at baseline and follow-up. None of the intervention groups significantly slowed cIMT progression compared to the reference group. A subgroup analysis showed that men in the diet group had significantly smaller cIMT progression than in the reference group (-0.078 mm, 95% CI: -0.146 to -0.009, p=0.02) and no significant increase in cIMT (p=0.23). No other group showed a slowed cIMT progression. None of the intervention groups significantly slowed cLD progression when compared to the reference group.

### Conclusions

Among guideline-based lifestyle interventions, only diet leads to a significantly smaller progression of cIMT in older men of a representative population sample. No other lifestyle intervention contributed to a slowing of the progression of structural carotid markers. It must be questioned whether the guideline-based recommendations for a lifestyle change that were in place until recently are adequate to decelerate the atherosclerotic process.



**KEYWORDS:** Lifestyle intervention, arteriosclerosis, carotid intima-media thickness, physical activity, diet

## Introduction

In Finland, the main cause of death is related to cardiovascular diseases, which accounted for approximately 33% of all deaths in 2020 [1]. With rising life expectancy in developed countries, it is therefore of high importance to assess cardiovascular risk and promote healthy lifestyles at an early stage to enable disease-free aging [2]. The World Health Organization (WHO) defined the most important lifestyle components as smoking status, physical activity, diet, and alcohol consumption [3]. As such, a meta-analysis that summarized the combined effects of these healthy lifestyle behaviors found that adherence to all four components defined by the WHO was associated with a lower risk of cardiovascular events, and 66% lower mortality [4]. The WHO further provides physical activity recommendations for adults, which were recently updated in 2020 [5] but until then, the recommendations were at least 150 minutes of moderate, or at least 75 minutes of vigorous physical activity to reduce the risk of non-communicable diseases, such as cardiovascular diseases [6]. A reduction of cardiovascular risk might further be achieved by reaching certain dietary goals [7]. Therefore, the Nordic diet was introduced in Scandinavian countries to improve dietary habits in Nordic populations [8]. It includes high consumption of whole-grain products, low-fat dairy products, berries, fruits and vegetables, rapeseed oil, and fish.

To stratify the risk of cardiovascular events (e.g., stroke, myocardial infarction), the assessment of carotid wall structure has been established as a highly reliable, non-invasive subclinical marker for the atherosclerotic process [9, 10]. The detection of pre-symptomatic and at-risk populations in clinical and epidemiological settings by carotid intima-media thickness (cIMT) to calculate cardiovascular risk has proven to be reliable and is therefore widely used [11-15]. While cIMT takes into account the inner two layers of the arterial wall, it is known that the adventitia, the most outer wall, plays a pivotal role in vascular remodeling [16]. Therefore, the assessment of carotid lumen diameter (cLD) has gained attraction in the last decade, as it has been even more closely linked to mortality than cIMT [17].

While cross-sectional studies showed plausible associations of carotid structure with various lifestyle factors [18-20], no adequately sized randomized controlled trial (RCT) has yet shown the medium-term effect of diet on the atherosclerotic process, and only one study with a duration of 6 years showed that the progression of cIMT could be reduced through aerobic exercise training in a group of middle-aged men not taking cholesterol-lowering medication [21]. As such, we aimed to fill this research gap regarding multi-year lifestyle interventions in the general population and the effects of such interventions on carotid structure conducting the DR`s EXTRA (Dose-Responses to EXercise TRAining) research project. The specific objective was to investigate whether guideline-based lifestyle interventions such as endurance or strength training, diet, or a combination of these components are able to slow the atherosclerotic process.

## Methods

### Design and participants

DR's EXTRA aims to quantify the health benefits of lifestyle interventions (regular aerobic exercise, strength training, healthy diet, or a combination of these) throughout a four-year randomized controlled trial (ISRCTN45977199, http://isrctn.org). At the time of recruitment (2002-2004), a random sample (stratified by sex) of 3,000 community-dwelling Finnish men and women aged 55-74 years old, was obtained from the people's registry of the city of Kuopio, Finland. Among them, 1410 completed baseline measurements (response rate 47%). The power calculations was based on the results from the 4-year increase in cIMT in the DNA Polymorphism and Carotid Atherosclerosis (DNASCO) study [21]. The primary outcome of the study was atherosclerotic progression, measured as cIMT change. The expected cIMT increase during the intervention was 0.13 mm (SD 0.25 mm). The required sample size was 200 participants per intervention group (significance level 5%, power 90%, drop-out rate 10% in 4 years) [21]. An ad-hoc power analysis based on the observed standard deviation of cIMT measurements at baseline (SD = 0.14 mm) revealed that our sample size provides 80% power to detect any differences of magnitude 0.059 mm between intervention groups and the control group using Dunnett's test. As differences of 0.059 mm are not clinically relevant, our sample size is sufficient to detect any clinically relevant group differences [12]. All participants were randomized into the study groups under surveillance of the principal investigator, who was blinded for the outcome measures. The randomization happened in blocks of 180 participants. The participants chose one of six opaque and sealed envelopes in sequential order to determine group allocation. All investigators who performed or evaluated the outcome measures were blinded to the study groups. The full study overview, including the recruitment process, has previously been published [22, 23]. All participants provided written consent. The study complied with the Declaration of Helsinki and was approved by the Research Ethics Committee of the Hospital District of Northern Savo, Finland.

### Interventions

After baseline measurements, the participants were randomly allocated to one of the six interventions (aerobic exercise, resistance exercise, diet, aerobic exercise and diet, and resistance exercise and diet, reference), all lasting four years each. For ethical reasons, the reference group received recommendations for regular physical activity and a healthy diet at baseline via an in-person visit with a clinician. The aerobic exercise group was prescribed an individualized training program. In the first 6 months, participants were asked to gradually increase exercise volume from 2 to 4 sessions per week, lasting 30-60 minutes each, at 40-50% of their maximal oxygen uptake, which had been measured in a maximal exercise test. From 6 months until the end of the study, the subjects were asked to perform the individualized aerobic exercise regimen 5 times per week for approximately one hour per training, at 60% of maximal oxygen uptake. The subjects in the aerobic group performed all training sessions unsupervised and on their own. They were asked to monitor training intensity either with a heart rate monitor

or through arterial pulse palpation and were asked to log their training sessions. For the resistance exercise group, training intensity was quantified based on entry-level assessments with one repetition maximum for the main muscle groups. Thereafter, training loads were adapted regularly (at 1, 3, 6, 24, 36, and 48 months) during the intervention. The subjects in the resistance training group were granted access to the gym of the local research center, where training was monitored via a smart card system (HUR Ltd, Finland).

The diet group was asked to follow 4 dietary goals, as described by the Finnish Nutrition Recommendations and American Diabetes Association, which consisted of ≥400 grams/day of vegetables, fruit, and berries, ≥2 servings of fish/week corresponding to ≥30 grams/day, ≥14 grams of fiber/1000 kilocalories, and ≤10 energy percentage of daily energy intake from saturated fatty acids [24]. The combined aerobic exercise and diet, and the resistance exercise and diet groups had identical goals and followed the same study protocols as the groups with a single intervention arm (i.e., diet, aerobic, or resistance training).

**Assessment of outcomes: carotid intima-media thickness and carotid lumen diameter**

Four expert sonographers assessed carotid properties using B-mode ultrasonographic imaging. The measurement was performed on the right and left common carotid artery (CCA) after the subjects had rested in a supine position for at least 15 minutes, following the Mannheim Consensus [25]. CCA measurements were performed at baseline and the end of the study. All measurements were recorded in full length, and expert ultrasound readers selected the frames where cIMT on the far wall could be best detected for further analyses. To ensure the validity of the cIMT calculations, four frames were selected for each subject (two frames at end-diastole directly before the ascent of R-wave of the ECG, on the left and right CCA, respectively). The frames were analyzed via the fully automatic and validated B-mode image analysis program DYARA (DYnamic ARtery Analysis) [10, 26]. The reader marked a one-centimeter measurement segment within the distal two centimeters of the CCA, and the program measured cIMT at the far wall as mean cIMT over one centimeter, as well as mean cLD. If the wall and/or outer lumen detection was insufficient for outer lumen diameter and the intima-media complex within the marked region, the reader manually corrected the wall detection. The results of each frame were then exported and the mean of four end-diastolic values was used for all analyses.

**Assessment of physical activity**

Physical activity was measured at baseline and follow-up via a 12-month retrospective questionnaire, which included the most commonly performed activities in Finland (e.g., cross-country skiing, walking, cycling) [27]. Only at-least moderate physical activity had to be considered and participants were asked to report the frequency of each type of physical activity, the mean duration, and mean intensity on a 1-4 scale, whereby 1 was considered light, and 4 very heavy physical activity.

**Compliance**

Compliance with the exercise interventions was measured from 0% to 100%. Participants in the aerobic group were considered to be 100% compliant if they exercised for 60 minutes, 5 times per week at moderate intensity (total of 300 minutes). In the resistance group, 100% compliance was defined as 2 training sessions per week or more, with 2 sets and 15 repetitions, at 60% or more of the estimated 1 repetition maximum [28]. For diet, participants were 100% compliant if they adhered to all 4 dietary goals they were given [24]. Calculation of compliance for the participants in the combined groups was done using the mean compliance of the respective interventions.

**Statistical analyses**

The primary outcome for this study was the progression of cIMT throughout the four-year intervention, with cLD as an additional accepted structural carotid outcome reflecting the arteriosclerotic process. In a second, more explorative step, we analyzed sex-specific differences between the groups. Continuous variables are shown as means ± SD and dichotomous variables as frequencies and percentages. To compare the effect of the treatments prescribed in the five intervention arms with the control treatment over the four-year intervention on cIMT and cLD, we used an analysis of covariance (ANCOVA) [29]. Both cIMT and cLD at follow-up were used as outcome variables in the models, whereas a group indicator, sex, and an interaction between the two served as predictors. In addition, the model was adjusted for the following baseline values: cIMT or cLD, respectively, systolic blood pressure, age, body mass index, cholesterol, maximal oxygen uptake ($\dot{V}O_{2max}$), and minutes of at least moderate physical activity per week. We compared all five groups with the control treatment by estimated marginal means and Dunnett's test. These comparisons were stratified by sex. We included an interaction between sex and the group indicator in the ANCOVA models. This allows us to test for heterogeneous treatment effects between sexes. We used residual diagnostic plots such as Q-Q plots of the residuals and residual-vs-fitted plots to check for potential violations of the model assumptions. The diagnostic plots did not indicate any violations of the model assumptions. Some participants had missing data in the variables included in the models. As the fraction of missing data was <5%, no imputation of missing data was done. Even when subjects are randomized into the groups, slight baseline imbalances are to be expected which could lead to regression to the mean. Therefore, we controlled for potential baseline imbalances using an ANCOVA with a baseline and a follow-up measurement [29]. Further, we included an interaction between sex and the group indicator in the ANCOVA models. This allows us to test for heterogeneous treatment effects between genders. Using the Dunnett test, we do not primarily control the false discovery rate (FDR), but the familywise error rate (FWER). We keep the probability of falsely rejecting at least one null hypothesis (i.e., type I error) at 5% for all comparisons. All statistical analyses were 2-sided, and adjusted p-values of $\leq 0.05$ were considered statistically significant. We used IBM SPSS Statistics for Windows, version 26.0 (IBM Corporation), for all analyses.

**Results**

In total, 1400 participants had valid baseline cIMT measurements and 1132 (567 men and 565 women) had valid post-intervention measurements. A total of 268 subjects (19.1%) either dropped out or did not have a valid measurement post-intervention, as visible in the flow chart (figure 1). The dropout rate did not significantly differ between any of the groups.

Baseline characteristics for each of the six study groups are presented in table 1. On average, 50.4% of all men and 52.7% of all women reached at least 300 minutes per week of at least moderate physical activity, as recommended by the WHO [6]. On average, 1.73 (±1.12) out of 4 dietary goals were met.

Following the intervention phase, none of the intervention groups had significantly lower increase in cIMT or cLD than the reference group (table 2).

In our secondary analysis, we found that men in the diet who received the dietary intervention were the only subgroup to not have a significant cIMT increase ($p=0.23$), adjusted for age (figure 2). Men in the resistance + diet group ($p=0.04$) and women in the aerobic ($p=0.03$) and resistance group ($p<0.01$), respectively, showed increased cLD after the intervention phase (figure 2).

Men in the diet group had a significantly higher diet score at follow-up compared to the reference group (0.53, 95% CI: 0.07 to 0.98, $p=0.01$) and the resistance group (0.54, 95% CI: 0.08 to 1.00, $p<0.01$). Women in the diet group (0.42, 95% CI: 0.15 to 0.68, $p<0.01$), aerobic + diet group (0.36, 95% CI: 0.11 to 0.61, $p<0.01$), and resistance + diet group (0.35, 95% CI: 0.10 to 0.59, $p<0.01$) significantly improved their diet scores.

The overall mean compliance in the aerobic group was 62%, 53% in the resistance group, 84% in the diet group, 71% in the aerobic + diet group (57% for exercise and 85% for diet), and 66% in the resistance + diet group (47% for exercise and 84% for diet).

High levels of physical activity did not slow cIMT or cLD progression (supplement). The lowest tertile achieved less than 175 weekly minutes of at least moderate physical activity, the middle tertile less than 435 minutes, and the highest tertile at least 435 minutes.

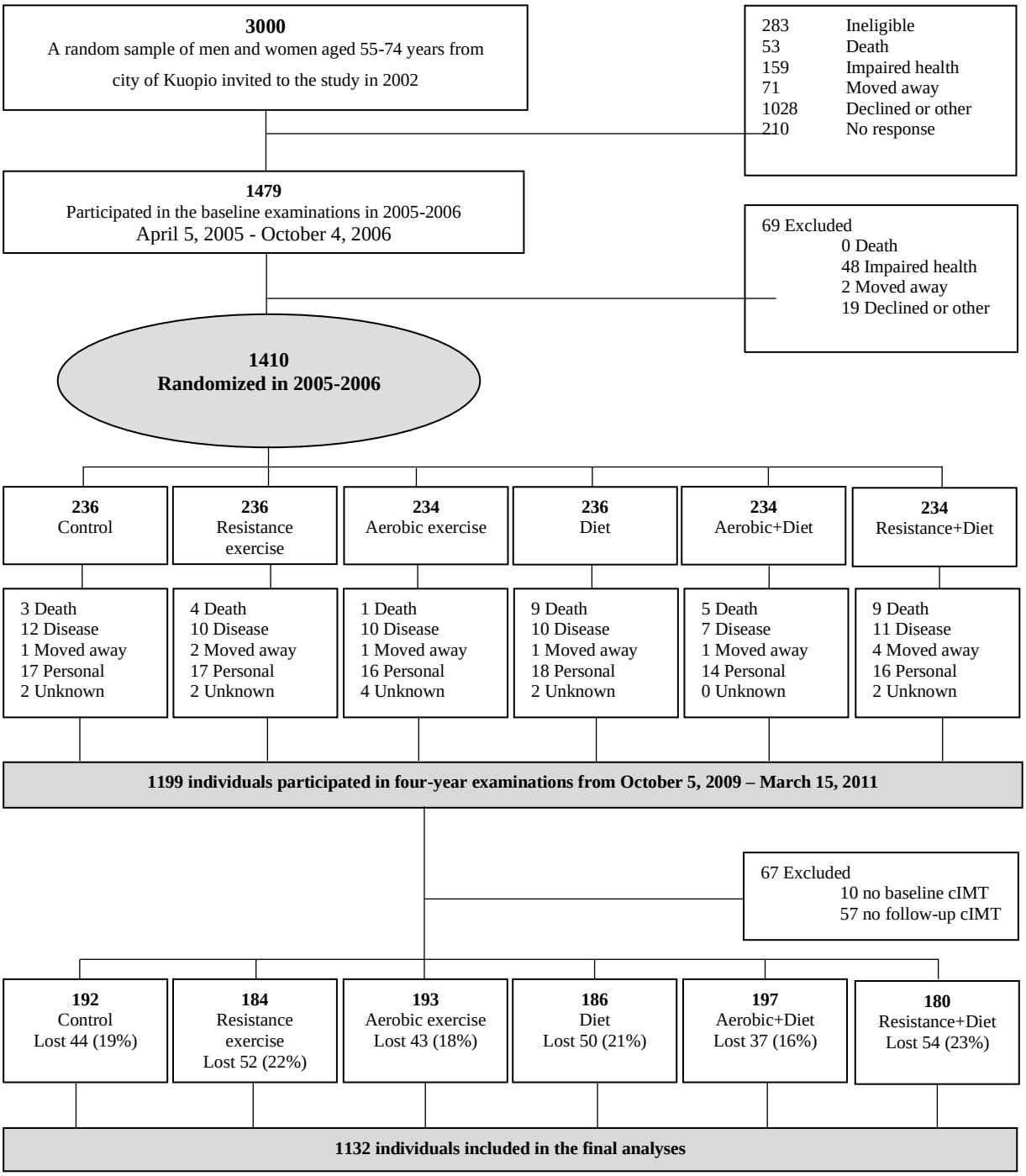


Figure 1: Flow chart of the DR's EXTRA study.

Table 1: Baseline characteristics of the participants after random group allocation, by sex.

| | Men | | | | | | Women | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Reference (n=124)** | **Aerobic (n=111)** | **Resistance (n=119)** | **Diet (n=122)** | **A. + Diet (n=109)** | **R. + Diet (n=107)** | **Reference (n=112)** | **Aerobic (n=122)** | **Resistance (n=115)** | **Diet (n=112)** | **A. + Diet (n=123)** | **R. + Diet (n=124)** |
| **Age (years)** | 65.9 (5.3) | 67.1 (5.4) | 66.7 (5.5) | 66.8 (5.6) | 65.8 (5.2) | 65.3 (5.5) | 66.6 (5.4) | 66.9 (5.2) | 66.5 (5.3) | 67.0 (5.3) | 66.4 (5.3) | 66.3 (5.3) |
| **BMI (kg/m2)** | 27.6 (3.7) | 28.0 (4.1) | 27.4 (4.0) | 27.2 (3.9) | 28.4 (4.2) | 27.5 (3.7) | 27.8 (5.1) | 28.4 (4.7) | 27.2 (4.7) | 27.4 (4.9) | 27.8 (5.3) | 27.1 (5.0) |
| **Waist (cm)** | 99.1 (11.1) | 99.4 (11.6) | 98.6 (11.1) | 97.2 (10.6) | 101.0 (11.2) | 98.5 (10.4) | 89.1 (12.8) | 90.2 (12.0) | 87.3 (11.7) | 89.2 (13.1) | 89.3 (14.4) | 87.6 (13.5) |
| **cIMT (mm)** | 0.83 (0.15) | 0.85 (0.16) | 0.84 (0.15) | 0.83 (0.15) | 0.82 (0.16) | 0.82 (0.13) | 0.82 (0.13) | 0.82 (0.14) | 0.79 (0.13) | 0.82 (0.13) | 0.81 (0.14) | 0.82 (0.13) |
| **Dia LD (mm)** | 7.49 (0.87) | 7.58 (0.89) | 7.73 (1.02) | 7.48 (0.80) | 7.59 (0.92) | 7.61 (0.82) | 6.86 (0.82) | 6.94 (0.82) | 6.82 (0.67) | 6.87 (0.78) | 6.97 (0.74) | 6.83 (0.72) |
| **Sys BP (mmHg)** | 145 (18) | 145 (19) | 143 (18) | 146 (18) | 143 (19) | 143 (19) | 147 (22) | 152 (20) | 150 (23) | 149 (20) | 148 (19) | 148 (21) |
| **Dia BP (mmHg)** | 84 (9) | 84 (10) | 83 (9) | 84 (10) | 85 (10) | 84 (8) | 81 (9) | 83 (8) | 82 (10) | 83 (9) | 82 (9) | 83 (9) |
| **Chol. (mmol/L)** | 4.9 (1.0) | 4.8 (1.0) | 4.9 (0.9) | 5.0 (0.8) | 4.9 (1.0) | 4.8 (0.8) | 5.3 (0.8) | 5.1 (0.9) | 5.3 (1.0) | 5.3 (0.9) | 5.2 (0.9) | 5.2 (1.0) |
| **Trig. (mmol/L)** | 1.4 (0.8) | 1.4 (0.8) | 1.4 (0.7) | 1.3 (0.7) | 1.4 (0.8) | 1.4 (0.7) | 1.2 (0.5) | 1.3 (0.5) | 1.3 (0.7) | 1.4 (0.7) | 1.3 (0.7) | 1.3 (0.7) |
| **HDL (mmol/L)** | 1.5 (0.4) | 1.5 (0.4) | 1.5 (0.4) | 1.6 (0.5) | 1.5 (0.4) | 1.5 (0.4) | 1.9 (0.4) | 1.8 (0.5) | 1.9 (0.5) | 1.8 (0.4) | 1.9 (0.6) | 1.9 (0.4) |
| **$\dot{V}O_{2max}$ (L/min)** | 2.2 (0.5) | 2.2 (0.5) | 2.2 (0.6) | 2.2 (0.4) | 2.2 (0.5) | 2.2 (0.5) | 1.5 (0.3) | 1.4 (0.3) | 1.5 (0.3) | 1.4 (0.3) | 1.5 (0.3) | 1.4 (0.3) |
| **Mod&Vig PA (min)** | 419 (371) | 498 (515) | 395 (339) | 371 (269) | 410 (341) | 443 (385) | 378 (258) | 409 (341) | 398 (345) | 357 (297) | 422 (371) | 444 (349) |
| **Diet Score** | 1.49 (1.11) | 1.75 (1.18) | 1.57 (1.11) | 1.86 (1.15) | 1.60 (1.19) | 1.63 (1.04) | 1.82 (1.14) | 1.80 (1.19) | 1.96 (1.20) | 1.66 (1.15) | 1.85 (1.22) | 1.75 (1.19) |
| **Diab. med n(%)** | 10 (8.1) | 13 (11.7) | 10 (8.4) | 11 (9.0) | 10 (9.2) | 10 (9.3) | 5 (4.5) | 5 (4.1) | 4 (3.5) | 8 (7.1) | 8 (6.5) | 5 (4.0) |
| **Chol. med n(%)** | 48 (38.7) | 47 (42.3) | 39 (32.8) | 41 (33.6) | 34 (31.2) | 35 (32.7) | 31 (27.7) | 44 (36.1) | 37 (32.2) | 39 (34.8) | 43 (35.0) | 52 (41.9) |
| **BP med n(%)** | 51 (41.1) | 51 (45.9) | 55 (46.2) | 47 (38.5) | 47 (43.1) | 36 (33.6) | 42 (37.5) | 60 (49.2) | 44 (38.3) | 42 (37.5) | 50 (40.7) | 62 (50.0) |

Values are means ± SD or n (%). *Legend: A + Diet, aerobic and diet; R + Diet, resistance and diet; BP med, antihypertensive medication; BMI, Body Mass Index; cIMT, carotid intima-media thickness; Chol, cholesterol; Chol. med, cholesterol-lowering medication; Dia BP, diastolic blood pressure; cLD, carotid lumen diameter; Diab. Med (diabetic medication); HDL, high-density lipoprotein; Mod&Vig PA, moderate and vigorous physical activity; Sys BP, systolic blood pressure; Trig, triglycerides; $\dot{V}O_{2max}$, maximal oxygen uptake; Waist, waist circumference.*

Table 2: Mean difference of the main outcome variables cIMT and cLD at follow-up, as well as physical activity, and diet. The difference is calculated as intervention group – reference group.

| Variable | Groups | | Estimated mean difference | 95% CI | | p-value |
|---|---|---|---|---|---|---|
| **cIMT (mm)** | Aerobic (n=184) | Reference (n=183) | -0.013 | -0.039 | 0.013 | 0.553 |
| | Resistance (n=186) | | -0.012 | -0.038 | 0.014 | 0.626 |
| | Diet (n=183) | | -0.006 | -0.032 | 0.020 | 0.938 |
| | Aerobic + Diet (n=185) | | -0.007 | -0.033 | 0.019 | 0.878 |
| | Resistance + Diet (n=171) | | -0.010 | -0.036 | 0.017 | 0.776 |
| **cLD (mm)** | Aerobic (n=178) | Reference (n=183) | 0.04 | -0.08 | 0.17 | 0.824 |
| | Resistance (n=178) | | 0.02 | -0.11 | 0.14 | 0.976 |
| | Diet (n=182) | | -0.01 | -0.14 | 0.11 | 0.993 |
| | Aerobic + Diet (n=181) | | 0.03 | -0.09 | 0.16 | 0.910 |
| | Resistance + Diet (n=165) | | 0.04 | -0.09 | 0.17 | 0.865 |
| **Moderate and vigorous physical activity (minutes/week)** | Aerobic (n=199) | Reference (n=197) | 67 | -14 | 148 | 0.146 |
| | Resistance (n=195) | | 75 | -6 | 155 | 0.082 |
| | Diet (n=191) | | 34 | -47 | 115 | 0.703 |
| | Aerobic + Diet (n=203) | | 54 | -27 | 136 | 0.315 |
| | Resistance + Diet (n=186) | | 2 | -80 | 83 | 0.999 |
| **Diet (score)** | Aerobic (n=227) | Reference (n=229) | -0.70 | -0.34 | 0.20 | 0.903 |
| | Resistance (n=228) | | -0.06 | -0.33 | 0.21 | 0.928 |
| | Diet (n=228) | | 0.32 | 0.05 | 0.59 | 0.014 |
| | Aerobic + Diet (n=221) | | 0.28 | -0.02 | 0.55 | 0.032 |
| | Resistance + Diet (n=223) | | 0.26 | -0.02 | 0.53 | 0.075 |

*Legend: cIMT, carotid intima-media thickness; cLD, carotid lumen diameter.*

Table 3: cIMT and cLD at baseline and follow-up, by group and sex. Adjusted for risk factors systolic blood pressure, age, BMI, cholesterol, $\dot{V}O_{2max}$, moderate and vigorous physical activity, and diet score.

| Group | Baseline cIMT (mm) | Follow-up cIMT (mm) | p-value | Baseline cLD (mm) | Follow-up cLD (mm) | p-value |
|---|---|---|---|---|---|---|
| **Reference (n=236)** | 0.825 ± 0.140 | 0.862 ± 0.123 | <0.001 | 7.19 ± 0.90 | 7.45 ± 0.76 | 0.190 |
| **Aerobic (n=233)** | 0.837 ± 0.149 | 0.857 ± 0.131 | <0.001 | 7.24 ± 0.91 | 7.56 ± 0.76 | 0.150 |
| **Resistance (n=234)** | 0.816 ± 0.138 | 0.844 ± 0.135 | <0.001 | 7.27 ± 0.93 | 7.60 ± 0.89 | 0.717 |
| **Diet (n=234)** | 0.828 ± 0.141 | 0.860 ± 0.145 | 0.232 | 7.19 ± 0.85 | 7.44 ± 0.73 | 0.397 |
| **Aerobic + Diet (n=232)** | 0.817 ± 0.150 | 0.846 ± 0.143 | 0.014 | 7.27 ± 0.89 | 7.62 ± 0.87 | 0.110 |
| **Resistance + Diet (n=231)** | 0.820 ± 0.132 | 0.844 ± 0.130 | 0.002 | 7.20 ± 0.86 | 7.59 ± 0.71 | 0.041 |
| **Total (n=1400)** | 0.824 ± 0.142 | 0.852 ± 0.135 | <0.001 | 7.23 ± 0.89 | 7.54 ± 0.79 | 0.002 |

Legend: cIMT, carotid intima-media thickness; cLD, carotid lumen diameter; $\dot{V}O_{2max}$, maximal oxygen uptake capacity.

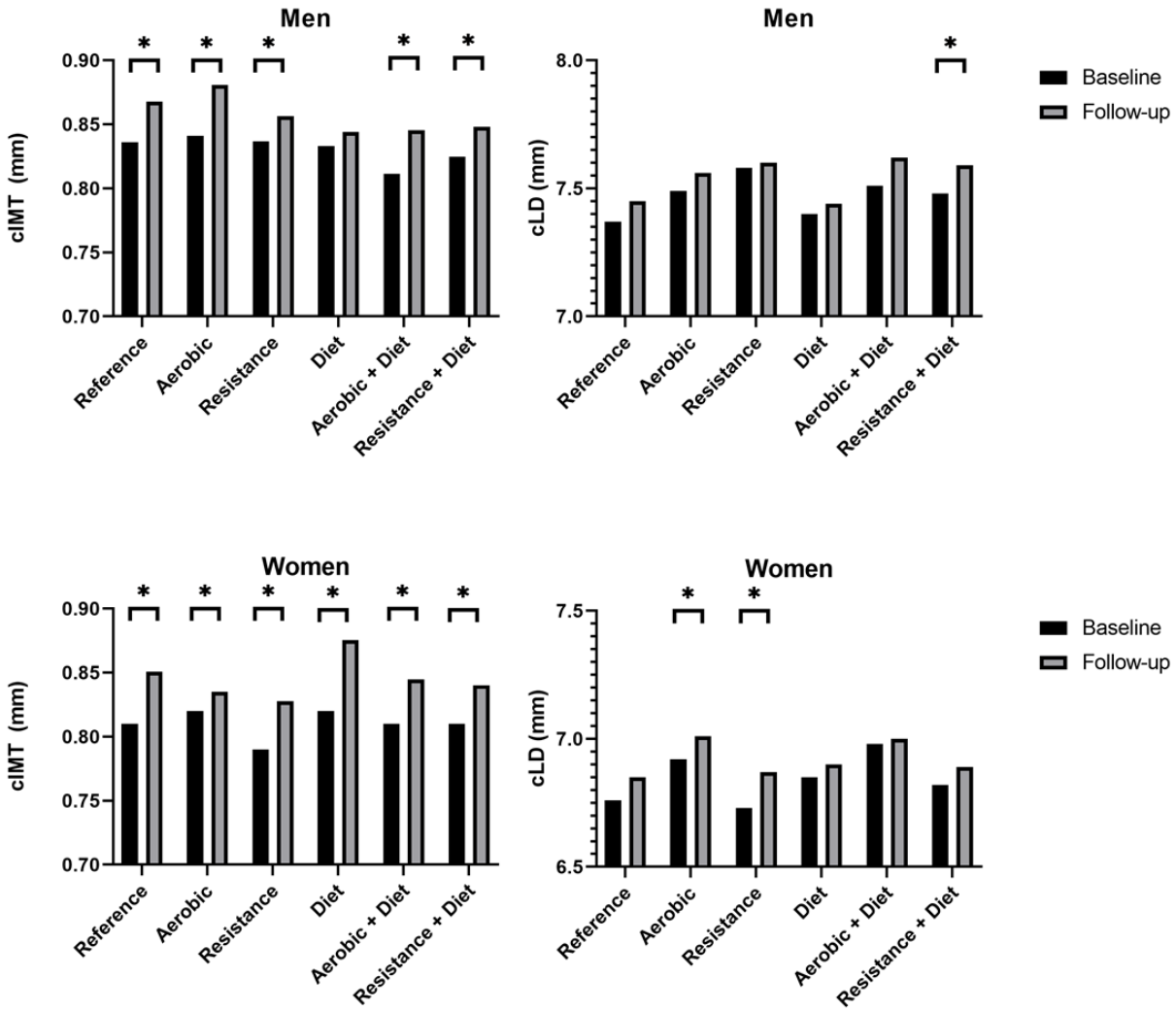


Figure 2: Mean 4-year progression of carotid intima-media thickness and carotid lumen diameter, divided by sex, and adjusted for age. Graphs show results by intervention group. Significant differences between baseline and follow-up are marked with a star. *Legend: cIMT, carotid intima-media thickness; cLD, carotid lumen diameter.*

## Discussion

The DR's EXTRA study investigated the effects of guideline-based aerobic training, resistance training, dietary goals, and the combination of diet and either aerobic or resistance training on the progression of atherosclerosis in middle-aged to elderly Finns. The main finding is that after the four-year randomized-controlled trial, none of the intervention groups significantly slowed atherosclerotic progression (measured as cIMT and cLD) when compared to the reference group. A secondary analysis revealed that men who were given dietary goals had a significantly lower increase in cIMT than the reference group. None of the other groups exerted a significant slowing on the atherosclerotic process. Our findings show that all physical activity-based interventions recommended in the WHO guidelines established until recently [6] do not slow

down the progression of the atherosclerotic process of the carotid artery.

Our study is the largest RCT to date that aimed at slowing the progression of atherosclerosis through lifestyle interventions. The objective was to reinforce and broaden the results of our much smaller, six-year intervention study and to show the possible additive effect of a combination of different lifestyle factors [21]. Similarly unsuccessful was a previous Scandinavian four-year study of drug-treated hypertensives, which did not show any effects of lifestyle change on the progression of cIMT [30]. A recent non-randomized lifestyle intervention trial lasting only 6 months was equally unsuccessful [31]. In contrast, a four-year lifestyle intervention only including women aged 44 to 50 showed that diet improvements and exercise could slow cIMT progression [32]. However, the authors noted that menopause accelerated carotid atherosclerosis and that the intervention was less effective in postmenopausal women. This is in line with our findings, that at least for women, lifestyle improvements may not slow atherosclerotic progression after the menopause.

Our study clearly demonstrates that interventions following the WHO physical activity guidelines that were in place until 2020 do not slow carotid atherosclerotic progression. The new WHO guidelines provide higher recommendations of physical activity and less sedentary time [5]. They aim at encouraging increased duration and frequency of moderate and vigorous physical activity. To date, its usefulness in lifestyle-based interventions to slow atherosclerotic progression remains to be proven. Training regimen going beyond the WHO guidelines including high-intensity interval training might be beneficial [33].

In this study, we examined cLD progression as a marker even more strongly linked to cardiovascular risk and all-cause mortality than cIMT [17, 34]. However, although we implemented guideline-based lifestyle interventions, none of the intervention groups had a significantly slower progression than the reference groups. This further consolidates our findings that the guidelines were not sufficient to induce any structural changes in the carotid artery.

**Physical activity**

In our study, people who were active before the intervention remained active and even reduced physical activity to meet the study criteria, whereas those who were less active, were also less compliant with the intervention. This could partially explain our results and highlight that adherence to physical activity guidelines is not enough to slow cIMT or cLD progression. In this community-based setting, the high level of physical activity in our population may be due to extensive governmental prevention programs in the 1960s and 1970s to tackle the high risk of cardiovascular disease in Finland [35]. In addition, recommendations for physical activity had been updated just a few years before the start of the intervention [36]. This may have led to a ceiling effect and highlights the importance of updated health promotion recommendations by the WHO since at baseline most of our participants were more physically active than similar populations [37].

### Diet

We found that only men who were given dietary goals had slower cIMT progression. However, this group had a significantly higher diet score at baseline than the reference group. In contrast, we found that women in all three groups that included dietary goals improved their diet scores significantly, but the dietary improvement did not alter cIMT and cLD progression. Our results are in line with the few intervention studies aiming at slowing cIMT progression through dietary changes [38, 39]. While the 1-year RCT by Murie-Fernandez et al. (2011) showed that subjects with above-average cIMT could benefit from a Mediterranean diet, such results could not be shown across their entire population [38].

### Limitations

The majority of our study population already followed the recommendations of the WHO for physical activity that were in place at the time of the study. This potentially negated the supposed effects of an additional guideline-based exercise-based intervention. Another limitation is that physical activity was calculated from a questionnaire and not assessed objectively. It has been shown that participants may overestimate the amount of physical activity in questionnaires (e.g., to fit social desirability) [40]. In addition, most training sessions were unsupervised for feasibility reasons. However, resistance training was monitored with a smart card system and aerobic training sessions were logged by the participants.

### Conclusions

To date, this was the largest and longest guideline-based, multi-arm lifestyle interventional study aiming at reducing the progression of cIMT and cLD in middle-aged to elderly seniors. However, over the course of the intervention based on exercise, diet, and the combination of exercise and diet in older adults, atherosclerotic progression could not be slowed. Since cardiovascular risk reduction is associated with reduced cIMT progression [41], there is a clear need for larger and longer intervention studies to prove the beneficial effect of lifestyle changes in the mid to long term. Even the adapted WHO guidelines 2020 have to prove its suggested beneficial effect on the progression of atherosclerosis.

**Author Contributions:** PK, KS, RM, MH, RR and AST were responsible for conceptualization and methodology. PK, KS, RM, MH, GN, AST and RR conducted the research. GN and DI performed the statistical analyses. GN and AST wrote the manuscript. DI, PK, KS, RM, MH, RR and AST provided expert advice for statistical analysis and critically reviewed the manuscript. RR was responsible for funding acquisition. All authors read and approved the final manuscript.

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**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** Data described in the manuscript, code book, and analytic code will not be made available because ethical permissions do not allow it.

**Conflicts of Interest:** The authors declare no conflict of interest.

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## Supplement

Table 1: Mean cIMT and cLD differences, by tertiles of at least moderate physical activtiy, and sex.

| cIMT (mm) | PA tertiles | Sex | Estimate | SE | 95% CI | | p-value |
|---|---|---|---|---|---|---|---|
| | Low - Medium | Men | 0.0004 | 0.0133 | -0.0308 | 0.0317 | 0.999 |
| | Low - High | Men | -0.0063 | 0.0130 | -0.0368 | 0.0242 | 0.878 |
| | Medium - High | Men | -0.0068 | 0.0128 | -0.0367 | 0.0232 | 0.857 |
| | Low - Medium | Women | 0.0020 | 0.0128 | -0.0281 | 0.0320 | 0.987 |
| | Low - High | Women | 0.0014 | 0.0131 | -0.0295 | 0.0322 | 0.994 |
| | Medium - High | Women | -0.0006 | 0.0126 | -0.0302 | 0.0291 | 0.999 |
| **cLD (mm)** | Low - Medium | Men | 0.033 | 0.079 | -0.152 | 0.218 | 0.907 |
| | Low - High | Men | 0.047 | 0.077 | -0.134 | 0.227 | 0.816 |
| | Medium - High | Men | 0.014 | 0.076 | -0.164 | 0.191 | 0.982 |
| | Low - Medium | Women | -0.012 | 0.076 | -0.189 | 0.166 | 0.987 |
| | Low - High | Women | -0.054 | 0.078 | -0.237 | 0.128 | 0.764 |
| | Medium - High | Women | -0.043 | 0.075 | -0.218 | 0.133 | 0.836 |

*Legend: CI, confidence intervals; cIMT, carotid intima-media thickness; cLD, carotid lumen diameter; PA, physical activity; SE, standard error.*

## Chapter 7 – Publication 4

# Ideal Life's Simple 7 score relates to macrovascular structure and function in the healthy population

Authors

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## Abstract

**Background:** Cardiovascular health scores such as the Life's Simple 7 from the American Heart Association and the assessment of arterial properties are independently used to determine cardiovascular risk. However, evidence of their association remains scarce, especially in healthy, middle-aged to older populations.

**Methods:** A healthy sample of the Swiss population aged 50-91 years as part of the COmPLETE cohort study was included. Carotid intima-media thickness (cIMT), carotid lumen diameter (cLD), carotid distensibility coefficient (DC), flow-mediated dilation (FMD), and brachial-ankle pulse wave velocity (baPWV) were used to determine arterial properties. The Life's Simple 7 cardiovascular health score was calculated using seven categories (body-mass index, cholesterol, systolic blood pressure, hemoglobin A1c, smoking status, physical activity, and diet). In accordance with the American Heart Association, for each category, two points were given for an ideal health metric level, intermediate scores 1 point, and poor scores 0 points. Intermediate and ideal health scores corresponded to a total of 5-9 and 10-14 points, respectively.

**Results:** 280 participants (50.7% male) were included. After adjusting for age and sex, an ideal health score was associated with lower cIMT (-0.038 mm, 95% CI: -0.069 mm – -0.007 mm, p=0.017, lower cLD (-0.28 mm, 95% CI: -0.46 mm – -0.11 mm, p=0.002), and lower baPWV (-0.05 m/s, 95% CI: -0.08 m/s – -0.02 m/s, p=0.003). No differences were found for FMD and DC.

**Conclusions:** Even in a healthy sample of middle-aged and older adults, individuals with an ideal cardiovascular health score showed more favorable biomarkers of vascular aging than those with an intermediate score. This stresses the relevance of promoting an optimal lifestyle even among the healthy population.

## Introduction

Age is the strongest independent risk factor for cardiovascular morbidity and mortality [1]. Maintaining good health until old age plays an important role [2]. Pursuing a healthy lifestyle is a key determinant in delaying the onset of cardiovascular disease. As such, the American Heart Association introduced the Life's Simple 7, consisting of 7 modifiable and easily applicable lifestyle metrics [3]. Adherence to the Life's Simple 7 has been linked to a longer and healthier life, but in the USA, the percentage of people pursuing a healthy lifestyle has steadily been on the decline [4]. In Switzerland, recent data suggest that physical activity is on the rise [5] and more people are pursuing a healthy diet [6]. However, improvements need to be made for tobacco smoking [7] and alcohol consumption [8].

Despite offering a simple approach to implementing lifestyle changes in a patient's life, adherence to Life's Simple 7 has been shown to influence vascular aging and cardiovascular morbidity and mortality. The Atherosclerosis Risk in Communities (ARIC) Study demonstrated a reduced incidence of arterial cardiovascular diseases [9], venous thromboembolism [10], and lesser arterial stiffness at higher age in those participants that adhere to the Life's Simple 7 [11]. Other studies demonstrate a high effect of Life's Simple 7, especially in a population with a generally low proportion of individuals with an optimal lifestyle, on carotid intima-media thickness (cIMT) [12-14] and coronary artery calcification [13]. However, to the best of our knowledge, there has been no study assessing the association between ideal cardiovascular health metrics on clinical biomarkers of vascular health in a healthy middle-aged to elderly population.

This is somewhat surprising, as extensive evidence on assessing cardiovascular risk using arterial structure and function has been published. cIMT is widely used as a surrogate for cardiovascular risk as it reflects subclinical atherosclerosis [15]. Recently, pooled analyses of four cohort studies in Europe suggest that carotid lumen diameter (cLD) might improve the prediction of stroke, cardiovascular disease, and mortality beyond cIMT [16]. While men have higher cIMT and cLD than women, previous studies suggest that progression is similar for both sexes [17, 18].

In addition, brachial flow-mediated dilation (FMD) has emerged as a valid noninvasive tool to detect early changes in cardiovascular functionality and thus to predict future cardiovascular events [19]. It has previously been shown that FMD decreases with age [20] and can be used to improve the classification of aging participants with low, intermediate, and high cardiovascular risk [19, 21].

Further, arterial stiffness measured by brachial-ankle pulse wave velocity (baPWV) is an independent predictor of cardiovascular events, even in healthy adults [22]. Arterial stiffening, a well-recognized indicator of early vascular aging, results from arterial wall remodeling driven by lifestyle factors, such as diet and physical activity [23].

In the general population, the Life's Simple 7, and various ultrasound-based assessment methods of arterial structure and function are independently known to be influenced by age and lifestyle. Both have been used separately to assess cardiovascular health, but evidence of their association is scarce, especially in healthy, middle-aged to elderly populations. Therefore, this study aims to assess the association between the Life's Simple 7 cardiovascular health score

and clinical biomarkers of structure and function of the main arteries in healthy community-dwelling participants.

## Methods

### Population and recruitment

The study consisted of participants between 50 and 91 years of age who were part of the COmPLETE Health study at the University of Basel, and tested between January 2018 and June 2019. The study was designed to identify healthy individuals who may serve as perfect examples to characterize healthy aging in the Swiss population. The full study protocol has been published previously [24]. Briefly, all participants were invited via letters sent to various districts of the city of Basel, Switzerland, and its suburbs. To be included in the COmPLETE Health Study, all participants were to be non-smokers for at least 10 years, have blood pressure values <160/100 mmHg, had to be free of any cardiovascular disease, and with a BMI <30 kg/m2. Exclusion criteria included drug or alcohol abuse, pregnancy, breastfeeding, history of cancer, or the inability to follow the study procedures. For the present study, only participants aged 50+ years old were included, as we did not measure carotid properties in those who were younger than 50 years. Participants were asked to refrain from any vigorous physical activity 24h before the measurement. Further, abstaining from alcohol consumption or caffeine on the day of the examination as well as fasting for three hours was requested. All the vascular screenings were performed in a supine position after the participants rested for at least 10 minutes in a dim-lighted, quiet room with ambient temperature. All participants were asked to wear noise-canceling headphones. At the start of the single visit, written informed consent was obtained from all participants before undergoing any study procedures. The study was approved by the Ethics Committee of Northwestern and Central Switzerland (EKNZ 2017-01451) and complied with the Declaration of Helsinki.

### Carotid properties

Carotid intima-media thickness (cIMT) measurement was performed using a standardized ultrasound scan protocol using the Fukuda UF 760 ultrasound scanner (Fukuda Denshi, Toyko, Japan) with a FUT-LA385-12P (8–13 MHz) transducer (Fukuda Denshi, Tokyo, Japan) [25]. The measurements were limited to the right common carotid artery to determine cIMT and carotid stiffness. Participants were asked to stay supine for 10 minutes with the head rotated at 45° to facilitate measurement. The ultrasound was performed along the common carotid artery and proximal to the carotid bifurcation over the course of two to six heart cycles. Software-based electrocardiogram-gated real-time quality control with automatic validation was used (Fukuda Denshi, Tokyo, Japan). This is based on a previously developed detection algorithm to assure accurate wall detection [26]. Inner carotid lumen diameter (cLD) and carotid distensibility coefficient (DC) were measured simultaneously. DC was calculated as $\frac{(2 \times \text{delta cLD} \times \text{diastolic cLD}) + (\text{delta cLD})^2}{(PP \times \text{diastolic cLD})^2}$, whereas delta cLD equated to systolic cLD minus diastolic cLD, and pulse pressure (PP) equated to systolic blood pressure minus diastolic blood pressure. In addition, blood pressure was measured oscillometrically on both arms (OMRON 705IT, OMRON Healthcare, Kyoto, Japan).

**Pulse wave velocity**

PWV was measured as brachial-ankle PWV (baPWV) using a noninvasive vascular screening device (VaSera VS-1500 N; Fukuda Denshi, Tokyo, Japan). Blood pressure cuffs were placed around the left ankle and left upper arm, and ECG recording was performed simultaneously. Using the foot-to-foot method for noninvasive measurement of baPWV, the pulse wave time from the heart to the ankle was calculated. The estimation of baPWV was done using a height-based formula by the VSS-30 software (Fukuda Denshi, Tokyo, Japan) [27]. The average of two measurements was taken to determine blood pressure and baPWV. If the difference between the two measurements was >10mmHg for systolic blood pressure, a third measurement was conducted. The average of the lowest two values was then used.

**Endothelial function**

FMD was measured semiautomatically using a high-resolution ECG-guided B-mode ultrasound system (UNEX EF 38G, UNEX Corp., Nagoya, Japan) according to current guidelines [28]. A 10-MHz H-shaped probe was used to generate short-axis and long-axis images of the right brachial artery and continuous automatic correction of the probe position during the whole measurement period. During the procedure, the arm was abducted at a 90° angle, and the insonation angle of the probe was fixed between 60 and 70°. The proximal edge of the cuff was placed 1–2 cm proximal to the cubital fossa and 5–10 cm distal to the probe. Precuff-inflation diameter was measured for 10 s immediately before the 5 min occlusion period. During the occlusion period, cuff pressure exceeded systolic blood pressure by 50 mmHg. During the last 60 s, precuff-deflation diameter was measured. Post deflation diameter and blood flow velocity were continuously measured during the 3 min post deflation period, and end-diastolic values (ECG-guided) were recorded for subsequent data analysis. After data collection, blinded semiautomatic quality control of all measurements was conducted using the UNEX EF 38G 1.0.14 software (UNEX Corp., Nagoya, Japan).

**Physical activity**

Physical activity was measured objectively via a wrist-worn triaxial accelerometer at a frequency of 50 Hz (GeneActive Activinsights Ltd., Kimbolton, UK) for 14 days. All participants were instructed to wear the accelerometer on the nondominant wrist during the measurement period and return it on the 15th day. The data was exported via the GENEActiv software v3.2 (GeneActiv Activinsights Ltd., Cambridgeshire, UK) and analyzed via the open-source Excel macro file "General Physical Activity" version 2 (Activinsights Ltd., Cambridgeshire, UK) [29]. The cutoffs for physical activity intensity were defined as 1.5-3.99 MET for low physical activity, 4.0-6.99 MET for moderate physical activity, and ≥7 MET for vigorous physical activity. Cutoffs for valid days were defined as follows: 1) at least ten hours of wear time over one day (midnight to midnight), 2) less than 18h of low physical activity, 3) less than eight hours of moderate physical activity, and 4) less than 2.5h of vigorous physical activity. Participants were only included in the analyses if at least five weekdays and two weekend days were valid [30]. For all valid days, the number of minutes of low, moderate, and vigorous physical activity was averaged.

**Nutrition**

Nutrition status was calculated using a validated dietary assessment tool [31]. Dietary assessment tools have been useful in estimating dietary habits, such as the amount of fruit and vegetables consumed. This dietary assessment tool displays the food pyramid of the Swiss Society for Nutrition (version 2005-2011). A portion size equivalent for various food items of the respective category is pictured in the middle, as well as five mealtimes (breakfast, snack #1, lunch, snack #2, and dinner). On the right side, there is a column for the sum of the five mealtimes. All participants were asked to fill out the dietary assessment tool for a typical day (e.g., normal workday, illness-free) and to write the number of portions per food item per mealtime. Subsequently, the kilocalories of the different food groups were calculated via a portion size equivalent.

**Other measurements**

Body height and mass were measured to the nearest 0.5 cm and 0.1 kg. Body-mass index was calculated as weight in kg divided by the squared value of height in meters. Smoking status was assessed before the visit via telephone interview. For hemoglobin A1c (HbA1c) and cholesterol, blood samples were collected by standard laboratory procedures with the Cobas analyzer (Cobas 8000; Roche Diagnostics, Basel, Switzerland).

**Cardiovascular health score**

As a surrogate for ideal cardiovascular health, we used the Life's Simple 7 score, introduced by the American Heart Association in 2010 [3]. The health score incorporates four modifiable behavioral metrics, namely physical activity, diet, smoking status, and BMI, as well as three biological metrics, namely systolic blood pressure, HbA1c, and cholesterol. For each health metric used for the score, a study participant could either score 0 (poor), 1 (intermediate), or 2 (ideal) points. Therefore, the health score ranged from 0 (poor) to 14 (ideal). The limits of each variable used in the health score were the same as those presented by the American Heart Association [3]. Scoring 0-4 points was considered poor, 5-9 intermediate, and 10-14 ideal. The dietary component of the Life's Simple 7 consists of 4 goals, which are ≥400 g of vegetables per day, ≥2 servings of fish per week (=30 g of fish per day), ≥14 g of fiber per 1000 kcal, and <10 energy percentage of daily intake of saturated fatty acids. The dietary assessment tool used in this study does not allow the assessment of all four components. Therefore, we used the daily servings of fruits and vegetables as a proxy for diet quality. Poor diet was considered ≤3 servings/day, 3-4.9 as intermediate, and ≥5 as ideal. In addition, we did not measure fasting blood glucose but HbA1c. We used the cutoff values for poor, intermediate, and ideal scores that were suggested by Ford et al. (2012), which determined ≥6.5% as poor, 5.7% to <6.5% as intermediate, and <5.7% as ideal [32].

**Statistical analyses**

Means and standard deviations were calculated for the descriptive section of our dataset. We performed independent t-tests to calculate the differences between the sexes.

We used multiple linear regression models to compare the outcomes (cIMT, cLD, DC, FMD, baPWV) between individuals with an ideal and an intermediate health score. All models were adjusted for sex and age. In addition, cLD was adjusted for height [33]. We included age using restricted cubic splines with four knots placed at specific data percentiles to account for

potential nonlinear associations [34] and an interaction between age and sex. Due to missing data, complete-case analyses would have resulted in the loss of 1% of observations for cIMT and 23%, 23%, 24%, and 0% for cLD, DC, FMD, and baPWV. Hence, we handled missing data using multiple imputation [35, 36]. Specifically, we used predictive mean matching to impute 50 datasets using all outcome variables, age, sex, health score, and height for prediction. Adequacy of model fits was checked using Q-Q-plots of the residuals and plots of fitted values versus residuals. As the residuals of the models for DC and baPWV showed a marked deviation from normality, we log-transformed those outcomes, after which the model assumptions were satisfied. For the log-transformed effects, we present the exponentiated estimates and confidence intervals which represent the ratio of the geometric means of the ideal outcomes to the intermediate health score.
Significance was set at $p<0.05$ in all tests; all tests were two-sided. All analyses were performed using SPSS version 26.0 for Windows (SPSS Inc., Chicago, Illinois, USA) and R version 4.2.0 for Windows (R Foundation for Statistical Computing, Vienna, Austria).

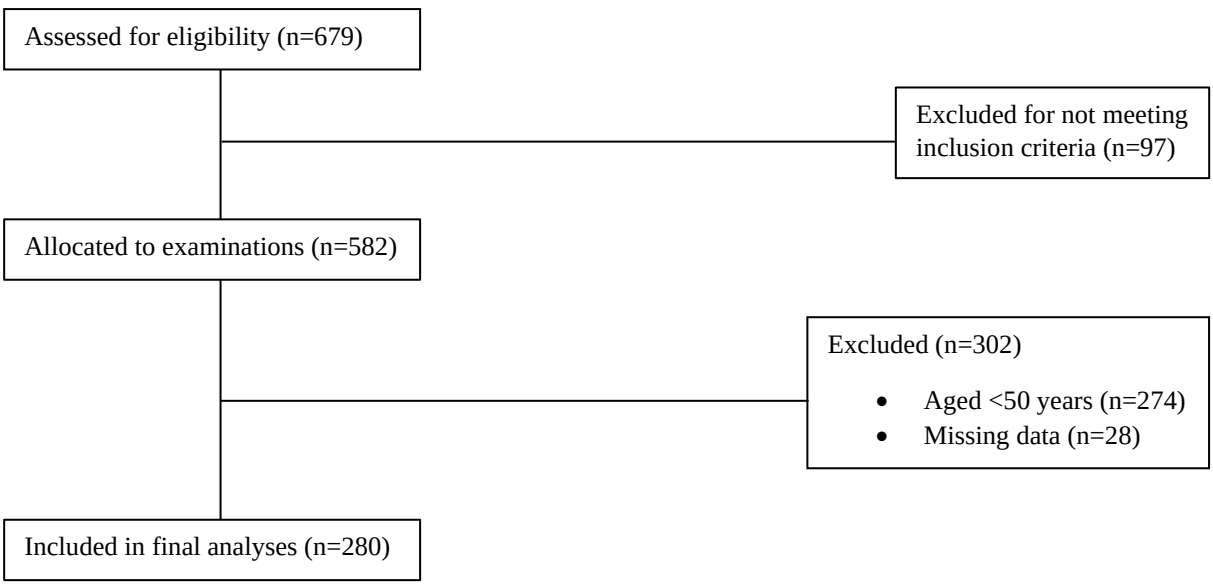


Figure 1: Flow-chart of participant recruitment and data acquisition.

## Results

Data from 280 participants (50.7% male) aged 50-91 years old were included in the analyses (figure 1). The participants' characteristics are described in table 1. In total, 125 participants (50.4% male) had an ideal cardiovascular health score, 155 participants (51.0% male) had an intermediate cardiovascular health score, and none of the participants had a poor cardiovascular health score.

All analyses of the arterial properties were adjusted for sex and age. As shown in table 2, those with an ideal health score had lower cIMT than those with an intermediate score (-0.038 mm, 95% CI: -0.069 mm – -0.007 mm, p=0.017). Similarly, those with an ideal health score had lower cLD than those with an intermediate score (-0.28 mm, 95% CI: -0.46 mm – -0.11 mm, p=0.002). Those with an ideal health score had a 5% lower geometric mean of baPWV (geometric mean ratio: 0.95) compared to those with an intermediate health score (95% CI: 0.92–0.98, p = 0.003). Figure s (see supplements) shows the absolute means of baPWV, by cardiovascular health score category. Those with an ideal health score had a 0.31% higher FMD than those with an intermediate health score on average (95% CI: –0.58% – 1.20%, p = 0.495). Finally, individuals with an ideal health score had a 6% lower geometric mean of DC (geometric mean ratio: 0.94) on average compared to individuals with an intermediate health score (95% CI: 0.85 – 1.05, p = 0.207). For both FMC and DC, our data provided little evidence for a true difference between the groups.

Table 1: Participants' characteristics, by cardiovascular health score category. P-value indicates the difference between the ideal and intermediate health scores.

| | N | **All** | N | **Ideal HS** | N | **Intermediate HS** | **p-value** |
|---|---|---|---|---|---|---|---|
| | | Mean (SD) | | Mean (SD) | | Mean (SD) | |
| **Male, n (%)** | 280 | 142 (50.7) | 125 | 63 (50.4) | 155 | 79 (51.0) | |
| **Age** | 280 | 68.1 (10.7) | 125 | 64.7 (10.1) | 155 | 70.9 (10.4) | <0.001 |
| **cIMT (mm)** | 277 | 0.772 (0.144) | 124 | 0.730 (0.127) | 153 | 0.807 (0.148) | <0.001 |
| **cLD (mm)** | 216 | 7.21 (0.77) | 103 | 6.96 (0.78) | 113 | 7.43 (0.68) | <0.001 |
| **DC (l/kPa)** | 216 | 905.5 (344.9) | 103 | 903.3 (308.0) | 113 | 907.6 (376.7) | 0.872 |
| **baPWV (m/s)** | 280 | 13.65 (2.49) | 125 | 12.77 (2.22) | 155 | 14.36 (2.48) | <0.001 |
| **FMD (%)** | 212 | 5.86 (3.66) | 98 | 6.27 (3.69) | 114 | 5.51 (3.62) | 0.122 |
| **Systolic BP (mmHg)** | 280 | 131 (13) | 125 | 125 (12) | 155 | 136 (12) | <0.001 |
| **Diastolic BP (mmHg)** | 280 | 81 (8) | 125 | 78 (8) | 155 | 83 (8) | <0.001 |
| **Body mass index (kg/m2)** | 280 | 24.0 (2.7) | 125 | 23.3 (2.4) | 155 | 24.5 (2.8) | <0.001 |
| **Waist to hip ratio** | 280 | 0.90 (0.08) | 125 | 0.88 (0.07) | 155 | 0.91 (0.08) | <0.001 |
| **Low PA (min)** | 272 | 107 (34) | 125 | 109 (36) | 155 | 104 (32) | 0.213 |
| **Moderate PA (min)** | 272 | 154 (65) | 125 | 177 (62) | 155 | 134 (60) | <0.001 |
| **Vigorous PA (min)** | 272 | 6 (9) | 125 | 9 (11) | 155 | 4 (7) | <0.001 |
| **Triglycerides (mg/dl)** | 278 | 121 (59) | 124 | 126 (55) | 154 | 125 (54) | 0.119 |
| **Total cholesterol (mg/dl)** | 278 | 238 (38) | 124 | 226 (34) | 154 | 248 (39) | <0.001 |
| **Fruits and vegetables (portions/d)** | 277 | 3.5 (1.7) | 124 | 4.2 (1.8) | 153 | 3.0 (1.4) | 0.024 |

*Legend: baPWV, brachial-ankle pulse wave velocity; BP, blood pressure; cIMT, carotid intima-media thickness; cLD, carotid lumen diameter; DC, distensibility coefficient; HS, Life's Simple 7 cardiovascular health score; PA, physical activity.*

Table *2*: Estimated mean difference or ratio of geometric means of the arterial properties between ideal and intermediate cardiovascular health score. Based on multiple imputation models. Adjusted for age and sex.

| Outcome variable | Health Score | Estimate | 95% Confidence Intervals | | p-value |
|---|---|---|---|---|---|
| **cIMT (mm)** | Ideal - Intermediate | -0.038 | -0.069 | -0.007 | 0.017 |
| **cLD (mm)** | Ideal – Intermediate | -0.28 | -0.46 | -0.11 | 0.001 |
| **DC (1/kPa)** [1] | Ideal – Intermediate | 0.94 | 0.85 | 1.05 | 0.267 |
| **FMD (%)** | Ideal – Intermediate | 0.31 | -0.58 | 1.20 | 0.495 |
| **baPWV (%)** [1] | Ideal - Intermediate | 0.95 | 0.92 | 0.98 | 0.003 |

*Legend: baPWV, pulse wave velocity; cIMT, carotid intima-media thickness; cLD, carotid lumen diameter; DC, distensibility coefficient; FMD, flow-mediated dilation.* [1] indicates geometrical mean ratios.

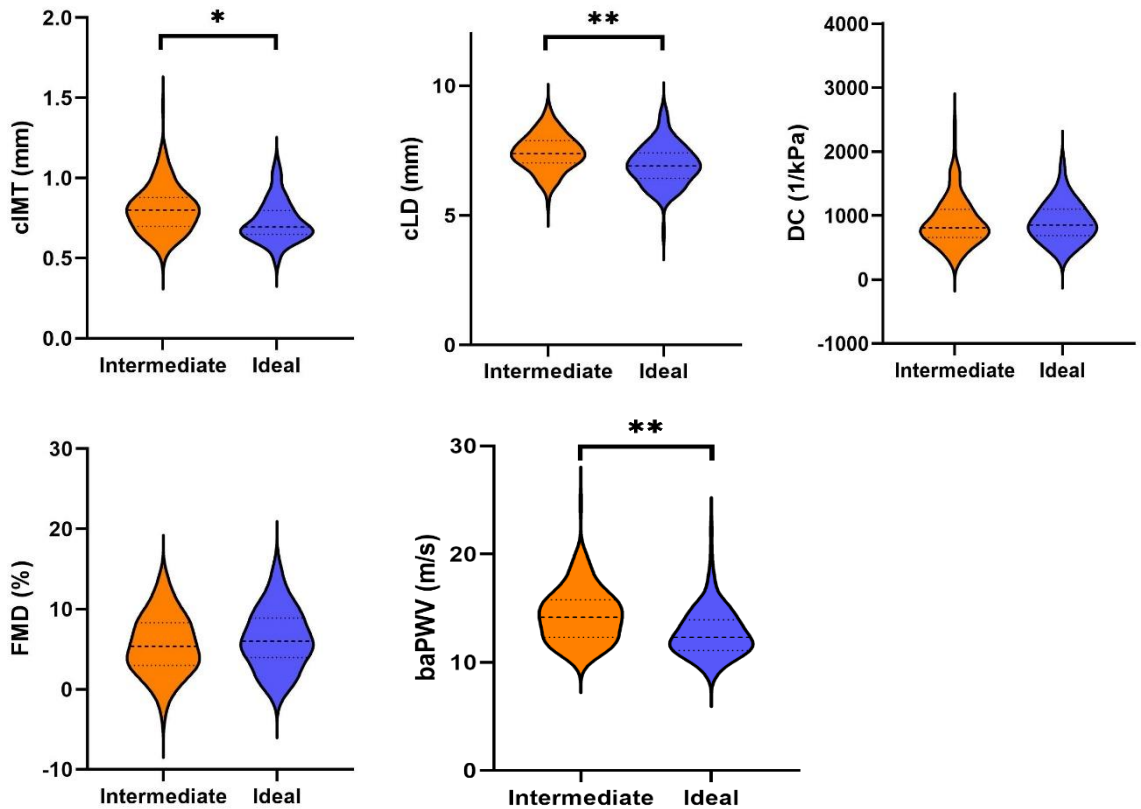


Figure *2*: Violin plots of the arterial properties, divided into intermediate and ideal health scores. *Legend: baPWV, pulse wave velocity; cIMT, carotid intima-media thickness; cLD, carotid lumen diameter; DC, distensibility coefficient; FMD, flow-mediated dilation.* * indicate p<0.05, ** indicate p<0.01.

**Discussion**

In this cross-sectional analysis that included participants aged 50-91 years old and free of any cardiovascular disease, we found that ideal cardiovascular health was associated with lower cIMT, cLD, and baPWV. We found no differences for FMD or DC.

While we found statistically significant differences between intermediate and ideal cardiovascular health, these results beg the question of whether they are clinically relevant. For cIMT, Lorenz et al. (2007) performed a meta-analysis comprising eight studies and over 37'000 adults [37]. Adjusted for sex and age, a 0.1 mm difference in cIMT equated to an increased risk of myocardial infarction of 10% to 15%. Similarly, the risk of stroke increases by 13% to 18% per 0.1 mm cIMT difference. Given that the cIMT difference between the intermediate and ideal cardiovascular health groups was considerably lower in our study, the benefits of ideal over intermediate cardiovascular health metrics in an otherwise very healthy population remain controversial. This is in line with recent results of the meta-analysis by Fritze et al. (2020) [18]. The authors concluded that cIMT provided little information on cardiovascular risk and non-cardiovascular mortality. However, they found that higher cLD was associated with a higher hazard ratio for coronary heart disease and cardiovascular disease. A 1 mm difference in cLD equated to an increased cardiovascular risk of 29%. As with cIMT however, the difference in cLD in our population was much lower than what can be considered clinically relevant.

As for baPWV, the meta-analysis by Vlachopoulos et al. (2012) showed that per 1 m/s increase, the risk of cardiovascular events, cardiovascular mortality, and all-cause mortality increased by 12%, 13%, and 6%, respectively. The baPWV difference found between the two groups in our study is considerably lower, again leaving the matter of its clinical relevance open to discussion.

Taken together, only three of the biomarkers of arterial structure and function showed statistically significant differences between the intermediate and ideal cardiovascular health groups in our study. Although these effects are relatively small, it seems noteworthy that ideal lifestyle-based cardiovascular health metrics still impact vascular aging even in such a healthy population. In accordance with studies on people with a broader range of cardiovascular risk and a higher proportion of individuals with poor cardiovascular health [12-14], these results demonstrate the importance of promoting the highest possible adherence to Life's Simple 7 in any population. However, reaching an intermediate cardiovascular health metric might be sufficient for healthy vascular aging in very healthy people with high fitness and low cardiovascular risk. This is in agreement with a recent meta-analysis, that showed that although meeting 5-7 of the Life's Simple 7 metrics offered the highest protection from cardiovascular events, meeting 3-4 metrics was already associated with a 47% risk reduction when compared to those with a score of 0-2 [38].

With this study, we consolidate the case for using Life's Simple 7 to assess cardiovascular health and make lifestyle recommendations. We demonstrated that in the healthiest part of the population aged 50+ years, meeting some health metrics was associated with similar vascular health to meeting most or all metrics. In contrast, other assessment tools, such as the Framingham Risk Score [39], heavily depend on non-modifiable metrics, such as age, sex, and race [40]. It is also well known that ultrasound-based cardiovascular risk predictors such as cIMT or FMD are profoundly influenced by those non-modifiable metrics. For example, recent findings in a healthy, elderly population showed that 28.2% of cIMT variance in men and 23.9%

in women was explained by age [41]. Similarly, using data from the same population as in the present study, we found that FMD decreased by 63.6% in men and 47.1% in women between the ages of 20-91 years [20]. Our results may therefore be of particular interest and motivating for the general population, as the Life's Simple 7 has the advantage of being more comprehensive and communicative than other risk scores and solely consist of modifiable metrics.

**Strengths and limitations**

This study population consisted of middle-aged and older subjects with no manifested cardiovascular disease or history of cardiovascular events. Exclusion criteria included cardiovascular risk factors, such as obesity, smoking, and high blood pressure. Therefore, our study population was unique, representing a healthy population sample. Further, we used state-of-the-art measuring techniques for arterial properties, physical activity, and blood analyses and strictly adhered to current guidelines. This study, however, has several limitations. As with all cross-sectional approaches, results must be interpreted accordingly, as causation cannot be determined. Second, the strength of having a population free of cardiovascular disease and with only very few cardiovascular risk factors can also be seen as a limitation, as our results cannot be translated to the general population. Third, the diet assessment was done using a questionnaire that did not allow to conclude on all dietary components used in the Life's Simple 7 score. Lastly, due to some missing data, we had to impute values, potentially leading to an overestimation of test statistics [42].

**Conclusions**

This study demonstrates that differences in arterial properties can be found even in a healthy population with either an intermediate or ideal cardiovascular health score. When maintaining a healthy lifestyle becomes more and more challenging, Life's Simple 7 offers a comprehensive and communicable approach to improving and preserving vascular health over the life course. Ultrasound-based measurements of arterial properties, such as FMD, cIMT, and baPWV, are feasible and highly valuable tools to monitor the effects of lifestyle modifications in general, especially cardiovascular morbidity and mortality.

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**Institutional Review Board Statement:** The studies involving human participants were reviewed and approved by the Ethics Committee of Northwestern and Central Switzerland (EKNZ 2017-01451).

**Informed Consent Statement:** All participants provided their written informed consent to participate in this study.

**Data Availability Statement:** The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

**Conflicts of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplement

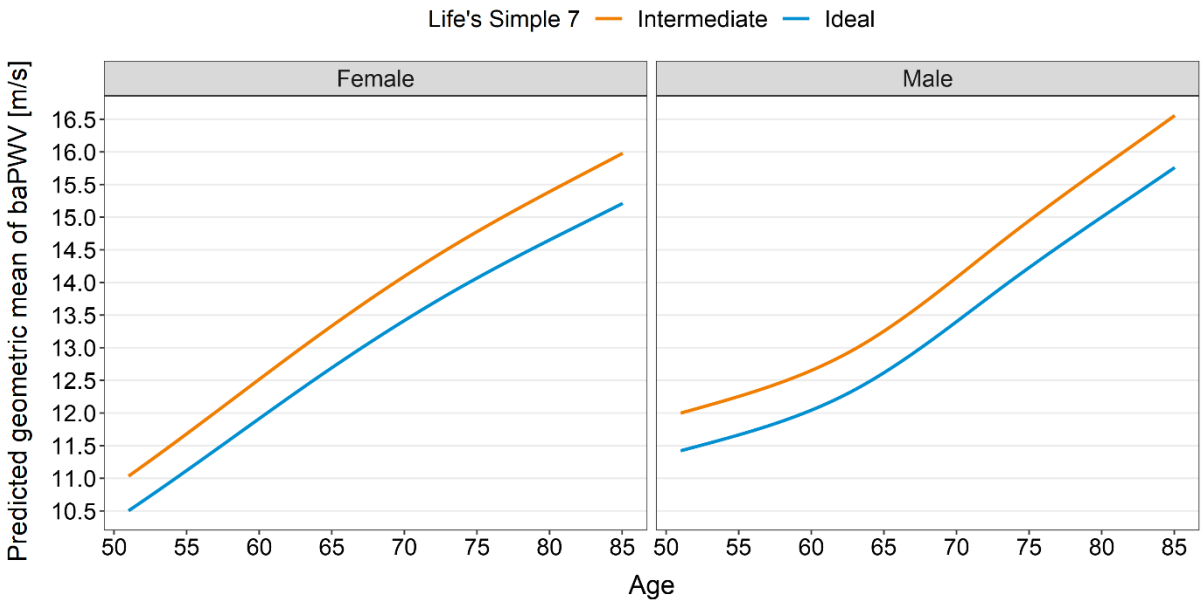


Figure 1: baPWV prediction by cardiovascular health score category.

# Chapter 8 – Discussion and outlook

My PhD project aimed at explaining the association between lifestyle factors and the properties of large human arteries. It was composed of several sections and multiple studies. Beginning with the completion of the data collection of the Swiss COmPLETE Health and COmPLETE Heart studies I continued with the analyses of the ultrasound data from the Finnish DR's EXTRA study. Afterwards, I validated the DAT used in the COmPLETE study. In the following chapters, I discuss the main results from the four publications. I will present the findings from the cross-sectional Swiss and Finnish data, as well as from the Finnish intervention. Lastly, I will provide an outlook on how the findings from my PhD project may be relevant for future studies and research projects.

## 8.1 Main findings

My PhD project sheds light on how lifestyle habits are associated with arterial properties and how lifestyle may influence them. On the one hand, this project shows that adherence to healthy lifestyle components was associated with healthier arteries. These findings were first described in a middle-aged to elderly Finnish population and focused on the carotid arteries. Then, the Finnish results were confirmed in a healthy sample of the Swiss population aged 50+ years old. To better see the differences between the Finnish and Swiss cohorts, table 2 shows the participants' characteristics of both studies. On the other hand, my project clearly displays that an intervention aiming at improving physical activity, diet, or a combination of both, did not effectively overcome the aging effect on carotid properties in a representative sample of middle-aged to elderly Finns. We made evident that atherosclerotic progression was not slowed in any of the intervention groups, when compared to the reference group. Lastly, my PhD project successfully demonstrated that a simple visually aided dietary assessment tool was valid to assess some dietary components, such as fruits and vegetables consumption. The dietary assessment tool showed that, especially the elderly population, was able to estimate fruits and vegetables consumption at an acceptable precision, when compared to the food record. This kind of tool may therefore be of high value in future studies, as the precise assessment of dietary habits is known to be burdensome and inaccurate for which dietary assessment is often left out in large study settings.

*Table 2: Participants' characteristics of the DR's EXTRA and COmPLETE studies.*

| | **DR's EXTRA (n=1400)** | **COmPLETE Health (n=280)** |
|---|---|---|
| **Male, n(%)** | 692 (49.4) | 142 (50.7) |
| **Age** | 66.4 (5.4) | 68.1 (10.7) |
| **Life's Simple 7** | 7.4 (1.8) | 9.3 (1.6) |
| Low | 82 (5.9) | 0 (0.0) |
| Intermediate | 1144 (82.0) | 155 (55.4) |
| Ideal | 170 (12.1) | 125 (44.6) |
| **Carotid intima media thickness (mm)** | 0.824 (0.142) | 0.772 (0.144) |
| **Carotid lumen diameter (mm)** | 7.23 (0.90) | 7.21 (0.77) |
| **Carotid distensibility ($kPa^{-1}$)** | 1170 (700.0) | 905.5 (344.9) |
| **Systolic blood pressure (mmHg)** | 147 (20) | 131 (13) |
| **Diastolic blood pressure (mmHg)** | 83 (9) | 81 (8) |
| **Body mass index ($kg/m^2$)** | 27.6 (4.5) | 24.0 (2.7) |
| **Light physical activity (min/week)** | 133 (216) | 107 (34) |
| **Moderate physical activity (min/week)** | 289 (314) | 154 (65) |
| **High physical activity (min/week)** | 112 (165) | 6 (9) |
| **Use of medication** | | |
| Antihypertensives n(%) | 587 (41.9) | 49 (17.5) |
| Antidiabetics n(%) | 99 (7.1) | 0 (0.0) |
| Statins n(%) | 490 (35.0) | 18 (6.4) |

## 8.2 Cross-sectional findings

The cross-sectional approaches of both the Finish DR's EXTRA and the Swiss COmPLETE study are relevant for several reasons. First, the analyses of cIMT as a well-established biomarker of vascular health allows for comparisons with other populations. We showed that the general Finnish population had higher cIMT than the healthy Swiss sample. Second, we added the measurement of cLD to our analyses, which has recently been suggested to be more strongly associated with cardiovascular risk than cIMT [41]. Until now, cLD has not yet been extensively examined in relation to the AHA's Life's Simple 7 health score. We therefore make a valuable contribution to the knowledge gap by including two different cohorts, with 1'400 and 280 participants, respectively.

Although not published in any of the four manuscripts, the explicit difference between carotid properties of Swiss and Finnish aging populations is striking, as the Swiss showed had lower cIMT, cLD, and higher carotid distensibility [33]. We hereby contribute to the existing evidence of a strong South – North gradient for cIMT in European populations. In the publication of the IMPROVE study by Baldassarre et al. (2010), data from Italy, France, the Netherlands, Sweden, and Finland were collected [33]. The IMPROVE study was the first to describe the North-South gradient of cIMT in the general population. The Finnish data of the IMPROVE study were taken in the same center as the DR's EXTRA study, therefore we were able to expand the database of Finnish cIMT data and add cLD measurements.

As for the COmPLETE study executed in Basel, Switzerland (latitude 47.6°N), it can be placed directly between the IMPROVE data from France (48°N) and Italy (45°N and 43°N). The results of COmPLETE and DR's EXTRA fit very well into the existing evidence on the matter, as inclusion criteria regarding age were comparable. The cIMT values measured in Finland were slightly lower (0.824 mm vs. 0.870 mm) than reported by Baldassarre et al. (2010), but still higher than what we measured in Switzerland (0.772 mm) [33]. Although latitude was the strongest predictor for cIMT in the IMPROVE study, and Switzerland is located further North than Italy, our cIMT data was lower than that measured in the two Italian IMPROVE study centers. While this may seem counterintuitive at first glance, the unique characteristics of the COmPLETE study and the different measuring techniques may likely be the reason for this phenomenon: As smoking [100], high BMI [101], and other lifestyle habits [102] are predictors for increased cIMT, it is hardly surprising that only including the healthiest individuals of the population will result in lower cIMT.

The COmPLETE data are very valuable nevertheless, as they show that not being obese, not smoking, and not suffering from arterial hypertension grade II or above (>160/100 mmHg) can positively impact healthy ageing. As to why latitude is such an important factor for cIMT, there are several potential explanations. Dietary habits are thought to impact cIMT, as evidence suggests that the Mediterranean diet (high in fruits and vegetables, olive oil, nuts and seeds, and fish) has protective effects on the cardiovascular system [103, 104]. In the IMPROVE study, however, while the Southern countries had better diet scores, the association with cIMT was non-significant. The authors argued that socioeconomic status and education may play a role, as those with higher purchasing power tend to adhere to healthier diets [105, 106]. Further, the Nordic diet also includes important elements of the Mediterranean diet (e.g., fruits, nuts, whole grains, and fish), with the fundamental difference being the source of fats [107]. Whereas the Mediterranean diet promotes the use of olive oil, the Nordic diet uses rapeseed and canola oil. Whether this small difference is partially responsible for the differences in cIMT is unclear. To the best of my knowledge, only one study has explored the association between cIMT and the Nordic diet [108], in which the authors did not find any evidence suggesting an association between the two factors. The IMPROVE study group also found that, as with dietary quality, the inclusion of socioeconomic status and education did not seem to explain the latitude differences for cIMT [33]. The authors also pointed at ethnicity and genetic factors that may influence carotid atherosclerosis. As they noted, genetic predispositions such as family history of coronary heart disease, are associated with higher cIMT, but the exact role of genetics is not fully understood yet. Genetic predisposition, such as family history, was not a focus of this PhD thesis. It is well known that those with positive family history of cardiovascular disease, have increased cIMT, even with low numbers of other cardiovascular risk factors [109]. Whether the influence of genetic factors on cIMT decreases with age, remains to be proven. As such, it is noteworthy, and very positive, that the data we collected show that healthy lifestyle habits may dampen the influence of genetic factors.

Regarding the Life's Simple 7 health score, the results of both DR's EXTRA and COmPLETE are worth a closer look. In the DR's EXTRA study, we found that none of the participants had a perfect score (14/14 points), while only one person (0.1%) scored 13/14 points (manuscript 2). For COmPLETE, one participant scored 14/14 points (0.4%), and an additional seven participants scored 13/14 points (2.5%) (manuscript 4). Our results are comparable with other populations. For example, a Spanish population-based study found that only 0.2% of adults obtained all 14 points [110]. In contrast, Benjamin et al. (2017) showed that only 3% of US American adults did not meet any of the ideal health metrics and 15% met only one of the 7 ideal metrics [79]. Further, less than 40% of the adults aged 60+ years old had ≥2 health metrics at ideal levels and at any age, with women showing higher cardiovascular health scores than men. In the DR's EXTRA study, only 0.6% of our population did not achieve a single ideal health metric, which is similar to findings from other European studies [88, 110]. In the COmPLETE population, due to the exclusion criteria, all participants had at least one perfect metric, namely being non-smoker or having stopped tobacco smoking >10 years ago. On average, the participants of the COmPLETE study had a Life's Simple 7 score of 9.3, whereas in DR's EXTRA, the average was at 7.4. This difference is hardly surprising but still highlights that even in the healthiest individuals of the population, the average health score was intermediate, not ideal. This shows that there is still great potential for lifestyle improvement interventional programs. A wider use of the Life's Simple 7 may help improve lifestyle in the general population, as it only includes modifiable health metrics, therefore being more comprehensive and motivational than traditional cardiovascular risk score. Having compared the results from COmPLETE and DR's EXTRA, the following chapters will discuss the specific findings of the respective studies.

### 8.2.1 The DR's EXTRA study

The DR's EXTRA cross-sectional publication is, to the best of my knowledge, only the second study to report on cIMT, carotid distensibility, and the Life's Simple 7, with only the Parisian PPS3 study reporting associations between the three in 2016 [88]. We did, however, have two non-negligible advantages over PPS3. First, our study cohort consisted of individuals from the general population, rather than patients of primary health care centers, which allows for a more representative overview of the population. Our results may therefore be closer to what can be expected in the general population and easier to compare with future general population-based cohorts. Second, in addition to measuring cIMT as a structural parameter of the carotid artery, we also included cLD, which reflects structural as well as functional aspects of the artery. While cIMT is a well-established and independent cardiovascular risk factor, the recent findings of the meta-analysis by Fritze et al. (2020) [41] recommend using cLD to assess cardiovascular risk, as it is a better predictor than cIMT. In our study, we were therefore able to follow a more holistic approach on carotid structure than PPS3, assessing both cIMT and cLD. Indeed, we were able to demonstrate that cIMT is not associated with the Life's Simple 7, whereas lower cLD is associated with a higher Life's Simple 7 score. We showed that those with an ideal score

(i.e., score 10+ points) had significantly lower cLD than the ones with an intermediate or poor health score. To the best of my knowledge, our study group was the first to do so, highlighting the importance of measuring cLD. Our results, however, somewhat contradict the PPS3 findings, which reported lower cIMT in those with a more favorable Life's Simple 7 score. As explained in the introduction of my PhD thesis, only little and contradicting evidence is available on cIMT and the Life's Simple 7 [89, 90]. On the one hand, two studies (namely the PPS3 from France, and a US-American twin study) showed an association between cIMT and the Life's Simple 7. On the other hand, the AGES-Reykjavik study from Iceland and the DR's EXTRA study, both from Northern Europe, failed to show an association between the two parameters. Whether that is a coincidence or not is too early to state, but we are adding to the evidence of cross-sectional analyses stating that in Northern Europe, cIMT may not be as easily influenced by a healthy lifestyle.

In addition to cIMT, as PPS3 did too, we measured carotid distensibility. We were able to find significantly lower distensibility (i.e., stiffer carotid arteries) in the group with a poor Life's Simple 7 health score, as opposed to those with an ideal score. To my knowledge, we are the only two study groups to have done so. That cIMT, a structural biomarker, is not associated with the Life's Simple 7, but cLD and carotid distensibility, both markers of functional capacity of the artery, are, is interesting. As not much information is published on that matter, the reason for this association remains speculative. The authors of the PPS3 study argued that functional parameters may better mirror the cumulative exposure to different cardiovascular risk factors, and therefore may be better to interpret together with the Life's Simple 7. To me, a potential explanation may lie in the compensatory mechanisms of the arteries due to age, and more specifically, lifestyle [111]. Large arteries, like the carotid artery, are composed of a considerable portion of elastin. Elastin is a matrix protein responsible for the distensibility of the arteries, giving them the ability to relax during systole and to lower the afterload on the heart, known as the Windkessel effect. Stiffer arteries reduce said effect, increasing the strain on the heart and reducing perfusion to the organs. Therefore, to counteract the impaired blood flow caused by a stiffer carotid artery, it has been proposed that the artery remains wider during diastole to compensate for the reduced expansion during systole [41]. As only few studies have reported that cLD may be more predictive for cardiovascular events than cIMT, more data are needed to confirm those claims. Our study group was the first to report the association between cLD and the Life's Simple 7, hopefully opening the door for more studies on that matter in the future. The association we found regarding carotid distensibility and the Life's Simple 7 seems to further strengthen the argument of the compensatory mechanisms of the carotid artery. As for all three carotid parameters that we used, however, there are not enough data available yet to make definitive statements.

### 8.2.2 The COmPLETE cohort study

With the COmPLETE study, we were able to extract valuable results from the unique dataset the cohort study offers. Indeed, the COmPLETE population provides novel information on healthy ageing. As such, none of the participants in the study had a poor Life's Simple 7 score. This is due to the strict inclusion criteria of COmPLETE, as current tobacco smoking and smoking cessation <10 years before the study, a BMI >30 kg/m$^2$, and blood pressure >160/100 mmHg or the usage of antihypertensive medication were exclusion criteria. As a result, any COmPLETE cohort study participant automatically scored at least 3 points (2 points for not smoking and at least 1 point for not being obese. Therefore, any participant only had to score one more point to have an intermediate cardiovascular health score. Even though we only included the healthiest individuals of the general population in the COmPLETE cohort study, we were able to find differences in arterial properties between those with an intermediate and those with an ideal Life's Simple 7 score. To my knowledge, we were the first ones to do so. This is of particular interest, since it clearly demonstrates that even in the apparently healthiest individuals of the population, there is an additional benefit to following and/or maintaining a healthy lifestyle.

As in the DR's EXTRA study, we measured carotid properties (i.e., cIMT, cLD and carotid distensibility). Confirming the Finnish findings, we showed that there was a significant difference in cLD between those with an intermediate and those with an ideal health score. What we could not confirm, however, is a difference in carotid distensibility. Further, we found that in COmPLETE those with an ideal health score had lower cIMT, which we did not find in DR's EXTRA.

As discussed in the previous chapter, the reason for the discrepancies in the results are speculative, as very few studies have tackled this topic. The differences may, however, very well lie in the nature of the study designs, where DR's EXTRA aimed at a population-based approach and COmPLETE included only the healthiest subjects. Therefore, the age- and/or lifestyle related compensatory mechanisms explained above may already have begun in both studies but may not have been as advanced in COmPLETE. This would at least partially explain why we did not find significant differences in carotid distensibility.

As opposed to the DR's EXTRA study, for the manuscript of the COmPLETE cohort study, we did not only analyze carotid properties but aimed at a more holistic approach towards the arterial system, including baPWV and FMD in our analyses. The advantage is that we included parameters that assessed arterial properties at various locations, providing information at various body sites. Our results showed that baPWV was significantly lower in those with an ideal health score, demonstrating that even the apparently healthiest individuals of the population, those with a healthier lifestyle had lower arterial stiffness. This is of particular interest, as baPWV is used to measure arterial stiffness and prospective studies of healthy populations have shown that higher baPWV is associated with higher cardiovascular risk [112]. Our approach could be an important milestone in the detection of individuals who are at higher

risk for a cardiovascular event. In a healthy population like COmPLETE, this would mean that baPWV may have additive value to the traditional risk factors. In manuscript 4, we argued that the differences in baPWV between the two groups is too small to be clinically relevant. However, if we were able to detect those at risk in a healthy population without history of any cardiovascular disease and with only very few cardiovascular risk factors, this would be a great argument to use baPWV in routine practice. These claims, of course, will have to be confirmed, ideally in longitudinal and intervention studies. As baPWV is mostly used in Asian countries [15] and only little data is available for European populations, it would further be interesting to see whether our findings are reproduced in similar settings.

Regarding FMD of the brachial artery, our study was not able to find significant differences between those with an ideal and those with an intermediate Life's Simple 7 health score. To my knowledge, we were the first to report the association between the Life's Simple 7 and FMD. One very recent meta-analysis by Heiss et al. (2022) found that, in healthy adults, those with higher cardiovascular risk had lower FMD [113]. The authors found that age, BMI, smoking, and brachial arterial parameters were the strongest independent predictors of FMD. Our results are not fully comparable to the results by Heiss et al. (2022) because they used a different tool to calculate cardiovascular risk and FMD was measured using different methods, but in their meta-analysis, they discussed that the risk factors age, BMI, and smoking only explained 19% of the variability of FMD. As this begs the question to which factors explain the remaining 81%, I performed a linear regression analysis with our data to see whether non-modifiable factors (i.e., age and sex) or modifiable risk factors (i.e., the Life's Simple 7 variables, except for smoking), explained FMD variability in the COmPLETE cohort study (data not published). I found that age and sex only explained 4.5% and adding the Life's Simple 7 explained 8.2% of FMD variability. Consequently, other factors seem better suited to explain FMD variability. Although speculative, this may at least partially illustrate why we did not observe significant differences in the COmPLETE population, as the variables used to describe those with an ideal or intermediate health score only explain very little of FMD.

To conclude, as argued in manuscript 4, it must be said that the statistically significant differences for cIMT, cLD and baPWV are likely not clinically relevant. This, however, does not diminish the relevance of our finding that even in the healthiest individuals of the population, differences in atherosclerotic progression and arterial function are visible depending on Life's Simple 7 variables fully achieved.

### 8.3 Interventional findings

The DR's EXTRA intervention study was the largest lifestyle-based intervention to date. Including and randomizing 1'400 participants at baseline and collecting follow-up data of almost 1'200 participants in four years is a unique achievement for a population-based RCT. Its aims were, amongst others, to determine whether various lifestyle interventions were able to slow atherosclerotic progression in the general middle-aged to elderly Finnish population. Its results, however, have been underwhelming. As part of this PhD project, I was not able to detect any attenuation of atherosclerotic progression of the carotid arteries, regardless of intervention group. This shows that neither improved diet, nor resistance exercise, nor aerobic exercise, nor a combination of a healthy diet and either resistance or aerobic exercise were superior to the reference group. That is an important finding. It shows that lifestyle improvements may not have the desired effects on carotid structure in a middle-aged to elderly population. The exact reason why remains unclear but there are several potential explanations. First, our population was very physically active at baseline. This may have led to a ceiling effect, where the participants were close to their maximal potential and some even had to reduce their levels of physical activity in order to fall within the WHO guidelines that were in place during the intervention [114]. While the WHO guidelines have recently been updated in 2020, they remain largely identical to the previous ones, with relatively vague recommendations, and high intensity interval training is missing, even though its benefits on cardiovascular health have been proven [115-117]. Whether the new guidelines are superior to the old ones is therefore uncertain. In at-risk patients, there is evidence suggesting that high intensity interval training may be beneficial to reduce cardiovascular risk [118]. In healthy, older adults, such as in the DR's EXTRA study, high intensity interval training does not seem to be superior to moderate continuous training, as recent results from Norway suggest [119]. The Norwegian study, however, used a different score than the Life's Simple 7, making a direct comparison difficult. I can therefore only speculate whether an intervention using high intensity interval training may have been superior to the protocol used in DR's EXTRA, which only comprised moderate continuous training. Second, the effects of resistance exercise to slow atherosclerotic progression have yet to be shown. This would at least explain why two of the five intervention groups did not show significant slowing of atherosclerotic progression. As for diet, we showed that men in the groups that included a dietary component did not improve their diet scores. In contrast, women in those groups improved their diet scores but consistently started with higher scores than men. Third, maybe the carotid walls of our population had already undergone irreversible alterations that could not be reversed via lifestyle improvement. Forth, it could be speculated that four years of intervention may be too short to detect differences in our population. For example, the DNASCO study, which preceded DR's EXTRA, found that in men aged 50-60 years old, after six years of aerobic training, atherosclerotic progression could only be slowed in the participants who were not taking cholesterol-lowering medication [42]. In a US-American based study, women aged from 44-50 years old and subject to a dietary and

exercise intervention for four years, the intervention group significantly slowed atherosclerotic progression of the carotid artery [120]. It may therefore be that the lack of significant impact of various interventions in DR's EXTRA is explained by the higher age of our population or that in older populations, longer interventions are necessary. Last, it may be that the parameters used in the DR's EXTRA study were not suited to detect the effects of this lifestyle intervention. Results on vascular biomarkers such as baPWV, FMD, or DC have not yet been reported. To conclude, our results show that atherosclerotic progression to the arterial walls happen either earlier, more pronounced, or both, in the Finnish population than in other European populations. Therefore, prescribing lifestyle-based interventions to Finns aged 55+ years old may be too late to result in any desirable changes in the arterial walls or need longer than four years to show effect.

### 8.4 Dietary Assessment tool

The validation of the visually aided DAT shows, first and foremost, that the DAT can be seen as an attractive alternative to multi-day food records because although less precise, the DAT is timesaving and can detect some dietary habits such as fruits and vegetables consumption. Second, we demonstrated that especially the elderly population was able to estimate total calorie intake and most macronutrients relatively well. Third, our analysis method with sliding limits of agreement constitutes a novel approach for dietary assessment analysis. This validation study therefore opens several doors. For example, future studies may be encouraged to use the DAT for dietary screening in elderly populations, as we demonstrated high correlations between the DAT and the gold standard for dietary assessment, the 7d-FR [92]. The main disadvantage of the DAT is its relative lack of precision (e.g., because of social desirability or recall bias) [121-123]. However, as we showed that healthy elderly subjects were able to adequately estimate food intake, it leaves the DAT with far more advantages than disadvantages, namely the low burden for the participants and study personnel, low cost, ease of use, and low time effort. It has to be recognized that for future studies, some improvements can be made for the DAT. First, the food pyramid was not the most up-to-date version used by the Swiss Society for Nutrition (Schweizerische Gesellschaft für Ernährung). Second, the color-coded pyramid is highly suggestive, showing healthy food items in green, whereas food items that are considered unhealthy are displayed in brown. Third, some food items are displayed in each layer of the pyramid and serve as examples, while many are left out. This may lead to a bias where food items that are often consumed are also better reported. The fact that a pyramid is displayed is also highly suggestive, showing the food items that should be consumed in high quantities at the bottom of a pyramid, suggesting optimal nutrition.

The novel statistical analysis of the validation study opens another door for dietary assessment approaches. In studies that include dietary assessment, the number of kilocalories consumed, or macronutrients, will most likely not be normally distributed. They will be skewed to the right (the mean is higher than the median), as values close to zero are very unlikely, especially in first-world countries where famine is almost eradicated. As such, we opted for a statistical

method that has been described previously [124] to interpret log-transformed data that was back-transformed and plotted on a Bland and Altman plot. While this approach is not innovative per-se, we were the first to apply it in the field of nutrition. The resulting sliding limits of agreement between the two methods shown in the Bland and Altman plot makes the interpretation of the results on the original scale easier and more precise. For example, in a normal Bland and Altman plot, the limits of agreement between two methods will be the same, regardless of the mean and the differences. With the sliding limits of agreement, the data with a smaller mean have smaller limits of agreement, whereas data with a higher mean have higher limits of agreement. As a result and keeping in mind that it is expected that other DAT validation studies will face the same issue of data not being normally distributed, I believe that our study will serve as an example and maybe even have a pioneering role in this field.

### 8.5 Strengths and limitations

A major strength of this PhD project lies in the holistic approach to determine the influence of lifestyle on arterial properties. Ageing populations from two parts of Europe were analyzed in a cross-sectional and in an interventional study design. A representative sample of the Finnish population was taken, but also a sub-sample of healthy Swiss individuals. State-of-the-art measuring techniques were applied for all main outcome variables.

Furthermore, the DR's EXTRA study was, to date, the largest multi-year RCT, that analyzed the effects of lifestyle-based interventions on carotid structure. This, in junction with the unique multi-armed intervention based on strength training, aerobic training, diet, and a combination of training and diet, gave insight on the effects of lifestyle interventions to slow atherosclerotic progression in an unprecedented depth. The setting of the DR's EXTRA study can be seen as a strength, as the different intervention groups were given realistic dietary and physical activity goals, which corresponded to the international guidelines that were in place during the study. For the groups who were prescribed strength training, monitoring of the sessions was done objectively via a smart card system, allowing for precise tracking of physical activity and progression during the study. For feasibility reasons, and to increase compliance, aerobic exercise was performed individually, and the participants were asked to keep a training log. This reflects a real-life setting, as likely only few participants would have had the capability to follow three guided sessions per week over four years.

An additional strength of my PhD project lies in the population and the protocol of the COmPLETE study. The inclusion and exclusion criteria, selecting only healthy subjects without any manifested or history of CVD, even in old age, allowed for a comprehensive view on healthy vascular ageing. The combination of various vascular biomarkers and state-of-the-art diagnostic measuring techniques allowed for the best possible discrimination of vascular health between those who are considered to live an ideal lifestyle and those who are not.

The combination of vascular biomarkers and a cardiovascular health score is another strength of my PhD project, as they allow two different perspectives on cardiovascular health. On the one hand, the vascular biomarkers offer a direct view into the arteries, allowing precise

statements on vascular ageing and arterial health. On the other hand, the Life's Simple 7 provide information on lifestyle-related metrics, which in turn provide information on which factors may influence vascular ageing and where individual lifestyle adjustments can be made to ensure optimal ageing.
Lastly, some strengths may also lie in simplicity. The DAT that was used in the COmPLETE study and validated as part of my PhD project offers a simple and acceptable metho to assess some dietary patterns in adults. While a full understanding of dietary habits is not possible, the DAT allows to make statements regarding fruit and vegetables consumption, which are an essential component of a healthy diet [125]. In addition, we found that subjects aged 50+ years were able to precisely estimate their food intake. This makes the DAT a suitable and easy-to-use method to evaluate food quantity in middle-aged and older populations. Last, we validated our DAT against the 7d-FR. Other studies have validated DAT against 4d-FR, which has the disadvantage of assessing the differences in dietary habits between weekdays and weekends less precisely [126-128].

While my PhD project has great strengths, I must acknowledge some limitations. First, in the COmPLETE study, the ultrasound measurement of the carotid arteries was performed only in subjects aged 50+ years. Had we measured carotid properties in the entire COmPLETE population, we would have had a more thorough view on the atherosclerotic progression in healthy Swiss adults and a larger sample size. Especially in apparently healthy adults, it would have been interesting to establish whether differences in cIMT, cLD, and DC could be detected depending on lifestyle habits. However, the reason why we did not measure carotid properties in the COmPLETE participants aged 49 years or less, is quite evident. As atherosclerotic progression is such a slow process that takes decades to manifest, it is easily imaginable that the additional value of including those aged less than 50 years would have been negligible. Especially for signs of early vascular ageing, there are better tools than carotid arterial properties [15].
As for the DR's EXTRA study, while to date it was the largest of its kind, the number of participants per intervention arm was too low to make group- and sex-specific statements on the effects of the four-year trial. The power and sample size calculations were based on the DNASCO study [42], and aimed at showing cIMT progression over the entire population, not the individual groups. For feasibility reasons though, it is apparent why the limit of participants per group was set to less than 250 – an unprecedentedly high number of participants for this kind of intervention. The assessment of physical activity via questionnaire in the DR's EXTRA study must be seen as a limitation. Although purely speculative, it is possible that the participants overestimated their physical activity at baseline and were able to estimate their levels of physical activity more precisely after being confronted with physical activity logs for four years. This would explain why so many of the participants decreased physical activity during the intervention, even though physical exercise was prescribed. It has been asserted to me by the principal investigator of the DR's EXTRA study that the physical activity levels are

reliable. Retrospectively, it would have been better to measure physical activity objectively in at least some participants, to show whether the questionnaires were accurate. As visible in table 2 (chapter 8.1), the differences in physical activity levels between the Finnish and Swiss cohorts are quite considerable.

Another limitation is the consistency of the reporting of each metric of the Life's Simple 7. For example, of the seven health metrics, only four were assessed in the same manner in the COmPLETE and DR's EXTRA study, namely smoking status, BMI, cholesterol, and systolic blood pressure. In COmPLETE, physical activity was assessed objectively, using triaxial accelerometry, whereas in DR's EXTRA it was estimated via questionnaire. In contrast, the DR's EXTRA study used a 4d-FR to evaluate diet, while COmPLETE used the visually aided DAT. Lastly, in DR's EXTRA, fasting blood glucose was measured, whereas COmPLETE measured HbA1c. While the 7 metrics are comparable nevertheless, the assessment methods remain a potential confounder.

### 8.6 Outlook and future studies

The publication by Enserro et al. (2018) clearly shows that while improvement of the Life's Simple 7 score reduces the risk for cardiovascular events, the promotion of these modifiable habits should be put in place with a specific focus on younger populations and ensuring that those habits are maintained for the longest possible time [81]. In contrast, adults benefit far less from a lifestyle improvement. Our results confirm this, as they show that in middle-aged to elderly populations, a lifestyle-based intervention is not sufficient to slow atherosclerotic progression and indirectly highlights the importance of Enserro et al.'s (2018) findings. If a future intervention study comparable to DR's EXTRA were to be planned, it would be important to consider excluding physically very active participants, even if the sample is representative of the general population. As visible in our analyses, most of the participants fulfilled or even exceeded physical activity recommendations (971 out of 1400 participants at baseline). In addition, most participants (n=784) adhered to at least 2 or more dietary targets. Therefore, they potentially displayed a ceiling effect for interventions aiming at performing physical activity according to international recommendations and improving diet. Further, the DR's EXTRA study was undertaken under the physical activity recommendations that were in place at the time. The WHO has since adapted the recommendations by increasing active time, which future studies will need to consider. A speculative but promising approach would be to include high intensity interval training in future RCT. This training method has shown that some cardiovascular risk factors such as BMI [129] or hypertension [130] could be improved, whereas some have shown no results [131-133].

The COmPLETE cohort study provides a unique dataset of healthy subjects, especially at old age. With COmPLETE, we showed that compared to other European cohorts, atherosclerosis seemed to be less advanced. Further, we showed that even in the healthiest part of the population, adherence to a healthy lifestyle was associated with better arterial properties. A study in the general population from Basel, Switzerland would be interesting, as we would

potentially see how big the differences in arterial properties are. This would allow to draw comparisons with those with a poor, an intermediate, and an ideal cardiovascular health score. It would also be very valuable to see which of the seven health metrics predicts the various vascular biomarkers best, or vice versa. With my PhD project, we make the case for the introduction of the Life's Simple 7 in the general Swiss population.

### 8.7 Conclusions

Lifestyle and vascular health are closely interconnected in middle-aged to elderly populations from Switzerland and Finland. Thanks to my PhD project, it became evident that there are visible differences regarding vascular properties between those who strictly adhere to a healthy lifestyle and those who do not. These findings were first found in a community-dwelling Finnish population and confirmed in a healthy sub-sample of the Swiss population. This highlights the importance of a healthy lifestyle. Further, the association between the Life's Simple 7 cardiovascular health score and arterial properties demonstrates that the Life's Simple 7 are a practical alternative to traditional cardiovascular risk scores. The Life's Simple 7 have the great advantage over the risk scores to be easily implementable and improvements in the score can quickly be made, as it is not influenced by non-modifiable risk factors.

With the new knowledge gained in my PhD project, it must however be questioned if lifestyle-based interventions in middle-aged to elderly community-dwelling populations are adequate to reduce atherosclerotic progression and cardiovascular risk. A secondary analysis of the data from the lifestyle intervention I used showed that there were only improvements in men who followed a dietary intervention. As this demonstrates that neither diet, nor physical activity, nor a combination of diet and physical activity were superior to the reference group, other interventions may be needed to slow atherosclerotic progression.

Great effort should therefore be made to improve lifestyle in younger populations to increase disease-free lifetime. In addition, the Life's Simple 7 could be implemented at our latitudes as an uncomplicated tool to assess cardiovascular health.

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## Appendix 1: Graduate Education

| Course title | Teacher(s) | ECTS |
|---|---|---|
| An introduction to systematic reviewing: From literature search to meta-analysis | Christian Appenzeller-Herzog | 1 |
| Effizient Wissenschaftlich Schreiben | Gabriela Venetz | 0 |
| Grundlagen fachdidaktischer Forschungsmethoden | Kirsten Schweinberger | 3 |
| Vorlesung mit Übungen: Einführung in die Statistik mit Beispielen aus der Biologie | Daniel Berner, Thomas Fabbro, Peter Stoll | 2 |
| Presentation Training | Gabriela Rockmann | 1 |
| Out of the Box – visualize your science | Evelyn Huber-Trutmann | 1 |
| Good scientific practice | Helga Nolte | 1 |
| Ethics of Science | Isabelle Wienand | 1 |
| Academic Writing in Health Sciences | Annegret Mündermann | 1 |
| Meine erste Lehrveranstaltung unter Berücksichtigung der digitalen Lehre | Ulrike Hanke | 1 |
| Kolloquium Research in Clinical Exercise Medicine and Physiology | Julia Kröpfl, Arno Schmidt-Trucksäss | 3 |
| Writing a research paper in 12 weeks or less | Maria Katapodi | 4 |
| What's next after your PhD? | Dagmar Engfer | 0 |
| Essentials in Health Research Methology | Various speakers | 2 |
| **Total** | | **20** |