Effect of the community-based intervention *Healthy Lifestyle Community Programme* on common carotid intima-media thickness and other cardiovascular markers

Von der Naturwissenschaftlichen Fakultät

der Gottfried Wilhelm Leibniz Universität Hannover

zur Erlangung des Grades

Doktor der Naturwissenschaften

Dr. rer. nat.

genehmigte Dissertation von

von

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2022

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Tag der Promotion: 12.07.2022

DOI: 10.15488/12536

URL: https://www.repo.uni-hannover.de/handle/123456789/12635

Abstract

Background and aims: Cardiovascular disease (CVD) is the leading cause of death globally. Apart from established CVD risk markers such as cholesterol and blood pressure, CVD risk can be assessed by anatomical markers of the arterial wall structure, including common carotid intima-media thickness (ccIMT). While unhealthy dietary patterns and other unfavourable lifestyle choices are major contributors to CVD risk, healthy plant-based diets are associated with decreased CVD risk. In this context, lifestyle programmes may constitute an effective tool for empowering individuals to make healthier lifestyle choices, but the effectiveness of such programmes should be assessed in controlled trials. Thus, it was the aim of this thesis to assess the effectiveness of the *Healthy Lifestyle Community Programme* in improving ccIMT and other CVD markers.

Methods: A community-based, non-randomized controlled trial was conducted, including mostly middle-aged and elderly participants from the general population (intervention: n = 114; control: n = 87). The intervention consisted of a lifestyle programme focusing on four key areas: a largely plant-based diet (strongest emphasis), physical activity, stress management, and community support. Parameters were assessed at baseline, 10 weeks, 6 months, and 1 year. The control group received no intervention. Furthermore, a subsequent uncontrolled trial (n = 115) with a very similar lifestyle programme was conducted to replicate the findings.

Results: From baseline to 1 year, mean ccIMT significantly increased in both the intervention (0.026 [95% CI 0.012, 0.039] mm) and control group (0.045 [95% CI 0.033, 0.056] mm). The 1-year trajectory of mean ccIMT was lower in the intervention group (between-group difference: -0.012 [95% CI -0.022, -0.002] mm; p = 0.022; adjusted for baseline). In a subgroup analysis of participants with high baseline mean ccIMT (≥0.800 mm), mean ccIMT non-significantly decreased in the intervention group (-0.016 [95% CI -0.050, 0.017] mm; n = 18) and significantly increased in the control group (0.065 [95% CI 0.033, 0.096] mm; n = 12), with a between-group difference of -0.051 [95% CI -0.075, -0.027] mm (p <0.001; adjusted for baseline). At 1 year, significant decreases (compared to control) were also observed for body weight, body mass index (BMI), waist circumference, resting heart rate (RHR), remnant cholesterol (REM-C), and high-sensitivity C-reactive protein (hs-CRP), with no adverse effect on homocysteine. The uncontrolled trial showed decreases in body weight, BMI, waist circumference, and measured LDL cholesterol but a potential increase in REM-C. In both studies, dietary intake significantly improved in line with the recommendations given.

Conclusion: The results indicate that healthy lifestyle changes may beneficially affect ccIMT within 1 year, particularly if baseline ccIMT is high. Furthermore, the controlled intervention successfully reduced body weight, BMI, waist circumference, RHR, REM-C, and hs-CRP. However, the uncontrolled trial did not confirm the results regarding RHR, REM-C, and hs-CRP, which requires further investigation.

Trial registration: German Clinical Trials Register, DRKS00018775 and DRKS00018846

Keywords: plant-based diet, carotid intima-media thickness, obesity prevention

Kurzzusammenfassung

Hintergrund und Ziel: Kardiovaskuläre Erkrankungen (CVD) sind die Haupttodesursache weltweit. Neben etablierten CVD-Risikomarkern wie Cholesterin und Blutdruck kann das CVD-Risiko über anatomische Marker der Arterienwand, einschließlich der Intima-Media-Dicke der *Arteria carotis communis* (ccIMT), erfasst werden. Während ungesunde Ernährungsmuster und andere ungünstige Lebensstilfaktoren in großem Ausmaß zu einem erhöhten CVD-Risiko beitragen, sind gesunde pflanzenbasierte Ernährungsweisen mit einem niedrigeren CVD-Risiko assoziiert. In diesem Zusammenhang stellen Lebensstilprogramme möglicherweise ein effektives Mittel dar, um Individuen dazu zu befähigen, ihren Lebensstil gesünder zu gestalten. Die Effektivität solcher Programme sollte jedoch in kontrollierten Studien überprüft werden. Ziel dieser Arbeit war es daher, die Wirksamkeit des *Healthy Lifestyle Community Programme* in Bezug auf die Verbesserung von ccIMT und anderen CVD-Markern zu testen.

Methodik: Eine community-basierte, nicht-randomisierte kontrollierte Studie wurde mit Teilnehmenden, die vornehmlich mittleren und höheren Alters waren sowie aus der Allgemeinbevölkerung stammten, durchgeführt (Intervention: N = 114; Kontrolle: N = 87). Die Intervention bestand aus einem Lebensstilprogramm mit vier Schwerpunkten: eine überwiegend pflanzenbasierte Ernährungsweise (stärkste Betonung), körperliche Aktivität, Stressmanagement und gegenseitige Unterstützung. Die Risikoparameter wurden zu Baseline, nach zehn Wochen, sechs Monaten und einem Jahr bestimmt. Die Kontrollgruppe nahm an keiner Intervention teil. Des Weiteren wurde eine unkontrollierte Studie (N = 115) mit einem sehr ähnlichen Lebensstilprogramm durchgeführt, um die Ergebnisse zu reproduzieren.

Ergebnisse: Innerhalb von einem Jahr stieg der Parameter *mean ccIMT* sowohl in der Interventionsgruppe (0,026 [95 % Konfidenzintervall 0,012; 0,039] mm) als auch der Kontrollgruppe (0,045 [0,033; 0,056] mm) signifikant an. Der 1-Jahresverlauf von *mean ccIMT* war in der Interventionsgruppe niedriger (Gruppenunterschied: -0.012 [95% CI -0.022, -0.002] mm; p = 0,022; adjustiert für Baseline). In einer Subgruppe bestehend aus Teilnehmenden mit hohem Baseline-*mean ccIMT* (≥0,800 mm) nahm *mean ccIMT* in der Interventionsgruppe nicht-signifikant ab (-0,016 [-0,050; 0,017] mm; n = 18) und stieg in der Kontrollgruppe signifikant an (0,065 [0,033; 0,096] mm; n = 12; Gruppenunterschied: -0,051 [-0,075; -0,027] mm; p <0,001; adjustiert für Baseline). Zudem zeigte sich nach einem Jahr eine signifikante Abnahme (im Vergleich zur Kontrolle) auch bei Körpergewicht, Body-Mass-Index (BMI), Bauchumfang, Ruhepuls (RHR), *remnant*-Cholesterin (REM-C) und dem hochsensitiven C-reaktiven Protein (hs-CRP), während eine ungünstige Veränderung von Homocystein ausblieb. In der unkontrollierten Studie zeigte sich eine Abnahme von Körpergewicht, BMI, Bauchumfang und gemessenem LDL-Cholesterin, aber auch eine potenzielle Zunahme von REM-C. In beiden Studien verbesserte sich das Ernährungsmuster gemäß der gegebenen Empfehlungen.

Schlussfolgerung: Die Ergebnisse deuten darauf hin, dass gesunde Lebensstilveränderungen ccIMT möglicherweise innerhalb von einem Jahr günstig beeinflussen können, insbesondere bei hohen ccIMT-Ausgangswerten. Des Weiteren reduzierte die kontrollierte Intervention erfolgreich Köpergewicht, BMI, Bauchumfang, RHR, REM-C und hs-CRP. Die unkontrollierte Studie bestätigte jedoch nicht die Ergebnisse in Bezug auf RHR, REM-C und hs-CRP, was zukünftig untersucht werden sollte.

Studienregistrierung: Deutsches Register Klinischer Studien, DRKS00018775 und DRKS00018846

Schlagworte: pflanzenbasierte Ernährung, Intima-Media-Dicke, Adipositas-Prävention

Scientific publications in association with this thesis

Original research papers

1) Christian Koeder, Andreas Hahn, Heike Englert: Effect of a 6-month controlled lifestyle intervention on common carotid intima-media thickness; **published in:** J Nutr Health Aging 25(7), 869–877 (2021). **DOI:** 10.1007/s12603-021-1628-0
2) Christian Koeder, Ragna-Marie Kranz, Corinna Anand, Sarah Husain, Dima Alzughayyar, Nora Schoch, Andreas Hahn, Heike Englert: Effect of a 1-year controlled lifestyle intervention on body weight and other risk markers (the Healthy Lifestyle Community Programme, cohort 2); **published in:** Obesity Facts 15(2), 228–239 (2022); **published online:** 17 December 2021. **DOI:** 10.1159/000521164
3) Christian Koeder, Corinna Anand, Sarah Husain, Ragna-Marie Kranz, Nora Schoch, Dima Alzughayyar, Norman Bitterlich, Andreas Hahn, Heike Englert: Effect of a controlled lifestyle intervention on inflammatory markers – the Healthy Lifestyle Community Programme (cohort 2); **submitted to:** BMC Nutrition
4) Christian Koeder, Sarah Husain, Ragna-Marie Kranz, Corinna Anand, Dima Alzughayyar, Nora Schoch, Andreas Hahn, Heike Englert: Effect of a controlled lifestyle intervention on common carotid intima-media thickness – the Healthy Lifestyle Community Programme (cohort 2); **published in:** Journal of Nutritional Science 11, e47 (2022); **published online:** 13 June 2022. **DOI:** 10.1017/jns.2022.46
5) Christian Koeder, Dima Alzughayyar, Corinna Anand, Ragna-Marie Kranz, Sarah Husain, Nora Schoch, Andreas Hahn, Heike Englert: Associations of changes in plant-based diet indices and cardiovascular risk markers (the Healthy Lifestyle Community Programme cohort 3); **submitted to:** Nutrition Journal

Book contributions

Heike Englert, Corinna Anand, Christian Köder: Das Healthy-Lifestyle-Community-Programm: ein Community-basiertes, ganzheitliches Lebensstil-Interventionsprojekt zum gesunden Leben und Arbeiten. In: Gesundheitsförderung und Versorgung im ländlichen Raum. Grundlagen, Strategien und Interventionskonzepte. Bern: Hogrefe, 295–308, 2021. **DOI:** 10.1024/85979-000

Conference contributions (poster presentations)

1. Christian Köder, Heike Englert: Einfluss eines community-basierten Lebensstilinterventionsprogramms mit pflanzenbasierter Ernährung auf kardiovaskuläre Risikoparameter. Lebensjahre in Gesundheit – was leistet die Ernährung? Abstractband zum 56. Wissenschaftlichen Kongress, Gunter P. Eckert, Uwe Wenzel (Eds.), 85–85 (2019) [Deutsche Gesellschaft für Ernährung, 56. Wissenschaftlicher Kongress: 19–21 March 2019, Gießen]
2. Christian Koeder, Heike Englert: Influence of a community-based lifestyle intervention program including recommendations for a plant-based diet on cardiovascular risk parameters. Nutrients 2019: Nutritional Advances in the Prevention and Management of Chronic Disease, Lluis Serra Majem, María Luz Fernández (Eds.), Program and Abstract Book, 185 (2019) [Nutrients 2019: Nutritional Advances in the Prevention and Management of Chronic Disease: 25–27 September 2019, Barcelona]
3. Christian Koeder, Andreas Hahn, Heike Englert: A plant-based diet and healthy lifestyle lower C-reactive protein levels. Complement Med Res, 6–7 (2021). **DOI:** 10.1159/000514476 [The Future of Food and Healthcare: VegMed Web 2021 – Scientific Congress for Plant-Based Nutrition and Medicine: 28 February – 2 March 2021; virtual congress]
4. Christian Koeder, Andreas Hahn, Heike Englert: No clear association of sleep duration or bedtime with common carotid intima-media thickness. Atherosclerosis, Volume 331, e150–e151 (1 August 2021). **DOI:** 10.1016/j.atherosclerosis.2021.06.452 [89th EAS (European Atherosclerosis Society) Congress: 30 May – 2 June 2021; virtual congress]
5. Christian Koeder, Sarah Husain, Ragna-Marie Kranz, Andreas Hahn, Heike Englert: How to achieve sustainable eating in the general population? The 9th World Sustainability Forum, Program and Abstract Book, Sciforum-032538 (2021) [The 9th World Sustainability Forum: 13–15 September 2021; virtual congress]
6. Christian Koeder, Andreas Hahn, Heike Englert: Is fruit intake associated with common carotid intima-media thickness? Eur J Public Health, Volume 31, Issue Supplement 3, October 2021. **DOI:** 10.1093/eurpub/ckab165.391 (published: 20 October 2021) [14th European Public Health Conference 2021, Public health futures in a changing world: 10–12 November 2021; virtual congress]
7. Christian Koeder, Andreas Hahn, Heike Englert: Healthy lifestyle changes can improve cardiovascular markers within 10 weeks. Atherosclerosis, Volume 355, P158 (1 August 2022). **DOI:** 10.1016/j.atherosclerosis.2022.06.682 [90th EAS (European Atherosclerosis Society) Congress: 22–25 May 2022; hybrid virtual/non-virtual congress]

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List of abbreviations

BMI: body mass index

BP: blood pressure

CAC: coronary artery calcium

CCA: complete case analysis

ccIMT: common carotid intima-media thickness

CHIP: Complete Health Improvement Program

CI: confidence interval

COVID-19: coronavirus disease 2019

CT: computed tomography

CVD: cardiovascular disease

DASH: Dietary Approaches to Stop Hypertension

ESC: European Society of Cardiology

HbA1c: haemoglobin A1c

HDL-C: high-density lipoprotein cholesterol

HGF: hepatocyte growth factor

HLCP-1: Healthy Lifestyle Community Programme, cohort 1

HLCP-2: Healthy Lifestyle Community Programme, cohort 2

HLCP-3: Healthy Lifestyle Community Programme, cohort 3

holoTC: holotranscobalamin

hPDI: healthful PDI

hs-CRP: high-sensitivity C-reactive protein

IMT: intima-media thickness

LDL-C: low-density lipoprotein cholesterol

MMA: methylmalonic acid

MMP-12: matrix metalloproteinase-12

MUFA: monounsaturated fatty acids

NAFLD: non-alcoholic fatty liver disease

non-HDL-C: non-HDL cholesterol

NT-proBNP: N-terminal pro-B-type natriuretic peptide

oxLDL: oxidized LDL particles

PDI: plant-based diet index

PUFA: polyunsaturated fatty acids

REM-C: remnant cholesterol

RHR: resting heart rate

SEM: standard error of the mean

SFA: saturated fatty acids

TAG: triglycerides

TC: total cholesterol

TNF-α: tumor necrosis factor alpha

uPDI: unhealthful PDI

WBC: white blood cell count

1. General introduction

Cardiovascular disease (CVD) is the leading cause of death in Germany, Europe, and worldwide [1]. At the same time, it is known that a large percentage of CVD morbidity and mortality could be prevented by adopting healthier lifestyle practices [2–5]. It has even been stated that the most important way to prevent atherosclerotic CVD is to promote a healthy lifestyle throughout life [3]. However, the implementation of this knowledge remains a considerable challenge, and evidence is lacking on how a societal transformation which would establish healthy lifestyle practices can be achieved [6].

Dietary composition is a crucial factor in terms of CVD risk modification, and it has been suggested that diet quality may be the most important modifiable risk factor for CVD prevention [2]. Current evidence indicates that a shift in dietary patterns from a typical Western diet towards a predominantly plant-based diet, with a focus on healthy plant-based foods, would lead to considerable reductions in CVD risk [7–9]. Furthermore, current evidence strongly suggests that regular physical activity, including sports-related exercise but also other forms of physical activity, can beneficially affect CVD risk markers as well as CVD event and mortality risk [10–12]. While a direct causal relationship between psychological stress and CVD risk is more controversial [13, 14], excessive psychological stress [15–18] and psychosocial factors such as depression [19, 20], loneliness [21], and anxiety [14] are associated with increased subclinical inflammation as well as increased CVD risk [22]. In addition, smoking [23, 24] and excessive alcohol intake [25, 26] are established CVD risk factors. Furthermore, a variety of unfavourable socioeconomic factors have been shown to be indirectly or directly associated with increased CVD risk [27, 28].

1.1. Aim of this dissertation

As described above, sufficient evidence justifies the assumption that healthy lifestyle choices can reduce CVD risk and that, consequently, such choices should be recommended to the general public [3, 29, 30]. The abovementioned lifestyle factors provide a framework of how great achievements could potentially be accomplished in terms of CVD prevention. However, to date, this potential remains largely untapped [31], i.e. there is a gap between evidence-based knowledge about CVD prevention and the implementation of CVD prevention as a public health practice [32]. Many individuals lack the necessary know-how or motivation to make healthier lifestyle choices [33]. Consequently, such healthy lifestyle practices are not applied by large segments of the population [6], and both in Germany and worldwide, unhealthy lifestyle practices remain widespread [34]. Therefore, there is an urgent need for action and for tools that can promote the transfer of evidence-based knowledge and practical skills from “science to society” [35]. One possible tool for this purpose are community-based lifestyle intervention programmes [36, 37], which have the aim of empowering citizens to successfully transform their lifestyles and to maintain healthy lifestyle practices in the long term [38].

However, to date, there is insufficient evidence from controlled trials regarding the effectiveness of lifestyle interventions to improve a number of important CVD risk markers. These biomarkers include common carotid intima-media thickness (ccIMT), a biomarker of anatomical changes in the arterial wall structure [39, 40], inflammatory biomarkers such as high-sensitivity C-reactive protein (hs-CRP) [41–44], homocysteine (Hcy) [45, 46], and adiponectin (Apn) [47, 48] as well as biomarkers of oxidative stress such as oxidized low-density lipoprotein (LDL) particles (oxLDL) [49]. In terms of ccIMT, evidence from controlled trials is particularly lacking regarding the potential effect of interventions with a plant-based diet on ccIMT in participants from the general population [50] (see **Chapter 1.4.** and **Appendix 1: Table 1**; **Appendix 2: Table 2**; **Appendix 3: Table 3**) as well as regarding potential associations of ccIMT changes with changes in inflammatory markers [51]. Furthermore, while there is observational evidence of an association between diet and hs-CRP, evidence from controlled trials is lacking regarding the effectiveness of plant-based diet interventions to lower hs-CRP, the most firmly established biomarker of inflammation (**Chapter 1.6.**) [52–54]. In addition, there is very little evidence from controlled trials regarding the effect of plant-based diet interventions on Hcy (which is an inflammatory marker as well as a marker of B-vitamin status [45]) and potential effects on Apn [55] (for which conflicting evidence has been published [48, 54]; **Chapter 1.6.**). Similarly, only a small number of controlled trials worldwide have explored the effect of plant-based diet interventions on oxLDL (**Chapter 1.5.**) [56–61]. In addition, even in terms of established CVD risk markers, there is very little evidence from controlled trials in Germany regarding the effectiveness of interventions with a strong focus on a plant-based diet in improving these markers. These established CVD risk markers include body weight, body mass index (BMI), waist circumference, blood pressure (BP), pulse pressure, resting heart rate (RHR), total cholesterol (TC), measured and calculated LDL cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TAG) as well as important (but less explored) lipid fractions such as non-HDL cholesterol (non-HDL-C) and remnant cholesterol (REM-C). In addition, established CVD risk markers include biomarkers of glycaemic control, particularly fasting blood glucose, haemoglobin A1c (HbA1c), and insulin (**Chapter 1.5.**) [62–65]. To date, the widely used dietary scores plant-based diet index (PDI), healthful PDI (hPDI), and unhealthful PDI (uPDI) have mostly been assessed in large cohort studies [66], and it has hardly been tested whether these dietary scores are suitable for intervention studies and whether changes in these scores correlate with changes in CVD risk markers [67]. Against this background, it can be justified to assess the effectiveness of the intervention programmes *Healthy Lifestyle Community Programme* cohort 2 (HLCP-2) and *Healthy Lifestyle Community Programme* cohort 3 (HLCP-3) in improving dietary behaviour and the abovementioned CVD risk markers.

Study objectives

With the aim of testing the effectiveness of the lifestyle intervention HLCP-2, data were collected by conducting a community-based controlled trial over the course of 1 year (originally intended to last 2 years), including multiple measurement time points. In this study, the following CVD risk markers were analysed: body weight, BMI, waist circumference, systolic and diastolic BP, pulse pressure, RHR as well as the blood parameters TC, measured and calculated LDL-C, non-HDL-C, REM-C, HDL-C, TAG, glucose, HbA1c, insulin, hs-CRP, Hcy, and Apn. In addition, ccIMT was assessed by means of ultrasound, and dietary intake, physical activity, and sociodemographic parameters were assessed with questionnaires.

Furthermore, the effectiveness of the lifestyle intervention HLCP-3 was assessed in an uncontrolled trial to replicate the findings of the HLCP-2 study. In the HLCP-3 study, the same biomarkers as in the HLCP-2 study were assessed, with the exception of ccIMT, Hcy, and Apn (which were not assessed in the HLCP-3 study) as well as oxLDL (which was only assessed in the HLCP-3 study). The following research questions form the basis of this dissertation and are addressed in the respective journal publications:

1) How does the controlled lifestyle intervention HLCP-2 affect ccIMT in the short term (within 6 months)? (**Paper 1**, **Chapter 2.1.**)
2) How does the controlled lifestyle intervention HLCP-2 affect body weight and other established CVD risk markers in the short and medium term (within 10 weeks and 1 year) and can correlations between changes in diet and risk markers be demonstrated? (**Paper 2**, **Chapter 2.2.**)
3) How does the controlled lifestyle intervention HLCP-2 affect the inflammatory markers hs-CRP and Hcy (within 10 weeks and 1 year) as well as Apn (within 10 weeks) and can correlations between changes in diet and risk markers be demonstrated? (**Paper 3**, **Chapter 2.3.**)
4) How does the controlled lifestyle intervention HLCP-2 affect ccIMT in the medium term (within 1 year) and can correlations between changes in diet and ccIMT be demonstrated? (**Paper 4**, **Chapter 2.4.**)
5) How does the uncontrolled lifestyle intervention HLCP-3 affect body weight, other established CVD risk markers, and hs-CRP (within 10 weeks, 6 months, and 16 months) as well as oxLDL (within 10 weeks) and can correlations between changes in diet and risk markers be demonstrated? (**Paper 5**, **Chapter 2.5.**)

1.2. Preliminary remark: definition of the term plant-based diet

While the term plant-based diet has been widely used in recent years, including in the scientific literature, there is no consensus definition of the term [68–70]. Some researchers use the term plant-based diet to refer to a type of diet that excludes all animal-source foods (i.e. a vegan diet [68, 69, 71]) or nearly all animal-source foods (for example, a diet that is vegan except for honey [72]). Others understand the term to refer to a dietary pattern that excludes meat and fish (i.e. a vegetarian diet) [68, 73]. However, many understand the term plant-based diet to also include (at least optionally) some animal-source foods in relatively moderate amounts [74, 75]. For example, the traditional Mediterranean diet is often included under the plant-based diet umbrella [54] as it involves a decreased meat intake (compared to a typical Western diet) and a high intake of fruit, vegetables, cereal grains, legumes, nuts, and extra virgin olive oil, while also including fish, poultry, and relatively small amounts of red meat [76]. Some authors have even included a Palaeolithic diet (rich in meat, fish, and eggs but also plant-based foods [77]) in the group of plant-based dietary patterns [54]. Frequently, the term plant-based diet is also used synonymously with a healthy plant-based diet, i.e. a dietary pattern which is not just characterized by a reduced intake of animal-source foods but also by a reduced intake of plant-based foods categorized as unhealthy (added sugars, refined grains, etc.) [71]. Although mushrooms are not plants, these are, for simplicity's sake, often included in the group of plant-based foods [68] (sometimes in the vegetable food group [78]).

For the purpose of this thesis, a plant-based diet is defined as a dietary pattern that is centred around foods of plant origin, with a concomitant reduced intake of animal-source foods compared to a typical Western diet [79]. Thus, this wider definition of plant-based diets includes, for example, diets with a relatively low meat intake [80] as well as semi-vegetarian/flexitarian (mostly but not entirely meat-free) [81–83], pescatarian (no meat but fish), lacto-ovo-vegetarian (no meat or fish), or vegan dietary patterns [68, 69, 79, 84, 85]. Plant-based diets typically place a strong emphasis on (partly) replacing high-protein animal-source foods (meat, fish, eggs, and dairy) with high-protein foods of plant origin (especially legumes) [84, 86]. Similarly, for the purpose of this thesis, the term plant-based diet is not equated with a healthy plant-based diet. In turn, a healthy plant-based diet is defined as any dietary pattern that is centred around a diversity of fruit, vegetables, whole grains, legumes, nuts, seeds, and healthy oils, with a concomitantly reduced intake of animal-source foods (particularly meat) as well as a reduced intake of unhealthy plant-based foods, including added sugars, salt, refined grains, hydrogenated oils, and deep-fried foods [87]. Furthermore, in order for a dietary pattern to be considered healthy, appropriate caloric and nutrient intakes must be ensured [73, 79]. Defined in this way, healthy plant-based diets may include, for example, the traditional Mediterranean diet [76], the Dietary Approaches to Stop Hypertension (DASH) diet, the Portfolio diet [85, 88], or any similar dietary pattern.

While the definitions above are still not highly specific (in terms of certain foods to be included or excluded), they are widely used in the literature [85], and more specific descriptions can be formulated if needed [69]. The wider definition of plant-based diets used here may also have the advantage of being more attractive to many people, while stricter definitions may be perceived as off-putting by many [89]. Other aspects of diet may be of certain additional relevance (such as food preparation techniques, meal times [90, 91], or farming methods used for growing plant-based foods [92, 93]).

1.3. CVD

CVD is defined as a group of disorders of the heart and blood vessels [94]. CVD includes atherosclerotic CVD, which is the typical cause of myocardial infarction, stroke, and peripheral arterial disease, as well as non-atherosclerotic CVD [95], which is much less common and can, for example, be caused by certain bacterial infections or birth defects [94].

CVD is the leading cause of premature mortality in Germany, Europe as well as worldwide, both in women and in men [1]. Among behavioural risk factors, unfavourable dietary patterns and tobacco use are the two leading causes of premature mortality, which again is the case in Germany, Europe, and worldwide, for both women and men [1]. When considering only dietary risk factors, it has been proposed that the most important factors which increase mortality in Germany are low intakes of whole grains and legumes as well as a high intake of red meat [1]. Compared to Germany, at the European level and especially worldwide, a high sodium intake plays a more crucial role in increasing mortality, while high intakes of red meat and processed meat are of slightly lower importance [1]. Furthermore, many common CVD risk factors are also risk factors for cancer and other chronic diseases. These risk factors include obesity [96], high intakes of added sugars, refined grains, fried foods [97–99], red meat, and processed meat [100, 101], low levels of physical activity as well as increased inflammatory processes associated with these foods [102, 103], which all contribute to premature mortality [1].

Atherosclerosis is a long-term process of inflammation-associated, unfavourable arterial wall changes which can affect most segments of the arterial tree, including the coronary, carotid, and cerebral arteries [104]. More advanced atherosclerosis is characterized by focal lesions which protrude into the vascular lumen [105], and at the population level, atherosclerosis is the most important aetiological factor for myocardial infarction and stroke [106]. Factors which contribute to atherosclerosis development include hyperlipidaemia, hypertension, a prothrombotic state, increased inflammation and oxidative processes as well as damage to the endothelial layer (for example, due to a lack of nitric oxide) [107]. Many common chronic inflammatory diseases, including psoriasis, Crohn's disease, chronic obstructive pulmonary disease as well as depression, are associated with increased CVD risk [108]. Atherosclerosis development depends on exposure to risk factors over the course of one's

lifetime, and early signs of atherosclerosis can already be detected in childhood [107]. Based on traditional risk scores, a large portion of the population is categorized as being at low-to-intermediate risk of CVD events (myocardial infarction and stroke) [106]. Consequently, the window of greatest opportunity for CVD prevention (early prevention) is frequently missed, and steps towards the prevention or deceleration of atherosclerosis progression are frequently taken only in later life stages [106, 109].

For some individuals (for example, in the case of certain LDL receptor polymorphisms), genetic predisposition is the main cause of increased CVD risk, but the majority of the population are not affected by such overbearing genetic predispositions [110]. Although for the majority of the population genetic predispositions can still considerably influence CVD risk, the phenotypical expression of genetic predisposition is strongly influenced by environmental factors, especially lifestyle behaviours [104].

1.4. ccIMT

ccIMT: definition and anatomy

The parameter ccIMT has been used in clinical trials since the 1980s [111] and is still widely used today [112]. The intima-media thickness (IMT), or intima-media complex, comprises the combined thickness of the inner layer (endothelium; *tunica intima*) and middle layer (mostly smooth muscle; *tunica media*) of the artery wall, excluding the outer layer (connective tissue, primarily collagen and elastin fibres; *tunica externa* or *tunica adventitia*; **Figure 1**) [113]. While thickening of the intima generally reflects atherosclerotic changes [114], thickening of the media is predominantly a consequence of smooth muscle growth (medial hypertrophy or hyperplasia) [115]. It has been suggested that ccIMT, as opposed to IMT in other segments of the carotid arteries, might particularly reflect media thickening [115].

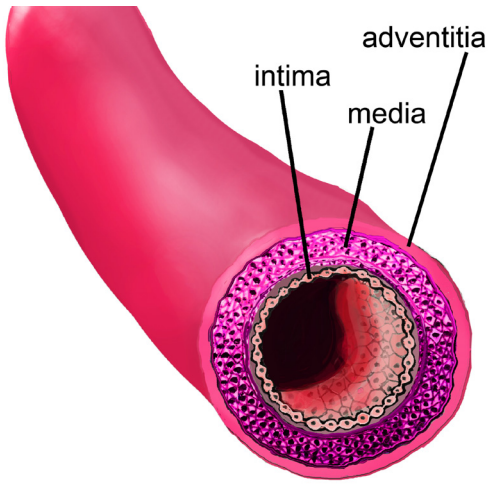

Figure 1. The three main layers of the arterial wall: intima, media, and adventitia

(Author's own diagram)

ccIMT as a marker of subclinical atherosclerosis

In recent years, it has been controversially discussed whether the term subclinical atherosclerosis should be used to describe ccIMT [116], as ccIMT is biologically and genetically distinct from atherosclerotic plaque burden [117]. However, while ccIMT is not equivalent to atherosclerosis, it does constitute a biomarker of the early stages of atherosclerosis [118]. Alternative terms to describe ccIMT have been suggested, such as arteriopathy or arterial injury [116]. However, it has been pointed out that these terms are not any more or less accurate at describing ccIMT than the term subclinical atherosclerosis and that, given the extensive literature already using the term subclinical atherosclerosis, there is little benefit of changing the established terminology [118].

ccIMT change (ccIMT progression)

Instead of assessing ccIMT values only cross-sectionally, measuring ccIMT change over time (ccIMT progression) has been increasingly used in intervention studies [119]. The parameter ccIMT change is controversial because concerns have been raised whether it can be measured precisely enough to detect true changes, i.e. whether the signal-to-noise ratio may be too small [112, 120–123]. For this reason, ccIMT is not recommended as a parameter for pre and post assessments in individuals [124]. However, when ccIMT change is assessed at the group level, the precision strongly increases [125], and a recent meta-analysis of 119 clinical trials including more than 100,000 patients (mean age: 62 years, 42% female) and an average follow-up of 3.7 years found that each 0.010 mm/year reduction in ccIMT change was associated with a CVD risk reduction of ~10%, with larger ccIMT reductions resulting in a more strongly reduced CVD risk [126]. Furthermore, the dietary interventions in this meta-analysis more consistently showed beneficial effects on ccIMT compared to medication-based interventions [126]. Thus, the parameter ccIMT change was demonstrated to be a useful surrogate marker of CVD risk in clinical trials, especially dietary interventions [126].

It should be distinguished between the usefulness of ccIMT change as a parameter in clinical trials [126] and the usefulness of cross-sectionally measured ccIMT as an additional parameter to be routinely assessed by general practitioners (family physicians) for the purpose of CVD risk categorization of patients [106, 117]. The latter is not recommended in the latest (2021) European Society of Cardiology (ESC) guidelines for CVD prevention, due to a lack of added value to standard risk parameters [127]. Similar recommendations have been made by the American College of Cardiology and the American Heart Association (AHA) [128].

ccIMT: associations with other risk markers (observational studies)

In observational studies, associations between mean ccIMT and a variety of non-classical CVD risk markers in blood have been observed, including positive correlations with arsenic [129, 130],

mercury [131], phosphorus [132], leptin, resistin, tumor necrosis factor-alpha (TNF-α), interleukin 6 [133], hs-CRP [134], myeloperoxidase [135], uric acid [136], allantoin [137], fibroblast growth factor-21 [138], alkaline phosphatase [139], trimethylamine N-oxide (TMAO) [140], and matrix metalloproteinase-12 (MMP-12) [141]. High exposure to lead [142, 143] has also been shown to be positively associated with ccIMT, while it is unclear whether there is an association with cadmium exposure [144].

On the other hand, inverse associations have been observed between mean ccIMT and a variety of markers in blood, including Apn [133], glucagon-like peptide-1 [145], apelin [146], fetuin-A [147], meteorin-like [148], total bilirubin [149], and soya isoflavones [150].

ccIMT: associations with dietary patterns (observational studies)

In cross-sectional studies, an association between a healthy Nordic dietary pattern and ccIMT could not be shown [151]. Similarly, no significant association between ccIMT and added sugar intake [152] or fruit and vegetable intake [152] could be demonstrated. However, an inverse association of ccIMT with cruciferous vegetable intake has been observed [153]. A small study indicates that long-term parenteral nutrition may be associated with lower ccIMT [154]. Another small study observed lower ccIMT values in individuals following a raw vegan diet compared to endurance runners on a typical Western diet [155, 156], while endurance running does not appear to affect ccIMT [157, 158]. In prospective cohort studies, no significant associations of moderate alcohol intake and ccIMT change could be shown [115, 159].

ccIMT: results from controlled trials with multimodal lifestyle interventions

To date, only a small number of controlled lifestyle trials have been conducted which have tested the effect of a combination of various lifestyle factors (diet, physical activity, etc.) on ccIMT (see **Appendix 1: Table 1**). Six such trials could be identified in the literature [50, 160–164]. The majority of these trials did not demonstrate a significant intervention effect on ccIMT [50, 161, 163, 164]. In two trials, however, a favourable intervention effect on ccIMT (compared to control) could be shown [160, 162]. In these two studies, conducted with individuals with hypertension and patients with type 2 diabetes, respectively, goals included weight loss, regular physical activity, and dietary changes [160, 162]. In one study, dietary recommendations included increasing fruit and vegetable consumption and reducing salt intake [160], while in the other study dietary recommendations were not specified [162].

ccIMT: results from controlled trials with interventions based on dietary patterns or foods

Only a small number of controlled trials with purely dietary interventions have assessed ccIMT (see **Appendix 2: Table 2**). About half of the controlled dietary interventions were unable to demonstrate a significant invention effect on ccIMT [165–169]. Two trials with a traditional Mediterranean diet showed a favourable effect on ccIMT (compared to a low-fat diet) [170, 171]. One study with participants with type 1 or type 2 diabetes demonstrated a favourable effect on ccIMT with the recommendation of increasing fruit, vegetable, and dairy intake (compared to usual diet) [51]. Furthermore, one study with elderly men with high cholesterol demonstrated a favourable effect on ccIMT with a moderate-fat, healthy Norwegian diet, including the recommendation to reduce the intake of meat and animal fats [172, 173]. In addition, one study with participants at high CVD risk demonstrated a favourable effect on ccIMT with a traditional Mediterranean diet (compared to a low-fat diet) only in a subgroup analysis of participants with high baseline ccIMT values [174].

ccIMT: results from controlled trials with dietary supplements

The majority of controlled trials with dietary supplements, including adults from the general population or individuals with diabetes or CVD (but without advanced kidney disease), have not been able to demonstrate a significant intervention effect on mean ccIMT [173, 175–188]. However, some clinical trials were successful in demonstrating such an effect. The supplements used in these trials were linseed oil [189–191], vitamin D [190], vitamin E [189, 192, 193], vitamin C [192, 193], vitamin B6 [194], folic acid [194, 195], vitamin B12 [194, 196], lutein [197], lycopene [197], garlic powder [198], and red ginseng [199] (all randomized controlled trials).

ccIMT: lack of evidence from controlled trials including a plant-based diet

Of the six abovementioned multimodal lifestyle trials [50, 160–164], only one included a strong focus on a plant-based diet [50] (**Appendix 1: Table 1**). The participants in this trial were coronary heart disease patients (United States), and the plant-based diet recommended was a very low-fat diet, excluding nuts and healthy oils (such as extra virgin olive oil) [50]. Thus, to date, no controlled trials have tested if a multimodal lifestyle intervention with a plant-based diet including nuts and healthy oils can beneficially affect ccIMT (**Appendix 1: Table 1**).

Similarly, no previous controlled trials (either multimodal lifestyle or purely dietary interventions [161, 163]) appear to have assessed the effect of a plant-based diet (rich in fruit, vegetables, whole grains, legumes, and nuts) on ccIMT in mostly clinically healthy individuals from the general population (**Appendix 1: Table 1**; **Appendix 2: Table 2**).

Furthermore, irrespective of study population, no controlled trials have correlated ccIMT changes with changes in the dietary scores PDI, hPDI, and uPDI [78], and no controlled intervention trials assessing

ccIMT have included dietary recommendations towards a plant-based diet in line with the hPDI, i.e. the recommendation to reduce both the intake of animal-source foods and of unhealthy plant foods while increasing the intake of healthful plant foods [78].

In addition, associations between changes in ccIMT and hs-CRP have hardly been explored. To date, only one controlled lifestyle/dietary trial appears to have reported correlations between ccIMT change and hs-CRP change [51], while no such trial has reported correlations of ccIMT change with Hcy change. Results regarding ccIMT are reported in **Paper 1** (**Chapter 2.1.**) and **Paper 4** (**Chapter 2.4.**).

Potential alternatives to ccIMT

It has been pointed out that IMT measured in other segments of the carotid arteries, i.e. the internal carotid artery or the carotid bulb/bifurcation (**Figure 2**), is more strongly associated with CVD risk compared to ccIMT, one reason being that the common carotid arteries are less prone to atherosclerosis [141, 200, 201]. However, repeatability is generally lower when assessing IMT in these other carotid segments (compared to ccIMT), and therefore, ccIMT appears to be more suitable for follow-up measurements in prospective studies (including interventions) [202]. In addition, according to the Mannheim consensus, the arterial segment in which IMT is to be measured (typically referred to as the region of interest) must be free of atherosclerotic plaque (focal lesions) [124]. As atherosclerotic plaque occurs less frequently in the common carotid arteries than in other carotid segments [117], the risk of having plaque in the region of interest (which can result in having to exclude participants) is lower when assessing ccIMT. This constitutes an additional reason why, for clinical trials adhering to the Mannheim consensus, the assessment of ccIMT is preferable to measuring IMT in other carotid segments. While IMT can also be assessed in other regions of the body (for example, femoral [203] or brachial artery IMT [204]), it is most commonly assessed in the carotid arteries [106]. Similarly, it has been found that three-dimensional measurement of carotid plaque burden (3D plaque score) may be more strongly associated with CVD risk than ccIMT [117]. However, currently the use of 3D plaque scoring is still seldomly used, it is a complex procedure that requires highly specialized knowledge, and results can be misleading as the position of the ultrasound probe strongly influences the measurements [118]. Consequently, 3D plaque score is a less practical parameter for clinical trials (and prospective studies in general) [118]. On the other hand, it has been suggested that the assessment of 3D plaque score may make it possible to reduce the sample size, thus making it a potentially superior alternative to ccIMT, if the necessary equipment and trained staff are available, if 3D plaque score is the primary parameter of the study, and if it is possible to reduce the sample size of the study [117, 200]. In addition, coronary artery calcium (CAC) scoring by computed tomography (CT) has been suggested as an alternative to ccIMT [106, 205]. However, while current evidence indicates that CAC scoring is superior to ccIMT for the purpose of CVD risk classification of

patients [30, 106], the equipment needed and the cost associated with these measurements can be prohibitive for their use in clinical trials [106]. While CT scans are non-invasive [106], they expose the participant to radiation [206], which is ethically concerning.

Some clinical trials have also used coronary angiography [207]. However, this method is more suitable for patients at high risk of CVD [208]. Furthermore, it is a technically difficult, invasive, and expensive method [209, 210] which involves radiation, and serious adverse effects for participants are possible [210], making it a less practical and an ethically more problematic alternative.

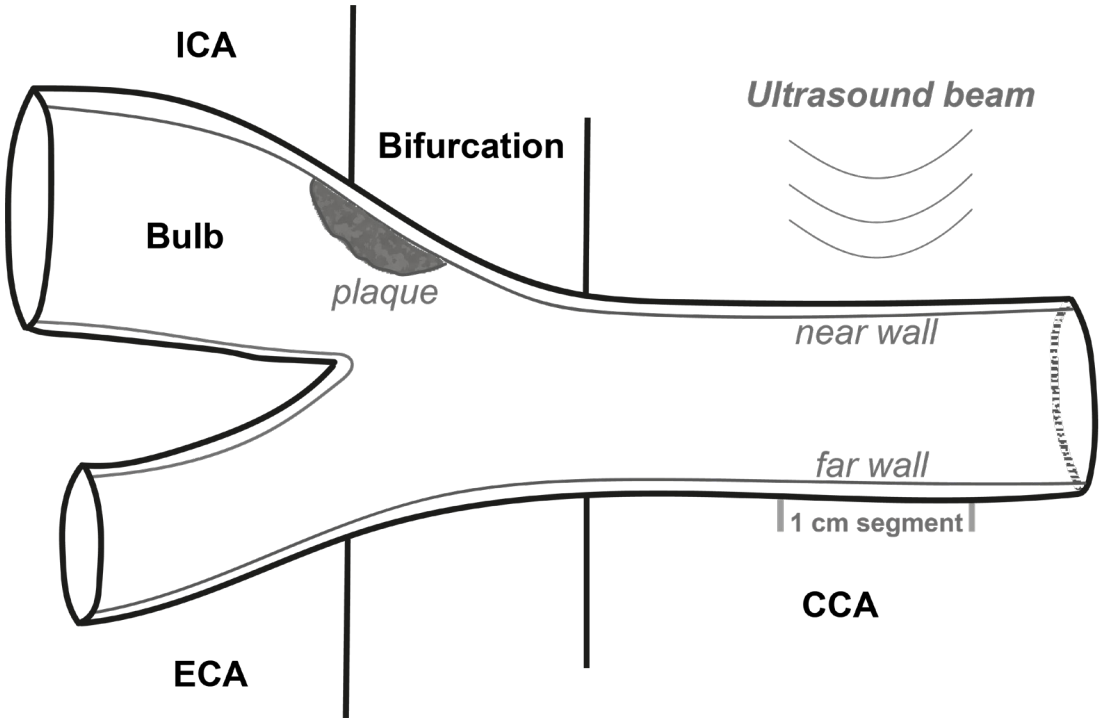

Figure 2. Segments of the carotid artery

Also pictured is the ultrasound beam coming from the ultrasonic probe and the 1 cm-segment of the common carotid artery (CCA) which constitutes the region of measurement (region of interest, ROI) according to the Mannheim consensus (a consensus paper on measuring carotid intima-media thickness); ICA: internal carotid artery; ECA: external carotid artery. (Author's own diagram, based on Touboul et al. 2012 [124])

1.5. Established CVD risk markers

The ESC guidelines (2021) on CVD prevention state that the main causal and modifiable risk factors for atherosclerotic CVD are circulating apolipoprotein-B-containing lipoproteins (of which LDL-C is the most abundant), high BP, cigarette smoking, and diabetes [30]. Consequently, it is of high relevance to assess cholesterol levels, BP, and parameters of blood glucose homoeostasis (glucose, HbA1c, insulin)

in lifestyle intervention trials [211]. In addition, the ESC guidelines list obesity and adiposity as important CVD risk factors [30]. Consequently, markers of obesity and adiposity (such as body weight, BMI, and waist circumference) should also be assessed in lifestyle intervention trials [212].

Cholesterol, TAG, and fasting glucose

Associations of certain circulating blood lipoproteins with CVD risk have been known since the 1950s [213, 214], while the existence of the genetic disorder of familial hypercholesterolaemia, characterized by extremely high LDL-C levels, was first described in the 1960s [215]. To date, little doubt remains regarding the causal nature of the association between increased LDL-C levels and increased CVD risk [30].

In contrast, the causal nature of the association of HDL-C with CVD risk is highly uncertain [216]. Inherited disorders causing low HDL-C are generally not associated with increased CVD risk [216]. In addition, pharmacological interventions that increase HDL-C levels have failed to decrease CVD risk [217–219]. Current evidence indicates that certain subfractions of HDL-C are positively associated with CVD risk, while other subfractions of HDL-C are negatively associated with this risk [219–221]. Furthermore, low-fat, plant-based dietary patterns (with a strong focus on fruit, vegetables, whole grains, and legumes) are associated with reduced HDL-C levels but also with reduced CVD risk [220]. Current evidence furthermore indicates that HDL functionality [222] may be of more relevance to CVD risk compared to total HDL-C quantity [118]. It has been proposed that the strong inverse association between HDL-C concentrations and CVD risk observed in cohort and case control studies may be simply due to the commonly found inverse association of HDL-C with atherogenic lipoproteins [216]. Recent observational evidence indicates that very high HDL-C levels are associated with an increased risk for CVD [30] as well as for infectious diseases, age-related macular degeneration, dementia, and mortality [216]. While evidence for a causal association between HDL-C and CVD risk is lacking, the ESC guidelines (2021) nevertheless describe HDL-C as useful biomarker to refine CVD risk estimation [30].

Similarly, despite decades of investigation into the potentially causal role of elevated TAG concentrations in CVD, there is insufficient evidence to demonstrate causality, and (like with HDL-C) the observed associations may be due to concomitant associations with atherogenic lipoproteins, inflammation, and/or coagulation [223].

While currently LDL-C is the main treatment (and preventative) target for decreasing CVD risk, it has been suggested that the calculated risk marker non-HDL-C (TC minus HDL-C) is equally important [30]. The ESC guidelines (2021) state that, unlike calculated LDL-C, which is frequently used in clinical practice as well as clinical trials [224], non-HDL-C is accurate even if TAG are high (≥400 mg/dl), that non-HDL-C may be more accurate that LDL-C in individuals with diabetes, and that non-HDL-C can be considered a treatment target for all patients as it captures the information regarding all

apolipoprotein-B-containing lipoproteins [30]. For LDL-C and non-HDL-C, the ESC guidelines suggest target goals of <100 mg/dl and <131 mg/dl, respectively [30]. Furthermore, recent evidence indicates that REM-C (TC minus LDL-C minus HDL-C) may be of equal or higher relevance to CVD risk than LDL-C [224, 225].

Similarly, oxLDL, i.e. LDL particles with oxidatively modified structural components, is deposited in the subendothelial space and is thus thought to contribute to atherosclerosis progression [226, 227]. Current evidence confirms that increased oxLDL levels are associated with an increased risk of CVD events [226] as well as other conditions such as non-alcoholic steatohepatitis [228] and more unfavourable coronavirus disease 2019 (COVID-19) outcomes [227].

Apart from being a marker of diabetes risk, high blood glucose levels are associated with an increased risk of hypertension and CVD (including stroke [229, 230]). Furthermore, high blood glucose [231] and HbA1c [232, 233] levels are also associated with poor COVID-19 outcomes.

Vital parameters (BP, pulse pressure, and RHR)

The ESC guidelines (2021) state that increased BP is a major cause of atherosclerotic CVD as well as non-atherosclerotic CVD (especially heart failure) [30]. The risk of death from myocardial infarction or stroke appears to increase linearly, even from levels as low as 90 mmHg systolic and 75 mmHg diastolic BP [30]. The 2021 ESC guidelines utilize the Systemic Coronary Risk Estimation 2 (SCORE2) risk algorithm to estimate an individual's 10-year risk of CVD events (fatal or non-fatal myocardial infarction or stroke). This risk algorithm includes age, sex, smoker status, systolic BP, and non-HDL-C [30].

Apart from BP, recent evidence confirms that pulse pressure (systolic BP minus diastolic BP) and particularly RHR are of high relevance as CVD risk markers [234]. Pulse pressure increases with aging, as a result of atherosclerosis progression and increased arterial stiffness [235, 236]. High pulse pressure is a sign of deteriorating cardiovascular health and is associated with increased CVD risk but also with an increased risk of chronic kidney disease [236], adverse postoperative outcomes [237], and cognitive decline [235]. Furthermore, higher RHR is not just associated with an increased risk of hypertension [238] and CVD (coronary heart disease, sudden cardiac death, heart failure, atrial fibrillation [239], stroke, and CVD in general) but also with an increased risk of diabetes [240], cancer mortality [241], and all-cause mortality [234, 242].

Adiposity markers (body weight, BMI, and waist circumference)

In non-smokers, there appears to be a linear relationship between BMI and mortality (with a J-shaped relation in ever-smokers), and in apparently healthy individuals all-cause mortality appears to be lowest at a BMI of 20–25 kg/m^2 (with a J-shaped or U-shaped relation) [30]. Both BMI and waist

circumference are strongly associated with atherosclerotic CVD risk [30, 243–246]. However, conflicting results have been published in terms of the association of BMI with CVD mortality in individuals with hypertension [247], type 2 diabetes [248], or pre-existing CVD [249], and even in healthy subjects [249]. These conflicting results may have been influenced by adverse effects of unintentional weight loss or beneficial effects of long-term CVD medication and other potential confounders [246, 250]. The association of CVD risk with BMI seems to be more prone to confounding, whereas the positive associations of waist circumference and other adiposity measures with CVD risk appear to be more clearly linear [246].

Waist circumference is the simplest measure of abdominal fat, and the 2021 ESC guidelines suggest waist circumference cut-off values of ≤94 cm for men and ≤80 cm for women [30]. However, different cut-off values may be suitable in different ethnicities [30]. While the concept of metabolically healthy obesity, i.e. the presence of obesity in the absence of metabolic risk factors, is controversially discussed in the literature [251–253], current evidence indicates that this usually is a transient phase that deteriorates into metabolically unhealthy obesity [30, 254]. Additionally, current evidence indicates that metabolically healthy obesity appears to be associated with increased CVD risk [245] and may also be associated with an increased risk of insulin resistance [255], non-alcoholic fatty liver disease (NAFLD) [256], gall stones [257], kidney disease [258], benign prostatic hyperplasia [259], cancer [260] as well as all-cause mortality (compared to metabolically healthy individuals with a BMI in the recommended range) [261, 262].

Evidence from controlled plant-based diet interventions regarding established CVD risk markers

In Germany, evidence from controlled trials including a plant-based diet is lacking, particularly from trials including participants from the general population as well as a no-intervention control group. Specifically, no controlled trials with a plant-based diet in line with the hPDI score [78] could be identified, and only a small number of controlled trials with similar characteristics (traditional Mediterranean, low-fat plant-based, or lacto-ovo-vegetarian diet) have been conducted [62–65]. As current evidence indicates that a plant-based diet in line with the hPDI would likely result in considerable benefits not just in terms of health promotion but also in terms of environmental sustainability [79, 263], it appears to be of great public health relevance to test the effectiveness of such an intervention (by conducting controlled trials) on established CVD risk markers, including the abovementioned biomarkers. Results regarding these biomarkers are described in **Paper 2** (**Chapter 2.2.**) and **Paper 5** (**Chapter 2.5.**).

Among established CVD risk markers, evidence from controlled trials is particularly lacking regarding potential effects of a plant-based diet on RHR [264, 265]. Furthermore, intervention studies assessing the effect of a plant-based diet on oxLDL in study populations in Germany are lacking, and only a small

number of such studies have been conducted in other countries (Spain, United States, and Canada) [56–61]. The results of these studies indicate that a healthy plant-based diet would likely result in lower oxLDL levels [56–61]. Results regarding the parameter oxLDL are described in **Paper 5** (**Chapter 2.5.**).

1.6. Inflammatory markers

hs-CRP: background

The ESC guidelines (2021) recommend against the routine assessment of hs-CRP (or other non-classical CVD biomarkers) for the purpose of CVD risk assessment in clinical practice because hs-CRP has limited additional value in reclassifying patients to a different CVD risk category [30]. However, hs-CRP is a sensitive marker for systemic low-grade inflammation and is a strong predictor of CVD risk [266]. It is also the most well-established and most thoroughly researched biomarker of inflammation and is, therefore, highly suitable to be assessed in the context of clinical trials [266, 267]. Transitioning from a typical Western diet to a healthy plant-based diet can be expected to lower hs-CRP levels [52, 53]. The assessment of hs-CRP can most reliably confirm whether an intervention is successful at reducing inflammation, thereby (presumably) reducing the risk of CVD (and other chronic diseases) [41, 266]. In addition, hs-CRP appears to be relatively stable, making it a reliable inflammatory marker to assess. For example, hs-CRP does not seem to be easily affected by a delay in specimen processing or by a change in storage temperatures, while, for example, the inflammatory marker TNF-α can strongly decrease in these cases [266]. Prospective assessments of hs-CRP should always be conducted at the same time of day (for example, in the morning) because hs-CRP levels appear to be higher at night. Due to potentially high within-subject variability it is a suitable parameter for prospective assessment in groups but not in individuals [266]. Different cut-off values for hs-CRP have been proposed [266, 268]. Frequently, the following categories are used to assess CVD risk based on hs-CRP levels: <1.0 mg/l (low risk), 1.0–3.0 mg/l (intermediate risk), and >3.0 mg/l (high risk) [266]. A cut-off value of <0.8 mg/l as the optimal range has also been suggested [268, 269]. The biomarker hs-CRP refers to CRP that is assessed by the use of assays with a low detection limit, which can reliably measure CRP at concentrations of <3 mg/l [266].

Increasing age, tobacco smoking, and a high alcohol intake are associated with increased hs-CRP levels [266]. In Individuals with obesity, metabolic syndrome, diabetes, autoimmune disease, arthritis, or depression, hs-CRP levels are also frequently increased [266].

hs-CRP and plant-based diets: results from controlled trials

Very few controlled trials including a no-intervention control group have assessed the effect of a plant-based diet on hs-CRP levels in participants from the general population [52–54]. A small number of

studies with a traditional Mediterranean diet [270–272], low-fat diet [273], or calorie-restricted diet [274] have been conducted. When looking at plant-based diets that were not Mediterranean, low-fat, or calorie-restricted, only two studies have been published comparing a plant-based diet group to a no-intervention control group, and both of these studies were conducted in young adults [275, 276]. Furthermore, the majority of the abovementioned controlled trials could not demonstrate a beneficial intervention effect on hs-CRP [270, 272–276]. Thus, while observational studies suggest that healthy plant-based diets are associated with significantly lower hs-CRP levels [52, 53], evidence from controlled trials is largely lacking that adopting such a dietary pattern would result in lower hs-CRP. Results of the HLCP-2 and HLCP-3 studies regarding hs-CRP are described in **Paper 3** (**Chapter 2.3.**) and **Paper 5** (**Chapter 2.5.**), respectively.

Hcy: background

Hcy is an biomarker associated with inflammation [277] and, in the context of a plant-based diet, also functions as a marker of vitamin B12 adequacy [278, 279]. High Hcy levels are associated with increased CVD risk [45]. However, the precise mechanisms by which elevated Hcy levels may adversely affect the vascular structure are not well understood [277]. It has been proposed that high Hcy concentrations may lead to mitochondrial dysfunction with an overproduction of reactive oxygen species, increased oxidative stress as well as increased thrombogenicity [46, 280–283]. Unfavourable effects of Hcy on all three layers of the arterial wall are possible, i.e. adverse effects on endothelial function (intima), medial remodelling, and adventitial inflammation [277]. Irrespective of the role of Hcy as an inflammatory and CVD risk marker, it is important to assess potentially unfavourable effects of adopting a plant-based diet on vitamin B12 status [284]. If the intake of animal-source foods is strongly decreased and at the same time vitamin B12-fortified foods or vitamin B12-containing supplements are not consumed, then the risk of not achieving recommended dietary intakes of this vitamin appears to be increased [285]. As a functional parameter of vitamin B12 status, the assessment of Hcy can fulfil the role of observing potential intervention effects on vitamin B12 status [285], while a more precise assessment of vitamin B12 status does not appear to be indicated in the context of a lifestyle programme without the recommendation of a strictly vegetarian or vegan diet [286].

Hcy and plant-based diets: results from controlled trials

Evidence from controlled trials regarding potential effects of a plant-based diet on Hcy is largely lacking. To date, no controlled trials including a no-intervention control group appear to have tested the medium-term (≥1 year) effect of a plant-based diet on Hcy levels in participants from the general population. Only one short-term controlled trial with these characteristics (plant-based diet, a no-intervention control group, participants from the general population) could be identified [287]. This

study was conducted in Germany and observed no effect of an unsupplemented vegan diet on Hcy levels after 4 weeks [287]. Consequently, controlled trials are needed to assess whether the transition towards a plant-based diet (even if non-vegetarian) could adversely affect Hcy levels in the medium term. Results of the HLCP-2 study regarding Hcy are described in **Paper 3** (**Chapter 2.3.**).

Apn: background

Apn is an inflammatory marker that is controversially discussed in the literature [288, 289]. Apn is a protein hormone produced in adipocytes, which has been described to improve insulin sensitivity [48, 290]. Low circulating Apn levels are frequently observed in individuals with obesity, diabetes, or CVD, and an increase in Apn levels is widely interpreted to be beneficial [291]. However, conflicting results have been reported, including a positive association of Apn with CVD mortality and all-cause mortality [288]. Thus, it is uncertain under which circumstances higher or lower Apn levels are beneficial [48]. Healthy plant-based diets may improve insulin sensitivity [9], and as Apn and insulin sensitivity appear to be closely associated [291], it is of interest to understand whether plant-based diets would affect Apn levels. To date, the potential effect of plant-based diets on Apn levels is still unclear [53, 54, 292].

Apn and plant-based diets: results from controlled trials

While one controlled trial with obese women in Italy indicates that a traditional Mediterranean diet may increase Apn [293–295], no controlled trials with a no-intervention control group have tested the effect of a plant-based diet on Apn levels in participants from the general population (no such trials, of any duration, could be identified) [54, 55, 293, 293–302]. Therefore, it is uncertain whether transitioning to a plant-based diet would affect Apn levels. Results of the HLCP-2 study regarding Apn are described in **Paper 3** (**Chapter 2.3.**).

1.7. CVD prevention with lifestyle: planning interventions that are safe and effective

The World Health Organisation (WHO) has stated that about 80% of premature heart disease and stroke are preventable [303]. Lifestyle factors that could greatly contribute to CVD prevention include non-smoking, a healthy dietary pattern, regular physical activity, and the avoidance of excessive alcohol intake [304]. Furthermore, maintaining a healthy body weight throughout life is an important component of CVD prevention, with both overweight [245, 305] and extensive body weight fluctuations [306] being associated with increased CVD risk. Achieving and maintaining a healthy body weight has been described as a lifelong challenge, for which individuals require ongoing support and encouragement [307]. While a variety of lifestyle factors are relevant for maintaining a healthy body weight, the most crucial factor appears to be diet [308]. In particular, a dietary pattern with a much

higher fibre content (compared to a typical Western diet) can help prevent overweight and obesity [73]. Thus, a fibre-rich, predominantly plant-based diet can be a decisive tool for making the challenge of lifelong healthy weight maintenance much more achievable [309].

Healthy dietary patterns

While there is ongoing controversy about which dietary patterns can be described as the healthiest [170, 310–312], current evidence indicates that designing a healthy dietary pattern, which also effectively contributes to CVD prevention, can be achieved in a variety of ways [313–316], leaving much room for adaptations based on regional and personal preferences, environmental and ethical concerns as well as metabolic requirements (for example, when diabetes or allergies are present) [79]. The plant-based diet approach has found increasing support in the current medical literature, but it should be noted that a healthy plant-based diet does not constitute a specific diet characterized by specific foods but rather a broad dietary category [9, 317]. Healthy plant-based dietary patterns have been found to be associated with improved CVD risk markers, including body weight, blood lipids, BP, fasting glucose, HbA1c, inflammatory and oxidative stress markers as well as a reduced risk of diabetes, coronary heart disease [66], CVD events, cancer, and reduced all-cause mortality [9].

Preliminary evidence indicates that lifestyle interventions including a healthy plant-based diet, may be able to stabilize or even reverse type 2 diabetes and CVD [9, 87, 318]. However, more evidence is not only needed regarding the effectiveness of such interventions [87] and how these can be optimized but also in terms of whether the general public would be willing to adopt such dietary patterns [9]. When designing plant-based diet interventions, it should also be taken into consideration that high intakes of unhealthy plant-based foods (added sugars, refined grains, etc.) appear to be associated with an increased CVD risk [87].

A large volume of research has been conducted regarding the traditional Mediterranean diet, and it has been claimed that the traditional Mediterranean diet is the most beneficial dietary pattern for cardiovascular health [170]. However, it has also been suggested that the cardiovascular benefits of the traditional Mediterranean diet may be due to high intakes of fruit, vegetables, cereals, legumes, and extra virgin olive oil, which are foods rich in phenolic compounds with anti-inflammatory and antioxidant properties [170]. More research is needed to determine whether other types of healthy plant-based diets would be equally or even more beneficial than the traditional Mediterranean diet approach [309].

Dietary components

Current evidence supports the recommendation of a healthy plant-based diet based on fruit, vegetables, whole grains [317, 319], and legumes as well as nuts, seeds, and healthy oils (such as extra

virgin olive oil or rapeseed oil [311, 320]). While higher fruit and vegetable intakes are known to support CVD prevention [321], it is uncertain whether consuming more than 5 portions of fruit and vegetables daily can confer additional cardiovascular benefits [322].

Higher intakes of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) have been shown to be associated with improvements in CVD risk markers [320] as well as reduced CVD risk [313, 323, 324]. However, a causal relationship between MUFA and PUFA intake and CVD risk has not been clearly demonstrated so far [313, 325]. Nevertheless, current evidence supports the recommendation of including healthy plant-based fat sources (nuts, seeds, and healthy oils [320]) into the diet, instead of recommending a reduction in total fat intake per se (a recommendation which used to be found, for example, in the United States national dietary guidelines until the year 2000) [315]. Current evidence also indicates that increasing the intake of long-chain omega-3 fatty acids (eicosapentaenoic acid and docosapentaenoic acid) may slightly reduce the risk of coronary heart disease mortality and CVD events [326]. Similarly, increasing the intake of the short-chain omega-3 fatty acid alpha-linolenic acid appears to slightly reduce the risk of CVD events and arrhythmia [326]. Furthermore, current evidence indicates that a higher intake of omega-6 fatty acids appears to reduce TC levels and may reduce the risk of myocardial infarction in individuals at high risk [327]. In contrast, higher intakes of saturated fatty acids (SFA) and trans-fatty acids are associated with increased CVD risk [323].

A high intake of meat (especially red and processed meat [328–330]) is associated with increased CVD risk [331]. While clear evidence from randomized controlled trials of a causal relationship between red meat and increased CVD risk is lacking (which is a general problem in nutritional epidemiology) [332], current evidence justifies the recommendation to reduce meat intake in populations in which meat intake is high [328, 329, 333, 334]. In contrast to red meat, a higher intake of poultry appears to not be associated with increased CVD risk [335]. However, in contrast to fish intake, there is no clear inverse association between red meat [332] or white meat [336] intake and CVD risk.

Fish oil has been shown to improve arterial stiffness (as measured by pulse wave velocity [337]). Lean fish intake may lower TAG levels [338], and a higher fish intake (compared to a commonly found low fish intake) is generally associated with reduced CVD risk [339]. It has been suggested that this beneficial effect may only apply to individuals with established CVD and not the general population [340]. In addition, some studies have observed a U-shaped association of fish intake and CVD risk, and a clear disadvantage of fish-free (vegetarian) diets compared to pescatarian diets has not been shown [309]. Most studies assessing the association of fish intake with mortality have not adjusted for meat intake, which is important in order to determine whether there is an independent relationship between fish intake and mortality [340–343]. It has also been suggested that part of the beneficial effects on CVD risk observed for fish intake may be due to substituting meat (particularly

red and processed meat) with fish [29, 343]. Furthermore, the intake of fried fish may increase CVD risk [342], and a high fish intake is typically associated with a higher intake of mercury [344], which in turn is associated with increased CVD risk and all-cause mortality [345].

Current evidence indicates that moderate fish intake can be a part of a healthy plant-based diet (such as the traditional Mediterranean diet [76]) and that a regular intake of plant-based foods high in omega-3 fatty acids (such as rapeseed oil [320], walnuts, ground linseeds, or linseed oil) should be recommended [346]. Furthermore, current evidence supports the general recommendation of reducing animal protein intake and increasing plant protein intake (i.e. particularly the intake of legumes, nuts, and seeds) [331].

There is no conclusive evidence for a beneficial or harmful effect of egg intake on CVD risk in general [347–351]. However, an increased risk of heart failure with high egg intake is possible [347]. Similarly, for dairy intake no consistent associations have been observed with CVD risk [352–354]. A modestly reduced CVD risk with higher dairy intake is possible [352, 355–357]. This effect may partly be due to a substitution of less healthy foods with dairy [358] as well as the high calcium content of many dairy products [359]. Yogurt and cheese intake have been shown to be associated with decreased CVD risk [360–362], but this has not been consistently shown [354]. In contrast, high-fat dairy products may increase CVD risk [353, 357].

Furthermore, while the association of sodium intake and CVD risk remains controversial, a general recommendation to reduce the intake of table salt, particularly in individuals with hypertension, appears justified [363, 364]. In this context, an increased intake of potassium-rich foods (such as legumes, nuts, and green leafy vegetables) should also be recommended [364]. Spices may be an overlooked plant-based food group, and while this has not been sufficiently tested in controlled trials, a beneficial effect of a regular intake of a variety of spices on CVD risk can be hypothesized, one potential mechanism being via a lowering of inflammatory markers [365].

The intake of 100% fruit juice is not clearly associated with beneficial or adverse effects on CVD risk [366]. The intake of coffee is not associated with an increased risk of CVD [367, 368], and beneficial effects on CVD risk (and the risk of other chronic diseases [369, 370]) have been suggested [371, 372]. However, unfiltered coffee appears to increase TC and LDL-C levels [373, 374], and episodic coffee intake may increase the risk of sudden cardiac death in susceptible individuals [375]. Long-term effects of coffee intake on arterial stiffness and endothelial function are uncertain [376]. The intake of tea may slightly lower BP [377] and CVD risk [378].

Apart from addressing the abovementioned food groups and fatty acids, it is useful to address certain key micronutrients that are of high relevance in the context of plant-based dietary patterns. While ensuring an adequate intake of vitamin B12 is of particular importance when following a diet that completely excludes animal-source foods (vegan diet) [73, 379, 380], evidence indicates that dietary

patterns that are largely plant-based while still including some animal-source foods (especially lacto-ovo-vegetarian diets) also frequently result in suboptimal vitamin B12 intakes and consequently low vitamin B12 status [73]. Furthermore, in the context of lifestyle intervention trials, it is important to address vitamin D intake, as low vitamin D status may adversely affect CVD risk markers, including cholesterol levels [381, 382], BP, and BMI, and may increase the risk of obesity, abdominal obesity, insulin resistance, and metabolic syndrome [383, 384].

Planning sustainable dietary patterns

Public health authorities increasingly incorporate a variety of sustainability aspects into dietary guidelines and call on others to do so as well [30, 385, 386]. Consequently, when designing an intervention with the aim of quantifiably improving health parameters, it is commendable to take into account not just the effectiveness of the intervention in terms of improving personal health but also in terms of potential effects on other sustainability dimensions, including environmental impacts and human rights (sometimes referred to as planetary health [84, 387, 388]).

A shift towards a plant-based diet would likely not just decrease CVD risk [9, 87] but also decrease some of the harmful impacts of modern society on the environment [389, 390]. An elaborate plan for healthy diets from sustainable food systems, including the recommendation of moving towards a plant-based diet [84], has been drawn up, for example, by the EAT-Lancet Commission (**Figure 3**) [79]. The EAT-Lancet Commission's report states that, overall, plant-based staples (fruit, vegetables, grains, and legumes) have smaller ecological footprints (considering various environmental indicators) compared to animal-source foods, irrespective of whether the assessment is made per unit weight, per serving, per calorie, or per protein weight [79]. Highly relevant environmental impacts which would likely be beneficially affected by a shift towards plant-based diets include greenhouse gas emissions, phosphorus [391] and nitrogen pollution in waterways, energy use, land use as well as the acidification of water and soils (among others) [79, 84, 309, 389, 392]. Accordingly, the position can be taken that when designing lifestyle interventions not only personal health outcomes but also foreseeable environmental and societal impacts should (ideally) be taken into account [385, 390]. Incorporating these aspects into educational materials may also provide additional motivation to at least some of the participants to move towards a plant-based diet [393–396].

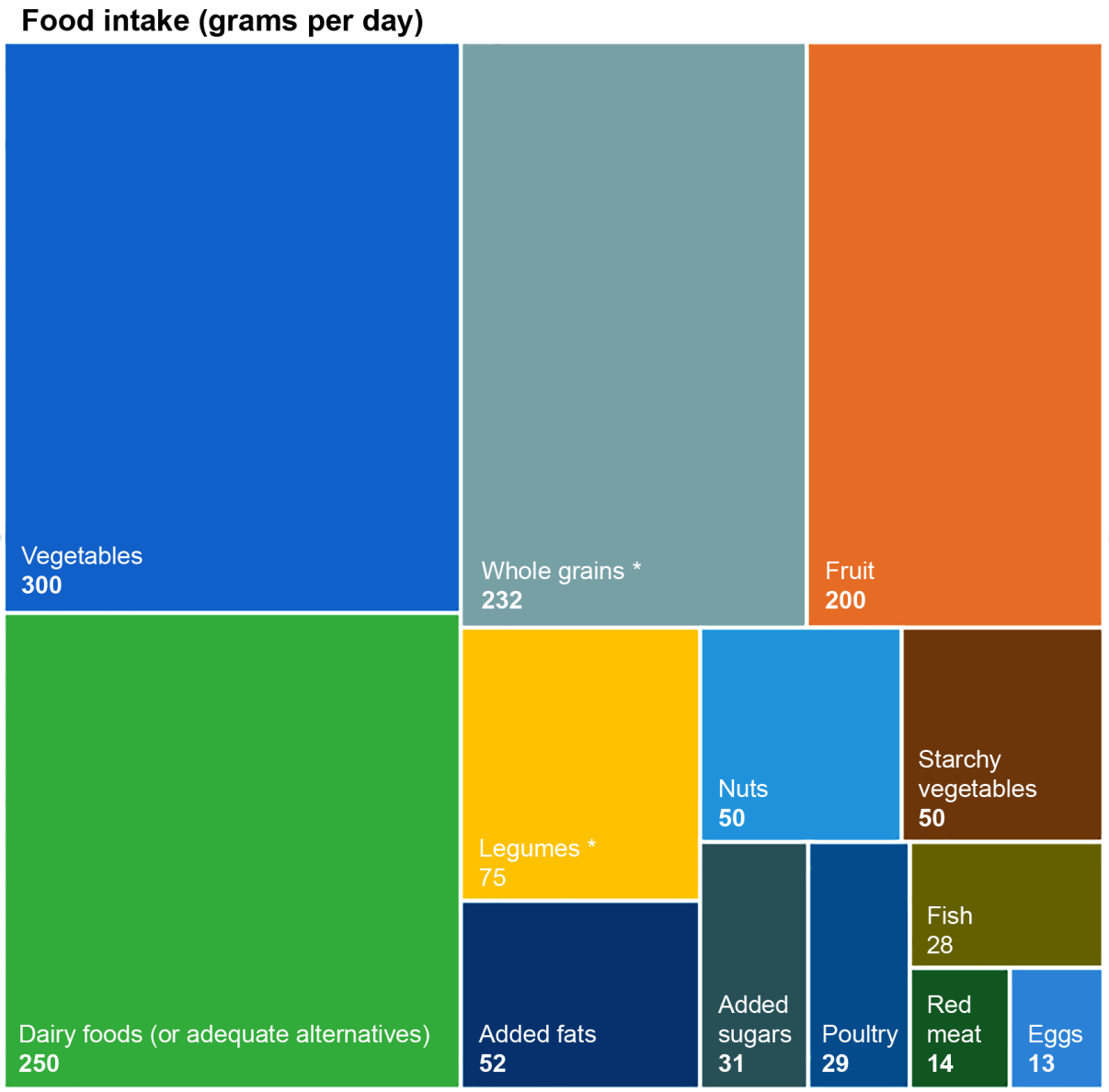

Figure 3. Generalized overview of a healthy and sustainable plant-based diet as per EAT-Lancet

Recommended food intake per day at the food group level (for an intake of 2500 kcal/day). The EAT-Lancet recommendations provide possible ranges (grams/day) for most food groups: starchy vegetables (0–100), vegetables (200–600), fruit (100–300), dairy foods (0–500), red meat (0–28), poultry (0–58), eggs (0–25), fish and shellfish (0–100), legumes (including soya foods: 0–150), nuts (including peanuts: 0–75), added fats ([predominantly] unsaturated oils: 20–80; [predominantly] saturated oils: 0–11.8), added sugar (0–31).

** The weight for grains and legumes refers to the weight of uncooked (dry) grains/legumes. It should be noted that the weight of cooked grains and legumes (as seen on one's plate) is about three times higher. (Author's own diagram, based on Woolston 2020 [84] and Willett et al. 2019 [79])*

Physical activity

Evidence indicates that regular physical activity increases vascular smooth muscle relaxation and reduces RHR, BP [397, 398], aortic valve calcification, vascular resistance, and CVD mortality [11]. Thus the recommendation to increase physical activity is non-controversial [10], including for individuals with preexisting coronary artery disease [399]. However, while it has been stated that safety concerns of increasing physical activity appear to be minimal [11], individuals with underlying CVD who are not accustomed to strenuous exercise should be guided to cautiously increase physical activity in accordance with advice by their family physician because strenuous exercise may otherwise increase the risk of acute cardiac events [400].

Furthermore, in the context of a highly heterogenous study population in community-based interventions, potentially including highly physically active individuals, it should be taken into account that excessive exercise may be associated with an increased risk of CVD mortality [401]. For example, an approximately U-shaped association has been reported between cycling and CVD mortality, with the lowest risk of CVD mortality at approximately 130 min/week [401]. Similarly, it has been reported that excessive levels of exercise appear to be associated with increased inflammation [402] and may increase coronary artery calcification as well as the risk of atrial fibrillation [403]. The optimal dose of exercise remains controversial [404].

Combined lifestyle factors

Apart from diet and physical activity, there are other lifestyle choices that can affect CVD risk. Current evidence indicates that healthy lifestyle choices including a combination of multiple lifestyle factors can strongly reduce both CVD and all-cause mortality and that these findings are largely consistent among individuals from different regions of the world, different ethnicities, and different socioeconomic backgrounds [405].

Smoking is a well-documented CVD risk factor, and the adverse cardiovascular effects of smoking also apply to passive smoking [392] as well as (with high probability) to water pipe smoking [406] and electronic cigarettes (heat-not-burn tobacco products) [407]. However, several unexpected findings, including a potentially elevated thrombotic risk related to smoking cessation, have been documented [408]. In this context, the concept of the smoker's paradox, a potentially decreased short-term mortality risk in smokers following myocardial infarction or stroke, has been controversially discussed since the 1990s [409, 410]. However, current evidence casts doubt on the causality of this association [410, 411] and supports the recommendation of smoking cessation for all current smokers [410], especially in conjunction with a healthy dietary pattern characterized by anti-inflammatory, anti-atherogenic, and anti-thrombotic properties (which can be assumed to be

attributes of a healthy plant-based diet) [412, 413]. Results from Mendelian randomization studies appear to confirm the causality of the association between smoking and increased CVD risk [217, 414]. Not only smoking and passive smoking but also other forms of air pollution (increased levels of fine particulate matter and gaseous pollutants) are strongly associated with the development of atherosclerotic CVD [30, 415–417]. Thus, the topic of air pollution is worth mentioning to participants of a lifestyle intervention programme [418].

For alcohol intake, a J-shaped association with overall CVD risk has been proposed, with light to moderate drinking (up to ~30–40 g/d; not uniformly defined [419–421]) being associated with a lower risk compared to non-drinking [419, 422]. However, the association of alcohol intake with stroke risk is less clear [419], with even moderate drinking potentially increasing stroke risk [423]. Furthermore, the potentially cardioprotective effects of moderate alcohol intake have been questioned [424, 425], not only because randomized controlled trials in this field are lacking (and hard to conduct) [422] but also because evidence from Mendelian randomization studies indicates that there may be no protective effect of moderate alcohol consumption on CVD risk [414, 425–428]. Furthermore, any alcohol intake appears to increase the risk of cancer in a variety of body regions [429–434], while the association is less certain for colorectal cancer [435] and prostate cancer [436, 437]. In addition, alcoholic drinks are high in calories and have a high addiction potential [422]. Thus, the recommendation to maintain alcohol intake in the non-drinking to moderate drinking range as well as the avoidance of daily alcohol consumption appears to be justified [420–422].

Furthermore, high levels of chronic psychological stress [15] or anxiety [14] as well as depression [438] are associated with increased CVD risk [30]. Thus, addressing psychological stress in the context of lifestyle interventions appears commendable [14, 403], especially in the case of survivors of CVD events [439].

Lifestyle factors are often interrelated and can affect each other [405], and a variety of social and economic aspects are associated with CVD risk. For many decades it has been suggested that factors such as community support and an active social life may lower CVD risk [440, 441]. However, the causality of this association is uncertain [317, 440]. It has also been suggested that the cardioprotective effects of the traditional Mediterranean diet may partly be mediated by social aspects commonly found in the Mediterranean region [442, 443]. In this context it has been proposed that conviviality (here defined as the process of enjoying shared meals) is considered an integral part of a traditional Mediterranean lifestyle [442–446]. Conviviality has been described as an important contributor to lower psychological stress levels and improved social health [443]. Conviviality may also be associated with healthier eating practices and may thus indirectly affect CVD risk [443, 447]. Furthermore, adverse social and environmental conditions, including financial insecurity, job insecurity, low levels of

education [448], long working hours, high job strain [28], and unfavourable socioeconomic surroundings are (indirectly or directly) associated with increased CVD risk [27].

Current CVD prevention guidelines

The 2021 ESC guidelines for CVD prevention recommend optimizing lifestyle behaviour by way of adopting a healthy dietary pattern, engaging in regular physical activity, avoiding alcohol excess, not smoking, maintaining a healthy body weight and body composition as well as managing psychological stress [30]. In terms of diet, the ESC guidelines recommend a plant-based diet, with reduced intakes of animal-source foods and highly processed foods. Specifically, a daily intake of at least 200 grams of fruit, at least 200 grams of vegetables, and 30 grams of unsalted nuts are recommended [30]. While the intake of processed meat should be minimized, the intake of red meat should be limited to a maximum of 350–500 grams per week, and the consumption of sugar-sweetened beverages should be discouraged [30]. The ESC guidelines also recommend to consume fish, particularly fatty fish, once to twice per week [30]. No specific recommendation regarding the intake of legumes (pulses) is provided. However, legumes are mentioned in the general recommendations of the guidelines which state that that "a more plant-based food pattern, rich in fibre, that includes whole grains, fruits, vegetables, pulses, and nuts" should be chosen [30]. Vegetarian dietary patterns are not mentioned in the ESC guidelines. The ESC guidelines describe a healthy dietary pattern as a cornerstone of CVD prevention in all individuals [30].

Similarly, in the United States, the AHA (2021) has published dietary guidelines to improve cardiovascular health [29]. In this publication, the AHA recommends a dietary pattern high in fruit, vegetables, and whole grains as well as healthy high-protein foods, with a strong emphasis on plant-based protein sources, i.e. "mostly protein from plants (legumes and nuts)" [29]. In agreement with the ESC guidelines, a regular intake of non-fried fish is recommended, with the benefits of fish intake attributed to both the omega-3 fatty acid content as well as the substitution effect that occurs when fish replaces other sources of animal protein (red and processed meat or full-fat dairy products) [29]. The AHA guidelines regard a variety of different healthy dietary patterns to be consistent with their recommendations, including a traditional Mediterranean diet, the DASH diet as well as healthy vegetarian diets (which is in line with the 2011 Dietary Guidelines for Americans) [29]. At the nutrient level, both the ESC and AHA guidelines recommend increasing the intake of fibre, PUFA, and MUFA as well as decreasing the intake of SFA, trans fatty acids, added sugars, added salt, and alcohol [29, 30].

To reduce all-cause and CVD mortality, the ESC guidelines recommend to engage in at least 150–300 min of moderate-intensity or 75–150 min of vigorous-intensity aerobic physical activity per week [30]. Similarly, the AHA guidelines recommend at least 150 min of moderate-intensity or 75 min of vigorous-intensity physical activity per week [3]. The ESC guidelines recommend to restrict alcohol

consumption to a maximum of 100 grams per week (i.e. ~14.3 g/d) [30], while the AHA guidelines recommend to limit alcohol intake to a maximum of 28 g/d for men and 14 g/d for women, respectively [3, 29]. The ESC guidelines recommend that all tobacco smoking should be stopped regardless of potential weight gain [30]. Similarly, the AHA guidelines recommend that all individuals that use tobacco should be strongly advised to stop tobacco use [3].

Thus, these guidelines reflect the current evidence regarding healthy dietary patterns and can be utilized in the context of lifestyle interventions to assure participants that the recommendation to move towards a plant-based diet is in accordance with the most recent guidelines by leading medical organizations. This may be useful because dietary recommendations towards a plant-based diet may be more controversially received by study participants compared to the long-established advice for regular physical activity, non-smoking, and moderation in alcohol intake [449–451].

Internationally, current guidelines for CVD prevention recommend that lifestyle interventions should be composed of multiple components, including education, counselling, motivation, and self-management support [6]. Furthermore, current guidelines state that controlled trials should be conducted in order to test the effectiveness of lifestyle programmes [452]. Consequently, controlled trials are needed to assess the effectiveness of lifestyle interventions in improving dietary behaviour (in line with a healthy plant-based diet) and the abovementioned CVD risk markers.

1.8. History and components of the HLCP intervention

The HLCP intervention was conducted in three different cohorts: while the present dissertation includes results from the controlled HLCP-2 (2018–2020) and uncontrolled HLCP-3 (2019–2020) intervention studies, these were preceded by the controlled HLCP-1 study (2017–2019) which included a previous and slightly different version of the lifestyle programme. Some results from the HLCP-1 study have been published [578].

The HLCP intervention was inspired by a healthy lifestyle programme from the United States named Complete Health Improvement Program (CHIP), the effectiveness of which has been assessed in a number of studies [579]. The abbreviation CHIP originally stood for Coronary Health Improvement Project as CHIP's predominant focus originally was CVD prevention. However, the programme resulted in considerable improvements also in terms of other chronic illnesses, including type 2 diabetes and depression, so that in 2012 the new name was chosen. CHIP was founded by Hans Diehl, in 1986, who had previously been the director of education and research at the Pritikin Longevity Center. Hans Diehl adapted this health centre's approach of residential lifestyle programmes (with a focus on a healthy plant-based diet and physical activity) and applied this concept to a community setting, thus creating CHIP as a community-based (and more affordable) intervention programme. The first version of CHIP was conducted in rural British Columbia (Canada) in 1988. It comprised 16 seminars and had a duration

of 4 weeks [579]. The first article in a medical journal that presented results from CHIP was published in 1998 [580]. In turn, the HLCP intervention was created by Heike Englert who had worked with Hans Diehl in Rockford, Illinois (United States) [581, 582], and who then applied CHIP's community-based concept to a variety of settings in Germany. With her working group, Heike Englert developed the current HLCP intervention with a focus on four lifestyle factors: a healthy predominantly plant-based diet, physical activity, stress management, and community support.

The HLCP-2 and HLCP-3 interventions consisted of an intensive phase (the first 10 weeks of the programme) and a less intensive phase (from 10 weeks until the end of the programme). The intensive phase included 14 seminars (HLCP-2) and 15 seminars (HLCP-3), respectively, as well as 8 additional workshops. The less intensive phase included monthly seminars and a monthly email newsletter. Furthermore, participants of the intervention group received two one-on-one coaching sessions (~15 min each) at the beginning and end of the intensive phase. In addition, participants of the intervention group received a variety of printed educational materials: a healthy lifestyle handbook ("my way to health"; ~270 pages), a recipe booklet with healthy plant-based recipes (~80 pages), a shopping guide ("healthy shopping, healthy eating"; ~20 pages), and a laminated, illustrated information sheet with an overview of the lifestyle recommendations.

The seminar topics (and the associated healthy lifestyle handbook chapters) were structured around the following topics: (1) "opportunities and limitations of modern medicine", (2) "a healthy lifestyle: the best medicine", (3) "eat yourself healthy", (4) "fibre: your good friend", (5) "with fork and knife against overweight and type 2 diabetes", (6) "concerns of the heart: fat & cholesterol", (7) "blood pressure: the silent threat", (8) "taking cancer prevention into your own hands", (9) "physical activity: running away from illness", (10) "manage stress – a life with more relaxation", (11) "healthy and fit at any age", (12) "an experiment: the contract between yourself and your health", (13) "change is possible", (14/15) "on the tracks of your personal strengths" and "from 'I' to 'we'". Furthermore, the seminars included practical interactive sessions (~10–30 min each; for example, cookery demonstrations, quick exercise or meditation routines, or short group work activities). The seminars, workshops, and printed materials employed tangible examples and utilized plain language (basic literacy level). In one seminar, a breakout session for women and men in separate groups was conducted addressing issues related to sex-specific conditions such as postmenopausal weight gain and prostate cancer.

The workshops included cooking classes, a "guided shopping tour" at a local supermarket (to demonstrate that healthy foods are widely available and can be affordable), archery and table tennis workshops as well as a "relaxation in nature" workshop. The one-on-one coaching sessions included a "my way to health" sheet on which notes were taken to address each participant's personal goals and determine potential strategies to achieve these goals.

The recipe booklet contained easy recipes (with pictures) for breakfast, main meals, dips/spreads, salads, and desserts. The "shopping guide" contained a variety of information regarding different food groups (fruit; vegetables; legumes; whole grains; nuts and seeds; dried fruit; vegetarian convenience products; spices, herbs, and salt; fish; meat; soya milk and other dairy alternatives; tea; coffee; alcohol; plant oils; margarine; sugar and sweeteners; soft drinks; fruit juice) as well as commentary on organic foods, fast food, essential nutrients, healthy plant-based alternatives, and nutrition labels commonly found on packaged foods, plus a shopping list. An English translation of the laminated, illustrated information sheet with an overview of the lifestyle recommendations can be found in **Appendix: Supplementary material Paper 4**. All seminars, workshops, and coaching sessions were conducted and all content was created by our research group. The HLCP interventions were based on the theoretical background of Prochaska's "model of change" and "transtheoretical model" [583].

In terms of diet, it was recommended that the participants consume more fruit, vegetables, whole grains, legumes (including healthy soya foods like tofu, tempeh, low-sugar soya milk, low-sugar soya yogurt, soya flour, etc.), nuts, seeds, and healthy oils (for example, cold-pressed olive and rapeseed oil) as well as good sources of plant-based omega-3 fatty acids. At the same time the recommendation was to decrease the intake of meat (including red meat and poultry), butter, full-fat dairy, eggs, salt, and added sugars as well as to avoid alcohol excess. No specific macronutrient ratio was recommended. Neither was there a recommendation to restrict calories. It was furthermore recommended to drink plenty of water, and participants were also informed about potentially critical nutrients in relation to plant-based diets, including vitamin B12, calcium, vitamin D, and iodine (and reliable sources of these nutrients were communicated).

In terms of physical activity, recommendations were to be physically active for at least 30 minutes per day (preferably 60 minutes and preferably outdoors), to reduce and break up sitting time, to form support groups for exercise, to choose types of physical activity that one enjoys, and to proceed at one's own pace. In addition, topics addressed included the interrelation of physical activity and quality of life, bone health, and health markers.

In terms of stress management, the recommendation was given to establish relaxation routines, to spend more time with supportive people (such as friends or family), to focus on helping others as well as to permit oneself to receive support and to enjoy nice gestures from others. It was also recommended to take 5 minutes each morning to consciously start the day, to prioritize time out for relaxation and rest, and to incorporate breaks into one's daily routine (for example, 5 minutes of paying attention to one's breathing, doing yoga stretches, or just lying down). Further recommendations were to consider joining a club or an association, to exercise as a form a stress relief, to learn how to accept things one cannot change, to try out audio books or courses for stress relief, to avoid unnecessary sources of stress (people, objects, or situations), to ignore false accusations, to avoid typical quarrels

with neighbours, to identify activities one enjoys and to experience them very consciously as well as to avoid common (but unhealthy) coping strategies (for example, unhealthy eating, smoking, and alcohol excess). In addition, topics addressed included mental overload, getting into arguments, pressure to perform, being pressed for time, harmful effects of stress, stress-associated eating, quality of life, and healthy sleep.

In terms of community support, participants were encouraged to support each other in their endeavour to make healthy lifestyle improvements, to form "support groups" (for example, for walking, running, cooking, or visiting restaurants), and to ask their friends, family, and work colleagues to be supportive of (rather than hostile towards) their healthy lifestyle choices. In addition, it was attempted to create a supportive environment in the town where the HLCP interventions were conducted by involving several local general practitioners, pharmacies, the mayor, the local press, and local shops (among others).

An overview of the preventative approach used in the HLCP-2 and HLCP-3 studies is shown in **Figure 4**.

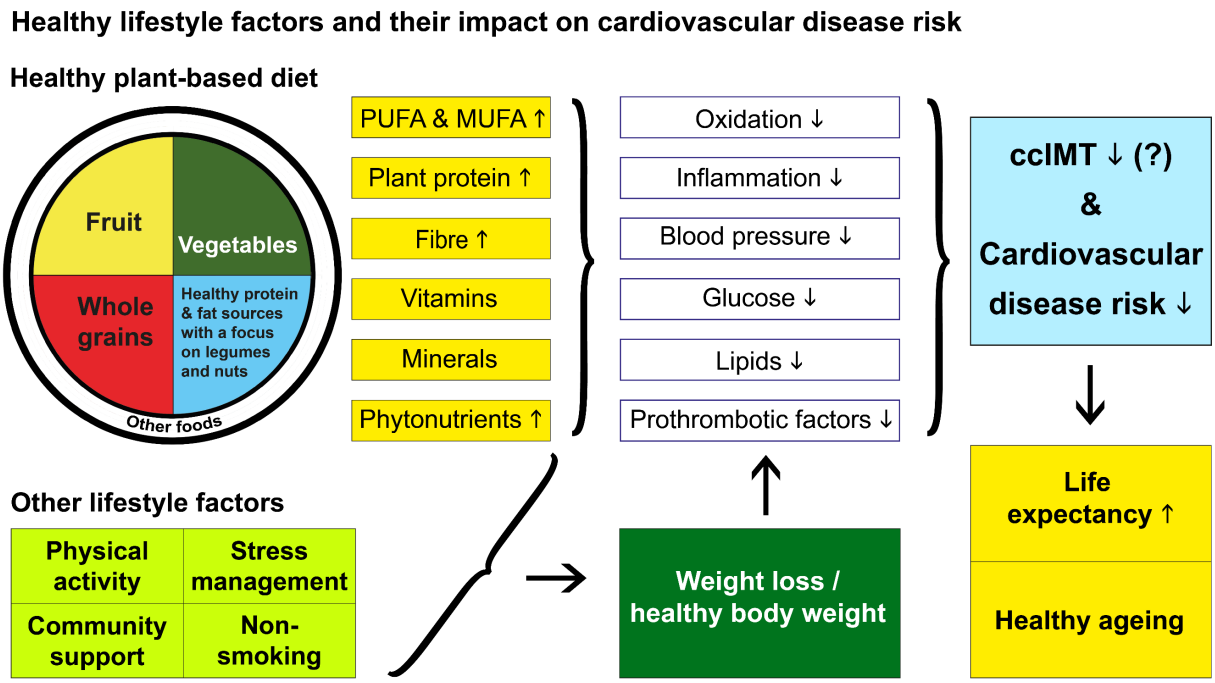

Figure 4. Healthy lifestyle factors and their impact on cardiovascular disease risk

PUFA: polyunsaturated fatty acids; MUFA: monounsaturated fatty acids; ccIMT: common carotid intima-media thickness (Author's own diagram, based on a diagram by Yannakoulia and Panagiotakos 2021 [307])

2. Scientific publications

2.1. Paper 1 (published)

Title: Effect of a 6-month controlled lifestyle intervention on common carotid intima-media thickness

Authors: Christian Koeder, Andreas Hahn, Heike Englert

Published in: The journal of nutrition, health & aging, volume 25 (2021), pages 869–877

Journal's impact factor: 4.1

DOI: 10.1007/s12603-021-1628-0

PubMed ID: 34409964

J Nutr Health Aging. 2021;25(7):869-877
Published online April 13, 2021, http://dx.doi.org/10.1007/s12603-021-1628-0

Original Research

Effect of a 6-Month Controlled Lifestyle Intervention on Common Carotid Intima-Media Thickness

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Abstract

OBJECTIVES: The intima-media thickness of the common carotid artery (ccIMT) is an established risk marker for cardiovascular disease (CVD). However, it is unclear whether lifestyle interventions can easily demonstrate an improvement in ccIMT. The objective was to test if our intervention would beneficially affect ccIMT (among other CVD markers).

DESIGN: Non-randomized controlled trial.

SETTING: Rural northwest Germany.

PARTICIPANTS: Middle-aged and elderly participants from the general population (intervention: n = 114; control: n = 87).

INTERVENTION: A community-based, 6-month controlled lifestyle intervention focusing on four areas of lifestyle change: a plant-based diet, physical activity, stress management, and an improved social life. A strong emphasis was on dietary change.

MEASUREMENTS: We tested whether ccIMT change from baseline to 6 months was different between groups.

RESULTS: With all participants included, no significant difference in mean ccIMT change between groups was observed (p = 0.708). However, in a subgroup analysis with participants with high baseline mean ccIMT (≥0.800 mm) a significant difference in mean ccIMT change between intervention (-0.023 [95% CI -0.052, 0.007] mm; n = 22; baseline mean ccIMT: 0.884 ± 0.015 mm) and control (0.041 [95% CI 0.009, 0.073] mm; n = 13; baseline mean ccIMT: 0.881 ± 0.022 mm) was observed (p = 0.004). Adjusting for potential confounders did not substantially alter the results.

CONCLUSION: The results indicate that healthy lifestyle changes can beneficially affect ccIMT within 6 months and that such a beneficial effect may be more easily demonstrated if participants with high baseline ccIMT are recruited. The observed effect is of relevance for the prevention of CVD events, including myocardial infarction and stroke.

Key words: Plant-based diet, healthy aging, preventive medicine, cardiovascular disease, cardiovascular health.

Introduction

It is widely accepted that healthy dietary and lifestyle choices can lower CVD risk (1). Furthermore, pathological arterial wall changes that lead to CVD events may even be reversed (2–4). However, very few intervention studies have been successful in demonstrating such a reversal (5, 6), and some of these studies were not controlled (7–11), making their results less reliable. Parameters employed in studies to demonstrate the reversal of arterial wall pathology (atherosclerosis and smooth muscle growth) have been, for example, coronary artery occlusion (assessed by coronary arteriography (4, 5, 10)) and angina pectoris symptoms (9). A non-invasive parameter that could fulfil this task in non-symptomatic populations is ccIMT (12).

The parameter ccIMT is an established (albeit controversial) (13) marker of the progression of arterial wall pathology, subclinical organ damage, and the risk of future CVD events, including myocardial infarction and ischaemic stroke (12, 14). The measurement of ccIMT via ultrasound allows the assessment of pathological arterial wall changes while still at a subclinical stage (15).

A recent meta-analysis of intervention studies (including mostly pharmaceutical and dietary supplement trials) indicates that ccIMT change is a valid surrogate marker for CVD risk and as such a useful parameter for intervention studies (16).

The objective of the study was to test if our lifestyle intervention would lead to measurable improvements in ccIMT (among other CVD risk markers).

Methods

Participants

For the intervention and control groups 114 and 87 participants were recruited, respectively. The number of evaluable participants included in the analysis of ccIMT change was 82 in the intervention and 61 in the control group (Figure 1).

All subjects gave their written informed consent prior to inclusion in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the ethics committee of the Medical Association of Westphalia-Lippe and of the University of Münster (Münster, Germany; reference: 2018-171-f-S; approved 4 April 2018).

The intervention group was recruited, parameters were assessed, and the intervention was conducted in a small town in northwest Germany. The control group was recruited and assessments were made in another small town, nearby in the same region.

For the intervention group, participants were recruited from the general population via a health market (February 2018)

Received January 13, 2021
Accepted for publication March 3, 2021

and by word of mouth, while for the control group, participants were recruited at a local public event (September 2018). As the intervention was conducted as a community-based programme, subjects were not strictly preselected, but a similar age group was targeted, and a similar male-to-female ratio was aspired to in both groups. The only inclusion criteria were the physical and mental ability to take part in the study and to be ≥18 years of age.

Study design

This controlled intervention study had a total duration of 18 months. Results of the first 6 months for the parameter ccIMT are presented in this article. The intervention consisted of a healthy lifestyle programme, while the control group received no intervention. The same parameters were assessed at equivalent time points in both groups: baseline, 10 weeks, 6, 12, and 18 months. The first 10 weeks of the intervention constituted the intensive part of the lifestyle programme. The parameter ccIMT was not assessed at 10 weeks as a significant change in such a short period of time was considered unlikely. Therefore, the first follow-up measurement of ccIMT was conducted at 6 months.

Participants were not randomized, and in lifestyle interventions, blinding participants to group allocation is not possible (17). Blinding of the ultrasound technician (who was assessing ccIMT) to group allocation was not feasible either. However, this technician was not involved in the implementation of any aspect of the intervention (17).

Both the intervention and control group study arms were conducted in parallel, but the control group study arm started and finished 6 months later than the intervention group (same duration of follow-up in both groups), as there were insufficient capacities to recruit and start both study arms at the same time.

Study hypothesis

Healthy dietary patterns and other healthy lifestyle factors are associated with lower ccIMT values (18). In addition, some lifestyle interventions have demonstrated a reduction in ccIMT (19–23) or at least a slowed down increase in ccIMT (24–26). We therefore hypothesized that participants of our lifestyle intervention would demonstrate a significant decrease in ccIMT values from baseline to 6 months and that this decrease would be significantly larger than in the control group. The primary outcome measure of the study was body weight change and results regarding this and the remaining outcome parameters of the study will be published shortly.

Lifestyle programme

The first 10 weeks (intensive phase) of the lifestyle intervention consisted of 14 consecutive seminars. The remainder of the intervention consisted of monthly seminars. Seminar topics focused on a healthy plant-based diet (27), healthy levels of physical activity, management of psychological stress, community support, and self-motivation, with a strong emphasis on dietary change. The seminars included short practical units such as cookery demonstrations or sessions with invited guests, including local general practitioners. Participants were given the opportunity to take part in eight additional workshops in smaller groups (~20 participants each; ~1-hour duration) which included cookery classes, a guided shopping tour, archery and table tennis workshops, and a relaxation workshop in nature. Dietary recommendations were to move towards a healthy, plant-based diet, i.e. to consume more healthy plant-based foods (fruit, vegetables, legumes including soya foods, whole grains, nuts, seeds, and healthy oils) and to consume less meat, butter, full-fat dairy, eggs, salt, added sugars, and highly processed foods and to avoid alcohol excess. Critical nutrients in plant-based diets, including vitamin B12, vitamin D, calcium, and iodine were discussed and adequate sources were communicated. No strict dietary rules or limits on portion sizes were given. Plant-based diets were defined as being predominantly based on plant foods. Such dietary patterns can be non-vegetarian, or they could be vegetarian or vegan as well. Dietary recommendations were monitored with semi-quantitative food logs. Apart from the seminars and workshops, participants received a healthy lifestyle handbook, a recipe booklet, a laminated one-page sheet with an overview of the lifestyle recommendations, and (after the intensive first 10 weeks) a monthly e-mail newsletter. The intervention group also received two one-on-one health coaching sessions (~15 min each), one at baseline and one at 10 weeks (before and after the intensive phase).

Carotid ultrasonography

Measurements of ccIMT were conducted following a strict protocol in accordance with the Mannheim consensus (28). Only the far wall was scanned. Measurements were taken at the time of the widest luminal distention during the cardiac cycle (29, 30). All measurements were made with the same ultrasound device (Mindray DC-N3, Mindray, Shenzhen, China), equipped with a high-resolution linear array transducer and automated digital edge detection software (Auto IMT). A frequency of 8.5 MHz and an image depth of 37 mm were used. Zoom was not used (31). The precision, provided by the manufacturer with which the intima-media thickness could be assessed was 0.01 mm. Within each 1 cm segment, the software automatically measured the intima-media thickness at 149 measurement point pairs and from these computed the mean (mean ccIMT) and maximal (max ccIMT) values. Two measurements were taken on each side. These four measurements resulted in four mean and four max values per person, per measurement time point. The data presented here are the (group) means of the (individual) means of each of these four mean values (mean ccIMT) or max values (max ccIMT), respectively. All measurements were made by the same technician, an internist with previous experience in ccIMT measurement with this device.

Statistical analyses

A sample size calculation was performed based on the primary outcome measure of the study, which was change in body weight. This calculation was based on data from a pilot study with a prototype version of the lifestyle programme (32). Assuming a dropout rate of at least 10%, a minimum sample size of 93 participants (intervention: 62; control: 31) was indicated to reach a global power of 0.8 and a global significance level of 0.05.

For the secondary end point of mean ccIMT change (from baseline to 6 months) an additional sample size calculation was performed using data from comparable studies (23, 26). Based on our expectation of a change in mean ccIMT of -0.100 mm from baseline to 6 months in the intervention group (effect size: ~0.5) (23), and no change in the control group, our actual sample size was adequate to detect a difference in mean ccIMT with a power of 0.8 and at a significance level of 0.05. Any detected differences in secondary end points, including ccIMT, are considered exploratory. Initially, actual sample size was moderately higher than required (intervention: n = 114; control: n = 87; Figure 1).

Shapiro-Wilk test was used to assess the data for non-normality, and $p < 0.05$ was defined as describing a non-normal distribution. For comparing baseline characteristics between groups, Fisher's exact test was used for categorical variables, while independent t-test was used for normally distributed and Mann-Whitney U test for non-normally distributed continuous variables (all tests were two-sided). To evaluate within-group mean ccIMT and max ccIMT changes, in the intervention and control groups, respectively, paired t-test was used for normally distributed and Wilcoxon test was used for non-normally distributed data (all tests were two-sided).

To evaluate the difference in ccIMT change (mean and max, respectively) between the two groups a one-way analysis of covariance (ANCOVA) was used, using the baseline ccIMT values (mean and max, respectively) as covariates (33, 34). The ANCOVA analyses were then repeated adjusting for several confounders gained from the literature [35–38] and from associations observed in this study population. Apart from baseline mean or max ccIMT, the covariates adjusted for were sex, age, smoker status, body mass index (BMI), total cholesterol, LDL cholesterol, HDL cholesterol, systolic and diastolic blood pressure, and HbA1c. ANOVA has been shown to be robust against non-normally distributed data [39]. Furthermore, exploratory subgroup analyses were conducted including only participants with baseline mean ccIMT ≥0.800 mm. In sensitivity analysis, results were additionally adjusted for changes in medication (blood pressure, diabetes, and cholesterol-lowering medication). In further sensitivity analyses, instead of adjusting for baseline values, we adjusted for the mean of the baseline and 6-month values (33, 40).

Correlations of baseline ccIMT with other CVD risk markers were assessed with Spearman's rho correlations (two-sided). All blood parameters, vital parameters, and anthropometric measurements were assessed in the fasted state. In addition, to determine the repeatability (within-assay precision) of repeated left and right measurements of ccIMT at one time point, Spearman's rho correlations (two-sided) were calculated.

All analyses were based on unimputed data (complete case analysis). Blinding was not feasible for statistical analysis. The analysis strategy was intention to treat (41). Statistical significance was consistently set at the 0.05 level. All analyses were conducted using IBM SPSS Statistics (Version 25.0. Armonk, NY). Baseline values are given as mean ± standard error of the mean (SEM).

Results

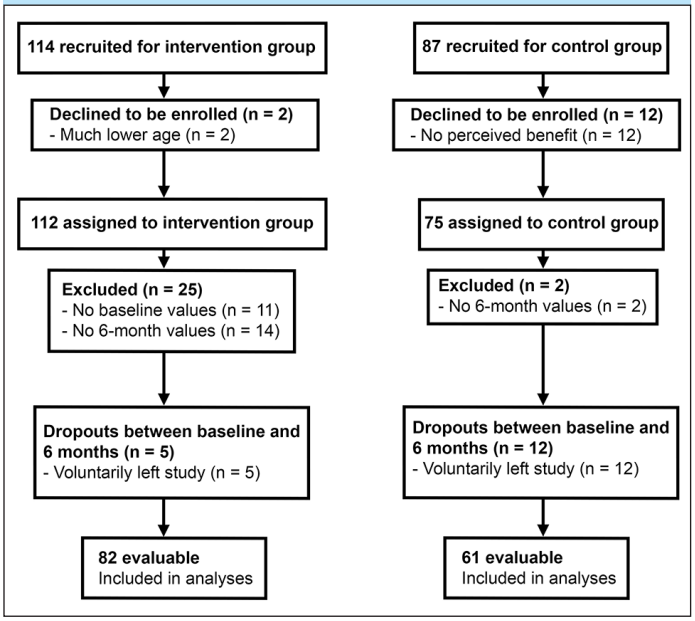
Compliance

In the intervention group, compliance, as defined by seminar attendance during the 10-week intensive phase of the lifestyle programme, was relatively high, with 61 out of the 82 evaluable participants (74.4%) attending ≥11 (out of 14) seminars.

Baseline characteristics

For a total of 143 participants (intervention: 82; control: 61) ccIMT values were available for both measurement time points (baseline and 6 months), and these participants were included in the analysis. The flow of participants through the study is shown in Figure 1.

Figure 1. Flow chart of participants through the study



The distribution of male and female participants was not significantly different between groups ($p = 0.378$). Mean age was higher in the intervention than in the control group ($p = 0.005$; Table 1). Baseline mean ccIMT and max ccIMT, systolic and diastolic blood pressure, body weight, BMI, and waist circumference were not significantly different between the two groups (Table 1). Equally, there were no statistically significant differences between the two groups in terms of

Table 1. Baseline characteristics of evaluable participants

Variable	Intervention group (n = 82)	Control group (n = 61)	p-value #
Men, n (%)	26 (31.7)	24 (39.3)	0.378 [a]
Age at baseline, years	59.4 ± 1.0	54.7 ± 1.4	0.005 [b]
Body weight, kg	81.9 ± 2.0	85.2 ± 2.5	0.244 [c]
BMI, kg/m^2	27.7 ± 0.6	28.3 ± 0.8	0.665 [c]
Waist circumference, cm	98.8 ± 1.6	97.8 ± 2.0	0.777 [c]
Systolic BP, mm Hg	133.3 ± 1.7	131.8 ± 2.1	0.568 [b]
Diastolic BP, mm Hg	80.3 ± 0.9	79.4 ± 1.3	0.648 [c]
Mean ccIMT, mm	0.698 ± 0.015	0.672 ± 0.018	0.277 [b]
Max ccIMT, mm	0.863 ± 0.018	0.823 ± 0.022	0.130 [c]
Smoker status, n (%)	Current/occasional: 8 (9.8) Ex: 27 (32.9) Never: 47 (57.3)	Current/occasional: 14 (23.0) Ex: 17 (27.9) Never: 30 (49.2)	0.105 [a]
Marital status, n (%)	Married: 69 (84.1) Partner, unmarried: 4 (4.9) Single (not widowed): 6 (7.3) Single (widowed): 3 (3.7) Missing data: 0 (0.0)	Married: 51 (83.6) Partner, unmarried: 3 (4.9) Single (not widowed): 3 (4.9) Single (widowed): 3 (4.9) Missing data: 1 (1.6)	0.952 [a]

Values are means ± SEM except for qualitative variables, expressed as n (%); BMI: body mass index; BP: blood pressure; ccIMT: common carotid intima-media thickness; SEM: standard error of the mean; # p-value for comparisons between groups by: a .Fisher's exact test (two-sided); b. independent t-test (two-sided); c. Mann-Whitney U test (two-sided)

baseline cholesterol (total, LDL, and HDL), triglycerides, fasting glucose, HbA1c, fasting insulin, or resting heart rate (unpublished results). In addition, the two groups did not differ in the distribution of their smoker status (p = 0.105) or marital status (p = 0.952; Table 1). Furthermore, there were no significant differences between the two groups in terms of alcohol intake frequency or the percentage of participants with any of a variety of diagnosed disease conditions assessed (hypertension, dyslipidaemia, heart disease, peripheral artery disease, diabetes, retinopathy, peripheral neuropathy, diabetic foot, kidney disease, allergies, gastrointestinal disease, thyroid disease, depression, rheumatoid arthritis, chronic pain, lung disease, bone disease as well as "other disease" or "free of diagnosed disease"). In addition, there were no significant differences between the two groups in terms of the percentage of participants with a history of stroke, a history of cancer, a family history (siblings, parents, grandparents) of myocardial infarction or stroke, or the percentage of participants who, based on baseline values, had hypertension, high total cholesterol, high LDL cholesterol, low HDL cholesterol, or high triglycerides. However, the percentage of participants with baseline HbA1c ≥6.5% was higher in the control group (4 individuals) than in the intervention group (0 individuals; p = 0.032; Supplementary table 1).

Baseline mean and max ccIMT stratified by risk factors

At baseline, for the overall study population (n = 143) mean ccIMT was 0.687 ± 0.012 mm, and max ccIMT was 0.846 ± 0.014 mm.

At baseline, mean ccIMT values were significantly higher in the left (0.701 ± 0.014 mm) compared to the right (0.677 ± 0.013 mm) carotid artery (n = 124; p = 0.009). Similarly, at baseline max ccIMT values were significantly higher on the left (0.857 ± 0.016 mm) compared to the right (0.824 ± 0.016 mm) side (n = 124; p = 0.015).

At baseline, men (n = 50) had higher ccIMT values than women (n = 93) (mean ccIMT: p = 0.005; max ccIMT: p = 0.011).

Bivariate correlations of ccIMT with other CVD risk factors

Combining participants of both the intervention and control groups, baseline mean ccIMT did not significantly correlate with baseline total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, insulin, diastolic blood pressure, resting heart rate, body height, body weight, or BMI. However, baseline mean ccIMT positively correlated with glucose (r = 0.304; p <0.001), HbA1c (r = 0.238; p = 0.004), systolic blood pressure (r = 0.309; p <0.001), waist circumference (r = 0.207; p = 0.013), and age (r = 0.618; p <0.001).

Out of the above-mentioned parameters, baseline max ccIMT positively correlated with glucose (r = 0.351; p <0.001), HbA1c (r = 0.285; p = 0.001), insulin (r = 0.184; p = 0.028), systolic blood pressure (r = 0.313; p <0.001), waist circumference (r = 0.296; p <0.001), BMI (r = 0.174; p = 0.037), and age (r = 0.584; p <0.001).

Combining participants of both the intervention and control groups, mean ccIMT change (progression from baseline to 6 months) did not significantly correlate with the progression values of cholesterol (total, LDL, or HDL), triglycerides, glucose, HbA1c, insulin, systolic or diastolic blood pressure,

Table 2. Changes in mean ccIMT and max ccIMT from baseline to 6 months

Variable	Intervention group (n = 82)	Control group (n = 61)	p-value #	p-value # (multivariable- adjusted)
Change in mean ccIMT (mm)	0.018 (95% CI 0.003, 0.032)	0.025 (95% CI 0.010, 0.039)	0.708 [a]	0.835 [b]
Change in max ccIMT (mm)	0.007 (95% CI -0.016, 0.029)	0.014 (95% CI -0.006, 0.035)	0.928 [c]	0.848 [d]

Values are means and 95% confidence intervals; ANCOVA: one-way analysis of covariance; BMI: body mass index; ccIMT: common carotid intima-media thickness.; # p-value for comparisons between groups by: a. ANCOVA, adjusted for baseline mean ccIMT; b. ANCOVA, adjusted for baseline mean ccIMT, sex, age, smoker status, BMI, total cholesterol, LDL cholesterol, HDL cholesterol, systolic and diastolic blood pressure, and HbA1c (intervention group: n = 81; control group: n = 61); c. ANCOVA, adjusted for baseline max ccIMT; d. ANCOVA, adjusted for baseline max ccIMT, sex, age, smoker status, BMI, total cholesterol, LDL cholesterol, HDL cholesterol, systolic and diastolic blood pressure, and HbA1c (intervention group: n = 81; control group: n = 61)

resting heart rate, body weight, waist circumference, or BMI. However, mean ccIMT change negatively correlated with baseline mean ccIMT (r = -0.272; p = 0.001).

Similarly, max ccIMT change did not correlate with any of the above-mentioned progression values, but max ccIMT progression negatively correlated with baseline max ccIMT (r = -0.317; p <0.001).

Repeatability

Repeatability (within-assay precision) of mean ccIMT and max ccIMT measurements at one time point, on the left and right side, respectively, was generally good (r ≥0.94 for mean ccIMT, and r ≥0.90 for max ccIMT).

Mean differences in repeated measurements were small for mean ccIMT (baseline left: 0.003 mm, n = 110; baseline right: 0.004 mm, n = 105; 6 months left: 0.002 mm, n = 131; 6 months right: <0.001 mm, n = 134), as well as for max ccIMT (baseline left: 0.007 mm, n = 110; baseline right: 0.002 mm, n = 105; 6 months left: 0.005 mm, n = 131; 6 months right: 0.003 mm, n = 134; occasionally there were individual missing values due to low image quality, usually related to anatomical factors, especially high body fat.)

Mean and max ccIMT change from baseline to 6 months

From baseline to 6 months, mean ccIMT significantly increased by 0.018 (95% CI 0.003, 0.032) mm in the intervention group (p = 0.005) and significantly increased by 0.025 (95% CI 0.010, 0.039) mm in the control group (p = 0.001). The difference between these two changes was not statistically significant (p = 0.708; adjusted for mean ccIMT baseline values).

From baseline to 6 months, max ccIMT non-significantly increased in both groups (intervention: p = 0.126; control: p = 0.133), and there was no significant difference between the two groups (p = 0.928; adjusted for max ccIMT baseline values; Table 2).

In a subgroup analysis including only the participants with baseline mean ccIMT ≥0.800 mm, in the intervention group (n = 22) there was a non-significant decrease in mean ccIMT of -0.023 (95% CI -0.052, 0.007) mm from baseline to 6 months (p = 0.205) while in the control group (n = 13) there was a significant increase in mean ccIMT of 0.041 (95% CI 0.009, 0.073) mm (p = 0.017).

The difference between these two changes was statistically significant (p = 0.004; adjusted for mean ccIMT baseline values; Table 3), and this constituted a between-group difference of 0.063 (95% CI 0.020, 0.107) mm.

This difference remained statistically significant after adjusting for baseline mean ccIMT, sex, and age (p = 0.004). Due to the low number of cases only these three covariates were adjusted for, but in a sensitivity analysis, additionally adjusting for smoker status, BMI, total cholesterol, LDL cholesterol, HDL cholesterol, systolic and diastolic blood pressure, and HbA1c did not change the result (p = 0.029). This result also remained significant after adjusting for baseline mean ccIMT, sex, age, and smoker status as well as for progression values (changes from baseline to 6 months) of BMI, total cholesterol, LDL cholesterol, HDL cholesterol, systolic and diastolic blood pressure, and HbA1c (p = 0.037; sensitivity analysis). Furthermore, this result remained significant after adjusting for baseline mean ccIMT, sex, age, and changes in medication (p = 0.009; sensitivity analysis).

In a further sensitivity analysis, instead of adjusting for baseline mean ccIMT, we adjusted for the mean of the baseline and 6-month mean ccIMT values. This did not substantially change the result (p = 0.008).

In this same subgroup (participants with a baseline mean ccIMT ≥0.800 mm), in the intervention group (n = 22) there was a decrease in max ccIMT of -0.034 (95% CI -0.067, -0.001) mm from baseline to 6 months (p = 0.047) while in the control group (n = 13) there was a non-significant increase in max ccIMT of 0.025 (95% CI -0.029, 0.079) mm (p = 0.333).

The difference between these two changes was statistically significant (p = 0.020; adjusted for max ccIMT baseline values; Table 3). This constituted a between-group difference of 0.059 (95% CI 0.001, 0.116) mm.

This difference remained statistically significant after adjusting for baseline max ccIMT, sex, and age (p = 0.019). In a sensitivity analysis, additionally adjusting for smoker status, BMI, total cholesterol, LDL cholesterol, HDL cholesterol, systolic and diastolic blood pressure, and HbA1c did not change the result (p = 0.042). Furthermore, this result remained significant after adjusting for baseline max ccIMT, sex, age, and medication change (p = 0.036; sensitivity analysis). This

Table 3. Subgroup analysis: changes in mean ccIMT and max ccIMT from baseline to 6 months in participants with baseline mean ccIMT ≥0.800 mm

Variable	Intervention group (n = 22)	Control group (n = 13)	p-value #	p-value # (multivariable- adjusted)
Change in mean ccIMT, mm	-0.023 (95% CI -0.052, 0.007)	0.041 (95% CI 0.009, 0.073)	0.004 [a]	0.004 [b]
Change in max ccIMT, mm	-0.034 (95% CI -0.067, -0.001)	0.025 (95% CI -0.029, 0.079)	0.020 [c]	0.019 [d]

Values are means and 95% confidence intervals; ANCOVA: one-way analysis of covariance; ccIMT: common carotid intima-media thickness. # p-value for comparisons between groups by: a. ANCOVA, adjusted for baseline mean ccIMT; b. ANCOVA, adjusted for baseline mean ccIMT, sex, and age; c. ANCOVA, adjusted for baseline max ccIMT; d. ANCOVA, adjusted for baseline max ccIMT, sex, and age

result was attenuated when adjusting for baseline max ccIMT, sex, age, and smoker status as well as for progression values of BMI, cholesterol (total, LDL, and HDL), blood pressure (systolic and diastolic), and HbA1c (p = 0.121; sensitivity analysis). However, except for sex, none of the covariates in the ANCOVA model had a significant influence on the model.

In a further sensitivity analysis, instead of adjusting for baseline max ccIMT, results were adjusted for the mean of the baseline and 6-month max ccIMT values. This also attenuated the results for max ccIMT change (p = 0.108) However, this covariate had no significant influence on the model.

Adverse events

No adverse events related to the study were observed in either study group.

Discussion

Contrary to our study hypothesis, with all participants included, mean ccIMT did not decrease in the intervention group but rather increased in both the intervention and control groups, highlighting the importance of a control group. Also contrary to our hypothesis, with all participants included, our results showed no significant difference in mean ccIMT change between intervention and control. This indicates that the short-term effect of lifestyle on ccIMT may not be detectable in non-symptomatic individuals from the general population without increased baseline ccIMT values. However, in subgroup analyses of participants with baseline mean ccIMT of ≥0.800 mm a significant difference in mean ccIMT change was observed between intervention and control, favouring the intervention group. This difference between intervention and control remained significant after multivariable adjustment, which suggests that it might have been a beneficial result of the intervention programme. This difference also remained significant when adjusting for progression values of several common CVD risk markers (BMI, cholesterol levels, blood pressure, and HbA1c), which indicates that this observed treatment effect was independent of changes in BMI (42), cholesterol (43), blood pressure (44), and long-term blood glucose levels (45), which are generally assumed to be major determinants of ccIMT. This suggests that, as with CVD risk in general (46), there are other factors that influence ccIMT apart from the classic CVD risk markers and that at least some of these factors can also be influenced by diet and other lifestyle factors.

In our subgroup analysis with participants with baseline mean ccIMT ≥0.800 mm the difference in mean ccIMT change between the intervention and control group was 0.063 (95% CI 0.020, 0.107) mm. This indicates that the observed difference may be clinically relevant. Willeit et al. (2020), in a large-scale meta-analysis of intervention studies, have shown that each 0.010 mm/year slower progression of ccIMT reduced the risk of myocardial infarction by 12% and of stroke by 8% (mean follow-up: 3.7 years), with the subgroup of dietary interventions showing more consistent results than the medication-based interventions (16).

In a prospective cohort study from Italy with a follow-up of 12 years, Olmastroni et al. (2019) showed that both mean and max ccIMT increase more rapidly with age in individuals who develop multifocal carotid atherosclerosis, which indicates that long-term ccIMT change is a marker of atherosclerosis development (36). It can therefore be hypothesized that significant differences in short-term ccIMT change between intervention and control, as seen in our subgroup, reflect real differences in pathological arterial wall changes.

The question remains why in our study, with all participants included, there was an increase in mean ccIMT in both the intervention (mean: 0.017 mm) and control groups (mean: 0.025 mm; Table 2) in only 6 months. In a large international analysis of 31 cohorts, Lorenz et al. (2018) reported a mean annualised mean ccIMT change of 0.01 mm, with a range of -0.10 to 0.05 mm (35), and our results fall within this range. This large variability in ccIMT change in different studies is possibly due to a combination of measurement error, random fluctuations (that cannot be controlled), interindividual differences, and actual ccIMT changes being relatively small and nonlinear (35, 36).

Comparison with results from the literature

In other studies, associations between mean ccIMT and a variety of non-classic CVD markers in blood have been observed, including a positive correlation with high-sensitivity C-reactive protein (hs-CRP) (47), which we also assessed in our study. However, in the subgroup of participants with baseline mean ccIMT ≥0.800 mm no significant difference

in hs-CRP changes was shown between intervention and control (unpublished results), which indicates that the observed difference in ccIMT change was unrelated to hs-CRP.

Similarly to our results, in the PREDIMED-Navarra study, a randomized controlled trial from Spain using a traditional Mediterranean diet with either virgin olive oil or nuts as the intervention, with all participants included, after 1 year no significant difference in mean ccIMT change was found between groups (48). However, in subgroup analyses including only participants with baseline mean ccIMT ≥0.9 mm, both in the olive oil and the nut intervention groups a significant mean ccIMT decrease of -0.093 mm and -0.086 mm, respectively, was observed, with no significant change in the control group (all three groups combined: n = 61) (48). In the analysis adjusted for age, sex, and hyperlipidaemia at baseline, the differences in mean ccIMT change between the intervention and control group were statistically significant (48). Like the PREDIMED-Navarra study, our results indicate that mean ccIMT may favourably respond to healthy lifestyle changes only (or more strongly so) in subjects with more advanced unfavourable arterial wall changes.

The majority of controlled lifestyle (including diet and/or exercise) interventions have failed to demonstrate a clear effect on ccIMT compared to control (24, 49), while only one of these unsuccessful studies reported a subgroup analysis with high-risk participants (50). However, some controlled trials using diet and/or exercise have been able to show a favourable effect on ccIMT (decrease or slowed progression), and when dietary recommendations were given, these included advice to adhere to a traditional Mediterranean diet (which is a plant-based dietary pattern) (51, 52) or to consume less salt and alcohol and more fruit and vegetables (53), and in one study, more dairy (20) (all randomized controlled trials).

In observational studies, a healthier, more plant-based diet (more fruit, vegetables, legumes, and whole grains, moderate alcohol intake, and less red meat) has been associated with more favourable mean ccIMT changes over time (54). In contrast, one study from Spain did not find an association between diet quality and ccIMT, but in terms of diet quality, vegetable oils (other than olive and sunflower oil) were rated unfavourable, breakfast flakes and daily alcohol consumption were rated favourable, and no differentiation was made between refined and whole grains (55).

Strengths and weaknesses

A strength of our study is the use of a strict standardized measurement protocol and the averaging of four measurements per person per time point. Moreover, all ultrasound scans were conducted by the same technician (and with the same device), which excludes confounding due to inter-operator variability (13). In addition, repeatability of ultrasound measurements was high and comparable to previous studies (36, 56).

Our study had several weaknesses: first, while ccIMT was intentionally not assessed at 10 weeks (the end of the intensive phase of the intervention programme), but only at 6 months, we did observe that cholesterol levels in the intervention group decreased from baseline to 10 weeks but increased again from 10 weeks to 6 months (unpublished results). This development likely influenced ccIMT and this influence could not be examined. Second, ccIMT was measured at the widest luminal distention, which is comparable to peak-systole measurements (when ccIMT is slightly smaller), while it is more common to assess ccIMT at end-diastole (when ccIMT is slightly larger) (29). However, both approaches are reliable if consistently used (29). Third, the control group started with a delay of 6 months (same follow-up duration) compared to the intervention group. Seasonal variations in vitamin D status, for example, could influence ccIMT, but this effect is uncertain (57). An analysis of all time points of the study, which will also include dietary intake, will be published shortly.

Due to the community-based nature of our study, participants were not randomized individually as participants of the control group were not supposed to be aware of the contents of the intervention (58). This was achieved by recruiting the intervention and control groups in two separate small towns. Cluster randomization of the two study centres was not conducted, as a large volume of preparatory work was necessary in the intervention community before the beginning of the study (obtaining support from the mayor, finding adequate premises for events, involving local general practitioners, health workers, and the local press). Cluster randomization would have meant making these preparations in two municipalities for which there was insufficient time, as the funding was received at short notice and for an immediate, specific time period. This would also have necessitated withholding all information about the interventions' contents from local stakeholders in both municipalities and then informing one group of stakeholders that their municipality had not been chosen because of randomization. Such an approach was not considered feasible or ethical and could have ruined our reputation and endangered compliance. Furthermore, it has been shown that any benefits of randomization to protect against selection bias in health care trials are uncertain (59).

Future research

Future controlled trials assessing ccIMT progression should report both the within-group effects as well as a between-group comparison. Uncontrolled studies should not be undertaken, and there should always be a control group which does not receive an intervention. The control group at baseline should be comparable to the intervention group. Journal articles should always clearly state which section of the carotid artery was assessed (common, internal, bifurcation, or a combination thereof). Dietary recommendations given should be well-designed (1). Well-designed dietary and lifestyle interventions assessing ccIMT progression, with participants with high baseline ccIMT values (such as mean ccIMT ≥0.800 mm), may be able to demonstrate a direct beneficial effect of healthy lifestyle changes on artery health and could as such facilitate and speed up much-needed changes in the health care system (16).

Conclusion

Our study failed to confirm our hypothesis that the participants of our lifestyle programme (recruited from the general population in rural northwest Germany) would show a significant decrease in ccIMT after 6 months and that this decrease would be significantly more favourable than in the control group. However, in a subgroup analysis of participants with baseline mean ccIMT ≥0.800 mm we observed a significant and clinically relevant difference in ccIMT change between intervention and control, favouring the intervention group. Our results indicate that mean ccIMT change can be a suitable CVD risk parameter for lifestyle intervention studies (60) if individuals with high baseline ccIMT can be included. We would like to encourage other working groups assessing mean ccIMT progression to conduct subgroup analyses with a cut-off value for high baseline ccIMT, as this could corroborate or contradict our conclusions.

Author contributions: Christian Koeder: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, writing – review and editing, visualization, project administration; Heike Englert: conceptualization, methodology, writing – original draft, writing – review and editing, supervision, funding acquisition; Andreas Hahn: methodology, formal analysis, writing – original draft, writing – review and editing, supervision.

Funding: This work was conducted as part of the project "münster.land.leben" and was funded by the German Federal Ministry of Education and Research (BMBF) and the Joint Science Conference (GWK) within the programme "Innovative Hochschule" (grant number: 03IHS062A). Heike Englert reports a grant from the German Federal Ministry of Education and Research (BMBF) during the conduct of the study. Christian Koeder and Andreas Hahn have nothing to disclose.

Acknowledgments: We would like to thank Dr Norman Bitterlich for his help with statistical analysis, Dominik Zeljko for conducting the ccIMT measurements, and the entire research group.

Trial registration: German Clinical Trials Register DRKS (reference: DRKS00018775; www.drks.de).

Conflict of interest: None.

Ethical standards: The experiments conducted for this research comply with the current laws of the country in which they were performed.

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How to cite this article: C. Koeder, A. Hahn, H. Englert. Effect of a 6-Month Controlled Lifestyle Intervention on Common Carotid Intima-Media Thickness. J Nutr Health Aging. 2021;25(7):869-877; http://dx.doi.org/10.1007/s12603-021-1628-0

2.2. Paper 2 (published)

Title: Effect of a 1-year controlled lifestyle intervention on body weight and other risk markers (the Healthy Lifestyle Community Programme, cohort 2)

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Published in: Obesity Facts, volume 15 (2022), pages 228–239

Journal's impact factor: 3.9

DOI: 10.1159/000521164

PubMed ID: 34923493

Obesity Facts

Research Article

Obes Facts
DOI: 10.1159/000521164

Received: June 10, 2021
Accepted: November 23, 2021
Published online: December 17, 2021

Effect of a 1-Year Controlled Lifestyle Intervention on Body Weight and Other Risk Markers (the Healthy Lifestyle Community Programme, Cohort 2)

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Keywords
Plant-based diet · Overweight · Lifestyle medicine · Preventive medicine · Cardiovascular disease

Abstract
Introduction: The prevalence of obesity is high and increasing worldwide. Obesity is generally associated with an increased risk of chronic disease and mortality. The objective of the study was to test the effect of a lifestyle intervention on body weight and other chronic disease risk markers. ***Methods:*** A non-randomized controlled trial was conducted, including mostly middle-aged and elderly participants recruited from the general population in rural northwest Germany (intervention: $n = 114$; control: $n = 87$). The intervention consisted of a 1-year lifestyle programme, focussing on four key areas: a largely plant-based diet (strongest emphasis), physical activity, stress management, and community support. Parameters were assessed at baseline, 10 weeks, 6 months, and 1 year. The control group received no intervention. ***Results:*** Compared to the control, in the intervention group, significantly lower 1-year trajectories were observed for body weight, body mass index (BMI), waist circumference (WC), total cholesterol, calculated LDL cholesterol, non-HDL cholesterol, remnant cholesterol (REM-C), glucose, HbA1c, and resting heart rate (RHR). However, between-group differences at 1 year were small for glucose, HbA1c, and cholesterol (apart from REM-C). No significant between-group differences were found for 1-year trajectories of measured LDL cholesterol, HDL cholesterol, triglycerides, insulin, blood pressure, and pulse pressure. ***Conclusion:*** The intervention successfully reduced body weight, BMI, WC, REM-C, and RHR. However, at 1 year, effectiveness of the intervention regarding other risk markers was either very modest or could not be shown.

Introduction

Worldwide, the prevalence of obesity has been increasing for several decades, and the situation has been described as a pandemic [1, 2]. In Germany, as in many other countries, more than 20% of adults have obesity [1]. Obesity is generally associated with an increased risk of chronic diseases and, in the general population, increased cardiovascular disease (CVD) and all-cause mortality [3, 4]. Complex reasons for obesity have been proposed, and

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there is a range of factors to consider in terms of the aetiology of obesity [1, 2, 5, 6]. While such environmental, psychological, and metabolic factors can constitute barriers to putting a healthy lifestyle into practice [1, 7], the actual implementation of healthy lifestyle patterns could drastically lower the public health and individual burdens of obesity [8]. Lifestyle intervention programmes can serve as effective tools for addressing such barriers [9].

While diet and physical activity both are important for weight loss, diet can be considered the most critical factor [1, 10]. An effective lifestyle approach to address obesity may be the recommendation to follow a plant-based diet, i.e., a dietary pattern that is centred around health-promoting foods of plant origin [11–13]. A traditional Mediterranean diet is one example of such a plant-based dietary pattern which is focused on high intakes of fruit, vegetables, whole grains, legumes, nuts, seeds, and healthy oils [14]. Unless alcohol or oil intake is excessive, such a dietary pattern is high in fibre, has a low caloric density (compared to a typical "Western" dietary pattern), and promotes a healthy body weight [15]. In contrast, dietary interventions that focus on caloric restriction [16–18] are frequently unsuccessful in the long term [1] and may leave the individual constantly hungry [19], which may then result in a relapse to previous dietary behaviours [20]. Furthermore, dietary weight loss strategies with a simplistic focus on low-fat [21, 22] and low-sugar products [1] may be flawed and may even have adverse effects [21]. Certain plant-based foods such as nuts/avocados (high-fat) and fruits (high-sugar), which are generally regarded as healthy, would be excluded, if solely macronutrient composition is considered, instead of considering both macronutrient content as well as overall food quality.

Lifestyle interventions can improve health behaviour, including diet [23], physical activity [24, 25], smoker status [26], and possibly sedentary behaviour [27, 28]. Lifestyle interventions have been shown to improve disease risk markers, including body weight, body mass index (BMI), blood pressure, fasting blood glucose, and HbA1c [23, 29, 30].

While education-based lifestyle interventions can be effective [25], it is likely that effectiveness can be increased if interventions also incorporate strategies for offering practical guidance [31, 32] and for increasing participants' motivation [33]. The use of nudging, goal setting, and progress monitoring, for example, can motivate participants and increase adherence to recommendations [34, 35]. Furthermore, social support can facilitate successful lifestyle modification [10]. In contrast, body weight stigma is harmful and may promote the psychological issues that are frequently related to unhealthy eating practises and obesity [36, 37].

Frequently, lifestyle interventions have been able to demonstrate only short-term successes [38]. In addition, many dietary interventions have only assessed short-term outcomes (~3–4 months), and many have exclusively focused on certain dietary components (fruit, vegetables, fat, etc.) rather than whole dietary patterns [32]. Furthermore, many controlled studies lack a no-intervention control group [32], and while high-risk individuals are often recruited for lifestyle programmes, community-based interventions are more inclusive, can reach individuals outside of conventional healthcare settings, can provide expertise not easily accessible otherwise to citizens, and can have a snowball effect on the wider community [39].

Against this background, we hypothesized that a lifestyle intervention would effectively improve body weight and other chronic disease risk markers in a community-based setting, i.e., in a heterogeneous sample of participants from the general population (most of whom were clinically healthy). The objective of the study was to test the effectiveness of the intervention in the context of community health promotion.

Materials and Methods

Study Design

We conducted a non-randomized, controlled intervention trial between April 2018 and October 2020. Measurements were taken at baseline, 10 weeks, 6 months, and 1 year. Planned measurements for 18 months and 2 years could not be included due to the COVID-19 pandemic (18-month time point: uneven time delays; intervention: September 2019, 17 months; control: June 2020, 20 months; results including the 18-month time point are shown in online supplementary Table 1 [for all online suppl. material, see www.karger.com/doi/10.1159/000521164]; 2-year time point: intervention: July 2020; no assessment in the control group). Participants were recruited from the general population in rural northwest Germany (intervention group: February 2018; control group: September 2018; as described previously [40]).

The intervention consisted of a healthy lifestyle programme, whereas the control group received no intervention. Participants were not blinded. Staff performing laboratory assessments were unaware of group allocation. Participants were not randomized because the intervention and control groups were recruited in two separate municipalities. The reason for this was that participants of the control group were meant to be unaware of the contents of the intervention. As we were unable to recruit and start both study arms at the same time, the control group study arm started and finished 6 months later (start: October 2018) than the intervention group (start: April 2018), with equivalent follow-up intervals in both groups. The study was registered in the German Clinical Trials Register (DRKS; reference: DRKS00018775; www.drks.de).

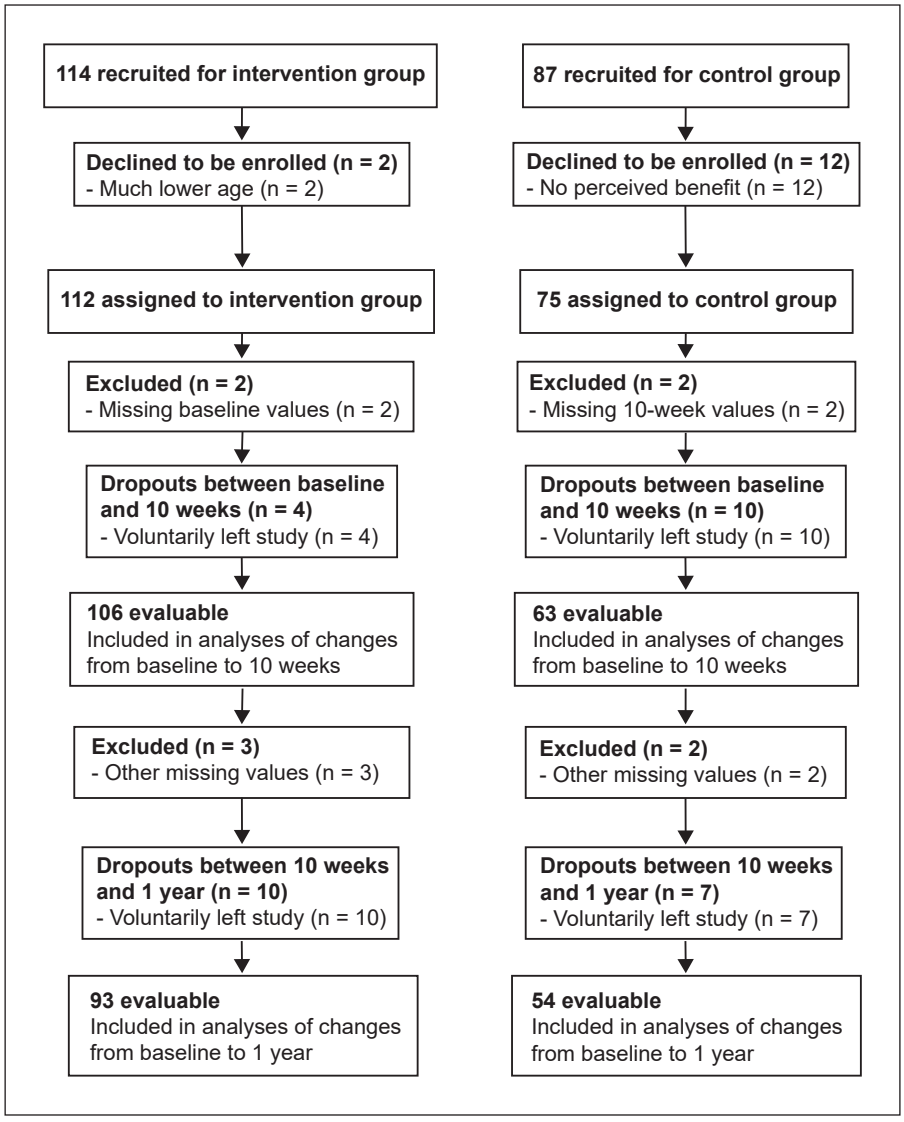

Fig. 1. Flow chart of participants through the study.

Participants

Participants were mostly middle-aged and elderly. The only inclusion criteria were the physical and mental ability to take part in the study (self-reported) and to be ≥18 years old. For the intervention, a total of 114 and for the control group, a total of 87 participants were recruited (shown in Fig. 1).

Lifestyle Programme

The lifestyle intervention (*Healthy Lifestyle Community*, cohort 2) consisted of an intensive phase (first 10 weeks) and a less intensive phase (remainder of the study). The intensive phase consisted of 14 seminars (each with ~70–100 participants and 3–4 health professionals) and 8 workshops (duration: ~2 h each, as described previously [40]). During the seminar breaks (~15 min), healthy snacks were provided (first by the research team, then increasingly by the participants), for example, whole-grain bread with plant-based spreads, salads, fruit, nuts, etc. Seminar topics included causes and development of lifestyle-related diseases, risk factors, lifestyle choices, and behaviour change (based on Prochaska's model of change [26, 41] and transtheoretical model [42, 43]). The seminars also included practical interactive sessions (~10–30 min; for example, cooking, exercise, meditation, and group work). Evidence-based content was presented using tangible examples and everyday language, while including vivid study materials (printed; basic literacy level) and employing the concepts of nudging [34] and gamification [44]. In one seminar, issues related to sex-specific diseases (such as postmenopausal weight gain and prostate cancer) were discussed in separate groups for women and men to provide a safe space for questions [45]. Seminars and workshops were conducted by our research group. Lifestyle choices were addressed in four key areas: diet, physical activity, stress management, and community support [13]. The strongest emphasis was on dietary change, with the recommendation of a healthy, plant-based diet [11, 16]. Specifically, recommendations were to consume more fruit, vegetables, whole grains, legumes (including soya foods), nuts, seeds, and healthy oils (such as cold-pressed ol-

ive or rapeseed oil) and to decrease the intake of meat (including red meat and poultry), butter, full-fat dairy, eggs, salt, and added sugars as well as to avoid alcohol excess [12]. There was no specific target in terms of the percentage of calories from different macronutrients. Non-dietary lifestyle recommendations included to be physically active for at least 30 min per day, to establish relaxation routines, and to spend more time with others (specific recommendations were not given in these areas).

Participants received a healthy lifestyle handbook, a recipe booklet, and a laminated information sheet with an overview of the lifestyle recommendations. They also took part in two one-on-one coaching sessions (~15 min each; at baseline and 10 weeks).

Assessment of Parameters

Measurements and blood sampling were all performed in the morning and in the fasted state. All analyses of blood samples were conducted at the University Hospital of Münster (measurement protocols are listed in online suppl. Table 2). Semi-quantitative 3-day protocols were used to assess dietary intake: these included tally sheets for individual foods (with portion sizes), categorized by food groups (cereals and cereal products; vegetables and mushrooms; legumes and legume products; fruit; dairy; meat and fish; potatoes, side dishes, and sauces; cakes, sweets, and snacks; ready-made meals; nuts and seeds; fats and oils; drinks; salt and sugar; and other foods/meals). The food protocols also contained several illustrated portion sizes (using examples such as "a handful" and "the size of your palm") and included two weekdays and one weekend day. The food protocols were tailored to the study population but were not validated. Adherence to dietary recommendations was assessed with the plant-based diet index (PDI), healthful PDI (hPDI), and unhealthful PDI (uPDI) [46] (due to the nature of our data, instead of reverse scores based on quintiles, as described by Satija et al. [46], we used positive and negative scores based on food portions; for example, for the calculation of the PDI score, all animal-source food portions were subtracted from all plant-based food portions). These indexes are based on 18 food groups. Higher scores for PDI and hPDI and lower scores for uPDI are considered favourable. Dietary recommendations in our study were partly based on the hPDI. Socio-demographic parameters and physical activity (in categories) were assessed using questionnaires.

Study Hypotheses

The primary hypothesis of the study was that in a heterogeneous sample from the general population (living lab approach), the intervention would lead to significantly reduced body weight, within the intervention group and compared to control, both at 10 weeks and at 1 year. Similarly, secondary hypotheses were that the intervention would significantly reduce (compared to control) BMI, waist circumference (WC), total cholesterol (TC), measured LDL cholesterol (LDL-C), calculated LDL-C, triglycerides (TAG), glucose, HbA1c, insulin, systolic and diastolic blood pressures (BPs), and resting heart rate (RHR). HDL cholesterol (HDL-C) was assessed exploratively. The analyses of non-HDL cholesterol (non-HDL-C), remnant cholesterol (REM-C), and pulse pressure (PP) were non-prespecified.

Statistical Analyses

A sample size calculation was performed based on change in body weight, the primary outcome parameter of the study. This calculation was based on data from a pilot study with a prototype version of the lifestyle programme (as described previously [40]). Assuming a dropout rate of at least 10%, a minimum sample size of 93 participants (intervention: 62; control: 31) was indicated to reach a global power of 0.8 and a global significance level of 0.05.

For between-group comparisons of baseline characteristics, Fisher's exact test was used for categorical variables. The independent *t* test was used for normally distributed variables, and the Mann-Whitney U test for non-normally distributed continuous variables. The Shapiro-Wilk test was used to assess data for non-normality, and $p < 0.05$ was defined as describing a non-normal distribution. To evaluate within-group changes, a paired *t* test was used for normally distributed data, and the Wilcoxon test was used for non-normally distributed data. All tests were two-sided.

For the analyses of changes from baseline to 10 weeks, between-group differences were assessed with a one-way analysis of covariance (ANCOVA). For the analyses of changes from baseline to 1 year, a one-way repeated-measures ANCOVA was used, using potential confounders as covariates.

Bivariate correlations were assessed with Spearman's rho correlations (two-sided). Analyses were based on unimputed data (complete case analysis [CCA]). In sensitivity analyses, imputed data (last observation carried forward [LOCF]) were used. Blinding was not feasible for statistical analysis. The analysis strategy was intention to treat (participants were included in analyses irrespective of compliance). Statistical significance was consistently set at the 0.05 level. All analyses were conducted using IBM SPSS Statistics (version 25.0; Armonk, New York, NY, USA).

Results

Baseline Characteristics

The flow of participants through the study is shown in Figure 1. These participants were included in the analyses (CCA).

Compared to the control, at baseline, the intervention group had a higher mean age ($p = 0.003$), higher educational level ($p = 0.002$), and higher mean REM-C ($p = 0.015$) (Table 1). In addition, the intervention group had a higher (more favourable) baseline hPDI score compared to the control ($p < 0.001$). Baseline alcohol intake frequency and physical activity levels were not significantly different between groups ($p > 0.05$). In terms of other characteristics, both groups were similar (Table 1).

For both groups combined, those with missing values (non-evaluable participants) were more likely to have obesity ($p = 0.046$), to be male ($p = 0.035$), or to be smokers ($p = 0.010$). They had a higher body weight ($p = 0.009$) and WC ($p = 0.028$), higher REM-C ($p = 0.020$), TAG ($p = 0.018$), and insulin ($p = 0.031$) as well as lower HDL-C ($p = 0.001$). In the intervention group, those with missing values were more likely to be smokers ($p = 0.009$) and had lower HDL-C ($p = 0.013$). In the control group, those with

Table 1. Baseline characteristics of evaluable participants (CCA)

Characteristics	Intervention group (n = 93)	Control group (n = 54)	p value[#]
Men, *n* (%)	29 (31.2)	19 (35.2)	0.716[a]
Age at baseline, years	59.3±0.9	55.1±1.3	**0.003**[b]
Body weight, kg	81.3±1.9	83.1±2.7	0.578[b]
BMI, kg/m^2	27.6±0.5	28.1±0.8	0.882[b]
Waist circumference, cm	98.7±1.5	96.1±2.2	0.327[c]
Overweight, *n* (%)	63 (67.7)	35 (64.8)	0.721[a]
Obesity, *n* (%)	25 (26.9)	14 (25.9)	1.000[a]
Smoker status, *n* (%)	Never: 52 (55.9)	Never: 27 (50.0)	
	Ex: 32 (34.4)	Ex: 17 (31.5)	0.319[a]
	Smoker: 9 (9.7)	Smoker: 10 (18.5)	
Marital status, *n* (%)	Married: 75 (80.6)	Married: 48 (88.9)	
	Partner (unmarried): 5 (5.4)	Partner (unmarried): 1 (1.9)	
	Single (not widowed): 9 (9.7)	Single (not widowed): 2 (3.7)	0.452[a]
	Single (widowed): 4 (4.3)	Single (widowed): 2 (3.7)	
	Missing data: 0	Missing data: 1 (1.9)	
Educational level, *n* (%)	Lower secondary school: 19 (20.4)	Lower secondary school: 20 (37.0)	
	Secondary school: 40 (43.0)	Secondary school: 15 (27.8)	
	University entrance qualification: 16 (17.2)	University entrance qualification: 16 (29.6)	**0.002**[a]
	University degree: 18 (19.4)	University degree: 2 (3.7)	
	Missing data: 0	Missing data: 1 (1.9)	
TC, mg/dL	207.4±4.0	208.3±6.2	0.901[c]
LDL-C (measured), mg/dL	132.9±3.8	139.5±6.0	0.327[c]
LDL-C (calculated), mg/dL	120.7±3.8	124.1±5.7	0.601[c]
Non-HDL-C, mg/dL	141.3±4.4	144.9±5.8	0.619[c]
REM-C, mg/dL	8.4±1.1	5.5±1.8	**0.015**[b]
HDL-C, mg/dL	66.1±1.9	63.3±2.4	0.433[b]
TAG, mg/dL	103.0±5.2	112.7±11.2	0.656[b]
Glucose, mg/dL	98.5±1.3	101.0±2.0	0.982[b]
HbA1c, %	5.4±0.0	5.4±0.1	0.498[b]
Insulin, µU/mL	12.3±1.9	12.0±1.1	0.234[b]
Systolic BP, mm Hg	133.8±1.6	132.2±2.3	0.538[c]
Diastolic BP, mm Hg	81.3±0.9	79.8±1.4	0.324[b]
Pulse pressure, mm Hg	52.5±1.2	52.3±1.7	0.915[c]
RHR, beats/min	68.3±1.1	69.9±1.2	0.380[c]
PDI, points	28.9±1.4	25.1±2.3	0.201[b]
hPDI, points	−7.0±2.2	−18.5±2.3	**<0.001**[b]
uPDI, points	−35.1±2.1	−27.8±2.6	0.050[b]

Values are means ± SEM, except for qualitative variables, expressed as *n* (%). TC, measured LDL-C, non-HDL-C, REM-C, HDL-C, TAG, glucose, HbA1c, and insulin: n = 92 (intervention), n = 53 (control); calculated LDL-C: n = 92 (intervention), n = 52 (control); systolic/diastolic BP, PP, and RHR: n = 52 (control); PDI, hPDI, and uPDI: n = 91 (intervention), n = 53 (control). SEM, standard error of the mean. [#] p value for between-group comparisons by [a] Fisher's exact test (two-sided) and the [b] Mann-Whitney U test (two-sided) and [c] independent t test (two-sided).

missing values were more likely to be male (p = 0.027), had a higher body weight (p = 0.008) and WC (p = 0.017), higher REM-C (p = 0.014), and lower HDL-C (p = 0.048).

Seminar Attendance

Seminar attendance during the 10-week intensive phase of the intervention was relatively high: 75 out of 106 participants evaluable for 10-week analyses (70.8%) and 69 out of 93 participants evaluable for 1-year analyses (74.2%) attended ≥11 (out of 14) seminars.

Changes in Body Weight and Risk Markers from Baseline to 10 Weeks (Intensive Phase; CCA)

Compared to the control, from baseline to 10 weeks in the intervention group, statistically significant reductions were observed for body weight, BMI, WC, TC, measured

Table 2. Baseline and follow-up measurements in evaluable participants (IN: $n = 93$; CON: $n = 54$)

Parameters	Grp	Baseline	10 weeks	6 months	1 year	Changes (baseline to 1 year)	p WG[§]	p BG[#]	
Body weight, kg	IN	81.3±1.9	77.9±1.7	76.9±1.6	77.4±1.7	−3.9 (−4.9, −2.9)	**<0.001**[a]	**<0.001**[c]	**<0.001**[d]
	CON	83.1±2.7	82.9±2.6	82.4±2.5	82.7±2.5	−0.4 (−1.6, 0.8)	0.473[b]		
BMI, kg/m^2	IN	27.6±0.5	26.4±0.5	26.1±0.5	26.3±0.5	−1.3 (−1.6, −1.0)	**<0.001**[a]	**<0.001**[c]	**<0.001**[d]
	CON	28.1±0.8	28.0±0.8	27.9±0.8	27.9±0.8	−0.1 (−0.5, 0.3)	0.662[a]		
WC, cm	IN	99±2	95±1	93±1	92±1	−6 (−7, −5)	**<0.001**[a]	**<0.001**[c]	**<0.001**[d]
	CON	97±2	97±2	96±2	96±2	−1 (−3, 1)	0.270[b]		
TC, mg/dL	IN	207±4	184±4	198±4	207±4	−1 (−6, 5)	0.806[b]	**0.035**[c]	**0.019**[d]
	CON	208±6	207±6	198±6	207±7	−1 (−8, 5)	0.725[b]		
Meas. LDL-C, mg/dL	IN	133±4	118±3	129±4	136±4	3 (−2, 8)	0.211[b]	0.142[c]	0.113[d]
	CON	140±6	136±6	138±6	138±6	−2 (−8, 4)	0.534[b]		
Calc. LDL-C, mg/dL	IN	121±4	103±3	110±4	120±4	−1 (−6, 4)	0.753[b]	**0.008**[c]	**0.005**[d]
	CON	124±6	121±6	115±5	126±6	2 (−3, 8)	0.534[a]		
Non-HDL-C, mg/dL	IN	141±4	123±4	131±4	141±5	0 (−5, 5)	0.987[b]	**0.005**[c]	**0.003**[d]
	CON	145±6	144±6	138±6	148±6	3 (−3, 10)	0.294[a]		
REM-C, mg/dL	IN	8±1	5±1	2±1	5±1	−3 (−5, −2)	**<0.001**[a]	**0.003**[c]	**0.002**[d]
	CON	6±2	8±2	0±2	11±1	5 (2, 8)	**<0.001**[a]		
HDL-C, mg/dL	IN	66±2	61±2	67±2	65±2	−1 (−3, 1)	0.418[a]	0.160[c]	0.208[d]
	CON	63±2	62±3	60±2	59±2	−5 (−7, −2)	**<0.001**[a]		
TAG, mg/dL	IN	103±5	102±5	105±5	107±5	4 (−3, 11)	0.168[a]	0.346[c]	0.245[d]
	CON	113±11	116±9	120±10	112±9	−1 (−18, 16)	0.485[a]		
Glucose, mg/dL	IN	99±1	94±1	96±1	99±1	0 (−2, 2)	0.990[b]	**0.007**[c]	**0.004**[d]
	CON	101±2	99±2	103±3	102±3	1 (−3, 5)	0.966[a]		
HbA1c, %	IN	5.4±0.0	5.4±0.0	5.4±0.0	5.4±0.0	0.0 (−0.1, 0.1)	0.790[a]	**0.002**[c]	**<0.001**[d]
	CON	5.4±0.1	5.5±0.1	5.6±0.1	5.6±0.1	0.2 (0.1, 0.2)	**<0.001**[a]		
Insulin, µU/mL	IN	12±2	10±1	13±3	11±1	−1 (−4, 1)	0.790[a]	0.143[c]	0.126[d]
	CON	12±1	12±1	12±1	12±1	0 (−2, 1)	**<0.001**[a]		
Systolic BP, mm Hg	IN	134±2	127±2	129±2	126±2	−8 (−11, −5)	**<0.001**[b]	0.518[c]	0.440[d]
	CON	132±2	127±2	127±2	127±2	−5 (−8, −2)	**0.005**[a]		
Diastolic BP, mm Hg	IN	81±1	78±1	79±1	77±1	−4 (−6, −2)	**<0.001**[a]	0.744[c]	0.639[d]
	CON	80±1	78±1	77±1	77±1	−3 (−5, −1)	**0.001**[b]		
PP, mm Hg	IN	53±1	50±1	50±1	49±1	−4 (−6, −2)	**0.001**[a]	0.609[c]	0.100[d]
	CON	52±2	49±2	50±2	51±2	−2 (−5, 1)	**0.133**[a]		
RHR, beats/min	IN	68±1	63±1	66±1	64±1	−4 (−6, −2)	**<0.001**[a]	**0.006**[c]	**0.009**[d]
	CON	70±1	69±2	69±2	69±2	−1 (−4, 1)	0.208[b]		

Values are means ± SEM, except for qualitative variables which are expressed as n (%). Changes are expressed as means and 95% CI; WC: $n = 53$ (CON); TC, measured LDL-C, non-HDL-C, REM-C, HDL-C, TAG, glucose, and insulin: $n = 92$ (IN), $n = 53$ (CON); calculated LDL-C: $n = 92$ (IN), $n = 52$ (CON); HbA1c: $n = 91$ (IN), $n = 53$ (CON); systolic/diastolic BP, PP, and RHR: $n = 52$ (CON). SEM, standard error of the mean; CI, confidence interval; p WG, p values for within-group changes from baseline to 1 year; p BG, p values for between-group differences in 1-year trajectories; IN, intervention; CON, control; Grp, group; Meas. LDL-C, measured LDL, cholesterol; Calc. LDL-C, calculated LDL-C. [§]p value for within-group comparisons by the [a] Wilcoxon test (two-sided) and [b] paired t test (two-sided). [#] p value for between-group comparisons by [c] repeated measures ANCOVA, adjusted for the baseline and [d] repeated measures ANCOVA, adjusted for baseline, age, and sex.

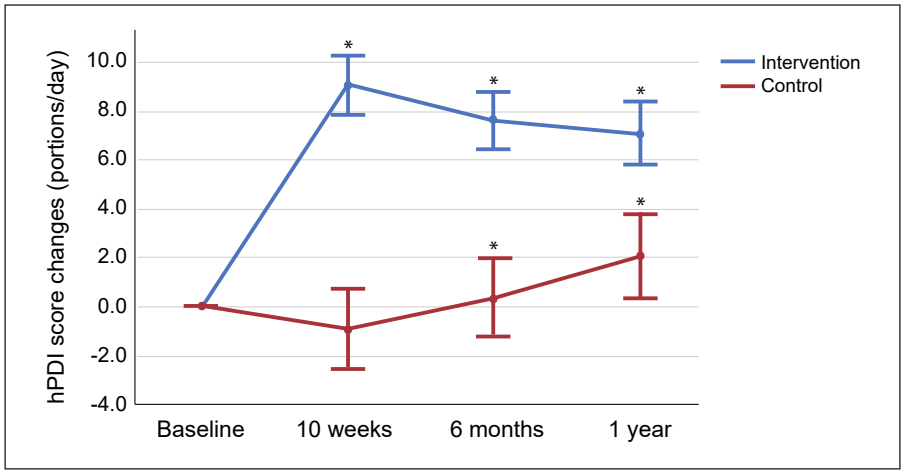

Fig. 2. One-year trajectories of hPDI score changes from baseline (portions/day). Values are means and 95% CIs of hPDI score changes (food portions/day; adjusted for baseline); *p value for differences from baseline: $p \leq 0.001$. CI, confidence interval.

and calculated LDL-C, non-HDL-C, REM-C, HDL-C, glucose, insulin, and RHR (all: $p \leq 0.025$; adjusted for baseline values; online suppl. Table 3). No significant between-group differences were observed for TAG, HbA1c, systolic or diastolic BP, or PP (adjusted for baseline values; online suppl. Table 3). In sensitivity analyses with imputed data (LOCF), these results were confirmed, except for TAG, which was significantly reduced in the intervention group compared to the control ($p = 0.044$; adjusted for baseline values, age, and sex).

Changes in Body Weight and Risk Markers from Baseline to 1 Year (CCA)

Compared to the control, in the intervention group, significantly lower 1-year trajectories (including all time points) were observed for body weight, BMI, WC, TC, calculated LDL-C, non-HDL-C, REM-C, glucose, HbA1c, and RHR (Table 2). No significant between-group differences were observed for the 1-year trajectories of measured LDL-C, HDL-C, TAG, insulin, systolic or diastolic BP, or PP (adjusted for baseline values; Table 2). In sensitivity analyses with imputed data (LOCF), these results were confirmed, except for TC and glucose, for which there were no significant between-group differences.

Adjustments for age and sex confirmed the results. Additional adjustments for smoker status, educational level, marital status, BMI, REM-C, and changes in alcohol intake confirmed these results.

In sensitivity analyses, including the 1½-year measurement time points, these results were largely confirmed. However, for glucose and RHR, there were no significant between-group differences (adjusted for baseline values; online suppl. Table 1).

Dietary Changes from Baseline to 10 Weeks (Intensive Phase)

In the intervention group, dietary scores significantly improved from baseline to 10 weeks and significantly more so than in the control: in the intervention group, PDI increased by 11 points (~4 portions/day) and hPDI increased by 23 points (~8 portions/day), while uPDI decreased by 11 points (~4 portions/day; unadjusted; all: $p < 0.001$).

Dietary Changes from Baseline to 1 Year

At 1 year, in the intervention group, PDI and hPDI were still increased (compared to baseline) by 9 points (~3 portions/day) and 19 points (~6 portions/day), respectively, while uPDI was still decreased by 11 points (~4 portions/day; unadjusted; all: $p < 0.001$). In Figure 2, changes in the hPDI score (adjusted for baseline) in both groups are shown.

Bivariate Correlations between Diet Score Changes and Risk Marker Changes (Baseline to 10 Weeks)

In bivariate correlations (including participants of both groups), PDI change inversely correlated with changes in body weight, WC, TC, LDL-C (measured and calculated), non-HDL-C, REM-C, and HDL-C (all: $p \leq 0.042$; online suppl. Table 4). Inverse correlations were observed between hPDI change and changes in body weight, BMI, WC, TC, LDL-C (measured and calculated), non-HDL-C, REM-C, HDL-C, insulin, and RHR (all: $p \leq 0.046$; online suppl. Table 4). Positive correlations were observed between uPDI change and changes in body weight, WC, glucose, and RHR (all: $p \leq 0.031$; online suppl. Table 4).

Bivariate Correlations between Diet Score Changes and Risk Marker Changes (Baseline to 1 Year)

Similar correlations were observed regarding changes from baseline to 1 year (expressed as the difference between baseline and the mean of the three follow-up time points): PDI change inversely correlated with changes in body weight, BMI, WC, TC, non-HDL-C, REM-C, glucose, HbA1c, and insulin (all: $p \leq 0.026$). Inverse correlations were also observed between hPDI change and changes in body weight, BMI, WC, TC, non-HDL-C, LDL-C (measured and calculated), HbA1c, and RHR (all: $p \leq 0.027$). Positive correlations were observed between uPDI change and changes in body weight, BMI, WC, HbA1c, and RHR (all: $p \leq 0.029$).

Bivariate Correlations between Diet Score Changes and Risk Marker Changes (10 Weeks to 1 Year)

For changes from 10 weeks to 1 year, inverse correlations were observed between hPDI change and changes in TC, HDL-C, and RHR (all: $p \leq 0.033$). No statistically significant correlations were observed between changes in PDI or uPDI and changes in any of the assessed risk markers.

Bivariate Correlations between Diet Score Changes and Risk Marker Changes (Food Group Level)

When diet score changes at the food group level were correlated with corresponding risk marker changes, the above associations (at the PDI/hPDI/uPDI level) were largely confirmed.

Physical Activity Changes (Baseline to 10 Weeks)

From baseline to 10 weeks, significant increases in intense physical activity (minutes/week; $p = 0.009$; sessions/week; $p < 0.001$), moderate physical activity (minutes/week; $p = 0.030$), and gentle physical activity (minutes/week; $p = 0.042$) were observed in the intervention group ($n = 105$) compared to the control ($n = 60$) (adjusted for baseline values, age, and sex).

Physical Activity Changes (Baseline to 1 Year)

The 1-year trajectory (including all time points) of intense physical activity (minutes/week) was still significantly higher in the intervention group ($n = 90$) compared to the control ($n = 50$; $p = 0.022$; adjusted for baseline values, age, and sex). However, this difference was due to a higher score in the intervention group at 10 weeks and 6 months. This higher score was not maintained at 1 year: in the intervention group, the difference in intense physical activity (minutes/week) between baseline and 1 year was not significant anymore ($p = 0.916$). No significant between-group differences were observed for the 1-year trajectories of moderate physical activity ($p = 0.239$), gentle physical activity ($p = 0.110$), or intense physical activity (sessions/week; $p = 0.053$; adjusted for baseline values, age, and sex).

Discussion

The present study aimed at clarifying the effect of a multimodal 1-year lifestyle intervention on body weight and weight-related risk parameters in middle-aged and older subjects in a community-based setting. Adherence to dietary recommendations was largely maintained at 1 year. In the intervention group, significant reductions in body weight, BMI, WC, REM-C, and RHR were observed at 10 weeks, and these favourable changes were maintained at 1 year. The Unites States Preventive Services Task Force (USPSTF) recommendations (2018) state that effective, intensive, behaviour-based weight loss interventions are typically designed to help adults with obesity achieve a weight loss of ≥5% (through changes in diet and physical activity) [47]. Furthermore, the USPSTF recommendations state that after 1 year, a weight loss of 2–3 kg (compared to the control) is typically observed in such interventions (with an absolute weight loss ranging from 1 to 9 kg in the intervention groups) [47]. The European Practical and Patient-Centred Guidelines for Adult Obesity Management in Primary Care (2019) suggest that a weight loss of 5–10% of initial body weight is a realistic target as well as a measure of successful weight loss [48]. Participants with obesity in our intervention group ($n = 24$) reduced their body weight (compared to baseline) by approximately 4 kg (4%), 7 kg (7%), and 5 kg (5%) within 10 weeks, 6 months, and 1 year, respectively (data not presented). This is in line with recommendations of 5–10% weight loss as a goal that can likely be maintained in the long term [18, 47, 48].

While 1-year trajectories of TC, calculated LDL-C, non-HDL-C, and glucose were also significantly lower in the intervention group, these differences were due to strong improvements from baseline to 10 weeks, and at 1 year, these improvements were no longer clinically meaningful. One important factor contributing to this reversal of previously achieved improvements was likely diet. While in the intervention group hPDI (the most relevant of the three diet scores assessed [46]) was still clearly increased at 1 year compared to baseline, we observed a significant decrease in hPDI from 10 weeks to 1 year

(i.e., after the end of the intensive phase; $p = 0.014$; $n = 89$; data not presented). Another relevant factor may have been that the significantly improved moderate and intense physical activity levels in the intervention group were maintained until 6 months (data not presented) but were not maintained at 1 year. This influence was likely small as in the intervention group, TC and LDL-C were seen to increase in the 10 weeks to 6 months timeframe. In addition, the influence of exercise on TC and LDL-C appears to be modest [49]. Dietary changes in the intervention group may also have been too moderate to achieve long-term cholesterol-lowering effects [50]. The results might also have been modestly influenced by a partial adaptation of endogenous cholesterol synthesis to dietary changes [51], seasonal changes [51, 52], and the relative imprecision of standard laboratory assessment methods [53]. It should be noted that seasonal changes may have influenced dietary intake: in the intervention group, hPDI greatly improved from spring to summer and decreased from summer to autumn and autumn to spring, while in the control group, hPDI slightly decreased from autumn to winter and increased from winter to spring and spring to autumn (Fig. 2). However, sensitivity analyses including the 1½-year measurement time points largely confirmed the results (online suppl. Table 1).

It is also noteworthy that baseline HDL-C values were comparatively high in both groups [54, 55]. At 1 year, the intervention group demonstrated a significant reduction in REM-C by about 3 mg/dL (a 38% reduction from baseline), which is a modest but potentially clinically relevant improvement [56–59]. REM-C is a strong independent risk factor for CVD events and CVD mortality [56, 57]. In our study, an increased PDI score was associated with a decrease in REM-C. A healthy, largely plant-based diet (traditional Mediterranean or low-glycaemic) has been shown to be associated with lower REM-C [60].

The RHR was significantly lowered by about 4 beats/min in the intervention group (compared to a decrease of 1 beat/min in the control group; Table 2), which is a clinically relevant effect [61]. The RHR is a risk marker for heart failure, stroke, cancer, and all-cause mortality [61, 62], and diet may be an underestimated factor for the RHR [63]. In our study, an increase in hPDI and a decrease in uPDI were associated with a decrease in the RHR. Regular aerobic exercise [63], consuming long-chain omega-3 fatty acids [63], relaxation, giving up smoking [61], and substantial weight loss [64] are associated with a decrease in the RHR. In our study, we did not observe significant correlations between changes (baseline to 1 year) in body weight or physical activity and changes in the RHR (data not presented).

Strengths and Limitations

A strength of our study was the assessment of a large variety of parameters, multiple measurement time points, and a no-intervention control group. However, assessing multiple parameters also increases the likelihood of significant findings. Another strength is that our statistical analyses (ANCOVA) could adjust for various potential confounders. A limitation of our study was that the control group started with a delay of 6 months (same follow-up duration), compared to the intervention group. Seasonal variations may have influenced the results [51, 52]. However, consistent seasonal trends in risk markers were not observed in either group (data not presented). A further limitation was that participants were not randomized (as described previously [40]). While both groups were comparable at baseline and we adjusted for potential confounders, some bias may have remained. While the dietary assessment method (3-day dietary record) provided relatively detailed data and avoided recall bias, it reflects only short-term food intake, and food intake may have been underreported, misreported, or adapted by the participants. In addition, our questionnaires were not validated for this population. To minimize this imprecision, we used food scores instead of assessing food intake at the food group level.

Future Research

While the obesogenic environment persists, consumers face a plethora of barriers to achieving a healthy body weight: food marketing, convenience of unhealthy choices, peer pressure, taste preference for calorie-dense foods, and many more [1]. Creating optimized interventions with a strong focus on diet, exercise, and factors that influence these can contribute to alleviating this problem [1]. These factors are easy to understand, and as first-line options for improving health, they are non-controversial [18, 65]. It can be the role of researchers not just to formulate what optimal lifestyle choices would look like but also to optimize interventions which can effectively translate evidence-based knowledge into real-life health improvement [46].

While a healthy, more plant-based diet may have several health benefits [11], losing weight [66] and shifting away from a diet rich in animal-source foods both may have detrimental effects on bone health (particularly hip fracture risk) [67], especially if this dietary shift is associated with a decreased intake of key nutrients for bone

health (including calcium, vitamin D, and protein) [68], which may be the case in near-vegan diets [67]. Therefore, adequate sources of these key nutrients should be communicated to participants, if a predominantly plant-based diet is recommended [68].

Conclusion

Our intervention succeeded in achieving relatively long-term (1-year) improvements in body weight, BMI, WC, REM-C, and RHR. It failed at maintaining meaningful long-term improvements in any of the other parameters assessed. Participants were recruited from the general population in rural northwest Germany, and the findings are likely applicable to similar populations. The results indicate that our intervention is effective in terms of body weight but that the programme requires further optimization to effect long-term improvements in other risk markers.

Acknowledgment

We would like to thank all participants, Alwine Kraatz who took part in the investigation, and Dr. Norman Bitterlich for his help with statistical analysis.

Statement of Ethics

All subjects provided written informed consent before participating in the study. The study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Medical Association of Westphalia-Lippe and of the University of Münster (Münster, Germany; reference: 2018-171-f-S; approved on 4 April 2018).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This work was conducted as part of the project "münster.land.leben" and was funded by the German Federal Ministry of Education and Research (BMBF) and the Joint Science Conference (GWK) within the programme "Innovative Hochschule" (grant no.: 03IHS062A). The funders had no role in the study design, data collection, analysis, interpretation, writing of the report, or the decision to publish the findings. The publication of this article was funded by the Open Access Fund of Leibniz Universität Hannover.

Author Contributions

Christian Koeder: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, writing – review and editing, visualization, and project administration; Ragna-Marie Kranz: conceptualization, methodology, validation, investigation, data curation, writing – review and editing, and project administration; Corinna Anand: conceptualization, methodology, validation, investigation, data curation, writing – review and editing, and project administration; Sarah Husain: conceptualization, methodology, validation, investigation, data curation, writing – review and editing, and project administration; Dima Alzughayyar: investigation, data curation, and writing – review and editing; Nora Schoch: conceptualization, methodology, investigation, data curation, writing – review and editing, and project administration; Andreas Hahn: methodology, formal analysis, writing – original draft, writing – review and editing, and supervision; Heike Englert: conceptualization, methodology, investigation, writing – original draft, writing – review and editing, project administration, supervision, and funding acquisition.

Data Availability Statement

The data are available from the corresponding author (C.K.) upon reasonable request.

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2.3. Paper 3 (submitted)

Title: Effect of a controlled lifestyle intervention on inflammatory markers – the Healthy Lifestyle Community Programme (cohort 2)

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Submitted to: BMC Nutrition

Title:

Effect of a controlled lifestyle intervention on inflammatory markers – the Healthy Lifestyle Community Programme (cohort 2)

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Abstract:

Background: Chronic low-grade inflammation is associated with an increased risk of chronic disease and mortality. The objective of the study was to test the effect of a healthy lifestyle intervention on biomarkers of inflammation (among other risk markers).

Methods: We conducted a non-randomized controlled trial with mostly middle-aged and elderly participants from the general population in rural northwest Germany (intervention: n = 114; control: n = 87). The intervention consisted of a 1-year lifestyle programme focusing on diet (largely plant-based; strongest emphasis), physical activity, stress management, and social support. High-sensitivity C-reactive protein (hs-CRP) was assessed at baseline, 10 weeks, 6 months, and 1 year. Homocysteine (Hcy) was assessed at baseline, 10 weeks, and 1 year. Adiponectin (Apn) was assessed at baseline and 10 weeks. An exploratory analysis of these inflammatory markers assessing the between-group differences with ANCOVA was conducted.

Results: The 1-year trajectory of hs-CRP was significantly lower in the intervention group compared to control (between-group difference: -0.8 (95% CI -1.2, -0.3) mg/l; p = 0.001; adjusted for baseline). The 1-year trajectory of Hcy was non-significantly higher in the intervention compared to control (between-group difference: 0.2 (95% CI -0.3, 0.7) µmol/l; p = 0.439; adjusted for baseline). From baseline to 10 weeks, Apn decreased significantly more in the intervention group compared to control (between-group difference: -1.6 (95% CI -2.7, -0.5) µg/ml; p = 0.004; adjusted for baseline).

Conclusions: Our study shows that healthy lifestyle changes can lower hs-CRP and Apn levels and are unlikely to significantly affect Hcy levels within 1 year.

Trial registration: German Clinical Trials Register (DRKS; reference: DRKS00018775, registered 12 September 2019; retrospectively registered; www.drks.de).

Keywords:

plant-based diet; subclinical inflammation; C-reactive protein; homocysteine; adiponectin; cardiovascular disease; lifestyle intervention

Background

Chronic low-grade inflammation is associated with an increased risk of a variety of chronic diseases, including cardiovascular disease (CVD) and cancer [1]. High-sensitivity C-reactive protein (hs-CRP) is an established peripheral biomarker of inflammation [2]. For every 1 mg/l increase in hs-CRP there appears to be an increase in all-cause mortality by >30% in men and >15% in women [1]. Higher hs-CRP levels are associated with increased inflammation and oxidative stress which are in turn associated with impaired endothelial function and increased CVD risk [3].

Shifting dietary patterns towards a healthy, largely plant-based diet [4] would likely decrease hs-CRP levels [5]. Most prominently, a traditional Mediterranean diet is associated with lower inflammatory markers, including lower hs-CRP levels [3, 6]. Similarly, vegetarian dietary patterns are associated with significantly lower hs-CRP values (~0.6 mg/l lower compared to non-vegetarian diets) [7]. In addition, increasing physical activity levels has been shown to lower hs-CRP [2]. While hs-CRP is

the most commonly assessed inflammatory marker, other inflammatory markers may also be of particular interest in connection with plant-based dietary interventions.

Homocysteine (Hcy) serves as a functional marker of vitamin B12, folate/folic acid, and vitamin B6 status, and a deficiency in any of these vitamins is associated with increased Hcy. In individuals with obesity [8] or hypothyroidism [9], Hcy levels are typically increased. Frequently, Hcy levels positively correlate with hs-CRP levels, and Hcy has been shown to stimulate CRP expression via downregulation of peroxisome proliferator-activated receptor γ (PPARγ) [10], a transcription factor with an important role in regulating glucose and lipid metabolism [11]. Increases in hs-CRP [12] and Hcy [13] are both associated with impaired insulin sensitivity, and increased Hcy levels are associated with an increased risk of hypertension and arterial wall damage [14]. Furthermore, Hcy is linearly associated with stroke risk, with each 1 μmol/l increase in Hcy being associated with a 6% increase in stroke risk [15]. When the recommendation of a predominantly plant-based diet is given, vitamin B12 intake is likely to decrease (unless fortified foods or supplements are consumed), with a probable increase in folate intake and an adequate intake of vitamin B6 [16]. In this context, due to a decrease in vitamin B12 intake, an increase in Hcy may occur [17].

Adiponectin (Apn) is a hormone secreted by adipose tissue and a controversial inflammatory marker [18]. Higher Apn levels are frequently interpreted to be beneficial, although conflicting results have been reported [18]. Higher Apn levels have been shown to be associated with increased insulin sensitivity, decreased oxidative stress, decreased inflammation, inhibited release of tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) as well as decreased activation of nuclear factor κB (NF-κB) [19]. In contrast, higher Apn levels are associated with increased all-cause mortality in haemodialysis patients [20] and individuals with heart disease [21]. Similarly, in individuals with a history of ischaemic stroke, higher Apn levels are associated with an increased risk of having another ischaemic stroke [22]. In addition, Apn levels are increased in a variety of chronic inflammatory diseases, including rheumatoid arthritis, chronic kidney disease, type 1 diabetes, and irritable bowel syndrome [23].

Inverse associations of Apn and hs-CRP have been documented [24], while some (but not all) studies also indicate an inverse association between Apn and Hcy [25]. Like in the case of Hcy, the potential effect of plant-based diets on Apn levels are unclear [26]. To date, controlled trials assessing the effect of healthy lifestyle changes including a predominantly plant-based diet on Apn levels in clinically healthy participants from the general population are lacking [26, 27].

Against this background, we hypothesized that our lifestyle intervention would lead to improvements in inflammatory markers (among other risk markers). The objective of the study was to test the effectiveness of the intervention in this regard.

Methods

Study design

We conducted a non-randomized, controlled intervention trial, with measurement time points at baseline, 10 weeks, 6 months, and 1 year. Hcy was not assessed at 6 months (as we did not expect such a short-term effect on vitamin B12 status). Apn was only assessed at baseline and 10 weeks (as a potential effect on Apn was uncertain, as not all time points could be assessed for financial reasons, and as the strongest effect was expected after the intensive phase of the programme, i.e. after 10 weeks). The study was intended to last 2 years, but due to the COVID-19 pandemic the last two time points could not be included in the present analyses (1½-year time point, included in sensitivity analyses: time delay in the control group; 2-year time point: not conducted in the control group; at these additional time points hs-CRP was assessed but not Hcy or Apn). The intervention consisted of a healthy lifestyle programme, and the control group received no intervention (as described previously [28]). We followed guidelines for non-randomized controlled trials [29]. The control group study arm started 6 months later (October 2018) than the intervention group (April 2018), with equivalent follow-up durations, because funding was granted at short notice and for a specific time period and there were insufficient capacities to recruit and start both study arms at the same time.

This study was registered in the German Clinical Trials Register (DRKS; reference: DRKS00018775, registered 12/09/2019; retrospectively registered; www.drks.de).

Participants

Participants were recruited from the general population at local public events in two separate small towns in northwest Germany (intervention group: February 2018; control group: September 2018 [28]). In each town, the mayor and a local physician helped with the recruitment of local citizens at these events. The only inclusion criteria were the physical and mental ability to take part in the study and to be ≥18 years old. A total of 114 and 87 participants were recruited for the intervention and control groups, respectively.

Lifestyle programme

The lifestyle intervention (Healthy Lifestyle Community Programme, cohort 2) consisted of an intensive phase (first 10 weeks) and a less intensive phase (from 10 weeks until the end of the study). The intensive phase consisted of 14 seminars and 8 workshops. The less intensive phase consisted of monthly seminars. Participants of the intervention group also took part in two one-on-one lifestyle coaching sessions (at baseline and 10 weeks) and received a healthy lifestyle handbook, a recipe booklet, and a laminated information sheet with an overview of the lifestyle recommendations.

The intervention programme and materials addressed healthy lifestyle choices in terms of diet (strongest emphasis), physical activity, stress management, and community support. Dietary recommendations were to move towards a healthy, predominantly plant-based diet, i.e. to increase the intake of fruit, vegetables, whole grains, legumes (including soya foods), nuts, seeds, and healthy oils and to decrease the intake of meat, eggs, butter, full-fat dairy, added sugars, refined grains, and salt as well as to avoid alcohol excess. Recommendations regarding physical activity, stress management, and community support were not specific but included suggestions to walk and cycle more, form a walking or jogging group with other participants, identify an enjoyable way to exercise

regularly, establish short daily “relaxation rituals”, practice mindfulness, spend more time in nature, and to form additional support groups with other participants (for example, for cooking and eating together).

Assessment of parameters

Biomarkers were assessed from blood samples. All blood samples were taken in the morning (6:00 to 11:00 am) and in the fasted state. Laboratory assays are shown in **Supplementary table 1**, **Additional file 1**. Dietary intake was assessed with semi-quantitative 3-day food protocols. Adherence to dietary recommendations was assessed using the plant-based diet index (PDI), healthful PDI (hPDI), and unhealthful PDI (uPDI) by Satija et al. [4]. Physical activity (in categories) and socio-demographic data and were assessed with questionnaires.

Study hypotheses

In terms of hs-CRP, Hcy, and Apn, the study hypotheses were that the intervention would significantly decrease hs-CRP (within-group and compared to control; from baseline to 10 weeks and from baseline to 1 year), that the intervention would increase Apn (within-group and compared to control; from baseline to 10 weeks) and that the intervention would not increase Hcy (within-group and compared to control; from baseline to 10 weeks and from baseline to 1 year). The three main hypotheses were regarding the between-group changes (hs-CRP and Hcy: 1-year changes; Apn: 10-week changes). Any detected differences in the secondary end points hs-CRP, Hcy, and Apn are considered exploratory.

Statistical analyses

A sample size calculation was performed based on changes in body weight (the primary outcome measure of the study [28]). However, for the secondary end point of hs-CRP change (from baseline to 1 year) an additional sample size calculation was performed using data from a comparable study

[30]. Based on the expectation of a hs-CRP decrease of ~30% from baseline to 1 year in the intervention group (effect size: ~0.38) [30], and no change in the control group, our sample size was adequate to detect a difference in hs-CRP change with a power of 0.65 and at a significance level of 0.05. Holm-Bonferroni correction was conducted to adjust for multiple comparisons.

Fisher's exact test was used for between-group comparisons of categorical variables. Independent t-test was used for normally distributed and Mann-Whitney U test for non-normally distributed continuous variables. Shapiro-Wilk test was used to assess data for non-normality ($p < 0.05$ was defined as describing a non-normal distribution). To evaluate within-group changes, paired t-test and Wilcoxon signed-rank test were used for normally and non-normally distributed variables, respectively. All tests were two-sided.

For the analyses of changes from baseline to 10 weeks, between-group differences were assessed with a one-way analysis of covariance (ANCOVA). For between-group comparisons of 1-year trajectories, a repeated measures ANCOVA was used, with potential confounders as covariates.

Bivariate correlations were assessed with Spearman's rho correlations (two-sided). Analyses were based on unimputed data (complete case analysis, CCA). In sensitivity analyses, imputed data (last observation carried forward, LOCF) were used. All analyses were conducted using IBM SPSS Statistics (Version 25.0. Armonk, NY).

Results

Baseline characteristics

For the analysis of hs-CRP changes (1-year trajectories), a total of 104 participants (intervention: 70; control: 34) were available (**Figure 1**). The analysis of Hcy changes (1-year trajectories) is based on a total of 120 participants (intervention: 68; control: 52). For the analysis of Apn changes (baseline to 10 weeks), a total of 141 participants (intervention: 80; control: 61) were available (**Supplementary figure 1**, **Additional file 1**).

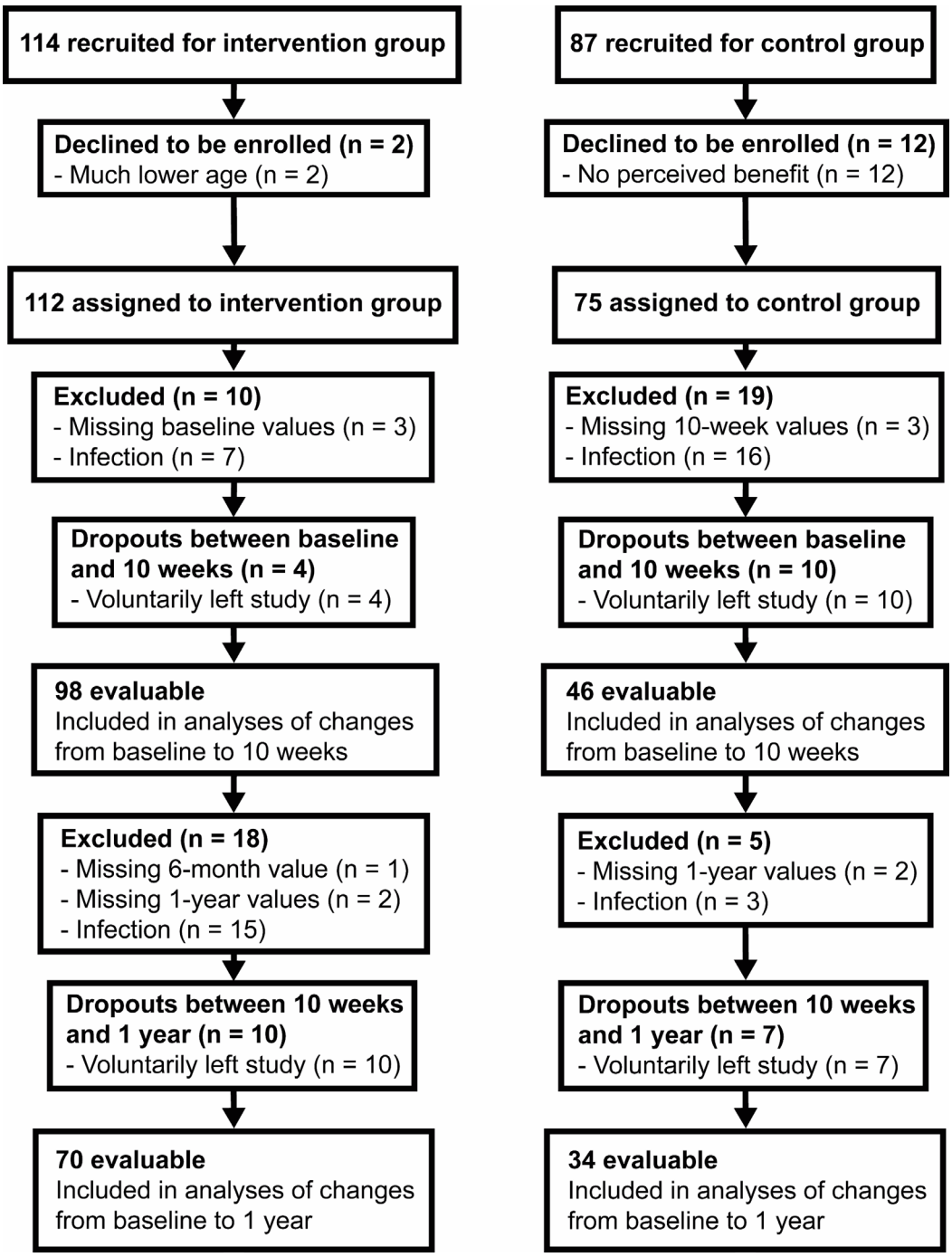

Figure 1. Flow chart of participants through the study (hs-CRP analysis)

hs-CRP: high sensitivity C-reactive protein

At baseline and compared to control, the intervention group had a higher age (p = 0.003), higher Apn levels (p = 0.001), and a higher (more favourable) hPDI (p = 0.006). Categories of educational levels were significantly different between groups (with neither group having a clearly higher education; p = 0.009; **Table 1**). There were no significant differences in PDI (p =

0.553), uPDI (p = 0.069), or other baseline characteristics (**Table 1**), including alcohol intake frequency and the percentage of participants with any of a variety of diagnosed disease conditions (as described previously[28]). Furthermore, there were no significant between-group differences in terms of the percentage of participants with a history of stroke, a history of cancer, a family history (siblings, parents, grandparents) of myocardial infarction or stroke, or the percentage of participants who (based on baseline values) had hypertension, high total cholesterol (TC), LDL-C, non-HDL-C, triglycerides (TAG), HbA1c, or low HDL-C.

Table 1. Baseline characteristics of evaluable participants (CCA)

Characteristics		**Intervention** (n = 70)		**Control** (n = 34)		**p-value**
		Means or n	**SEM or %**	**Means or n**	**SEM or %**	
Men, n (%)		24	34.3	13	38.2	0.827 [a]
Age at baseline, years		59.7	1.0	53.9	1.7	**0.003** [b]
hs-CRP, mg/l		1.3	0.2	2.6	0.7	0.667 [b]
Hcy, µmol/l *		12.4	0.4	11.8	0.4	0.635 [b]
Apn, µg/ml †		10.5	0.6	7.7	0.6	**0.001** [b]
Body weight, kg		81.4	2.0	82.3	3.7	0.936 [b]
BMI, kg/m^2		27.2	0.5	27.5	1.1	0.771 [b]
WC, cm		98.7	1.6	94.5	2.6	0.162 [c]
Overweight, n (%)		49	70.0	21	61.8	0.504 [a]
Obese, n (%)		16	22.9	8	23.5	1.000 [a]
Smoker status, n (%)	Never	41	58.6	17	50.0	0.262 [a]
	Ex	23	32.9	10	29.4	
	Smoker	6	8.6	7	20.6	
Marital status, n (%)	Married	59	84.3	31	91.2	0.945 [a]
	Partner (unmarried)	5	7.1	1	2.9	
	Single (not widowed)	3	4.3	1	2.9	
	Single (widowed)	3	4.3	1	2.9	
Educational level, n (%)	Lower secondary school	15	21.4	11	32.4	**0.009** [a]
	Secondary school	32	45.7	10	29.4	
	University entrance qualification	10	14.3	12	35.3	
	University degree	13	18.6	1	2.9	

CCA: complete case analysis; hs-CRP: high-sensitivity C-reactive protein; Hcy: homocysteine; Apn: adiponectin; BMI: body mass index; WC: waist circumference; SEM: standard error of the mean;
p-value for between-group comparisons by:
[a] Fisher's exact test (two-sided)
[b] Mann-Whitney U test (two-sided)
[c] independent t-test (two-sided)
* Intervention: n = 68; control: n = 52; † Intervention: n = 80; control: n = 61

Changes in hs-CRP (baseline to 10 weeks)

From baseline to 10 weeks, hs-CRP significantly decreased in the intervention group (-0.5 [95% CI -0.9, -0.1] mg/l; p <0.001; n = 98), with no significant changes in the control group (0.3 [95% CI -0.6, 1.2] mg/l; p = 0.956; n = 46). This constituted a between-group difference in hs-CRP changes of -1.0 (95% CI -1.7, -0.3) mg/l (p = 0.006; adjusted for baseline). Adjusting for potential confounders (baseline hs-CRP, age, sex, education level, marital status, alcohol intake, smoker status, BMI, REM-C, HDL-C, HbA1c, systolic BP, and resting heart rate (RHR)) confirmed this result (p = 0.002). Adjusting for baseline hs-CRP, age, sex, education level, marital status, and changes in alcohol intake, smoker status, BMI, REM-C, HDL-C, HbA1c, systolic BP, and RHR also confirmed this result (p = 0.004; **Supplementary table 2**, **Additional file 1**). Furthermore, results were confirmed in a sensitivity analysis using log-transformed (lg10) hs-CRP values (p = 0.002; adjusted for baseline), and sensitivity analyses including participants with an infection or common cold (self-reported at either measurement time point; non-log-transformed: p = 0.036; log-transformed: p = 0.004; adjusted for baseline; intervention: n = 105; control: n = 62).

Changes in hs-CRP (baseline to 6 months)

From baseline to 6 months, hs-CRP significantly decreased in the intervention group (p = 0.002; n = 83) and non-significantly increased in the control group (p = 0.905; n = 42). The 6-month trajectory of hs-CRP was significantly lower in the intervention group compared to control (between-group difference: -0.7 [95% CI -1.2, -0.2] mg/l; p = 0.003; adjusted for baseline). Adjusting for baseline hs-CRP, age, sex, education level, marital status, smoker status, alcohol intake, BMI, and HbA1c confirmed this result (p = 0.002). Adjusting for baseline hs-CRP, age, sex, education level, marital status, and changes (Δ[baseline, 6 months]) in smoker status, alcohol intake, BMI, and HbA1c also confirmed this result (p = 0.001). Furthermore, this result was confirmed by a sensitivity analysis using log-transformed (lg10) hs-CRP values (p = 0.002), a sensitivity analysis including participants with an infection or common cold (self-reported at any measurement time point; non-log-

transformed: p = 0.007; log-transformed: p = 0.002; adjusted for baseline; intervention: n = 99; control: n = 60), and a sensitivity analysis using imputed data (LOCF; p = 0.001; adjusted for baseline; intervention: n = 91; control: n = 55).

Changes in hs-CRP (baseline to 1 year)

From baseline to 1 year, hs-CRP significantly decreased in the intervention group (p = 0.002) and non-significantly decreased in the control group (p = 0.735; **Table 2**). The 1-year trajectory of hs-CRP was significantly lower in the intervention group compared to control (between-group difference: -0.8 [95% CI -1.2, -0.3] mg/l; p = 0.001; adjusted for baseline; **Figure 2**). This result remained significant after Holm-Bonferroni correction. Adjusting for baseline hs-CRP, age, sex, education level, marital status, smoker status, alcohol intake, BMI, and HbA1c confirmed this result (p = 0.001; **Table 2**). Adjusting for baseline hs-CRP, age, sex, education level, marital status, and changes (Δ[baseline, 1 year]) in smoker status, alcohol intake, BMI, and HbA1c also confirmed this result (p = 0.006; **Table 2**). Furthermore, this result was confirmed by a sensitivity analysis using log-transformed (lg10) hs-CRP values (p = 0.006; adjusted for baseline), a sensitivity analysis including participants with an infection or common cold (self-reported at any measurement time point; non-log-transformed: p = 0.007; log-transformed: p = 0.007; adjusted for baseline; intervention: n = 92; control: n = 53), a sensitivity analysis including the 1½-year measurement time points (p = 0.004; adjusted for baseline; intervention: n = 59; control: n = 29; CCA), a sensitivity analysis using imputed data (LOCF; p = 0.005; adjusted for baseline; intervention: n = 84; control: n = 49), and a further sensitivity analysis using imputed data (LOCF) as well as including the 1½-year measurement time points (p = 0.013; adjusted for baseline; intervention: n = 79; control: n = 49). Another sensitivity analysis, exchanging the time points for the control group to achieve a comparable sequence of seasons (spring, summer, autumn, autumn), confirmed the results, with lower hs-CRP in the intervention group (between-group difference: -0.8 [95% CI -1.3, -0.3] mg/l; p = 0.003; adjusted

for baseline hs-CRP, age, sex, education level, marital status, smoker status, alcohol intake, BMI, and HbA1c; intervention: n = 66; control: n = 32).

Table 2. hs-CRP and Hcy changes from baseline to 1 year in evaluable participants (CCA)

Parameters	**hs-CRP, mg/l**				**Hcy, µmol/l**			
Group	**IN** (n = 70)		**CON** (n = 34)		**IN** (n = 68)		**CON** (n = 52)	
	Mean	SEM or 95% CI	Mean	SEM or 95% CI	Mean	SEM or 95% CI	Mean	SEM or 95% CI
Baseline	1.3	0.2	2.6	0.7	12.4	0.4	11.8	0.4
10 weeks	0.9	0.1	3.0	0.8	12.3	0.4	12.2	0.4
6 months	1.0	0.1	2.6	0.6	-	-	-	-
1 year	1.0	0.2	2.1	0.6	11.4	0.4	10.4	0.4
Δ(baseline, 1 year)	-0.3	-0.7, 0.1	-0.5	-1.7, 0.8	-1.0	-1.8, -0.1	-1.4	-2.0, -0.8
p WG *	**0.002** [a]		0.735 [a]		0.060 [a]		**<0.001** [b]	
p BG †	**0.001** [c]				0.439 [c]			
p BG † (multivariable-adjusted)	**0.001** [d]				0.912 [d]			
	0.006 [e]				0.825 [e]			

CCA: complete case analysis; hs-CRP: high-sensitivity C-reactive protein; Hcy: homocysteine; IN: intervention; CON: control; SEM: standard error of the mean; CI: confidence interval; p WG: p-values for within-group changes from baseline to 1 year; p BG: p-values for between-group differences in 1-year trajectories; BMI: body mass index;
* p-value for within-group comparisons by:
[a] Wilcoxon test (two-sided)
[b] paired t-test (two-sided)
† p-value for between-group comparisons by:
[c] repeated measures ANCOVA, adjusted for the baseline values (hs-CRP and Hcy, respectively)
[d] repeated measures ANCOVA, adjusted for baseline values (hs-CRP and Hcy, respectively) as well as baseline age, sex, education level, marital status, smoker status, alcohol intake, BMI, and HbA1c
[e] repeated measures ANCOVA, adjusted for baseline values (hs-CRP and Hcy, respectively), baseline age, sex, education level, marital status as well as changes (Δ[baseline, 1 year]) in smoker status, alcohol intake, BMI, and HbA1c

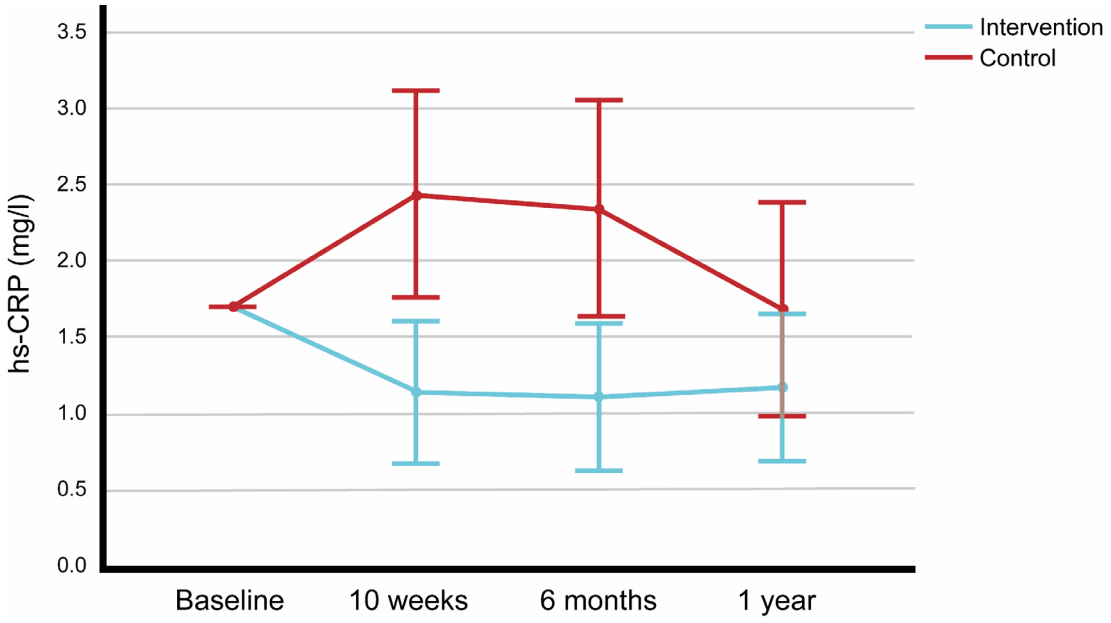

Figure 2. 1-year trajectories of hs-CRP

Values are means and 95% confidence intervals (adjusted for baseline); hs-CRP: high sensitivity C-reactive protein.

Changes in Hcy (baseline to 10 weeks)

From baseline to 10 weeks, Hcy did not significantly change in either the intervention (-0.4 [95% CI -1.3, 0.5] µmol/l; p = 0.366; n = 80) or control group (0.2 [95% CI -0.6, 1.1] µmol/l; p = 0.736; n = 61). There was no significant difference in Hcy changes between the intervention and control group (between-group difference: -0.2 [95% CI -1.3, 0.8] µmol/l; p = 0.656; adjusted for baseline).

This result was confirmed when adjusting for baseline Hcy, age, sex, education level, and marital status as well as alcohol intake, smoker status, and HbA1c (p = 0.450) or changes in alcohol intake, smoker status, and HbA1c (p = 0.259; **Supplementary table 3**, **Additional file 1**). Furthermore, a sensitivity analysis using log-transformed (lg10) Hcy values confirmed this result (p = 0.570; adjusted for baseline).

Changes in Hcy (baseline to 1 year)

From baseline to 1 year, Hcy non-significantly decreased in the intervention group (p = 0.060) and significantly decreased in the control group (p <0.001; **Table 2**). The 1-year trajectory of Hcy was non-significantly higher in the intervention group compared to control (between-group difference: 0.2 [95% CI -0.3, 0.7] µmol/l; p = 0.439; adjusted for baseline). This result was confirmed when adjusting for baseline Hcy, age, sex, education level, and marital status as well as smoker status, alcohol intake, BMI, and HbA1c (p = 0.912; **Table 2**) or changes (Δ[baseline, 1 year]) in smoker status, alcohol intake, BMI, and HbA1c (p = 0.825; **Table 2**). Furthermore, this result was confirmed by a sensitivity analysis using log-transformed (lg10) Hcy values (p = 0.393; adjusted for baseline) and a sensitivity analysis using imputed data (LOCF; p = 0.328; adjusted for baseline; intervention: n = 92; control: n = 74).

Changes in Apn (baseline to 10 weeks)

From baseline to 10 weeks, Apn significantly decreased in the intervention group (-2.5 [95% CI -3.5, -1.5] µg/ml; p <0.001; n = 80), with no significant changes in the control group (0.2 [95% CI -0.5,

0.8] µg/ml; p = 0.595; n = 61). Apn changes were significantly lower in the intervention group compared to control (between-group difference: -1.6 (95% CI -2.7, -0.5) µg/ml; p = 0.004; adjusted for baseline). This result remained significant after Holm-Bonferroni correction. This result was also confirmed when adjusting for baseline Apn, age, sex, education level, and marital status as well as smoker status, alcohol intake, BMI, TC, HDL-C, insulin, diastolic BP, and RHR (p = 0.002) or changes in smoker status, alcohol intake, BMI, TC, HDL-C, insulin, diastolic BP, and RHR (p = 0.003; **Supplementary table 3**, **Additional file 1**). Furthermore, a sensitivity analysis using log-transformed (lg10) Apn values confirmed this result (p = 0.018; adjusted for baseline).

Dietary changes

PDI and hPDI changes from baseline to 10 weeks as well as the 6-month and 1-year trajectories were significantly higher (more favourable) in the intervention group, while uPDI changes were lower (more favourable) in the intervention group (all: p <0.001; adjusted for baseline). 1-year trajectories of hPDI changes are shown in **Figure 3**. Including the 1½-year time points confirmed these results (all: p ≤0.001; adjusted for baseline). The dietary intake data (including changes at the food group level) confirmed that participants of the intervention group were following the dietary recommendations given.

Physical activity changes

Changes in physical activity from baseline to 10 weeks were significantly higher in the intervention group (intense: sessions/week, p = 0.006; moderate: minutes/week, p = 0.039; gentle: minutes/week, p = 0.042; adjusted for baseline values, age, and sex). However, no significant between-group difference was observed when changes in intense physical activity were assessed as minutes per week (p = 0.102). For the 6-month trajectories of physical activity, higher intense physical activity (when assessed as sessions/week; p = 0.012) and higher moderate physical activity (p = 0.049) were observed in the intervention group (adjusted for baseline values, age, and sex). For the 1-year

trajectories of physical activity, no significant between-group differences were observed (adjusted for baseline values, age, and sex).

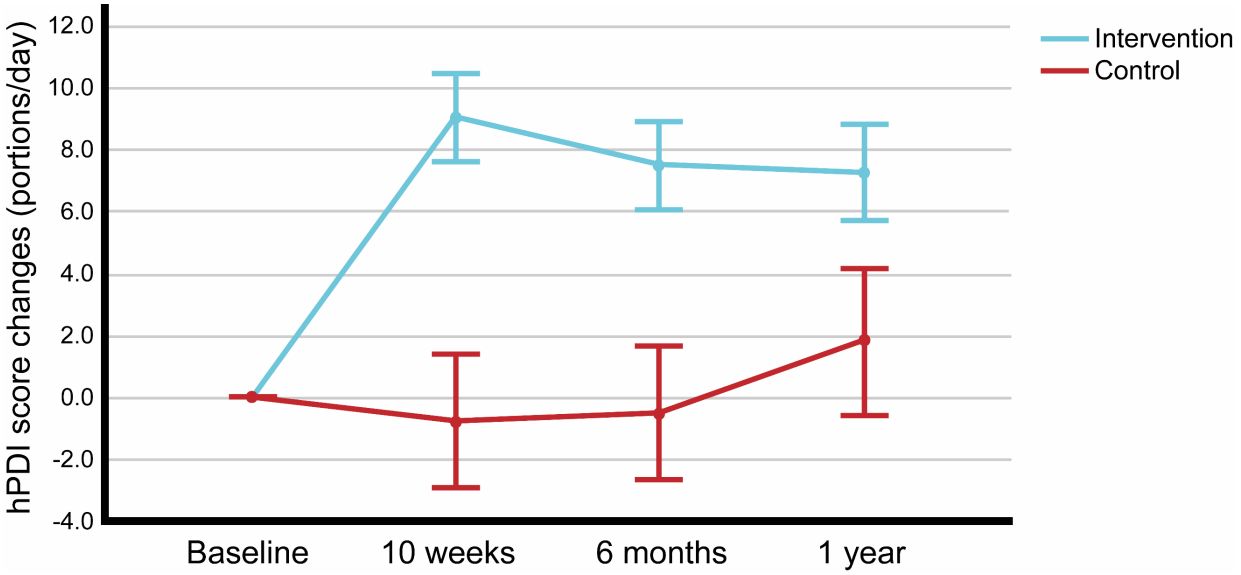

Figure 3. 1-year trajectories of hPDI changes (from baseline; portions/day)

Values are means and 95% confidence intervals (adjusted for baseline); hPDI: healthful plant-based diet index.

Bivariate correlations of hs-CRP, Hcy, and Apn changes with changes in other markers

Bivariate correlations of changes in hs-CRP, Hcy, and Apn with changes in other CVD markers as well as changes in dietary scores are shown in **Table 3**. 1-year hs-CRP changes positively correlated with body weight, BMI, waist circumference, and glucose and inversely correlated with Hcy. 1-year Hcy changes inversely correlated with REM-C (**Table 3**). 10-week Apn changes positively correlated with body weight, BMI, waist circumference, diastolic BP, TC, calculated LDL-C, non-HDL-C, REM-C, and insulin and inversely correlated with the plant-based diet scores PDI and hPDI (**Table 3**). At the food group level, most correlations between dietary intake and biomarkers were non-significant.

Table 3. Bivariate correlations of changes in hs-CRP, Hcy, and Apn with changes in other markers									
Parameter changes	**Correlations with hs-CRP change** (Δ[baseline, 1 year])			**Correlations with Hcy change** (Δ[baseline, 1 year])			**Correlations with Apn change** (Δ[baseline, 10 weeks])		
	r	**p-value**	**n**	**r**	**p-value**	**n**	**r**	**p-value**	**n**
Body weight	0.231	**0.018**	104	-0.132	0.152	120	0.254	**0.002**	141
BMI	0.241	**0.014**	104	-0.130	0.156	120	0.267	**0.001**	141
WC	0.415	**<0.001**	104	-0.163	0.077	119	0.256	**0.002**	141
Systolic BP	0.031	0.755	104	0.065	0.479	120	0.064	0.449	140
Diastolic BP	-0.075	0.448	104	0.006	0.945	120	0.183	**0.031**	140
Pulse pressure	0.070	0.483	104	0.073	0.426	120	0.002	0.982	140
RHR	0.077	0.438	104	-0.100	0.276	120	0.019	0.821	140
TC	0.010	0.923	104	-0.009	0.923	120	0.251	**0.003**	141
LDL-C (meas.)	-0.035	0.726	104	0.003	0.977	120	0.159	0.059	141
LDL-C (calc.)	0.029	0.767	103	-0.033	0.720	119	0.200	**0.018**	140
non-HDL-C	0.045	0.653	104	-0.048	0.601	120	0.251	**0.003**	141
REM-C	0.191	0.052	104	-0.186	**0.041**	120	0.203	**0.016**	141
HDL-C	-0.138	0.161	104	0.075	0.416	120	0.133	0.115	141
TAG	0.137	0.167	104	-0.133	0.148	120	0.092	0.280	141
Glucose	0.216	**0.028**	104	-0.083	0.370	120	-0.071	0.405	141
HbA1c	0.131	0.185	104	0.136	0.137	120	0.104	0.219	141
Insulin	0.066	0.503	104	-0.051	0.582	120	0.174	**0.039**	141
hs-CRP	-	-	-	-	-	-	0.122	0.187	119
Hcy	-0.264	**0.011**	92	-	-	-	-0.052	0.542	141
PDI	-0.045	0.660	100	0.110	0.244	114	-0.178	**0.044**	129
hPDI	0.042	0.676	100	-0.064	0.497	114	-0.195	**0.027**	129
uPDI	-0.006	0.956	100	-0.116	0.218	114	0.134	0.130	129

Participants of both the intervention and control groups are combined.
hs-CRP: high-sensitivity C-reactive protein; Hcy: homocysteine; Apn: adiponectin; r: Spearman correlation coefficients; BMI: body mass index; WC: waist circumference; BP: blood pressure; RHR: resting heart rate; TC: total cholesterol; LDL-C (meas.): measured LDL cholesterol; LDL-C (calc.): calculated LDL-C; non-HDL-C: non-HDL cholesterol; REM-C: remnant cholesterol; HDL-C: HDL cholesterol; TAG: triglycerides; PDI: plant-based diet index; hPDI: healthful PDI; uPDI: unhealthful PDI

Discussion

In line with our study's hypothesis, the 1-year trajectory of hs-CRP was significantly lower in the intervention group compared to control. Our results confirm that moving towards a healthier lifestyle (including a healthy plant-based dietary pattern) can decrease inflammation, as indicated by lower hs-CRP levels, even in subjects with relatively low baseline levels [5]. In the intervention group, significant increases in physical activity were achieved at 10 weeks, but these were not maintained at 1 year. In contrast, dietary improvements were largely maintained at 1 year. A reason for this was likely that our intervention placed a strong emphasis on dietary recommendations. This also indicates that diet was likely a relevant factor in improving hs-CRP levels, which is in line with previously reported associations between healthy plant-based dietary patterns and lower hs-CRP [3, 7]. The observed 1-year improvement in hs-CRP of -0.8 mg/l (compared to control) indicates a clinically

relevant effect [1] that is comparable to the effect of vegetarian diets documented in a recent meta-analysis (-0.6 mg/l) [7].

From baseline to 1 year, Hcy decreased in both groups (**Table 2**), with no significant difference between groups. In accordance with our study hypothesis, this indicates that our recommendations did not adversely affect vitamin B12 status, as indicated by Hcy levels within 1 year. However, we also observed an inverse correlation between Hcy changes (Δ[baseline, 1 year]) and changes in hs-CRP (**Table 3**). One could hypothesize that stronger adherence to plant-based dietary recommendations may more effectively lower hs-CRP but may at the same time adversely affect Hcy (due to potentially decreased vitamin B12 intake [16]). There is a lack of medium-term (≥1 year) controlled trials assessing the effect of lifestyle changes including a plant-based diet on Hcy levels in participants from the general population and using a no-intervention control group. One short-term controlled trial with these characteristics could be identified. This study, with healthy participants in Germany, demonstrated no effect of an unsupplemented vegan diet on Hcy levels after 4 weeks [31]. Controlled trials are needed to assess whether adopting a largely plant-based diet is associated with an increase in Hcy levels in the medium and long term. At 1 year, our intervention group had a mean Hcy plasma level of 11.4 µmol/l (**Table 2**). While some consider that Hcy values above 10 or 11 µmol/l may justify Hcy-lowering intervention [32], there is no consensus on adequate Hcy cut-off values, and a cut-off level of 14–15 µmol/l is also frequently used [14, 33]. Increased Hcy levels are associated with chronic disease risk [33]. Therefore, dietary recommendations should include strategies to ensure adequate intakes of vitamin B12, vitamin B6, and folate/folic acid.

While Apn significantly decreased in the intervention group from baseline to 10 weeks, Apn changes were not associated with changes in hs-CRP or Hcy (**Table 3**). However, Apn changes positively correlated with changes in body weight, BMI, waist circumference, cholesterol (TC, non-HDL-C, REM-C, calculated LDL-C), insulin, and diastolic BP and inversely correlated with changes in PDI and hPDI (**Table 3**). In observational studies, healthier, less inflammatory dietary patterns (including a traditional Mediterranean diet) tend to be associated with higher Apn levels, but these associations

have not been consistently shown [34]. For example, vegetarian diets are not clearly associated with altered Apn levels [35].

In participants with metabolic syndrome, some lifestyle interventions have resulted in significant [27] (or non-significant [36]) Apn increases. However, apart from our study, no other controlled trials could be identified which have assessed the effect of a dietary or lifestyle intervention including a strong focus on a predominantly plant-based diet on Apn levels in mostly clinically healthy participants from the general population [26, 27]. Thus, our results cannot be compared to highly similar studies.

A recent review on dietary influences on Apn levels concluded that healthy dietary patterns (including a traditional Mediterranean or a Dietary Approaches to Stop Hypertension diet) as well as higher dietary intakes of fibre, monounsaturated and omega-3 fatty acids, polyphenols, alcohol, and dairy products are associated with higher Apn levels and that, in contrast, higher intakes of saturated and trans fatty acids, added sugars, and red meat as well as high glycaemic and high-carbohydrate low-fat diets are associated with lower Apn levels [37]. Our results appear to largely be in contrast with these findings. While our intervention advocated for moderation in alcohol and dairy intake, we did not observe significant correlations between Apn changes and changes in alcohol or dairy intake. In addition, other studies to date do not consistently confirm associations of Apn with alcohol or dairy intake: while a cross-sectional study with apparently healthy adults in Spain (aged ≥55 years) found a positive association of wine intake with Apn levels, there was no significant difference in Apn levels between alcohol abstainers and moderate drinkers in this study [38]. Similarly, a prospective cohort study with >2800 participants in the United Kingdom (mean age: ~50 years) found that alcohol intake was not associated with Apn changes over time [39]. A recent meta-analysis of randomized controlled trials found that a high intake of dairy products was associated with higher Apn levels (~2.4 μg/ml higher compared with low or no dairy intake) [40]. However, other studies (not included in this meta-analysis) found that 400 ml/d of low-fat milk for 6 weeks had no significant effect on Apn (compared to control: habitual diet) [41] and that, in a 6-week crossover study, Apn significantly decreased in

both the dairy intervention group (3.2 servings/d of 2% fat milk per 2000 kcal; ~11% Apn decrease) and the non-dairy control group (diet without dairy; ~13% Apn decrease), with no significant between-group difference [42]. In our study, Apn significantly decreased by ~24% in the intervention group (with no significant change in control; **Supplementary table 3**, **Additional file 1**). Furthermore, a recent study found that kefir or milk supplementation for 3 weeks did not significantly affect Apn (with no significant difference between kefir and milk) [43]. Thus, it appears uncertain whether changes in alcohol or dairy intake influenced our results to a relevant extent. While some studies have observed weight loss to be associated with an increase in Apn [35] and increased Apn levels have been observed in individuals with anorexia nervosa (in whom body fat mass is drastically decreased) [44], our study showed that a decrease in Apn was associated with a decrease in body weight, BMI, and waist circumference (**Table 3**). While the effects of exercise training on Apn are also uncertain [45], a slight majority of controlled trials with adults indicate that exercise is associated with significantly higher Apn levels [24]. In contrast, in our study, increased exercise levels after 10 weeks were associated with decreased Apn levels.

Results from Mendelian randomization studies suggest that blood Apn concentrations are unlikely to be causally associated with metabolic disease, including type 2 diabetes [46], coronary artery disease [47], and obesity-related cancer [48]. Based on Mendelian randomization, higher Apn levels may, however, adversely affect osteoarthritis risk [49] and bone mineral density (in the femoral neck and forearm) [50].

Taken together these results indicate that the decrease in Apn observed in our study may not constitute an unfavourable effect. Although it has been proposed that the Apn pathway is a highly relevant mediator of the beneficial effects of a healthy dietary pattern [51], currently the association of Apn with the beneficial effects of healthy lifestyle changes appears unclear [34]. Our results do not confirm the common interpretation that Apn increases observed in intervention studies constitute a beneficial effect.

Strengths and limitations

A strength of the present study is the use of a no-intervention control group (which allows for comparison with a group in which no effect is expected) and multiple measurement time points (which allowed us to confirm that hs-CRP was consistently more decreased in the intervention group at each follow-up time point). Two relevant limitations are the non-randomized design and the 6-month delay in starting the control group (although the follow-up durations were equivalent). While our findings indicate a more favourable 1-year trajectory of hs-CRP in the intervention group, this result may have been influenced by seasonal changes [52], although seasonal effects on hs-CRP are uncertain [53]. While a significant hs-CRP decrease from baseline to 1 year was observed in the intervention group and no significant change was observed in the control group (even though baseline levels in the control group were higher), seasonal influences remain a potential confounder. However, sensitivity analyses comparing 1½-year trajectories or exchanging the time points (of the control group) to achieve comparable seasons confirmed that the hs-CRP trajectory was significantly lower in the intervention group. This indicates that the results are not strongly confounded by seasonal effects. Although both groups were comparable at baseline and we adjusted for potential confounders, some bias due to non-randomization may have remained. Other limitations are the small study sample and the high proportion of participants who dropped out or were excluded from the analysis (although sensitivity analyses with imputed data confirmed the results).

Conclusions

Over the course of 1 year, the lifestyle intervention lead to a significant improvement (decrease) in hs-CRP levels in a sample of indviduals without strongly elevated baseline values, without adversely affecting Hcy. The widespread theory that an increase in Apn constitutes a beneficial health effect could not be confirmed in our study. Our results are in accordance with findings from recent Mendelian randomization studies which also indicate that this assumption should be reconsidered. Further studies should investigate how lifestyle interventions can be optimized to efficiently lower

subclinical inflammation and thereby reduce disease risk. The parameters hs-CRP, Hcy, and Apn are secondary end points and our results should be considered exploratory.

List of abbreviations

Apn: adiponectin

BMI: body mass index

BP: blood pressure

CCA: complete case analysis

CI: confidence interval

CVD: cardiovascular disease

Hcy: homocysteine

HDL-C: HDL cholesterol

hPDI: healthful PDI

hPDImod: modified hPDI

hs-CRP: high-sensitivity C-reactive protein

LDL-C: LDL cholesterol

non-HDL-C: non-HDL cholesterol

PDI: plant-based diet index

PP: pulse pressure

PPARγ: peroxisome proliferator-activated receptor γ

REM-C: remnant cholesterol

RHR: resting heart rate

SEM: standard error of the mean;

TAG: triglycerides

TC: total cholesterol

uPDI: unhealthful PDI

Declarations

Ethics approval and consent to participate

All subjects provided written informed consent before participating in the study. The study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the ethics committee of the Medical Association of Westphalia-Lippe and of the University of Münster (Münster, Germany; reference: 2018-171-f-S; approved 4 April 2018).

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was conducted as part of the project “münster.land.leben” and was funded by the German Federal Ministry of Education and Research (BMBF) and the Joint Science Conference (GWK) within the programme “Innovative Hochschule” (grant number: 03IHS062A). The funders had no role in the study design, data collection, analysis, or interpretation, writing of the report, or the decision to publish the findings.

Authors' contributions

Christian Koeder: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, writing – review and editing, visualization, project administration; **Corinna Anand:** conceptualization, methodology, validation, investigation, data curation, writing – review and editing, project administration; **Sarah Husain:** conceptualization, methodology, validation, investigation, data curation, writing – review and editing, project administration; **Ragna-Marie Kranz:** conceptualization, methodology, validation, investigation, data curation, writing – review and editing, project administration; **Nora Schoch:** conceptualization, methodology, investigation, writing – review and editing, project administration; **Dima Alzughayyar:** investigation, data curation, writing – review and editing; **Norman Bitterlich**: methodology, formal analysis, writing – review and editing; **Andreas Hahn**: methodology, formal analysis, writing – original draft, writing – review and editing, supervision; **Heike Englert**: conceptualization,

methodology, investigation, writing – original draft, writing – review and editing, project administration, supervision, funding acquisition.

Acknowledgements

We would like to thank all participants, Alwine Kraatz who took part in the investigation, and Dr Reinhold Gellner for his valuable contributions in the application process for ethical approval and his medical advice during the study.

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2.4. Paper 4 (published)

Title: Healthy lifestyle changes favourably affect common carotid intima-media thickness: the Healthy Lifestyle Community Programme (cohort 2)

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Published in: Journal of Nutritional Science, online (13 June 2022), volume 11 (2022), e47

Journal's impact factor: 3.0

DOI: 10.1017/jns.2022.46

PubMed ID: 35754985

JNS
JOURNAL OF NUTRITIONAL SCIENCE

RESEARCH ARTICLE

Healthy lifestyle changes favourably affect common carotid intima-media thickness: the Healthy Lifestyle Community Programme (cohort 2)

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(Received 16 February 2022 – Final revision received 18 May 2022 – Accepted 20 May 2022)

Journal of Nutritional Science (2022), vol. 11, e47, page 1 of 12 doi:10.1017/jns.2022.46

Abstract

Common carotid intima-media thickness (ccIMT) progression is a risk marker for cardiovascular disease (CVD), whereas healthy lifestyle habits are associated with lower ccIMT. The objective of the present study was to test whether a healthy lifestyle intervention can beneficially affect ccIMT progression. A community-based non-randomised, controlled lifestyle intervention was conducted, focusing on a predominantly plant-based diet (strongest emphasis), physical activity, stress management and social health. Assessments of ccIMT were made at baseline, 6 months and 1 year. Participants had an average age of 57 years and were recruited from the general population in rural northwest Germany (intervention: *n* 114; control: *n* 87). From baseline to 1 year, mean ccIMT significantly increased in both the intervention (0·026 [95 % CI 0·012, 0·039] mm) and control group (0·045 [95 % CI 0·033, 0·056] mm). The 1-year trajectory of mean ccIMT was lower in the intervention group ($P = 0·022$; adjusted for baseline). In a subgroup analysis with participants with high baseline mean ccIMT (≥0·800 mm), mean ccIMT non-significantly decreased in the intervention group (−0·016 [95 % CI −0·050, 0·017] mm; *n* 18) and significantly increased in the control group (0·065 [95 % CI 0·033, 0·096] mm; *n* 12). In the subgroup, the 1-year trajectory of mean ccIMT was significantly lower in the intervention group (between-group difference: −0·051 [95 % CI −0·075, −0·027] mm; $P < 0·001$; adjusted for baseline). The results indicate that healthy lifestyle changes may beneficially affect ccIMT within 1 year, particularly if baseline ccIMT is high.

Key words: Cardiovascular prevention: Plant-based diet: Lifestyle medicine: Carotid intima-media thickness: Cardiovascular disease: Cardiovascular health: Preventive medicine

Introduction

Carotid intima-media thickness (cIMT) is a marker of pathological arterial wall changes (arterial injury)[1–3]. Often cIMT is referred to as subclinical atherosclerosis[4], although this is controversially discussed[2,3]. Increased cIMT is a considerable public health concern[5], and it has been estimated that the worldwide prevalence of increased cIMT (≥1·0 mm) is >25 % in individuals aged 30–79 years, equivalent to >1 billion individuals[5].

Most frequently, cIMT is assessed in the common carotid artery[6]. Common cIMT (ccIMT) is an established risk marker for cardiovascular disease (CVD) such as myocardial infarction, sudden cardiac death[7] and stroke[8]. Positive correlations have been shown between ccIMT and calcification scores of the coronary arteries and the aorta[9] as well as between ccIMT and epicardial adipose tissue[10].

The prospective assessment of ccIMT in clinical trials offers the possibility to test the effect of the intervention on the arterial structure, and this constitutes a more direct measure of artery health compared to serological markers, while at the same time avoiding invasive, complicated and very expensive procedures[1,3,11]. A recent meta-analysis of clinical trials, including dietary interventions, has shown that changes in ccIMT progression are able to predict changes in CVD risk,

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that even relatively small changes are relevant, and that ccIMT progression can thus be considered a suitable parameter for intervention studies[12].

Current evidence indicates that a dietary pattern with a strong emphasis on healthy plant-based components, a high water intake and lower intakes of unhealthy plant-based foods and of meat is associated with lower ccIMT[4,13–15].

Thus, the objective of the present study was to test if a healthy lifestyle intervention would beneficially affect ccIMT within 1 year (among other CVD risk markers[16]).

Materials and methods

Study design

The study was a non-randomised controlled trial, and ccIMT was assessed at baseline, 6 months and 1 year. The study had been planned with a duration of 2 years, but due to the COVID-19 pandemic we did not include the 1½-year time point (uneven time delays between groups; included in sensitivity analyses) or the 2-year time point (no assessment in the control group).

The intervention consisted of a lifestyle programme, and the control group received no intervention. Randomisation was not feasible, as described previously[17]. Both groups were recruited in two separate and comparable small towns in order to keep the participants of the control group unaware of the lifestyle recommendations given to the intervention group. The intervention group was recruited at a public market (February 2018) and by word of mouth, while the control group was recruited at a local public event (September 2018). The funding was provided for a specific time period, at relatively short notice and there were insufficient resources (time and staff) to recruit and start both study arms at the same time. Therefore, the intervention group started 6 months earlier (April 2018) than the control group study arm (October 2018), with equivalent follow-up intervals. The study was registered in the German Clinical Trials Register (DRKS; reference: DRKS00018775; www.drks.de).

Participants

For the intervention and control groups, a total of 114 and 87 participants, respectively, were recruited. Participants were mostly middle-aged and elderly individuals from the general population in rural northwest Germany. As a community-based intervention, the only inclusion criteria were the physical and mental ability to participate and to be ≥18 years old. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the ethics committee of the Medical Association of Westphalia-Lippe and of the University of Münster (Münster, Germany; reference: 2018-171-f-S; approved 4 April 2018). Written informed consent was obtained from all subjects.

Lifestyle programme

The lifestyle intervention has been described previously[17]. In brief, the intervention included an intensive phase (first 10 weeks; 14 seminars and 8 workshops) and a less intensive phase (monthly seminars). The focus of the intervention was on four areas of lifestyle change: a healthy, predominantly plant-based diet (strongest emphasis), physical activity, stress management and social health. Dietary recommendations were to consume more healthy plant foods (fruit, vegetables, whole grains, legumes, including soya foods, nuts, seeds and healthy oils) and to reduce the intake of unhealthy plant foods (added sugars, salt, refined grains, alcohol excess) and of animal-source foods (particularly meat)[18]. Participants also received a healthy lifestyle handbook, a recipe booklet and a laminated information sheet with an overview of the lifestyle recommendations[17] (Supplementary material).

Assessment of parameters

Measurements of ccIMT were conducted in accordance with the Mannheim consensus[19], as described previously[17] (Supplementary material). Dietary intake was assessed with semi-quantitative 3-d food protocols. Adherence to dietary recommendations was assessed with the plant-based diet index (PDI), healthful PDI (hPDI) and unhealthful PDI (uPDI)[18] (as described previously[16]). As the association of the intakes of potatoes, fish, eggs and dairy with ccIMT are less certain, whereas hPDI includes these food groups as negatives, we also conducted a *post hoc* analysis with a modified hPDI (excluding the food groups potatoes, fish, eggs and dairy). Physical activity (in categories) and socio-demographic parameters were assessed using questionnaires.

Study hypotheses

In terms of mean and max ccIMT, the study hypotheses were that the intervention would significantly decrease mean and max ccIMT (within-group and compared to control; from baseline to 1 year and from baseline to 1½ years). The two main hypotheses were regarding the between-group changes (mean and max ccIMT: 1-year changes). Any detected differences in the secondary end points mean and max ccIMT are considered exploratory.

Sample size calculation

The primary outcome parameter of the study was body weight[16], for which a sample size calculation was conducted, and a further sample size calculation was conducted for the secondary parameter ccIMT (as described previously[17]).

Statistical analyses

Between-group differences in baseline characteristics were assessed using Fisher's exact test for categorical variables and independent *t* test and Mann–Whitney *U* test for normally and non-normally distributed continuous variables, respectively (as described previously[17]). To evaluate within-group changes, paired *t* test and Wilcoxon signed-rank test were used for normally and non-normally distributed data,

respectively. Correlations were assessed with Spearman's rho correlations. All tests were two-sided.

To evaluate between-group differences in 1-year trajectories of ccIMT, a repeated measures analysis of covariance (ANCOVA) was used, with baseline ccIMT and potential confounders (especially age and sex) as covariates. Holm–Bonferroni correction was conducted to adjust for multiple comparisons. As it has been shown that baseline ccIMT is inversely associated with ccIMT change[20] and ccIMT may thus more strongly decrease in those with high baseline ccIMT[21], subgroup analyses were conducted, including only participants with high baseline mean ccIMT (≥0·800 mm). Analyses were based on unimputed data (complete case analysis, CCA). In sensitivity analyses, imputed data (last observation carried forward, LOCF) were used. Sensitivity analyses using log-transformed (lg10) ccIMT values were also conducted. All analyses were conducted using IBM SPSS Statistics (Version 25.0. Armonk, NY).

Results

Baseline characteristics

For a total of 126 participants (intervention: 71; control: 55), ccIMT values were available for all three measurement time points (baseline, 6 months and 12 months). The flow of participants through the study is shown in Fig. 1.

At baseline, the intervention group had a significantly higher age and educational level (Table 1). Baseline hPDI was also significantly higher in the intervention group (by 4·3 [95 % CI 2·0, 6·5] portions/day; $P < 0·001$), while PDI and uPDI were not significantly different between groups. Frequency of intense physical activity was higher in the control group ($P = 0·031$) when assessed by categories, but physical activity (gentle, moderate and intense) was not significantly different between groups when assessed by minutes/week.

Furthermore, a higher percentage of participants with a history of cancer (intervention: 9·9 %; control: 0 %; $P = 0·018$) and a higher percentage of participants with a family history (parents) of myocardial infarction or stroke (intervention: 56·3 %; control: 36·4 %; $P = 0·031$) were observed in the intervention group.

At baseline, there were no significant between-group differences in terms of ccIMT and a variety of other CVD parameters (Table 1). Similarly, there were no significant between-group differences in terms of alcohol intake frequency or the percentage of participants with (based on baseline values) hypertension, high values for total cholesterol (TC), LDL cholesterol (LDL-C; measured and calculated), non-HDL cholesterol (non-HDL-C), triacylglycerols (TAG), glucose or HbA1c or who had low HDL cholesterol (HDL-C). Furthermore, there were no significant between-group differences in terms of the percentage of those with a history of stroke, with a family history (siblings, grandparents) of myocardial infarction or stroke, or with any of a large variety of diagnosed disease conditions (hypertension, dyslipidaemia, heart disease, stroke, peripheral artery disease, diabetes, retinopathy, peripheral neuropathy, diabetic foot, kidney disease, allergies, gastrointestinal disease, thyroid disease, depression, rheumatoid arthritis, chronic pain, lung disease, bone disease as well as 'other disease' and 'free of diagnosed disease'). There were no significant differences in any of the baseline characteristics (as listed in Table 1) between the group of participants with complete ccIMT data and with incomplete ccIMT data ($P > 0·07$).

Seminar attendance

Compliance in the intervention group, defined as seminar attendance during the 10-week intensive phase of the lifestyle intervention, was relatively high: 60 out of the 71 evaluable participants (84·5 %) attended ≥10 (out of 14) seminars.

Repeatability of ccIMT measurements

Repeatability (within-assay precision) of the two repeated measurements of mean and max ccIMT was good at all time points (mean ccIMT: $r > 0·94$; max ccIMT: $r > 0·88$). Mean differences in repeated measurements were small for both mean ccIMT (between 0·000 and 0·005 mm) and max ccIMT (between 0·003 and 0·007 mm).

Mean ccIMT changes from baseline to 1 year

From baseline to 1 year, mean ccIMT significantly increased in both the intervention (0·026 [95 % CI 0·012, 0·039] mm; $P = 0·001$; n 71) and control group (0·045 [95 % CI 0·033, 0·056] mm; $P < 0·001$; n 55; Fig. 2; Supplementary Table S1). The 1-year trajectory of mean ccIMT was significantly lower in the intervention group compared to control (between-group difference: –0·012 [95 % CI –0·022, –0·002] mm; $P = 0·022$; adjusted for baseline mean ccIMT). This result remained significant after Holm–Bonferroni correction. This result was also confirmed when adjusting for baseline mean ccIMT, age and sex ($P = 0·038$; Fig. 2; Supplementary Table S1) or additionally for educational level, history of cancer, family history (parents) of myocardial infarction or stroke, and baseline hPDI ($P = 0·045$). Furthermore, this result was confirmed after adjusting (in addition to baseline mean ccIMT, age and sex) for baseline smoker status and alcohol intake ($P = 0·040$), for changes in smoker status and alcohol intake ($P = 0·028$), for baseline glucose, HbA1c, systolic BP and pulse pressure ($P = 0·029$), for baseline homocysteine (Hcy; $P = 0·041$) or for changes (Δ[baseline, 1 year]) in diastolic BP ($P = 0·039$). Apart from baseline mean ccIMT, none of the covariates had a significant influence on the models. Using log-transformed covariates confirmed the results.

However, in a sensitivity analysis comparing the 1½-year trajectories of mean ccIMT, the between-group difference was non-significant (–0·010 [95 % CI –0·022, 0·003] mm; $P = 0·119$; adjusted for baseline; intervention: n 63; control: n 47; Supplementary Table S2). Similarly, a sensitivity analysis with imputed data (LOCF) demonstrated no significant between-group difference in 1-year trajectories of mean ccIMT ($P = 0·815$; adjusted for baseline; intervention: n 101; control: n 75; Supplementary Table S3). A sensitivity analysis with imputed data (LOCF) comparing the 1½-year trajectories

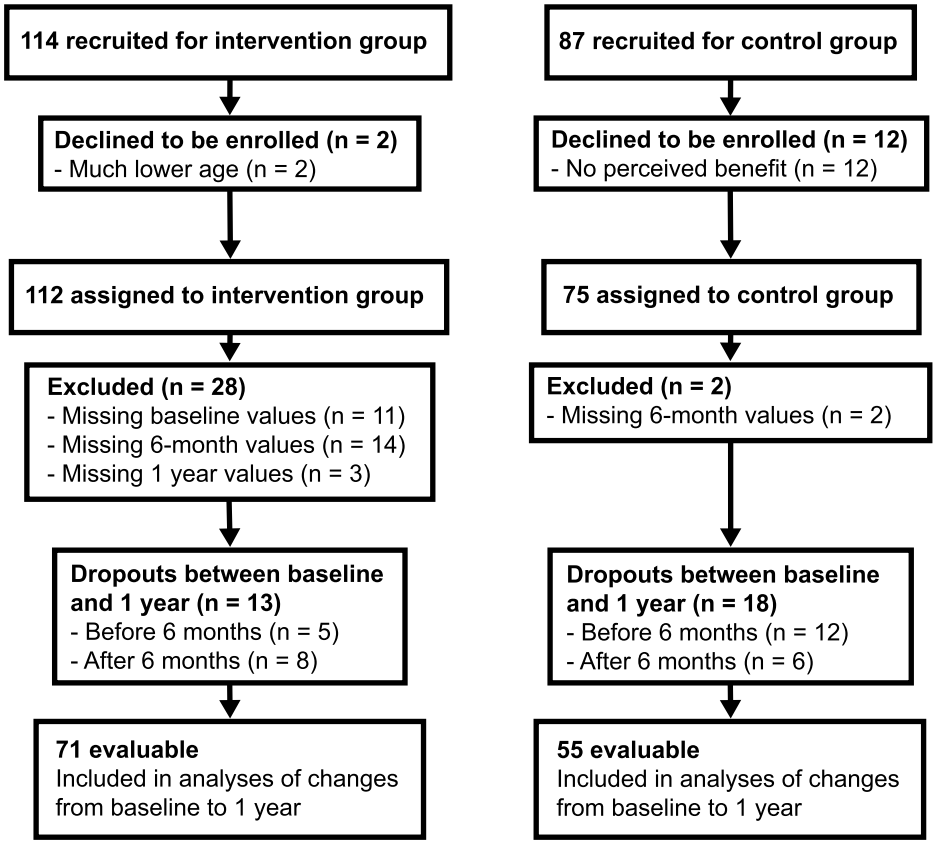

Fig. 1. Flowchart of participants through the study.

also attenuated the results ($P = 0{\cdot}853$; adjusted for baseline). In addition, the favourable effect of the intervention was also attenuated in sensitivity analyses using log-transformed (lg10) mean ccIMT values (1-year trajectories: $P = 0{\cdot}051$; 1½-year trajectories: $P = 0{\cdot}277$; adjusted for baseline).

Max ccIMT changes from baseline to 1 year

From baseline to 1 year, max ccIMT non-significantly increased in the intervention group (0·006 [95 % CI −0·015, 0·028] mm; $P = 0{\cdot}165$; *n* 71) but significantly increased in the control group (0·034 [95 % CI 0·015, 0·053] mm; $P = 0{\cdot}001$; *n* 55; Fig. 2; absolute values at each time point are can be found in Supplementary Table S1). The 1-year trajectory of max ccIMT was not significantly different between the intervention and control groups (between-group difference: −0·013 [95 % CI −0·030, 0·003] mm; $P = 0{\cdot}117$; adjusted for baseline max ccIMT). This between-group difference remained non-significant after adjusting for baseline max ccIMT, age and sex ($P = 0{\cdot}098$; Fig. 2; absolute values at each time point can be found in Supplementary Table S1). In contrast, the 1-year trajectory of max ccIMT was significantly lower after adjusting for baseline max ccIMT, age, sex, educational level, history of cancer, family history (parents) of myocardial infarction or stroke, and baseline hPDI ($P = 0{\cdot}041$) or for baseline max ccIMT, age, sex and changes in smoker status and alcohol intake ($P = 0{\cdot}045$). This result was again attenuated when adjusting (in addition to baseline max ccIMT, age and sex) for baseline smoker status and alcohol intake ($P = 0{\cdot}093$), for baseline TAG, glucose, HbA1c, systolic BP, pulse pressure and waist circumference ($P = 0{\cdot}061$), for baseline hs-CRP ($P = 0{\cdot}154$), for baseline Hcy ($P = 0{\cdot}093$) or for changes (Δ[baseline, 1 year]) in body weight and BMI ($P = 0{\cdot}164$). Apart from baseline max ccIMT, none of the covariates had a significant influence on the models. Using log-transformed covariates confirmed the results.

Similarly, this result was confirmed by a sensitivity analysis comparing the 1½-year trajectories of max ccIMT (−0·014 [95 % CI −0·034, 0·006] mm; $P = 0{\cdot}164$; adjusted for baseline; Supplementary Table S2), a sensitivity analysis with imputed data (LOCF; $P = 0{\cdot}756$; adjusted for baseline; Supplementary Table S3) as well as a sensitivity analysis with imputed data (LOCF) comparing the 1½-year trajectories of max ccIMT ($P = 0{\cdot}846$; adjusted for baseline). Similarly, this result was confirmed in sensitivity analyses using log-transformed (lg10) max ccIMT values (1-year trajectories: $P = 0{\cdot}200$; 1½-year trajectories: $P = 0{\cdot}295$; adjusted for baseline).

Mean ccIMT changes from baseline to 1 year (subgroup analysis)

In a subgroup analysis including only participants with mean ccIMT ≥0·800 mm, from baseline to 1 year, mean ccIMT non-significantly decreased in the intervention group (−0·016 [95 % CI −0·050, 0·017] mm; $P = 0{\cdot}311$; *n* 18) and

Table 1. Baseline characteristics of evaluable participants (CCA)

Characteristics	Intervention (*n* 71)		Control (*n* 55)		
	Mean or *n*	SEM or %	Mean or *n*	SEM or %	*P*-value*
Women, *n* (%)	50	70·4	34	61·8	0·344[a]
Age, years	59·4	1·0	55·3	1·3	**0·015**[b]
Mean ccIMT, mm	0·695	0·016	0·674	0·019	0·363[c]
Max ccIMT, mm	0·856	0·019	0·825	0·023	0·263[c]
Body weight, kg	81·4	2·2	84·4	2·5	0·269[c]
BMI, kg/m^2	27·6	0·6	28·3	0·8	0·524[c]
WC, cm	98·2	1·8	97·5	2·1	0·957[c]
Overweight, *n* (%)	46	64·8	38	69·1	0·704[a]
Obese, *n* (%)	20	28·2	15	27·3	1·000[a]
Systolic BP, mmHg	132·0	1·7	133·2	2·1	0·658[b]
Diastolic BP, mmHg	79·9	0·9	80·1	1·3	0·998[c]
Pulse pressure, mmHg	52·1	1·4	53·1	1·6	0·626[b]
RHR, beats/min	68·1	1·3	69·4	1·2	0·508[b]
TC, mg/dl	207·5	4·7	208·9	6·0	0·863[b]
LDL-C (measured), mg/dl	133·0	4·4	140·6	5·9	0·292[b]
LDL-C (calculated), mg/dl	121·0	4·5	125·5	5·6	0·526[b]
non-HDL-C, mg/dl	141·1	5·2	146·8	5·7	0·465[b]
REM-C, mg/dl	8·0	1·3	6·1	1·8	0·112[c]
HDL-C, mg/dl	66·5	2·2	62·1	2·4	0·184[b]
TAG, mg/dl	100·1	5·7	114·6	10·8	0·301[c]
Glucose, mg/dl	98·2	1·4	101·4	1·9	0·679[c]
HbA1c, %	5·4	0·0	5·5	0·1	0·816[c]
Insulin, µU/ml	10·9	0·8	12·4	1·1	0·223[c]
hs-CRP, mg/dl	0·12	0·02	0·30	0·08	0·304[c]
Hcy, µmol/l	12·5	0·5	11·9	0·4	0·614[c]
Smoker status					
Never	43	60·6	27	49·1	0·238[a]
Ex	20	28·2	16	29·1	
Smoker	8	11·3	12	21·8	
Marital status					
Married	58	81·7	49	89·1	0·499[a]
Partner (unmarried)	4	5·6	1	1·8	
Single (not widowed)	6	8·5	2	3·6	
Single (widowed)	3	4·2	2	3·6	
Educational level					
Lower secondary school	15	21·1	22	40·0	**0·002**[a]
Secondary school	30	42·3	15	27·3	
University entrance qualification	11	15·5	15	27·3	
University degree	15	21·1	2	3·6	
PDI, points	29·0	1·8	25·4	2·3	0·205[b]
hPDI, points	–6·2	2·4	–19·1	2·3	**<0·001**[c]
uPDI, points	–35·6	2·5	–27·8	2·5	0·070[c]

Values are means ± SEM except for qualitative variables which are expressed as *n* (%).
CCA, complete case analysis; ccIMT, common carotid intima-media thickness; BMI, body mass index; WC, waist circumference; BP, blood pressure; RHR, resting heart rate; TC, total cholesterol; LDL-C, LDL cholesterol; non-HDL-C, non-HDL cholesterol; REM-C, remnant cholesterol; HDL-C, HDL cholesterol; TAG, triacylglycerols; hs-CRP, high-sensitivity C-reactive protein; Hcy, homocysteine; PDI, plant-based diet index; hPDI, healthful PDI; uPDI, unhealthful PDI; SEM, standard error of the mean; IN, intervention; CON: control.
P-values in bold are values below 0.05.
* *P*-value for between-group comparisons by:
a Fisher's exact test (two-sided).
b Independent *t* test (two-sided).
c Mann–Whitney *U* test (two-sided).
TC, measured LDL-C, non-HDL-C, REM-C, HDL-C, TAG, glucose, HbA1c, insulin: *n* 70 (IN); calculated LDL-C, PDI, hPDI, uPDI: *n* 70 (IN), *n* 54 (CON); hs-CRP: *n* 54 (IN), *n* 36 (CON); Hcy: *n* 57 (IN), *n* 54 (CON); Marital status, educational level: *n* 54 (CON).

significantly increased in the control group (0·065 [95 % CI 0·033, 0·096] mm; $P = 0·001$; *n* 12; Fig. 2; Supplementary Table S4). The 1-year trajectory of mean ccIMT was significantly lower in the intervention group than in control (between-group difference: −0·051 [95 % CI −0·075, −0·027] mm; $P < 0·001$; adjusted for baseline mean ccIMT). This result remained significant after adjusting for baseline mean ccIMT, sex and age ($P < 0·001$; Fig. 2; absolute values at each time point can be found in Supplementary Table S4). Due to the low number of cases in the subgroup, only these covariates were adjusted for. However, in sensitivity analyses, further covariates were added: the between-group difference remained significant after adjusting for baseline mean ccIMT, age, sex, educational level, history of cancer, family history (parents) of myocardial infarction or stroke and baseline hPDI ($P < 0·001$). Similarly, this result was confirmed when adjusting (in addition to baseline mean ccIMT, age and sex) for baseline smoker status and alcohol intake ($P < 0·001$), for changes in smoker status and alcohol intake ($P < 0·001$), for baseline glucose, HbA1c, systolic BP and pulse pressure ($P < 0·001$), for baseline Hcy ($P < 0·001$) or for changes (Δ[baseline, 1 year]) in diastolic BP ($P < 0·001$). Apart from baseline mean ccIMT, age and sex, none of the covariates had a significant influence on any of the models. Using log-transformed covariates confirmed the results.

Furthermore, this result was confirmed in a sensitivity analysis comparing the 1½-year trajectories ($P = 0·001$; adjusted for baseline; Supplementary Table S5), a sensitivity analysis with imputed data (LOCF) comparing the 1-year trajectories ($P < 0·001$; adjusted for baseline; intervention: *n* 23; control: *n* 15; Supplementary Table S6), and a sensitivity analysis with imputed data (LOCF) comparing the 1½-year trajectories ($P = 0·002$; adjusted for baseline; $P < 0·001$, adjusted for baseline, age and sex).

In addition, the favourable effect of the intervention was confirmed in sensitivity analyses using log-transformed (lg10) mean ccIMT values (1-year trajectories: $P < 0·001$; 1½-year trajectories: $P = 0·001$; adjusted for baseline). Furthermore, a sensitivity analysis with a cut-off value of 0·790 mm (the 75th percentile of baseline mean ccIMT in our study population) confirmed the results (intervention: *n* 19; control: *n* 12; between-group difference: −0·059 [95 % CI −0·082, −0·035] mm; $P < 0·001$; adjusted for age and sex).

Max ccIMT changes from baseline to 1 year (subgroup analysis)

In the same subgroup, from baseline to 1 year, max ccIMT non-significantly decreased in the intervention group (−0·023 [95 % CI −0·071, 0·025] mm; $P = 0·327$; *n* 18) and non-significantly increased in the control group (0·041 [95 % CI −0·020, 0·102] mm; $P = 0·168$; *n* 12; Fig. 2; absolute values at each time point can be found in Supplementary Table S4). The 1-year trajectory of max ccIMT was significantly lower in the intervention group than in control (between-group difference: −0·046 [95 % CI −0·085, −0·007] mm; $P = 0·023$; adjusted for baseline max ccIMT). Adjusting for baseline max ccIMT, age and sex confirmed this result ($P = 0·003$).

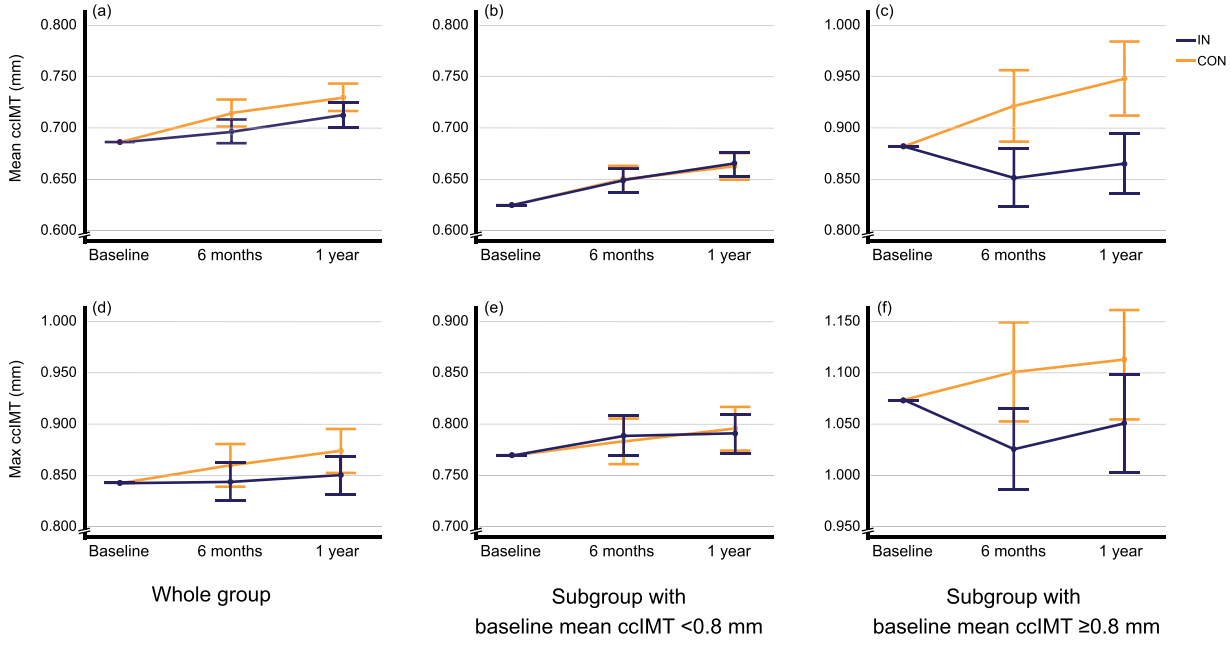

Fig. 2. 1-year mean and max ccIMT trajectories. Values are means and 95 % confidence intervals (adjusted for baseline). Whole group: (a and d) (IN: *n* 71; CON: *n* 55); subgroup with baseline mean ccIMT <0·8 mm: (b and e) (IN: *n* 53; CON: *n* 43); subgroup with baseline mean ccIMT ≥0·8 mm: (c and f) (IN: *n* 18; CON: *n* 12); *P*-values for between-group difference in 1-year trajectories of ccIMT (by ANCOVA; adjusted for baseline): (a) $P = 0{\cdot}022$; (b) $P = 0{\cdot}970$; (c) $P < 0{\cdot}001$; (d) $P = 0{\cdot}117$; (e) $P = 0{\cdot}965$; (f) $P = 0{\cdot}023$. ccIMT, common carotid intima-media thickness; IN, intervention group; CON, control group.

In sensitivity analyses, this result was confirmed when adjusting (in addition to baseline max ccIMT, age and sex) for educational level, history of cancer, family history (parents) of myocardial infarction or stroke and baseline hPDI ($P = 0{\cdot}007$), for baseline smoker status and alcohol intake ($P = 0{\cdot}001$), for changes in smoker status and alcohol intake ($P = 0{\cdot}004$), for baseline TAG, glucose, HbA1c, systolic BP, pulse pressure and waist circumference ($P = 0{\cdot}003$), for baseline hs-CRP ($P = 0{\cdot}027$), for baseline Hcy ($P = 0{\cdot}008$) or for changes (Δ[baseline, 1 year]) in body weight and BMI ($P = 0{\cdot}001$). Apart from baseline max ccIMT, age and sex, none of these covariates had a significant influence on any of the models. Using log-transformed covariates confirmed the results.

A sensitivity analysis comparing the 1½-year trajectories of max ccIMT attenuated the result when adjusting for baseline max ccIMT ($P = 0{\cdot}061$; adjusted for baseline; Supplementary Table S5) but confirmed the result when adjusting for baseline max ccIMT, age and sex ($P = 0{\cdot}011$; Supplementary Table S5). This result was also confirmed by a sensitivity analysis with imputed data (LOCF) comparing the 1-year trajectories ($P = 0{\cdot}017$; adjusted for baseline; Supplementary Table S6) and a sensitivity analysis with imputed data (LOCF) comparing the 1½-year trajectories of max ccIMT ($P = 0{\cdot}023$, adjusted for baseline; $P = 0{\cdot}002$, adjusted for baseline, age and sex).

In addition, the favourable effect of the intervention was also confirmed in sensitivity analyses using log-transformed (lg10) max ccIMT values (1-year trajectories: $P = 0{\cdot}004$; 1½-year trajectories: $P = 0{\cdot}020$; adjusted for baseline and age). Furthermore, a sensitivity analysis with a cut-off value of 0·790 mm (the 75th percentile of baseline mean ccIMT in our study population) confirmed the results (intervention: *n* 19; control: *n* 12; between-group difference: −0·065 [95 % CI −0·104, −0·026] mm; $P = 0{\cdot}002$; adjusted for age and sex).

Changes in diet scores and physical activity from baseline to 1 year

Compared to control, in the intervention group, the 1-year trajectories of PDI and hPDI were higher by 2·7 (95 % CI 1·7, 3·6) food portions/day and 3·9 (95 % CI 2·7, 5·0) food portions/day, respectively, while the 1-year trajectory of uPDI showed a decrease of −2·7 (95 % CI −3·7, −1·7) food portions/day (between-group differences: $P < 0{\cdot}001$; adjusted for baseline). Sensitivity analyses confirmed that the favourable dietary changes were maintained at 1½ years (between-group differences: $P < 0{\cdot}001$; adjusted for baseline).

A *post hoc* analysis showed that in the intervention group the 1-year trajectory of a modified hPDI (excluding the food groups potatoes, fish, eggs and dairy) was higher by 3·7 (95 % CI 2·7, 4·6) food portions/day (compared to control; between-group difference: $P < 0{\cdot}001$; adjusted for baseline). These results were maintained at 1½ years (between-group differences: $P < 0{\cdot}001$; adjusted for baseline).

At the food group level, results confirmed that participants of the intervention group were adhering to the recommendations, with significantly increased intakes (1-year trajectories) of fruit, vegetables, whole grains, legumes and nuts (approximately half a food portion/day higher, compared to control) as well as small increases in vegetable oil and fish intake. Conversely, in the intervention group, decreased intakes of meat, sweets/desserts and refined grains (approximately −0·3 to −0·4 portions/day) were observed, with small decreases also in the intakes of margarine, eggs and miscellaneous animal-source foods (compared to control). There were no significant between-group differences regarding the intakes (1-year trajectories) of tea, coffee, fruit juice, potatoes, sugar-sweetened beverages, animal fats, dairy or alcohol. No

Table 2. Bivariate correlations of changes (Δ[baseline, 1 year]) in ccIMT and other markers

	Correlation with mean ccIMT change		Correlation with max ccIMT change		
Parameter changes	Correlation coefficient	*P*-value	Correlation coefficient	*P*-value	*n*
Body weight	0·165	0·066	0·207	**0·020**	125
BMI	0·169	0·059	0·206	**0·021**	125
WC	0·077	0·393	0·144	0·109	125
Systolic BP	0·169	0·059	0·114	0·204	125
Diastolic BP	0·182	**0·042**	0·057	0·531	125
Pulse pressure	0·051	0·571	0·117	0·193	125
RHR	−0·065	0·472	−0·044	0·629	125
TC	−0·048	0·599	0·012	0·893	124
LDL-C (measured)	−0·060	0·505	−0·009	0·922	124
LDL-C (calculated)	−0·066	0·470	−0·005	0·959	123
Non-HDL-C	−0·074	0·415	−0·013	0·882	124
REM-C	0·015	0·865	0·033	0·715	124
HDL-C	−0·006	0·948	0·008	0·934	124
TAG	−0·006	0·947	0·007	0·941	124
Glucose	−0·019	0·833	0·074	0·417	124
HbA1c	0·032	0·728	0·116	0·199	124
Insulin	−0·109	0·227	−0·019	0·831	124
hs-CRP	−0·062	0·541	0·013	0·901	98
Hcy	−0·084	0·384	−0·140	0·144	110
PDI	0·103	0·262	−−0·031	0·741	120
hPDI	−0·043	0·638	−0·145	0·113	120
uPDI	0·075	0·413	0·240	**0·008**	120

Correlations coefficients: spearman's rho; ccIMT, common carotid intima-media thickness; BMI, body mass index; WC, waist circumference; BP, blood pressure; RHR, resting heart rate; TC, total cholesterol; LDL-C, LDL cholesterol; non-HDL-C, non-HDL cholesterol; REM-C, remnant cholesterol; HDL-C, HDL cholesterol; TAG, triacylglycerols; hs-CRP, high-sensitivity C-reactive protein; Hcy, homocysteine; PDI, plant-based diet index; hPDI, healthful PDI; uPDI, unhealthful PDI.
P-values in bold are values below 0.05.

significant between-group differences were observed for the 1-year trajectories of intense, moderate or gentle physical activity ($P > 0{\cdot}09$). This was confirmed for the 1½-year trajectories of physical activity ($P > 0{\cdot}10$).

Bivariate correlations of changes (Δ[baseline, 1 year]) in ccIMT and other markers

Bivariate correlations of changes in mean and max ccIMT with changes in other CVD markers as well as changes in dietary scores are shown in Table 2. For mean ccIMT change, a significant (although weak) correlation was only observed with changes in diastolic BP (Table 2). For max ccIMT change, significant correlations were only observed for changes in body weight, BMI and uPDI (Table 2). In addition, mean ccIMT change inversely correlated with baseline mean ccIMT ($r = -0{\cdot}179$; $P = 0{\cdot}044$), and max ccIMT change inversely correlated with baseline max ccIMT ($r = -0{\cdot}259$; $P = 0{\cdot}003$).

In the subgroup of participants with baseline mean ccIMT ≥0·800 mm, changes in uPDI positively correlated with changes in mean ccIMT ($r = 0{\cdot}370$; $P = 0{\cdot}048$) and max ccIMT ($r = 0{\cdot}539$; $P = 0{\cdot}003$) [*n* 29], while no significant correlations between changes in mean or max ccIMT and changes in PDI, hPDI or CVD markers were observed.

A *post hoc* analysis showed that changes in the modified hPDI (excluding the food groups potatoes, fish, eggs and dairy) inversely correlated with max ccIMT change ($r = -0{\cdot}229$; $P = 0{\cdot}012$). This result was confirmed in the subgroup of participants with high baseline ccIMT ($r = -0{\cdot}460$; $P = 0{\cdot}012$). Mean ccIMT change did not significantly correlate with changes in modified hPDI.

Mean and max ccIMT changes stratified by baseline risk factors

1-year trajectories of mean ccIMT and max ccIMT did not significantly differ between men and women ($P = 0{\cdot}313$; $P = 0{\cdot}208$) or between participants with or without overweight ($P = 0{\cdot}365$; $P = 0{\cdot}707$) or with or without obesity ($P = 0{\cdot}940$; $P = 0{\cdot}938$). Similarly, 1-year trajectories of mean ccIMT and max ccIMT did not significantly differ by smoker status ($P = 0{\cdot}467$; $P = 0{\cdot}449$), categories of alcohol intake ($P = 0{\cdot}547$; $P = 0{\cdot}591$), marital status ($P = 0{\cdot}921$; $P = 0{\cdot}931$) or educational level ($P = 0{\cdot}578$; $P = 0{\cdot}579$; all adjusted for baseline).

Discussion

The present study had the aim of examining the effect of a community-based lifestyle intervention on changes in mean and max ccIMT (among other CVD markers[16]). While the intervention resulted in significant dietary improvements, in line with the recommendations given, physical activity did not significantly increase over the course of 1 year. Previously we reported that, with all participants included, the intervention had no significant effect on ccIMT within 6 months in the whole study group but that in a subgroup of participants with high baseline ccIMT (mean ccIMT ≥0·800 mm) the intervention resulted in significantly lower (more favourable) mean and max ccIMT changes compared to control[17]. The present 1-year analyses (and 1½-year sensitivity analyses) confirm this result of significantly slowed down mean ccIMT and max ccIMT progression in the subgroup with high baseline ccIMT. This result is in line with the results of the PREDIMED-Navarra study, a 1-year controlled

intervention with a traditional Mediterranean diet, which showed significant effects on ccIMT only in the subgroup with high baseline ccIMT (mean ccIMT ≥0·9 mm)[21]. Comparable subgroup results from other studies seem to not have been published[22].

A recent meta-analysis suggests that every 0·010 mm reduction in mean ccIMT progression can reduce CVD event risk by 10–15 %[12]. Therefore, the between-group difference (−0·051 [95 % CI −0·075, −0·027] mm) observed in the high-baseline subgroup of the present study appears to be clinically relevant. In contrast to the previously reported 6-month results[17], with all participants included, the present 1-year analysis showed a significantly lower (slowed down) mean ccIMT progression in the intervention group. However, this between-group difference (−0·012 [95 % CI −0·022, −0·002] mm) was small and was attenuated in sensitivity analyses comparing the 1½-year trajectories. Nevertheless, it can be hypothesised that the clinical benefit of this effect may increase over time, if participants maintain the achieved lifestyle changes in the future[20,23].

To date, only a small number of controlled trials have assessed the effect of a combined lifestyle intervention (including diet and other lifestyle factors) on ccIMT[22,24–28], and only two of these trials were conducted with generally healthy participants[27,28]: in a suburban population in Japan, Okada *et al.* observed no significant effect of a 2-year lifestyle modification on mean ccIMT change (compared to control; dietary recommendations were based on the goals of the National Cholesterol Education Program; <30 % total fat, <7 % saturated fat and <200 mg dietary cholesterol per day)[27]. Similarly, in a study with perimenopausal women in the United States, Wildman *et al.* observed no significant effect of a 4-year diet (≤25 % total fat, ≤7 % saturated fat, ≤100 mg dietary cholesterol and 1300 kcal/d) and exercise intervention on mean ccIMT change (compared to control)[28]. In terms of diet-only interventions (i.e. interventions focused solely on a change in dietary pattern), no controlled trials assessing ccIMT change seem to have been conducted with generally healthy participants. Thus, our study appears to be the first controlled trial (lifestyle or dietary) to have shown a significant intervention effect on ccIMT in a community-based sample of mostly generally healthy participants from the general population.

Apart from our study, only one controlled trial appears to have assessed the effect of a combined lifestyle intervention including a strong focus on a predominantly plant-based diet on ccIMT[25]. In the present study, Aldana *et al.* observed no effect of a 1-year intervention including a very low-fat plant-based diet (Ornish Program) on ccIMT in patients with clinically confirmed coronary artery disease (compared to usual care)[25]. In contrast to our study, healthful plant-based high-fat foods (such as nuts, extra virgin olive oil or cold-pressed rapeseed oil) were not recommended in the Ornish Program[25], whereas more recent evidence suggests that these foods may not just improve cardiovascular health in general[29] but may also beneficially affect ccIMT[20,21]. In terms of diet-only interventions, five controlled trials have assessed the effect of moving towards a more plant-based diet on ccIMT: four of these studies used a Mediterranean diet (in Spain[20,21,30] and Italy[23]), and one study used a Mediterranean-like diet (in Norway[31,32]). All of these studies were conducted with participants at higher CVD risk, with four out of these five studies showing a significant favourable effect on ccIMT[20,21,23,31,32]. In contrast, two controlled trials observed that a low-carbohydrate diet had no significant effect on ccIMT in type 2 diabetes patients after 1 year (United States[33]) and 1½ years (Taiwan[34]), respectively. As no comparable studies could be identified, our study appears to be the first controlled trial (lifestyle or dietary) which tested the effect of recommending a predominantly plant-based diet (centred around fruit, vegetables, whole grains, legumes, nuts/seeds and healthy oils) on ccIMT in a study sample of mostly generally healthy participants[31].

Similarly, our study appears to be the first controlled trial (lifestyle or dietary) to assess correlations between ccIMT changes and changes in PDI, hPDI and uPDI as well as between ccIMT changes and Hcy changes. Apart from a dietary intervention study by Petersen *et al.*[35], our study also appears to be the only controlled trial (lifestyle or dietary) to have correlated ccIMT changes with hs-CRP changes. While we observed that uPDI change positively correlated with max ccIMT change (and in the subgroup analyses with both mean and max ccIMT changes), we did not observe significant correlations of ccIMT change with changes in Hcy or hs-CRP. As in our study, Petersen *et al.* found no significant correlation of mean ccIMT change with hs-CRP change[35]. The positive correlations of uPDI change with changes in mean ccIMT (r = 0·370) and max ccIMT (r = 0·539), which we observed in the high-baseline subgroup, indicate that the favourable effects on ccIMT were partially mediated by the observed decrease in uPDI. The results do not indicate a strong role of PDI or hPDI changes in the observed favourable effects. However, the significant inverse correlation of a modified hPDI (excluding the food groups potatoes, fish, eggs and dairy) with max ccIMT change which we observed indicates that an increased intake of healthful plant foods and a decreased intake of certain animal-source foods may also contribute to lower max ccIMT progression, although we observed a significant beneficial effect on max ccIMT change only in the subgroup analysis.

Among diet-only interventions, Jimenez *et al.* and Maiorino *et al.* observed a favourable effect on mean ccIMT in their intervention groups following a traditional Mediterranean diet after 5 and 7 years (coronary heart disease patients; Spain[20]) and after 4 and ~8 years (type 2 diabetes patients; Italy[23]), respectively. It should be noted that both studies compared their intervention groups to control groups following healthy low-fat diets[20,23], which may underestimate[36] but may also overestimate the effect (e.g. Jimenez *et al.* observed a decrease in Mediterranean diet adherence in the low-fat control[20]). In addition to the significant effects at 4 and ~8 years, Maiorino *et al.* observed a non-significant, favourable effect on mean ccIMT change at 2 years (between-group difference: P = 0·050[23]), which indicates that longer study durations may increase the likelihood of observing significant effects on ccIMT. While in our study dietary improvements

(compared to control) were similarly maintained at 1 year and 1½ years, it should be considered that in lifestyle and dietary interventions, adherence to recommendations may decrease over time, especially in generally healthy participants, and that dietary changes may occur in the no-intervention control group. Furthermore, our study indicates that dropout rates can be high in study populations of generally healthy individuals, potentially making long-term (≥4 years)[23] follow-up less feasible. We observed that the small but significant intervention effect on mean ccIMT at 1 year ($P = 0{\cdot}022$) became non-significant at 1½ years ($P = 0{\cdot}119$), which may have been influenced by the lower number of participants available at 1½ years. In their study with type 1 and type 2 diabetes patients in Australia, Petersen *et al.* were able to demonstrate a significant favourable effect of repeated counselling from a dietitian on mean and max ccIMT after 1 year (compared to usual diet)[35]. Petersen *et al.* observed that mean ccIMT change was ~0·016 mm lower in the intervention group (compared to control)[35], whereas our study found that mean ccIMT was lower by 0·012 mm in the intervention group. While the dietary instructions given to the intervention group by Petersen *et al.* were to increase the intakes of fruit (not fruit juice), vegetables and dairy (milk or yoghurt, not cheese) and at 3 months increased intakes of fruit (+179 g/d), vegetables (+46 g/d) and yoghurt (+38 g/d) were observed (compared to control), these increases were not maintained at 1 year[35]. In comparison, we observed increased intakes (1-year trajectories) of about half a food portion/day for fruit, vegetables, whole grains and legumes (equivalent to an increase of about 50–75 g/d each) as well as additional dietary changes (including a reduction in the intakes of meat, refined grains and sweets) in the intervention group (compared to control).

Our subgroup analyses confirm that significant effects on ccIMT may be more easily demonstrated in individuals with higher baseline ccIMT[21], and such higher ccIMT values are more likely to be present in study samples of individuals with confirmed CVD risk factors such as hypercholesterolaemia[31] or diabetes[23,35]. Nevertheless, it is of high public health relevance to develop tools (such as our intervention programme) which already initiate CVD prevention measures in individuals who are still at low to moderate CVD risk[37]. Consequently, it appears justifiable to conduct further lifestyle/dietary interventions assessing ccIMT change in generally healthy participants. It should also be considered that with participants at higher CVD risk a control group for which no effect (no intervention) or a lower effect can be expected may be ethically problematic[38]. Like Petersen *et al.*[35] (and no other controlled lifestyle/dietary trial), our study showed a significant effect on mean ccIMT change after 1 year. At a follow-up of <1 year, significant favourable effects of lifestyle/dietary pattern modification on ccIMT change (compared to control) have only been shown in type 2 diabetes patients (after 6 months; South Korea)[26] as well as in our subgroup analysis at 6 months[17].

A cut-off value for mean ccIMT of 0·8 mm, as used in our study, has been widely utilised as a threshold for describing what constitutes elevated mean ccIMT values[39]. It has furthermore been proposed that the age-, sex- and race-specific 75th percentile of mean ccIMT (derived from large cohort studies) should be used as a cut-off value[1,40]. In our study population (evaluable participants; *n* 126), the 75th percentile of baseline mean ccIMT was 0·786 mm, which confirms the usefulness of 0·800 mm as a cut-off value in our study. Sensitivity analyses with a cut-off value of 0·790 mm confirmed the results.

Only a small number of controlled trials have tested the effect of exercise-only interventions on ccIMT[41–46]. One small study demonstrated a significant favourable effect of exercise training on ccIMT (compared to control)[41,43], and one other study observed a significant beneficial effect only in a subgroup analysis of patients without identified carotid plaques[42]. The other studies did not demonstrate significant effects compared to control.

Age-related ccIMT increase is partly mediated by increased sympathetic nerve activity in vascular smooth muscle[47]. Psychological stress is associated with increased sympathetic activity[48], blood pressure[49] and ccIMT[50], and this highlights the importance of psychological stress management as a component of healthy lifestyle recommendations[51,52]. In the present study, we observed a significant reduction in body weight in the intervention group (compared to control). Weight loss is associated with decreased sympathetic activity[53] and decreased ccIMT[54], and in the present study a positive correlation was observed between changes in body weight and changes in max ccIMT ($P = 0{\cdot}020$) and mean ccIMT ($P = 0{\cdot}066$). However, in the high-baseline subgroup, changes in body weight did not significantly correlate with changes in mean ccIMT ($P = 0{\cdot}387$) or max ccIMT ($P = 0{\cdot}640$). Similarly, adjusting for body weight or BMI changes did not significantly influence the ANCOVA models ($P > 0{\cdot}69$; unpublished results). This indicates that body weight reduction was not a main driver of the favourable effects on ccIMT which were observed in the subgroup.

Strengths and limitations

A strength of the present study is the use of a no-intervention control group, a strict standardised measurement protocol[1,19,55], several follow-up time points, high repeatability[39] and having all ccIMT measurements conducted by the same technician and with the same device[1,40].

Two relevant limitations are the non-randomised design and that the intervention study arm started 6 months earlier than the control group (same follow-up durations). While our findings in the subgroup with high baseline ccIMT seem robust, residual confounding may have remained, particularly since our trial was non-randomised and, as a community-based study, the study sample was non-homogeneous[12,56]. However, baseline characteristics indicate that both study groups were comparable. Furthermore, seasonal changes may have influenced the results, for example by way of improved vitamin D status during the summer[57]. However, in the subgroup, results of the 6-month[17], 1-year and 1½-year analyses consistently demonstrated lower mean and max ccIMT trajectories in the intervention group. This indicates that these results are not strongly confounded by

seasonal effects. A further limitation is that the relatively small sample size did not allow for a complex statistical analysis of all potential confounders.

Conclusion

The results indicate that healthy lifestyle changes, as they were addressed in the Healthy Lifestyle Community Programme (cohort 2), can effectively reduce mean and max ccIMT if baseline ccIMT is above a value of 0·800 mm, indicating an elevated risk. The observed favourable effect of the intervention in participants with high baseline ccIMT likely constitutes a true deceleration of mean and max ccIMT progression. These results appear to be robust and are likely applicable to similar populations. Although in the present study most participants did not have elevated baseline ccIMT values, we still observed a significant and relevant beneficial intervention effect on ccIMT in the analysis including all participants. While the clinical benefit of the observed effect is likely greater in those with elevated ccIMT, it is equally an advantage to maintain normal ccIMT values within the normal range for as long as possible, if one takes ccIMT as a predictor of CVD risk. In the subgroup of individuals with low baseline ccIMT (<0·800 mm), lifestyle changes alone may not be sufficient or the lifestyle changes observed in the present study may not have been substantial enough to significantly improve ccIMT in the short term. As mean and max ccIMT are secondary end points, the results should be considered exploratory.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/jns.2022.46

Acknowledgements

We would like to thank all participants as well as Alwine Kraatz who took part in the investigation, Norman Bitterlich for his help with statistical analysis, Dominik Zeljko for conducting the ccIMT measurements and Reinhold Gellner for his valuable contributions in the application process for ethical approval and his medical advice during the study.

This work was conducted as part of the project 'münster. land.leben' and was funded by the German Federal Ministry of Education and Research (BMBF) and the Joint Science Conference (GWK) within the programme 'Innovative Hochschule' (H.E., grant number: 03IHS062A). The funders had no role in the study design, data collection, analysis or interpretation, writing of the report or the decision to publish the findings.

C. K.: conceptualisation, methodology, validation, formal analysis, investigation, data curation, writing – original draft, writing – review and editing, visualisation, project administration; S. H.: conceptualisation, methodology, validation, investigation, data curation, writing – review and editing, project administration; R.-M. K.: conceptualisation, methodology, validation, investigation, data curation, writing – review and editing, project administration; C. A.: conceptualisation, methodology, validation, investigation, data curation, writing – review and editing, project administration; D. A.: investigation, data curation, writing – review and editing; N. S.: conceptualisation, methodology, investigation, data curation, writing – review and editing, project administration; A. H.: conceptualisation, methodology, formal analysis, writing – original draft, writing – review and editing, supervision; H. E.: conceptualisation, methodology, investigation, writing – original draft, writing – review and editing, project administration, supervision, funding acquisition.

There are no conflicts of interest.

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2.5. Paper 5 (submitted)

Title: Associations of changes in plant-based diet indices and cardiovascular risk markers (the Healthy Lifestyle Community Programme cohort 3)

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Submitted to: Nutrition Journal

Associations of changes in plant-based diet indices and cardiovascular risk markers (the Healthy Lifestyle Community Programme cohort 3)

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Number of Tables: 3

Number of Figures: 1

Abstract

Background: A large number of cohort studies have shown associations between the plant-based diet index (PDI), healthful PDI (hPDI), and unhealthful (uPDI) and cardiovascular disease (CVD) risk. However, evidence is lacking on whether changes in these diet scores are associated with improved CVD risk markers in short- to medium-term intervention studies.

Methods: An uncontrolled lifestyle intervention was conducted with mostly middle-aged and elderly participants from the general population (n = 115). The intervention consisted of a 16-month lifestyle programme with the strongest focus on moving towards a healthy, largely plant-based diet, in addition to more general recommendations regarding physical activity, stress management, and community support. Dietary adherence was assessed with PDI, hPDI, and uPDI. Parameters were assessed at baseline, 10 weeks, 6 months, and 16 months. Waist circumference and dietary intake could not be assessed at 16 months. Oxidized LDL (oxLDL) was only assessed at baseline and 10 weeks. Data were analysed with paired t-test, Wilcoxon test, and Spearman's rho correlations (two-sided).

Results: From baseline to 10 weeks, dietary scores significantly improved, and body weight, body mass index (BMI), waist circumference, total cholesterol (TC), measured and calculated LDL cholesterol (LDL-C), oxLDL, non-HDL cholesterol, remnant cholesterol (REM-C), glucose, insulin, blood pressure (systolic and diastolic), and pulse pressure significantly decreased, with a small increase in HbA1c and no significant changes in HDL cholesterol, triglycerides, C-reactive protein, resting heart rate (RHR), or REM-C when based on calculated LDL-C. Comparing baseline and 16 months, significant decreases were seen in body weight (-1.8 [-2.6, -1.0] kg), BMI (-0.6 [-0.8, -0.3] kg/m^2), and measured LDL-C (-12.2 [-16.8, -7.7] mg/dl). However, a significant increase in REM-C (when based on measured LDL-C but not when based on calculated LDL-C) was also observed (9.9 [8.0, 11.8] mg/dl). Increases in hPDI consistently correlated with decreased body weight and BMI.

Conclusions: While the intervention was associated with beneficial changes in body weight, BMI, and measured LDL-C, a potential increase in REM-C was observed.

Trial registration: German Clinical Trials Register (reference: DRKS00018846, registered 18 September 2019; retrospectively registered; www.drks.de).

Keywords: plant-based diet; obesity prevention; cardiovascular disease; community-based interventions

Background

The plant-based diet index (PDI), healthful PDI (hPDI), and unhealthful PDI (uPDI) have been widely used since they were first published by Satija et al. in 2016 [1]. To date, these plant-based diet scores have been used mostly in large cohort studies [2]. Very few intervention studies have assessed whether changes in these indices are related to changes in established cardiovascular risk markers [3].

Results from cohort studies support the theory that a higher intake of healthy plant-based foods and concomitant lower intakes of unhealthy plant-based foods (such as added sugars and refined grains) and of animal-source foods are associated with a reduced risk of cardiovascular disease (CVD) [2]. However, evidence is lacking whether associations between changes in these easy to use indices (PDI, hPDI, and uPDI), which are based on food groups, and changes in CVD markers can also be shown in smaller intervention studies [3]. A secondary analysis of the PREVIEW intervention study (a large, 3-year, international, multicentre randomized controlled trial) found beneficial effects of PDI increases on body weight maintenance as well as beneficial effects of higher nut, fruit, and vegetables intakes on several CVD markers [3]. This indicates that the use of PDI, hPDI, and uPDI may be feasible for intervention studies, particularly those including dietary recommendations which are similar to what the hPDI score indicates and measures, i.e. to consume fewer animal-source and unhealthy plant-based foods and more healthy plant-based foods [4, 5].

While current evidence increasingly indicates that moving from a typical Western diet towards a more plant-based dietary pattern can favourably affect body weight [6], cholesterol levels, blood pressure (BP) [7] as well as markers of glycaemic control [8] and inflammation [9], it is uncertain how this knowledge can be applied, as a public health measure, to reach a wider audience of citizens and to encourage and enable them to change their food habits towards a well-planned, healthier, and more plant-based diet [4]. Adopting such a dietary pattern would also be in line with current guidelines for CVD prevention [15, 16] and could potentially reduce health care spending [17].

Against this background, we hypothesized that the community-based lifestyle intervention Healthy Lifestyle Community Programme cohort 3 (HLCP-3) would be effective in improving body weight and other CVD markers in a heterogenous sample of middle-aged and elderly participants (most of whom were clinically healthy). The objective of the study was to test the effectiveness of the intervention in improving CVD risk markers and to assess potential associations between changes in these biomarkers and changes in plant-based diet indices.

Methods

Study design

An uncontrolled lifestyle intervention was conducted between March 2019 and July 2020. Assessments were made at baseline (March 2019), 10 weeks (June 2019), 6 months (October 2019), and 16 months (July 2020). The 16-month measurement time point had originally been planned to take place after 12 months but was delayed due to the COVID-19 pandemic. The time points which had originally been planned for 18 and 24 months were cancelled due to the pandemic. Participants were recruited from the general population in rural northwest Germany.

Participants

Participants were mostly middle-aged and elderly. The only inclusion criteria were the physical and mental ability to take part in the study (self-reported) and to be ≥18 years old. For the intervention, a total of 117 participants were recruited (**Figure 1**).

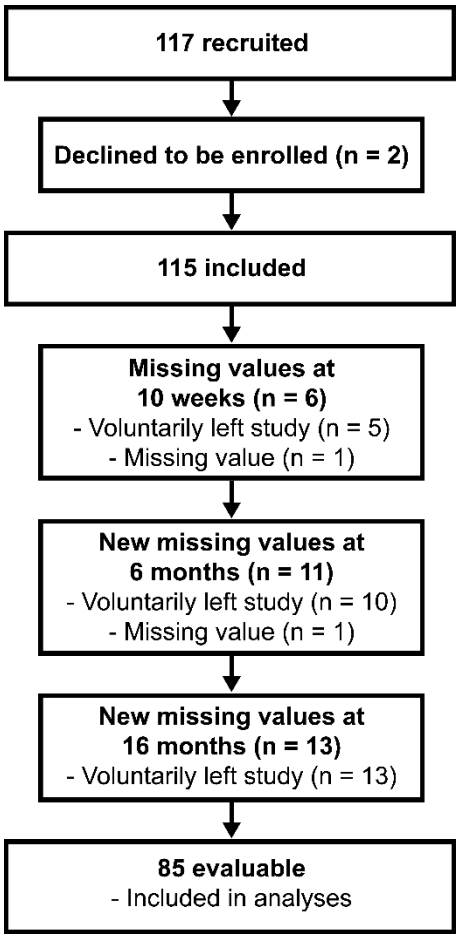

Figure 1. Flow chart of participants through the study

Lifestyle intervention

The lifestyle intervention HLCP-3 was similar to the HLCP-2 intervention, which has been described previously [4, 20]. It consisted of an intensive phase (10 weeks; 15 evening seminars plus 8 workshops) and a less intensive alumni phase (for the remainder of the study; with monthly seminars). Healthy lifestyle recommendations were given regarding diet, physical activity, stress management, and community support, with the strongest emphasis and the most detailed instructions in terms of diet. Dietary recommendations were to move from a typical German dietary pattern towards a more plant-based diet, increasing the intake of plant-based foods categorized as healthy (fruit, vegetables, whole grains, legumes, nuts, seeds, cold-pressed oils such olive oil, rapeseed oil, and linseed oil as well as spices) and decreasing the intake of animal-sources foods (particularly meat but also eggs and high-fat dairy products) as well as reducing the intake of plant

foods categorized as unhealthy (such as added sugars, salt, refined grains, and excessive amounts of alcohol). There was no recommendation to necessarily consume a smaller volume of food or to adhere to a certain macronutrient ratio. Dietary and other lifestyle recommendations were summed up on a laminated sheet of paper which was given to the participants. Apart from diet, the lifestyle recommendations were to engage in regular physical activity (≥30 min/d) and to be more mindful of taking time out to relax and of spending more time with supportive people (for example, friends and family).

Assessment of parameters

All assessments and blood sampling were performed in the morning (6:00 to 11:00 am) after an overnight fast. Laboratory assays have been published previously [4], except for high-sensitivity C-reactive protein (hs-CRP; analysed in serum; spectrometry: immunonephelometry; Siemens BN 2) and oxidized LDL particles (oxLDL; analysed in EDTA plasma; colorimetric: human oxidized LDL ELISA, Novus Biologicals; with a Grifols Diagnostic Triturus). For hs-CRP, participants with an infection or common cold (self-reported at either measurement time point) were excluded from the analyses if at the time point with an infection hs-CRP was above optimal (≥0.8 mg/l [21, 22]). Calculated LDL cholesterol (LDL-C) was calculated with the Friedewald formula. Remnant cholesterol (REM-C) was calculated as total cholesterol (TC) minus measured LDL-C minus HDL cholesterol (HDL-C) [23, 24]. As a sensitivity analysis, REM-C was also calculated based on calculated LDL-C minus (i.e. using calculated instead of measured LDL-C). Non-HDL cholesterol (non-HDL-C) was calculated as TC minus HDL-C. Waist circumference was not assessed at 16 months (due to the COVID-19 protocol). Similarly, food intake could not be assessed at 16 months (due to the COVID-19 pandemic), and oxLDL was only assessed at baseline and 10 weeks (due to the high cost). Semi-quantitative 3-day food protocols (based on portions of different food groups) were used to assess dietary intake. Adherence to dietary recommendations was assessed with the diet scores PDI, hPDI, and uPDI [5] (as described previously [4]). In addition, a post hoc analysis was conducted with a modified hPDI

(hPDImod), which was equivalent to hPDI except that the food groups potatoes, fish, eggs, and dairy were excluded. The rationale for this was that, although hPDI counts these food groups as "negatives", potential adverse cardiovascular effects of potatoes [25–28], fish [29], eggs [30–34], and dairy [27, 35, 36] (compared to plant-based protein sources) are uncertain [37]. In the context of this intervention, increases in PDI, hPDI, and hPDImod as well as a decrease in uPDI are considered desirable. Socio-demographic parameters were assessed using questionnaires.

Study hypotheses

The primary hypothesis of this study was that the intervention would be associated with significantly reduced body weight from baseline to 10 weeks, from baseline to 6 months, and from baseline to 12 months (which was delayed to 16 months). Similarly, secondary hypotheses were that the intervention would be associated with significant reductions in BMI, waist circumference, TC, measured LDL-C, calculated LDL-C, oxLDL, triglycerides (TAG), glucose, HbA1c, insulin, systolic and diastolic BP, and resting heart rate (RHR). HDL-C was assessed exploratively.

Statistical analyses

The sample size was based on our previous study (HLCP-2 study [4]). To evaluate within-group changes, paired t-test was used for normally distributed and Wilcoxon test for non-normally distributed data (two-sided tests). Shapiro-Wilk test was used to assess the data for non-normality, and $p < 0.05$ was defined as describing a non-normal distribution. Bivariate correlations were assessed with Spearman's rho correlations (two-sided). Analyses were based on unimputed data (complete case analysis, CCA). Statistical significance was consistently set at the 0.05 level. All analyses were conducted using IBM SPSS Statistics (Version 27.0. Armonk, NY). Changes are reported as means and 95% confidence intervals.

Results

Baseline characteristics

The flow of participants through the study is shown in **Figure 1**. These participants were included in the analyses (body weight; CCA). Sociodemographic baseline characteristics, smoker status, and prevalence of overweight and obesity are shown in **Table 1**. Baseline values of risk markers are shown in **Table 2**.

Table 1. Baseline characteristics of evaluable participants (CCA; n = 85)		
Women, n (%)		59 (69.4)
Age at baseline, years		58.7 ± 0.9
Overweight, n (%)		48 (56.5)
Obesity, n (%)		17 (20.0)
Smoker status, n (%)	Never	45 (52.9)
	Ex	33 (38.8)
	Smoker	6 (7.1)
	Missing data	1 (1.2)
Marital status, n (%)	Married	73 (85.9)
	Partner (unmarried)	2 (2.4)
	Single (not widowed)	5 (5.9)
	Single (widowed)	4 (4.7)
	Missing data	1 (1.2)
Educational level, n (%)	Lower secondary school	9 (10.6)
	Secondary school	29 (34.1)
	University entrance qualification	20 (23.5)
	University degree	26 (30.6)
	Missing data	1 (1.2)
Age is given as mean ± standard error of the mean. CCA: complete case analysis		

Changes in risk markers from baseline to 10 weeks (intensive phase)

From baseline to 10 weeks, significant decreases were observed for body weight, BMI, waist circumference, TC, measured and calculated LDL-C, oxLDL, non-HDL-C, REM-C, glucose, insulin, systolic and diastolic BP as well as pulse pressure. When REM-C was based on calculated LDL-C, no significant changes were observed. Despite decreases in glucose and insulin, a small increase in HbA1c was observed. No significant changes were observed for HDL-C, TAG, hs-CRP, and RHR.

Changes in risk markers from baseline to 6 months

From baseline to 6 months, significant decreases were observed for body weight, BMI, waist circumference, TC, measured and calculated LDL-C, non-HDL-C, glucose, HbA1c as well as systolic and

diastolic BP. In contrast to the REM-C decrease from baseline to 10 weeks, REM-C significantly increased from baseline to 6 months, which was, however, not observed when REM-C was based on calculated LDL-C. No significant changes were observed for HDL-C, TAG, insulin, hs-CRP, pulse pressure, or RHR.

Changes in risk markers from baseline to 16 months

From baseline to 16 months, significant decreases were observed for body weight, BMI, and measured LDL-C (**Table 2**). However, a small increase was observed for HbA1c. REM-C significantly increased, which was, however, not the case when REM-C was based on calculated LDL-C (**Table 2**). No significant changes were observed for TC, calculated LDL-C, non-HDL-C, HDL-C, TAG, glucose, insulin, hs-CRP, systolic or diastolic BP, pulse pressure, or RHR.

Table 2. 16-month analysis: baseline and follow-up measurements in evaluable participants (CCA)

Parameters	**n**	**Baseline**	**10 weeks**	**6 months**	**16 months**	**Δ(baseline, 16 months)** #	**p-value** §
Body weight, kg	85	80.0 ± 1.6	77.5 ± 1.5	77.2 ± 1.5	78.3 ± 1.6	-1.8 (-2.6, -1.0)	**<0.001** a
BMI, kg/m^2	85	26.7 ± 0.5	25.9 ± 0.5	25.8 ± 0.5	26.1 ± 0.5	-0.6 (-0.8, -0.3)	**<0.001** b
WC, cm	85	92.2 ± 1.5	88.8 ± 1.4	-	-	-3.4 (-4.3, -2.5)	**<0.001** b
TC, mg/dl	80	207.1 ± 4.5	190.6 ± 3.6	198.5 ± 3.9	206.0 ± 3.9	-1.1 (-6.1, 4.0)	0.424 b
LDL-C meas., mg/dl	80	140.4 ± 4.1	128.7 ± 3.6	126.7 ± 3.3	128.2 ± 3.3	-12.2 (-16.8, -7.7)	<**0.001** b
LDL-C calc., mg/dl	79	124.1 ± 3.8	109.5 ± 3.2	117.1 ± 3.2	121.9 ± 3.3	-2.2 (-6.5, 2.2)	0.240 b
oxLDL, pg/ml	73	1481.5 ± 48.5	1123.8 ± 49.3	-	-	-357.6 (-462.9, -252.4)	<**0.001** b
HDL-C, mg/dl	80	63.5 ± 2.0	62.2 ± 1.9	61.5 ± 1.8	64.7 ± 1.9	1.3 (-0.4, 2.9)	0.084 b
non-HDL-C, mg/dl	80	143.6 ± 4.1	128.4 ± 3.6	137.0 ± 3.6	141.3 ± 3.5	-2.4 (-7.0, 2.3)	0.248 b
REM-C, mg/dl	80	3.2 ± 1.1	-0.3 ± 1.2	10.3 ± 0.9	13.1 ± 1.1	9.9 (8.0, 11.8)	<**0.001** b
REM-C based on LDL-C calc., mg/dl	79	19.6 ± 1.0	18.4 ± 0.9	19.7 ± 0.8	18.9 ± 0.7	-0.7 (-2.1, 0.7)	0.817 b
TAG, mg/dl	80	99.7 ± 5.3	94.9 ± 5.2	100.6 ± 4.7	99.5 ± 6.0	-0.2 (-9.7, 9.3)	0.973 b
Glucose, mg/dl	80	102.2 ± 2.0	99.7 ± 1.7	97.2 ± 1.2	101.0 ± 1.4	-1.2 (-4.2, 1.9)	0.632 b
HbA1c, %	80	5.4 ± 0.1	5.6 ± 0.1	5.3 ± 0.0	5.5 ± 0.0	0.1 (0.0, 0.2)	**<0.001** b
Insulin, µU/ml	80	10.0 ± 0.8	9.0 ± 0.7	9.6 ± 0.7	10.1 ± 0.7	0.1 (-0.9, 1.1)	0.586 b

hs-CRP, mg/l (excl. inf.)	59	1.4 ± 0.2	1.5 ± 0.3	1.3 ± 0.2	2.3 ± 0.7	0.9 (-0.5, 2.2)	0.222 b
Systolic BP, mmHg	73	127.4 ± 1.6	117.3 ± 1.5	122.9 ± 1.8	126.7 ± 1.7	-0.7 (-3.7, 2.4)	0.642 b
Diastolic BP, mmHg	73	78.0 ± 1.1	72.8 ± 0.9	75.5 ± 0.9	77.3 ± 1.0	-0.7 (-2.6, 1.1)	0.417 a
PP, mmHg	73	49.4 ± 1.3	44.5 ± 1.1	47.4 ± 1.4	49.5 ± 1.4	0.1 (-2.5, 2.6)	0.949 a
RHR, beats/min	73	66.3 ± 1.1	67.1 ± 1.3	65.3 ± 1.2	64.2 ± 1.1	-2.1 (-4.1, 0.0)	0.062 b

Values are means ± SEM. Changes are expressed as means and 95% CI; CCA: complete case analysis; SEM: standard error of the mean; CI: confidence interval; BMI: body mass index; WC: waist circumference; TC: total cholesterol; LDL-C meas.: measured LDL cholesterol; LDL-C calc.: calculated LDL-C; oxLDL: oxidized LDL particles; non-HDL-C: non-HDL cholesterol; REM-C: remnant cholesterol; HDL-C: HDL cholesterol; TAG: triglycerides; hs-CRP: high-sensitivity C-reactive protein; BP: blood pressure; PP: pulse pressure; RHR: resting heart rate;
except for WC (changes from baseline to 6 months) and oxLDL (changes from baseline to 10 weeks)
§ p-value for within-group comparisons by:
[a] paired t-test (two-sided)
[b] Wilcoxon test (two-sided)

Dietary changes from baseline to 10 weeks (intensive phase)

Dietary scores significantly improved from baseline to 10 weeks: PDI increased by 15 points (5.1 [3.8, 6.5] food portions/d), hPDI increased by 32 points (10.8 [9.1, 12.5] food portions/d), and hPDImod increased by 26 points (8.6 [7.1, 10.1] food portions/d), while for uPDI a decrease of -12 points (-3.9 [-5.3, -2.4] food portions/d) was observed (all: $p < 0.001$; n = 85).

Dietary changes from baseline to 6 months

The improvements in dietary scores from baseline to 6 months were still significant but smaller than those from baseline to 10 weeks. From baseline to 6 months, there were significant increases in PDI (13 points; 4.3 [2.9, 5.7] food portions/d), hPDI (24 points; 7.8 [6.1, 9.6] portions/d), and hPDImod (19 points; 6.2 [4.7, 7.8] food portions/d), while for uPDI a decrease of -7 points (-2.5 [-3.9, -1.0] food portions/d) was observed (all: $p < 0.001$; n = 85).

Bivariate correlations between diet score changes and risk marker changes (baseline to 10 weeks)

There were significant inverse correlations between body weight and BMI changes and changes in PDI, hPDI, and hPDImod (**Table 3**). Changes in TC and measured LDL-C positively correlated with uPDI change. REM-C change inversely correlated with uPDI change (but no significant correlations were

observed when REM-C was based on calculated LDL-C). Insulin change positively correlated with uPDI change and inversely correlated with hPDImod change (**Table 3**). Diastolic BP change weakly but positively correlated with hPDImod change (**Table 3**). No other significant correlations were observed.

Table 3. Bivariate correlations of 10-week changes in PDI, hPDI, uPDI, and hPDImod with other markers

Parameter changes	PDI change		hPDI change		uPDI change		hPDImod change		n
	r	p-value	r	p-value	r	p-value	r	p-value	
Body weight	-0.279	**0.010**	-0.360	**<0.001**	0.178	0.103	-0.342	**0.001**	85
BMI	-0.289	**0.007**	-0.389	**<0.001**	0.197	0.071	-0.372	**<0.001**	85
WC	-0.063	0.568	-0.145	0.186	0.102	0.353	-0.173	0.114	85
TC	-0.067	0.552	-0.068	0.546	0.241	**0.031**	-0.214	0.057	80
LDL-C meas.	-0.037	0.743	-0.043	0.705	0.259	**0.020**	-0.203	0.071	80
LDL-C calc.	-0.040	0.726	0.029	0.797	0.121	0.290	-0.108	0.343	79
oxLDL	-0.073	0.539	-0.097	0.412	0.099	0.404	-0.033	0.781	73
HDL-C	0.014	0.902	-0.097	0.390	0.196	0.082	-0.148	0.189	80
non-HDL-C	-0.099	0.384	-0.044	0.697	0.174	0.123	-0.189	0.093	80
REM-C	0.002	0.986	0.120	0.290	-0.221	**0.049**	0.117	0.303	80
TAG	-0.054	0.637	-0.046	0.684	0.155	0.169	-0.158	0.161	80
Glucose	-0.072	0.523	-0.101	0.375	0.147	0.192	-0.196	0.082	80
HbA1c	0.103	0.361	-0.017	0.880	0.030	0.792	0.028	0.805	80
Insulin	0.061	0.591	-0.164	0.147	0.238	**0.033**	-0.230	**0.040**	80
hs-CRP	-0.070	0.596	-0.080	0.547	-0.012	0.927	-0.152	0.250	59
Systolic BP	0.058	0.625	0.035	0.767	-0.004	0.973	0.065	0.587	73
Diastolic BP	0.210	0.075	0.207	0.079	-0.109	0.360	0.251	**0.032**	73
Pulse pressure	-0.014	0.904	-0.035	0.771	0.005	0.967	-0.019	0.871	73
RHR	-0.069	0.563	-0.145	0.220	0.021	0.862	-0.178	0.132	73

PDI: plant-based diet index; hPDI: healthful PDI; uPDI: unhealthful PDI; hPDImod: modified hPDI; r: Spearman correlation coefficient; BMI: body mass index; WC: waist circumference; TC: total cholesterol; LDL-C meas.: measured LDL cholesterol; LDL-C calc.: calculated LDL-C; oxLDL: oxidized LDL particles; non-HDL-C: non-HDL cholesterol; REM-C: remnant cholesterol; HDL-C: HDL cholesterol; TAG: triglycerides; BP: blood pressure; RHR: resting heart rate;

Bivariate correlations between diet score changes and risk marker changes (baseline to 6 months)

There were significant inverse correlations of changes in body weight and BMI with changes in hPDI and hPDImod (**Table 4**). For changes in TC, measured LDL-C, calculated LDL-C, and non-HDL-C, significant inverse correlations were observed with changes in PDI, hPDI, and hPDImod (**Table 4**). In addition, HDL-C change inversely correlated with changes in hPDI and hPDImod and positively correlated with uPDI change. Systolic BP change inversely correlated with PDI change (**Table 4**). No other significant correlations were observed.

Table 4. Bivariate correlations of 6-month changes in PDI, hPDI, uPDI, and hPDImod with other markers

Parameter changes	PDI change		hPDI change		uPDI change		hPDImod change		n
	r	p-value	r	p-value	r	p-value	r	p-value	
Body weight	-0.191	0.079	-0.265	**0.014**	0.125	0.254	-0.245	**0.024**	85
BMI	-0.201	0.065	-0.278	**0.010**	0.129	0.240	-0.256	**0.018**	85
WC	-0.056	0.608	-0.096	0.380	0.013	0.908	-0.077	0.486	85
TC	-0.256	**0.022**	-0.363	**<0.001**	0.169	0.133	-0.355	**0.001**	80

LDL-C meas.	-0.298	**0.007**	-0.343	**0.002**	0.173	0.124	-0.345	**0.002**	80
LDL-C calc.	-0.327	**0.003**	-0.397	**<0.001**	0.168	0.138	-0.396	**<0.001**	79
HDL-C	-0.169	0.133	-0.243	**0.030**	0.293	**0.008**	-0.260	**0.020**	80
non-HDL-C	-0.237	**0.034**	-0.359	**0.001**	0.119	0.293	-0.337	**0.002**	80
REM-C	0.115	0.308	-0.025	0.826	-0.145	0.201	0.021	0.852	80
TAG	0.128	0.257	-0.006	0.958	-0.005	0.963	0.021	0.851	80
Glucose	-0.168	0.136	-0.149	0.186	0.187	0.096	-0.211	0.060	80
HbA1c	-0.007	0.953	-0.155	0.171	0.147	0.193	-0.122	0.281	80
Insulin	0.113	0.318	-0.058	0.612	0.051	0.652	-0.055	0.629	80
hs-CRP	-0.026	0.844	-0.066	0.618	-0.125	0.346	-0.047	0.725	59
Systolic BP	-0.234	**0.047**	-0.072	0.545	-0.022	0.853	-0.132	0.267	73
Diastolic BP	-0.190	0.107	-0.145	0.221	0.068	0.567	-0.100	0.398	73
Pulse pressure	-0.134	0.257	0.031	0.797	-0.103	0.387	-0.040	0.738	73
RHR	0.007	0.953	-0.145	0.220	-0.020	0.868	-0.076	0.521	73

PDI: plant-based diet index; hPDI: healthful PDI; uPDI: unhealthful PDI; hPDImod: modified hPDI; r: Spearman correlation coefficient; BMI: body mass index; WC: waist circumference; TC: total cholesterol; LDL-C meas.: measured LDL cholesterol; LDL-C calc.: calculated LDL-C; non-HDL-C: non-HDL cholesterol; REM-C: remnant cholesterol; HDL-C: HDL cholesterol; TAG: triglycerides; BP: blood pressure; RHR: resting heart rate;

Bivariate correlations between changes in food intake (food group level) and risk marker changes

Correlations of biomarker changes with changes of dietary intake at the food group level largely confirmed the associations that were observed at the diet score level. The following correlations with correlation coefficients of r ≥0.3 and p ≤0.001 were observed: from baseline to 10 weeks, changes in legume intake inversely correlated with changes in body weight (r = -0.346) and BMI (r = -0.351; n = 85), while changes in the intake of sweets and desserts positively correlated with changes in TC (r = 0.399), and HDL-C (r = 0.371). TAG changes inversely correlated with changes in vegetable intake (r= -0.398; n = 80; the same was true for REM-C based on calculated LDL-C; r = -0.383; n = 79). From baseline to 6 months, changes in nut intake inversely correlated with changes in systolic BP (r = -0.394). No other highly significant (p ≤0.001) correlations at the food group level were observed.

Discussion

The present study had the aim of assessing potential effects of the HLCP-3 intervention on body weight and other CVD risk markers in a sample of mostly middle-aged and elderly individuals in rural northwest Germany (most of whom were clinically healthy) and of assessing potential correlations between changes dietary scores and risk markers. During the intensive phase of the intervention (baseline to 10 weeks), the majority of parameters significantly improved, including body weight,

BMI, waist circumference, TC, measured and calculated LDL-C, oxLDL, non-HDL-C, REM-C, glucose, and insulin. While most of these parameters were still significantly decreased at 6 months, this was not the case for insulin and REM-C.

Contrary to the significant REM-C decrease from baseline to 10 weeks (-3.5 mg/dl), a significant REM-C increase was observed from baseline to 6 months (+7.1 mg/dl), and this increase was even clearer when looking at changes from baseline to 16 months (+9.9 mg/dl; **Table 2**). This unexpected finding is in contrast to the significant 1-year REM-C decrease (-3 mg/dl) which we had observed in our previous study, one year earlier, with a nearly identical intervention programme (HLCP-2 study) [4]. However, like in the present study, in the HLCP-2 study we also observed considerable fluctuations of REM-C levels at the four measurement time points (baseline: 8 mg/dl; 10 weeks: 5 mg/dl; 6 months: 2 mg/dl; 12 months: 5 mg/dl) [4]. This could suggest that REM-C levels may be prone to considerable fluctuations. Should this be the case, REM-C would constitute a less reliable parameter to assess in small intervention studies. Furthermore, in the present study, correlations between REM-C changes and dietary changes could not explain the observed REM-C increase (**Table 3** and **Table 4**). The PREDIMED study, a large randomized controlled trial with a traditional Mediterranean diet in Spain, found REM-C to be a highly relevant CVD marker in overweight/obese subjects at high CVD risk, with high REM-C (≥30 mg/dl) being associated with an increased risk of major adverse cardiovascular events [38]. However, in the PREDIMED analysis, REM-C was based on calculated LDL-C (Friedewald formula; LDL-C was not measured) [38]. In contrast, in the present study, REM-C was based on measured LDL-C [38]. The differences between REM-C based on calculated vs. measured LDL-C is evident in **Tables 2**, **3**, and **4**. While it has been suggested that the use of measured LDL-C (rather than calculated LDL-C) makes estimating REM-C more precise [38], there may still be considerable imprecision. REM-C changes are subject to potential measurement inaccuracies in three different markers (TC, HDL-C, and LDL-C) at two different time points. In contrast, REM-C based on calculated LDL-C is equivalent to TAG/5 (in mg/dl) [23, 24], as combining the REM-C and Friedewald formulas eliminates the other parameters (TC and HDL-C) from the equation. Thus, REM-C based on calculated

LDL-C is subject to measurement error in only one parameter (TAG). Our study illustrates that REM-C based on measured LDL-C and REM-C based on calculated LDL-C are not necessarily similar and should be considered two separate parameters. Furthermore, new assays are now available to directly measure REM-C, and while directly measured REM-C appears to strongly correlate with calculated REM-C, they are not equivalent and may not have the same relevance to CVD risk [23].
In addition, the PREDIMED analysis included a large number of participants (n = 6901) [38], while our study sample was relatively small, potentially making measurement imprecisions more relevant [39]. Thus, one may hypothesize that analytic and biologic variation [39] could partly explain our unexpected finding that REM-C first decreased (10 weeks) and then increased (6 months; 16 months), while other cholesterol markers (TC, measured and calculated LDL-C, and non-HDL-C) were decreased (significantly or non-significantly) at all follow-up time points (**Table 2**). Although TC and LDL-C are the more established CVD markers (compared to REM-C), recent evidence from cohort [40] as well as Mendelian randomization studies indicates a high clinical relevance of REM-C [41]. However, a differentiation of REM-C subfractions may be important as the associations of different REM-C subfractions with CVD risk appear to be heterogenous [41]. While, in the fasting state, "actual REM-C" (REM-C without lipoprotein(a) cholesterol) can be defined as the sum of cholesterol in very-low density (VLDL) and intermediate-density lipoproteins (IDL) [40], to date, there appears to be no consensus definition of REM-C [42].
Higher REM-C levels appear to be associated with a more strongly activated immune system and a higher white blood cell count (WBC) [43], while largely plant-based diets appear to be associated with a lower WBC [44] (although this may be influenced by the fat content of the diet [45]). Cholesterol-enriched remnant particles can enter the intima layer of arteries and thus contribute to atherosclerosis [23], and higher REM-C levels have been associated with higher common carotid intima-media thickness (ccIMT) [46]. Accordingly, in the HLCP-2 study we showed that our lifestyle intervention appeared to decrease REM-C and to also beneficially affect ccIMT (particularly in those with high baseline values [20]).

Non-HDL-C includes REM-C as it is composed of LDL-C, REM-C, including lipoprotein(a) cholesterol [23]. Thus, non-HDL may be an alternative way of incorporating REM-C into CVD risk assessment [23]. The calculation of non-HDL-C relies on only two parameters (TC and HDL-C; without LDL-C), and non-HDL-C appears to be reliable in both the fasting and non-fasting state [23]. Furthermore, it has been suggested that cholesterol levels in the non-fasting state may be more relevant to CVD risk [23, 40]. Thus, future trials should assess whether non-HDL-C may be a more reliable parameter for small intervention studies than calculated REM-C. Non-HDL-C appears to be a superior CVD risk marker to LDL-C particularly in individuals with non-fasting blood samples, high TAG, and/or low LDL-C (≤70 mg/dl) [23].

In the present study, we observed that a lower intake of sweets and desserts, lower uPDI as well as higher hPDI and hPDImod were associated with lower HDL-C. This confirms the observation in our previous study (HLCP-2) [4] as well as results from the literature that some plant-based diets [52] as well as higher intakes of alpha-linolenic acid (ALA) [53] or whole grains [54] appear to be associated with small decreases in HDL-C. However, the clinical relevance of this is uncertain [55] as the quantity of HDL-C may be less important as a determinant of CVD risk than HDL function [56].

In the present study, no consistent changes in markers of glycaemic control (glucose, HbA1c, or insulin) were observed. Although the relevance of the small increase in HbA1c from baseline to 16 months (5.4 to 5.5%; **Table 2**) seems uncertain and may be due to measurement error, it can be hypothesized that short-term psychological stress may have a small adverse effect on HbA1c [57]. It should also be noted that the 16-month time point (July 2020) was already after the start of the COVID-19 pandemic (which may have been associated with increased stress for the participants) [58].

While some plant-based diets are associated with lower hs-CRP values [59], and our previous study (HLCP-2) demonstrated a significant 1-year decrease in hs-CRP (unpublished data), the present study showed no significant changes in hs-CRP. A particular focus on foods with anti-inflammatory effects (such as certain spices [60]) may be useful for future studies [61].

The improvements in systolic and diastolic BP as well as pulse pressure observed in the present study at 10 weeks were not maintained at 16 months (**Table 2**). In contrast, our previous study (HLCP-2) observed significant decreases in BP and pulse pressure from baseline to 1 year in the intervention group (which was not significantly different from control, however) [4]. Similarly, while results from our previous study (HLCP-2) showed a 1-year RHR decrease (-4 bpm) [4], the present study could not confirm this result (**Table 2**)

With a mean baseline BMI of 27 kg/m^2, the present study successfully reduced body weight, BMI, and waist circumference, which could be shown at all time points (**Table 2**; waist circumference was not assessed at 16 months), although these favourable changes were smaller than in our previous study (HLCP-2), with a similar baseline mean body weight and BMI in both studies [4].

While some authors have suggested that a very strong focus on plant-based foods with a low degree of processing and a concomitant strongly reduced intake of animal-source foods is an optimal way of improving body weight and CVD risk [64], more studies are needed to ascertain this and to assess what such an optimized dietary pattern should look like [29, 65].

The present study shows that most of the parameters assessed were improved at 10 weeks and 6 months but that at 16 months these improvements could only be shown for body weight, BMI, and measured LDL-C. This lack of maintenance may have been due to decreasing compliance over time. While our data indicate that in the present study, adherence to the dietary recommendations given was similar compared to our previous study (HLCP-2) [4], dietary data were not available for the 16-month time point.

Strengths and limitations

A strength of the present study is the assessment of a variety of CVD risk markers and multiple measurement time points. However, multiple assessments also increase the risk of significant findings. A strong limitation of our study is that it is an uncontrolled study. Thus, causality cannot easily be inferred. Events unrelated to the intervention, such as seasonal influences [66, 67], may have

influenced the results. The COVID-19 pandemic may also have influenced the results of the 16-month time point, although the number of new COVID-19 cases in Germany appears to have been relatively low at the time (July 2020) [68]. Dietary intake was assessed with a 3-day dietary record at each time point, which we had tested in our previous study (HLCP-2) [4]. However, the dietary questionnaires were not validated, and some misreporting of food intake is possible. In order to minimize this imprecision, the focus of dietary evaluation was on the food scores (PDI, hPDI, and uPDI).

Conclusions

The present study (HLCP-3) was able to replicate the findings of our previous study (HLCP-2) in terms of significant improvements in dietary adherence, body weight, BMI, and waist circumference (but not RHR or REM-C) at the end of the study [4]. Increases in hPDI consistently correlated with decreased body weight and BMI. In contrast to a significant 1-year decrease in REM-C in the HLCP-2 study, the present study showed a decrease in measured LDL-C (-12 mg/dl) with a concomitant increase in REM-C (+10 mg/dl) at 16 months. The suitability of REM-C for small intervention studies should be assessed in future studies. The present study indicates that further optimization is needed to make the transfer of plant-based dietary recommendations and practices to the general population safe and effective in terms of CVD prevention.

List of abbreviations

BMI: body mass index

BP: blood pressure

CCA: complete case analysis

ccIMT: common carotid intima-media thickness

CI: confidence interval

CVD: cardiovascular disease

HDL-C: HDL cholesterol

HLCP-2: Healthy Lifestyle Community Programme, cohort 2

HLCP-3: Healthy Lifestyle Community Programme, cohort 3

hPDI: healthful PDI

hPDImod: modified hPDI

hs-CRP: high-sensitivity C-reactive protein

LDL-C calc.: calculated LDL-C

LDL-C meas.: measured LDL cholesterol

non-HDL-C: non-HDL cholesterol

oxLDL: oxidized LDL particles

PDI: plant-based diet index

PP: pulse pressure

REM-C: remnant cholesterol

RHR: resting heart rate

SEM: standard error of the mean

TAG: triglycerides

TC: total cholesterol

uPDI: unhealthful PDI

WBC: white blood cell count

Declarations

Ethics approval and consent to participate

All subjects provided written informed consent before participating in the study. The study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the ethics committee of the Medical Association of Westphalia-Lippe and of the University of Münster (Münster, Germany; reference: 2019-142-f-S; approved 12 March 2019).

Consent for publication

Not applicable

Availability of data and materials

The dataset used during the current study is available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was conducted as part of the project “münster.land.leben” and was funded by the German Federal Ministry of Education and Research (BMBF) and the Joint Science Conference (GWK) within the programme “Innovative Hochschule” (grant number: 03IHS062A). The funders had no role in the study design, data collection, analysis, or interpretation, writing of the report, or the decision to publish the findings.

Authors' contributions

CK: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, writing – review and editing, project administration; **DA:** conceptualization, methodology, investigation, data curation, writing – review and editing; **CA:** conceptualization, methodology, investigation, data curation, writing – review and editing, project administration; **RK:** conceptualization, methodology, investigation, data curation, writing – review and editing, project administration; **NS:** conceptualization, methodology, investigation, data curation, writing – review and editing, project administration; **AH**: methodology, formal analysis, writing – original draft, writing – review and editing, supervision; **HE**: conceptualization, methodology, investigation, writing – original

draft, writing – review and editing, project administration, supervision, funding acquisition. All authors read and approved the final manuscript.

Acknowledgements

We would like to thank all participants, Alwine Kraatz who took part in the investigation, Dr Norman Bitterlich for his help with statistical analysis, and Dr Reinhold Gellner for his valuable contributions in the application process for ethical approval and his medical advice during the study.

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3. General discussion

The aim of this dissertation was to test the effectiveness of the lifestyle intervention HLCP-2 by conducting a community-based controlled trial over the course of 1 year (originally intended to be 2 years). The lifestyle intervention can be considered effective if the results indicate significant improvements in CVD risk markers compared to control.

In order to demonstrate potential beneficial effects on the arterial wall structure, ccIMT was assessed (**Paper 1** and **Paper 4**). To demonstrate potential effects on circulating biomarkers, cholesterol levels (TC, LDL-C, non-HDL-C, REM-C, HDL-C), TAG, glucose, HbA1c, and insulin (**Paper 2**) as well as the inflammatory markers hs-CRP, Hcy, and Apn (**Paper 3**) were assessed. To demonstrate potential effects on anthropometric and vital parameters, the markers body weight, BMI, waist circumference, systolic and diastolic BP, pulse pressure, and RHR were assessed (**Paper 2**). In addition, dietary intake was assessed with questionnaires in order to correlate changes in CVD markers with changes in dietary scores (PDI, hPDI, and uPDI). Sociodemographic parameters were assessed to be able to compare groups and to adjust for potential confounders (especially age and sex). Furthermore, the effectiveness of the HLCP-3 lifestyle intervention was assessed in a follow-up cohort by means of an uncontrolled trial (starting one year after the HLCP-2 study; **Paper 5**). Participants in the HLCP-2 and HLCP-3 studies were mostly middle-aged and elderly individuals, recruited from the general population in rural northwest Germany. The results of this dissertation are thus presented in five scientific articles (**Chapter 2**).

3.1. ccIMT (Paper 1 and Paper 4)

Effectiveness of the HLCP-2 lifestyle programme in improving ccIMT

Results from **Paper 1** and **Paper 4** indicate a beneficial effect of the intervention on ccIMT, with a deceleration (i.e. lower values) of ccIMT progression in the intervention group (compared to control; **Paper 4**).

After 6 months and with all participants included, results from **Paper 1** demonstrate only a small and statistically non-significant difference in mean ccIMT change between the two groups, favouring the intervention group (intervention: 0.018 mm; control 0.025 mm; between-group difference: $p = 0.078$). However, the subgroup analysis of participants with high baseline mean ccIMT (≥0.800 mm) demonstrated a mean ccIMT decrease (-0.023 mm) in the intervention and an increase in the control group (0.041 mm) within 6 months (between-group difference: $p = 0.004$; adjusted for baseline), indicating that already after 6 months beneficial effects on ccIMT may be possible in individuals with high baseline ccIMT (**Paper 1**).

The 1-year analysis, presented in **Paper 4**, confirmed the results of the 6-month analysis (**Paper 1**). The 1-year analysis demonstrated that with all participants included the non-significant between-group

difference observed at 6 months became significant at 1 year, indicating lower (more favourable) mean ccIMT change values in the intervention group (intervention: 0.026 mm; control: 0.045 mm; between-group difference: p = 0.022). Furthermore, in the subgroup analysis (participants with high baseline ccIMT), mean ccIMT decreased in the intervention (-0.016 mm) and increased in the control group (0.065 mm), with a between-group difference of -0.051 mm (p <0.001; adjusted for baseline), favouring the intervention group.

Consequently, the study's hypothesis that the HLCP-2 intervention would beneficially affect ccIMT in the medium term (within 1 year) could be confirmed. It should be noted that the observed beneficial and clinically meaningful effect was considerably more evident in the subgroup analyses, while the beneficial effect appears less certain in the analyses with all participants included. Based on these results, it can be concluded that the healthy lifestyle recommendations given in the HLCP-2 intervention appear to have beneficially affected the arterial wall structure of the common carotid arteries, particularly in participants with higher baseline mean ccIMT, i.e. those who are at higher CVD risk. The parameter ccIMT change has been confirmed to be a relevant marker of CVD event risk [126] and higher ccIMT values reflect early structural deterioration of the carotid artery wall [121]. In addition, an increased atherosclerotic burden in the carotid arteries is associated with atherosclerosis progression in other vascular territories [121]. Therefore, these results permit the hypothesis that the beneficial intervention effect on ccIMT observed in the HLCP-2 study may be associated with beneficial effects not just in the common carotid arteries but in the arterial tree in general (although this could not be assessed in the study).

The results do not permit conclusions as to whether the observed favourable effect on ccIMT was primarily mediated by effects on atherosclerotic wall changes or on medial hypertrophy [124]. Although BP moderately decreased from baseline to 1 year, this occurred in both groups (intervention and control), with no significant between-group differences (**Paper 2**). Pulse pressure, which may impact ccIMT [453], significantly decreased only in the intervention group, but this result was not significantly different compared to control (**Paper 2**). In contrast, RHR significantly decreased in the intervention group compared to control (**Paper 2**). One may, therefore, hypothesize that the beneficial effect on ccIMT may be related to the observed beneficial effect on RHR. However, a significant correlation between RHR changes and ccIMT changes could not be demonstrated (**Paper 4**). To date, evidence from intervention studies for an association between RHR and ccIMT is lacking [454, 455]. However, a cohort study with generally healthy, middle-aged and elderly individuals in China (n = 1157) found that each 10 beats/min increase in RHR was associated with a 47% increased risk of having elevated combined carotid IMT values (a combined marker of ccIMT, bifurcation IMT, and internal carotid IMT) [456].

Associations between changes in ccIMT and diet in the HLCP-2 study

In terms of diet, only a weak positive correlation between max ccIMT change and uPDI change (r = 0.240; p = 0.008; **Paper 4**) could be shown, which indicates an association of a more frequent intake of unhealthy plant-based foods (including added sugars and refined grains) with increased max ccIMT. Again, this result was clearer in the subgroup with high baseline ccIMT (**Paper 4**). In a post hoc analysis, it was possible to demonstrate that changes in a modified hPDI (equivalent to hPDI except for excluding the food groups potatoes, fish, eggs, and dairy) inversely correlated with max ccIMT change (r = -0.229; p = 0.012), which was confirmed in the subgroup analysis (r = -0.460; p = 0.012; **Paper 4**), indicating that increased intakes of healthy plant-based foods and/or a decreased meat intake may also have been partly responsible for the observed beneficial effects on ccIMT.

Thus, it can be concluded that the results provide at least some evidence for an association between the observed dietary changes and the beneficial effects on ccIMT. Potential reasons for the lack of a demonstrable effect of hPDI improvements on mean or max ccIMT may include the following: the three-day food protocols used in the study cannot precisely reflect actual dietary changes [457], and the HLCP-2 study may have been underpowered for demonstrating such associations [78]. Furthermore, the dietary scores hPDI and uPDI categorize the following plant foods as unhealthy: fruit juices, refined grains, potatoes, sugar-sweetened beverages as well as sweets and desserts (including chocolate) [78]. However, recent meta-analyses indicate that moderate fruit juice intake [321, 366, 458–460] and the intake of non-fried potatoes [331, 461] may not be associated with an increased CVD risk. Similarly, although the effect of individual foods is likely small, recent meta-analyses indicate a potentially beneficial effect of chocolate (especially dark chocolate) intake on CVD risk markers [462–466] and CVD risk [467, 468]. While the sugar in chocolate may have adverse effects on CVD risk [467], there may be concomitant beneficial cardiovascular effects of cocoa [462, 465, 467]. While more studies are needed, recent meta-analyses also suggest that it is uncertain whether refined grain intake (especially compared to animal-source food intake) is associated with adverse or neutral effects on CVD risk [331, 469–471]. However, given the observed association of decreased uPDI with decreased max ccIMT (**Paper 4**), our results confirm that an emphasis on reducing dietary intake of added sugars and refined grains in dietary recommendations appears to be justified [451].

While the PDI, hPDI, and uPDI scores are widely used and their associations with CVD risk has been repeatedly shown in cohort studies [66], such associations are more difficult to demonstrate in relatively small intervention studies, and our approach of using these dietary scores in the context of a small intervention study was novel (also see **Chapter 3.4.**) [67].

ccIMT: Results from other controlled dietary interventions

The HLCP-2 study is novel in that it assessed the effect of a lifestyle intervention, including a plant-based diet and including a no-intervention control group, on ccIMT in a community-based setting with participants from the general population (most of whom were clinically healthy). The only other lifestyle trial which advocated a strong reduction in animal-source foods was conducted with coronary heart disease patients and was not able to demonstrate a significant intervention effect on ccIMT [50] (see **Appendix 1: Table 1**). The results of the present study indicate that not just the traditional Mediterranean diet, which includes specific foods such as olive oil, fish, red wine, and *sofrito* (a tomato-based sauce that typically contains olive oil, onion, and garlic) [472, 473] but also other dietary patterns which are largely based on healthful plant-based foods (fruit, vegetables, legumes, whole grains, nuts, and seeds) may slow down age-related unfavourable arterial wall changes (**Paper 4**; but further research is needed to confirm this) [172].

The HLCP-2 study appears to be the only controlled lifestyle/dietary intervention trial to date (irrespective of the dietary pattern recommended) which has shown a significant intervention effect on ccIMT in a study sample of mostly clinically healthy participants from the general population [161, 163]. Although the results from the HLCP-2 study confirm that it appears to be more probable to detect a significant effect on ccIMT when recruiting only individuals at higher CVD risk (as demonstrated by the subgroup analyses with participants with high baseline ccIMT; **Paper 1** and **Paper 4**), it is crucially important to already initiate CVD prevention measures in individuals who are not yet at high risk of CVD events [474, 475]. Although the HLCP-2 study indicates that it is possible to demonstrate a beneficial intervention effect on ccIMT in a sample of mostly middle-aged individuals most of whom are clinically healthy, the beneficial intervention effect in the group including all participants was modest and was attenuated at 1½ years (**Paper 4**). It can be hypothesized that larger beneficial effects may be possible with more considerable dietary (and other lifestyle) changes [318]. Thus, future studies may opt to preferentially recruit participants at higher CVD risk [170, 174] and/or seek out highly motivated individuals who are willing to commit to more intensive lifestyle changes.

Results from the HLCP-2 study appear to be confirmed by an uncontrolled trial with a healthy plant-based diet in the United States, conducted with participants at risk for or with coronary artery disease, which observed a non-significant mean ccIMT decrease (-0.011 mm) within 1 year and demonstrated that higher adherence to a healthy plant-based diet was associated with favourable effects on ccIMT (although not statistically significant) [476].

ccIMT: ideal follow-up duration

Typically, the age-related increase of ccIMT is a relatively slow process [117]. Consequently, it has been suggested that controlled trials assessing the intervention effect on ccIMT should have a minimum

sample size of 200–300 participants per group, with a follow-up duration of 2 years [117, 477]. However, the HLCP-2 study demonstrates that smaller sample sizes and shorter follow-up durations can be sufficient to detect significant intervention effects. This confirms the results of a small number of previous controlled lifestyle [160, 162] and diet-only [51, 174] trials which were successful at demonstrating a beneficial intervention effect on ccIMT. Furthermore, longer follow-up durations may pose the risk of insufficient long-term compliance to the lifestyle recommendations given [478–480]. The HLCP-2 study showed that adherence to dietary recommendations started to decline after the intensive phase of the study (after 10 weeks; **Paper 2**) and that the beneficial intervention effect on ccIMT was significant at 1 year but became non-significant at 1½ years. Previous studies have found that self-efficacy, high motivation to change one's diet, and perceived competence to follow the recommendations were predictors of improved long-term maintenance of healthy eating [478]. Furthermore, perceived social support and a sense of coherence have been shown to improve long-term adherence to lifestyle changes [481]. These components were also included in the HLCP-2 intervention (**Paper 1**) [482].

The effect of exercise on ccIMT

Not many controlled trials assessing the effect of exercise on ccIMT have been conducted (see **Appendix 3: Table 3**). Out of five identified trials [483–488], only one trial (published in two articles [486, 487]) was able to show a significantly more favourable effect on ccIMT in the intervention group compared to control, while one other trial was able to show a beneficial effect (only) in a subgroup analysis of patients without identified carotid plaques [483].

When conducting exercise interventions, it should be taken into account that high-intensity endurance exercise can lead to cardiac remodelling (including a larger myocardial mass, cardiac volume, and left ventricular mass; "athlete's heart" [157, 489]), and can also result in changes in the vascular structure [490]. It is uncertain whether these alterations constitute pathological changes or solely non-pathological adaptations to exercise [491]. On the other hand, high-intensity endurance athletes often demonstrate beneficial vascular changes (such as lower aortic stiffness, as assessed by carotid-femoral pulse-wave velocity, and higher carotid compliance [157]), with no apparent difference in resting cardiac function, compared to (more moderately) physically active controls [157]. While one can hypothesize that high-intensity endurance exercise (for example, ultramarathon running) may potentially increase ccIMT [491], only a few studies have been conducted in this area, and these studies do not indicate that high-intensity endurance exercise increases ccIMT [157, 489, 492–495]. While studies indicate that resistance exercise may adversely affect carotid arterial compliance and arterial stiffness [496–499], current evidence does not indicate that resistance training increases ccIMT [483, 486, 496].

It should also be taken into account that relevant acute effects of exercise on ccIMT are possible. For example, Bianchini et al. (2019) demonstrated a significant mean ccIMT decrease from 0.537 mm to 0.495 mm, in 28 male triathletes participating in an Ironman triathlon competition, within 20 min after the competition [500]. Studies are needed to replicate such effects in a larger number of participants and to assess whether such effects are true effects (rather than due to measurement error), whether these effects are maintained for a longer period of time (for example, 24 hours after the competition) and whether these effects can also occur with lower-intensity exercise.

Considerations regarding ccIMT assessment in future studies

Current evidence indicates that ccIMT is a relevant predictor of CVD event risk [126] as well as a parameter for which repeatability is sufficiently high (at the group level) [126, 202]. Previous evidence had suggested that repeatability for ccIMT is higher compared to IMT in other segments of the carotid arteries (carotid bulb or internal carotid artery) [202]. However, recent evidence indicates that IMT of the carotid bulb (bulb-IMT) may be more closely related to incident CVD events (stroke or myocardial infarction) than ccIMT [141]. Consequently, it should be investigated if, when measuring bulb-IMT, an equivalent (or higher) repeatability can be achieved compared to ccIMT. If this could be confirmed, then bulb-IMT may be a superior parameter (including for intervention trials) compared to ccIMT. However, the Mannheim consensus suggests that interindividual variability for bulb-IMT is high and that high bulb-IMT may mostly be due to non-pathological interindividual anatomic variation [124]. Furthermore, a meta-analysis of three Swedish cohorts by Lind et al. (2020) showed that ccIMT and bulb-IMT were differently associated with a variety of plasma proteins considered candidate CVD risk markers [141]. However, after adjusting for major CVD risk factors, both ccIMT and bulb-IMT were positively associated with MMP-12, which itself is positively associated with the occurrence of carotid atherosclerotic plaque and CVD event risk [141]. In addition, only ccIMT (and not bulb-IMT) was associated with N-terminal pro-B-type natriuretic peptide (NT-proBNP), which is a well-known CVD risk marker [141] as well as a potential risk marker for kidney disease [501, 502] and adverse COVID-19 outcomes [503]. Similarly, only ccIMT (and not bulb-IMT) was associated with hepatocyte growth factor (HGF) [141], an inflammatory marker which is frequently increased in individuals with obesity, insulin resistance, or liver disease [504] and is associated with increased CVD risk [505], including the risk of heart failure [506]. It should be noted that the association between HGF and ccIMT may vary dependent on CD34-positive cell levels [507]. Furthermore, it has been shown that aerobic exercise can beneficially affect (decrease) levels of NT-proBNP [508] and possibly HGF [509]. Lowering sodium intake may also lower NT-proBNP [510], and it has been suggested that coffee consumption (compared to no coffee) may also be associated with lower NT-proBNP [511].

In terms of plant-based diets, it should also be taken into account that these dietary patterns should be well-planned (particularly in the long term) and should include adequate sources of potentially critical nutrients (such as vitamin B12, vitamin D, calcium, zinc, and iodine), which are of relevance for CVD prevention as well as general health [73]. For example, subclinical hypothyroidism, which may be related to both iodine deficiency or excess [512] and which is a relatively common condition [513], appears to be associated with increased ccIMT [514]. Similarly, vitamin B12 deficiency is associated with increased ccIMT [196, 379]. It should also be noted that, despite the known differences between ccIMT and IMT in other carotid segments (and although the Mannheim consensus states that IMT from different sites should be documented separately [124]), many peer-reviewed articles (including meta-analyses) still only report results for an unspecified carotid IMT [209, 515].

3.2. Obesity, established CVD risk markers, REM-C, and RHR (Paper 2 and Paper 5)

Results from **Paper 2** demonstrate that, after 1 year, body weight, BMI, waist circumference, REM-C, and RHR were significantly improved (decreased) to a clinically relevant extent. In contrast, 1-year changes of glucose, HbA1c, cholesterol (apart from REM-C), TAG, insulin, BP, and pulse pressure were very small or non-significant (compared to control). Thus, it can be concluded that the HLCP-2 study could successfully demonstrate a medium-term improvement in its primary parameter (body weight) and at least some other important CVD risk markers.

A recent meta-analysis of randomized controlled trials found that higher viscous fibre intake reduced mean body weight, BMI, and waist circumference independently of calorie restriction [516], and this may have been one mechanism which contributed to the results of the HLCP-2 study. When conducting similar weight loss intervention trials it should be noted that a large number of widely used medications can have adverse effects on weight gain and body fat distribution [517]. This appears to be the case particularly with antipsychotic and antiretroviral drugs (used in the treatment of HIV infection) but also with antidepressants, anti-seizure drugs, beta-blockers, and corticosteroids [517].

In terms of different dietary approaches, a recent meta-analysis and guideline paper by the European Association for the Study of Obesity documented that a very low-calorie (600–800 kcal/d) ketogenic diet can effectively reduce body weight, BMI, and waist circumference in individuals with obesity [518]. The authors of this guideline paper concluded that this type of dietary regimen may be particularly suitable for individuals with severe obesity and/or comorbidities who urgently require substantial weight loss and that this regimen should be conducted under medical surveillance [518]. After the weight target is achieved, long-term lifestyle strategies (including dietary counselling and physical activity) are recommended [518]. The very low-calorie ketogenic diet approach often includes meal replacements including protein isolates (for example, whey, pea, or soya protein) and meals based on animal-source foods (meat, fish, or eggs) as well as, potentially, synthetic amino acid

supplements [518]. Multivitamin and multimineral supplementation is also recommended by the authors [518].

Established CVD risk markers

While the HLCP-2 study showed a significant decrease in REM-C (**Paper 5**) and the HLCP-3 study a significant decrease in measured LDL-C (**Paper 5**), both studies indicate that the lifestyle programme was ineffective at significantly improving TC, markers of glycaemic control (glucose, HbA1c, and insulin), or BP in the medium-term. Recent meta-analyses indicate that certain plant-based diets (such as vegetarian diets [519–521], the traditional Mediterranean diet [522, 523], and the DASH diet [524, 525]) are associated with improved markers of glycaemic control. Thus, it can be hypothesized that the dietary (and exercise) improvements may not have been substantial enough to improve these markers or that the significant favourable lifestyle changes which were observed in both studies (**Paper 2** and **Paper 5**) may have to be sustained for a longer period of time to beneficially affect these markers [309].

REM-C

Recent evidence confirms the relevance of REM-C and indicates that higher levels of REM-C are associated with increased arterial stiffness [526] and an increased risk of NAFLD [527], chronic kidney disease [528], diabetes [529], peripheral artery disease [530], coronary artery disease [531], myocardial infarction, and ischaemic stroke [530]. The association between elevated REM-C levels and increased atherosclerosis appears to be independent of LDL-C and apolipoprotein B levels [532]. Furthermore, recent evidence indicates that, in individuals with type 1 diabetes, REM-C levels predict progression of diabetic nephropathy and retinopathy [533]. However, it should be pointed out that the formula for calculating REM-C (TC minus LDL-C minus HDL-C) includes HDL-C and thus assumes that the quantity of HDL-C is an important predictor of CVD risk, when recent studies point out that, rather than total HDL-C quantity, HDL structure and function appear to be key factors for the anti-atherogenic effects of HDL particles [534].

While in the HLCP-2 study a significant favourable intervention effect on REM-C was observed (**Paper 2**), the follow-up uncontrolled trial (HLCP-3 study) showed a REM-C decrease during the intensive phase (first 10 weeks), followed by an unexpected increase in REM-C (although measured LDL-C remained considerably and significantly reduced; **Paper 5**). This potentially adverse effect on REM-C in the HLCP-3 study was only observed when REM-C was based on measured LDL-C but not when REM-C was based on calculated LDL-C (both are standard ways of estimating REM-C [534]). In terms of REM-C changes, measurement error may play a considerable role (as described in **Paper 5**). Current evidence and results from the HLCP-2 and HLCP-3 studies indicate that REM-C changes

observed in small controlled trials should be interpreted with caution and that the most appropriate method for estimating REM-C remains to be determined [534, 535]. The current ESC guidelines on CVD prevention do not include cut-off levels for high REM-C [30].

Some studies suggest that there may be an association between low vitamin D status and high REM-C [536–538]. Thus, future studies should assess the possibility of lowering REM-C by improving vitamin D status. While in the HLCP-2 study no significant correlations between REM-C and ccIMT were observed (**Paper 4**), recent cross-sectional studies have observed that high REM-C is associated with an increased risk of having high ccIMT in patients with ischaemic stroke (including in those with optimal LDL-C levels) [539] as well as in children and adolescents [540]. These observations confirm results from several earlier studies [541–544].

RHR

In terms of RHR, recent meta-analyses confirm the high relevance of this parameter and indicate that higher RHR values are associated with an increased risk of hypertension [238], heart failure [234, 545], atrial fibrillation, coronary heart disease, sudden cardiac death, stroke, CVD in general, total cancer [234] as well as increased cancer mortality [241] and all-cause mortality [234, 546]. Despite considerable observational evidence for the relevance of RHR as a chronic disease risk marker, evidence for a causal role of RHR in disease risk is lacking and there is currently no established reference range for healthy RHR values [547]. However, preliminary cut-off values for healthy RHR of ≤70 beats/min for men and ≤80 beats/min for women have been suggested, with the established cut-off for tachycardia being >100 beats/min [547]. While the HLCP-2 study demonstrated a clinically relevant decrease in RHR (**Paper 2**), only a non-significant decrease was observed in the uncontrolled trial (HLCP-3 study; **Paper 5**). Thus, the effectiveness of the programme in this regard seems uncertain.

oxLDL

The HLCP-3 study showed a significant 24% decrease in oxLDL from baseline to 10 weeks, confirming the study hypothesis that the intervention would beneficially affect oxLDL (**Paper 5**). Although observational studies have shown some conflicting results regarding the association of oxLDL and CVD risk, the most recent meta-analysis found that high circulating oxLDL levels are associated with a clearly increased risk of atherosclerotic CVD [226, 548]. Increased oxLDL levels have been described to increase endoplasmic reticulum stress, which is thought to be a major contributor to the adverse cardiovascular effects of oxLDL [49], and it has been suggested that increasing age and BMI are two important contributors to increased oxLDL levels [549]. While LDL particles also contain antioxidant components, LDL particles are exposed to pro-oxidants such as iron and enzymes (lipoxygenase and metalloproteinase) on the arterial wall [49]. Highly oxidized LDL has been described to attract

scavenger receptors on macrophages which take up oxLDL and form macrophage foam cells which in turn contribute to atherosclerosis progression [49]. It can be hypothesized that the high antioxidant content of healthy plant-based diets (such as high contents of vitamin C, vitamin E, and carotenoids) may be responsible for the observed decrease in oxLDL [73]. However, significant correlations of oxLDL changes with changes in the dietary scores PDI, hPDI, or uPDI could not be shown in the HLCP-3 study (**Paper 5**). Taken together, the decreased oxLDL observed in the HLCP-3 study can be interpreted to be a beneficial effect. However, the fact that this was an uncontrolled trial and that oxLDL was only assessed at baseline and 10 weeks make the clinical significance of this finding uncertain.

3.3. Inflammatory markers (Paper 3)

hs-CRP

Results from **Paper 3** demonstrate that the HLCP-2 intervention resulted in relevant improvements in hs-CRP, which is the most established parameter of subclinical inflammation [267]. Apart from its relevance regarding CVD risk, recent meta-analyses present new evidence that elevated hs-CRP levels are associated with poorer COVID-19 outcomes [550] as well as a potentially increased risk (at levels >3.0 mg/l) of developing psychosis [551], which confirms the high relevance of decreasing hs-CRP.

In terms of hs-CRP-lowering lifestyle factors, two recent meta-analyses indicate that while Tai Chi appears to be ineffective at lowering hs-CRP (but may decrease TNF-α) [552], chromium supplementation may beneficially affect hs-CRP [553]. To date, the association of dietary chromium intake with different dietary patterns appears uncertain [554–556], and variability of chromium content in foods appears to be very high [557, 558]. However, cereal grains typically are an important contributor to chromium intake [559–563]. Similarly, legumes can be a rich source [558, 564].

A recent cross-sectional analysis of UK Biobank data including >400,000 participants found that higher meat (particularly processed meat) intake was associated with higher serum hs-CRP levels [565]. Consequently, one may hypothesize that a decrease in meat intake could have been partly responsible for the hs-CRP-lowering effect observed in the HLCP-2 study (**Paper 3**). However, the UK Biobank study also showed that the observed associations between meat intake and hs-CRP were largely due to higher body weight and adiposity [565]. The HLCP-2 study confirmed a significant positive correlation of hs-CRP change with changes in body weight, BMI, and waist circumference (**Paper 3**).

Hcy

Results of the HLCP-2 study showed no significant intervention effect on Hcy levels, thus confirming that the recommendation to move towards a plant-based diet, in conjunction with informing participants about reliable sources of vitamin B12 and in the context of the participants typically not following an entirely vegetarian diet, did not adversely affect vitamin B12 status in the medium

term (1 year). Although addressing vitamin B12 is of higher importance in the context of vegetarian (and particularly vegan [566]) diets [73], informing participants about this vitamin should ideally not be neglected even in the case of more moderate recommendations for a reduction in animal-source food intake, as low vitamin B12 status is not a rare occurrence in the general population [285, 567–569]. A recent Mendelian randomization study showed that higher genetically predicted Hcy levels are associated with an increased risk of subarachnoid haemorrhage and stroke, which confirms that even modestly elevated Hcy may be an important risk factor for stroke [570]. Thus, an adequate intake of B vitamins (particularly B12, folate, and B6) should be a goal for all diets [571].

While methylmalonic acid (MMA) is the most sensitive marker of vitamin B12 status and is not affected by folate or vitamin B6 status, MMA reflects liver vitamin B12 stores and responds more slowly than Hcy (but more quickly than serum vitamin B12) to changes in vitamin B12 intake [569]. Furthermore, MMA is relatively expensive to assess, levels increase with aging and renal dysfunction (which is not uncommon in elderly individuals), and the clinical relevance of slightly increased MMA levels is uncertain [569]. Holotranscobalamin (holoTC) is also used as a marker of vitamin B12 status, but it is not a functional indicator of vitamin B12 status. HoloTC reflects recent vitamin B12 intake and absorption and does not indicate if liver stores are low [569]. Thus, Hcy appears to be a suitable marker for future studies comparable to the HLCP-2 study.

Apn

The HLCP-2 study observed an unexpected significant decrease in Apn within 10 weeks (**Paper 3**), adding to the conflicting results that have been reported for Apn and its association with disease risk [572]. A recent review article by Kalkman (2021) has attempted to explain the so-called Apn paradox by the observation that overnutrition and obesity can, by separate mechanisms, result in increases or decreases in circulating Apn [48]. In obesity, the amount of hypertrophic white adipose tissue is increased, and (as this tissue is poorly perfused) hypoxia occurs, which decreases Apn synthesis in adipocytes, thus lowering circulating Apn levels [48]. At the same time, obesity may result in decreased cellular uptake of Apn, which can be expected to increase circulating Apn levels [48]. As it has been proposed that there is an overall consensus that obesity reduces Apn secretion from adipocytes (likely associated with decreased Apn synthesis) [573], it seems unlikely that the HLCP-2 intervention decreased Apn synthesis or secretion (as the adiposity markers body weight, BMI, and waist circumference were decreased by the intervention). Thus, the second pathway proposed by Kalkman [48], in reverse, i.e. decreasing adiposity (weight loss) resulting in increased cellular uptake of Apn (increased “Apn sensitivity”), which may in turn decrease circulating Apn, can be hypothesized to be a potential mechanism which could explain our results. Kalkman bases his theory on the assumption that both low and high Apn levels reflect a situation of insulin resistance [48].

While a recent review included a proposed normal range of circulating Apn of 3–30 µg/ml [573], there appear to be no established reference ranges for Apn. The current ESC guidelines on CVD prevention do not mention Hcy or Apn [30]. Taken together, our results and the most recent published evidence on hs-CRP, Hcy, and Apn indicate that the HLCP-2 intervention achieved a meaningful improvement in subclinical inflammation, as indicated by lowered hs-CRP, and appears to have caused no harm, as indicated by unaffected Hcy levels and the very uncertain relevance of Apn level changes. In contrast to the favourable intervention effect on hs-CRP in the controlled trial (HLCP-2 study; **Paper 3**), no significant changes in hs-CRP were observed in the follow-up uncontrolled trial (HLCP-3 study; **Paper 5**), indicating that further research is needed in this regard.

3.4. Correlations of risk marker changes with changes in PDI, hPDI, and uPDI

It should be noted that, in the HLCP-2 and HLCP-3 studies, we used a novel approach to calculate PDI, hPDI, and uPDI to make it suitable for the smaller sample size (compared to large cohort studies). While the original scores by Satija et al. (2016) [574] are calculated from positive and inverse scores based on quintiles of dietary intake [78], like Zhu et al. (2021) [67] we used positive and negative scores for the respective food groups described by Satija et al. [78] (**Paper 2**). Our results confirm that this novel approach appears to be feasible even for relatively small intervention studies. The HLCP-2 and HLCP-3 studies showed significant associations of decreasing body weight, BMI, and waist circumference with favourable dietary changes as indicated by at least some of the dietary scores (PDI, hPDI, and uPDI; **Paper 2** and **Paper 5**).

Increases in PDI and hPDI were associated with decreases in cholesterol (TC, measured and calculated LDL-C, non-HDL-C, REM-C, HDL-C), HbA1c, and insulin (**Paper 2**). Additionally, increased hPDI was associated with decreased RHR (**Paper 2**), and decreased uPDI (which indicates a decreased intake of unhealthy plant-based foods) was associated with decreases in glucose, HbA1c, and RHR (**Paper 2**). Most of these associations were consistent for changes from baseline to 10 weeks as well as changes from baseline to 1 year (**Paper 2**).

For changes in hs-CRP and Hcy, no significant correlations with the dietary score changes (PDI, hPDI, and uPDI) were observed, but decreased Apn levels were associated with increases in PDI and hPDI (**Paper 3**). For max ccIMT change (but not mean ccIMT change), a positive correlation could be shown with uPDI change, while an inverse correlation was observed with the novel modified hPDI (**Paper 4**).

The HLCP-3 study (**Paper 5**) confirmed that favourable dietary changes (in line with the recommendations given) were not just associated with decreased body weight, BMI, and waist circumference but (at 6 months) also with decreased TC, measured LDL-C, and non-HDL-C. Furthermore, the HLCP-3 study found that increases in hPDI and the novel modified hPDI as well as a decrease in uPDI were associated with decreased HDL-C (6-month changes; **Paper 5**). This confirms the

inverse association of hPDI change with HDL-C change which was observed in the HLCP-2 study (baseline to 10 weeks; 10 weeks to 1 year; **Paper 2**).

In both the HLCP-2 and HLCP-3 studies, the changes in PDI, hPDI, and uPDI could successfully demonstrate that dietary behaviour was consistently improved and that these improvements were maintained to a large extent at the end of the studies (**Paper 2** and **Paper 5**). In a much larger study sample, Zhu et al. (2021) found that, during a 3-year weight-loss maintenance phase of an international, multicentre intervention study including 710 participants with overweight or obesity and pre-diabetes (aged 26–70 years), an increase in PDI was associated with lower weight regain but not with changes in other CVD risk markers [67]. Apart from this study and the HLCP-2 and HLCP-3 studies, no other intervention studies appear to have assessed associations between PDI/hPDI/uPDI changes and changes in risk markers.

While a considerable number of cohort studies have shown significant associations between improvements in these dietary scores and reduced CVD risk [66, 575], even large cohort studies have not always observed the expected associations. For example, a recent large cohort study from South Korea (n = 118,577; age: 40–69 years) observed no significant association between hPDI and CVD mortality [576]. However, higher PDI and lower uPDI were associated with decreased mortality [576]. Similarly, another analysis from the same study in South Korea, including almost 150,000 individuals aged ≥40 years (of which ~33% had dyslipidaemia) found that a higher (more unfavourable) uPDI was associated with a significantly increased risk of having dyslipidaemia [577]. This association was stronger in participants aged ≥55 years [577]. Thus, these results provide some evidence that the abovementioned dietary scores may be useful not only in Western but also in East Asian populations, in spite of large differences in typical dietary intakes between Western and East Asian populations [577].

Taken together, these results indicate that the assessment of changes in PDI, hPDI, uPDI as well as the novel modified hPDI can be helpful for demonstrating adherence to the dietary recommendation of transitioning towards a healthy plant-based diet. The HLCP-2 and HLCP-3 studies also indicate that significant associations between improved dietary scores (PDI, hPDI, uPDI, and modified hPDI) and improved CVD risk markers can particularly be shown for markers of adiposity (body weight, BMI, and waist circumference) as well as, to a large extent, for cholesterol levels (**Paper 2** and **Paper 5**). Significant associations could also be shown between improved dietary scores and beneficial effects on ccIMT (**Paper 4**) but not hs-CRP (**Paper 3** and **Paper 5**). Recent evidence from large cohort studies confirms the clinical relevance of these dietary scores [66, 575].

4. General conclusions and perspectives

Firstly, this thesis provides evidence that the lifestyle programmes assessed (HLCP-2 and HLCP-3) are effective at improving dietary intake in line with the recommendations given, which are in turn largely in line with current guidelines for CVD prevention. In terms of ccIMT, the present thesis contributes the finding that it appears to be possible to demonstrate a beneficial effect of healthy lifestyle changes on ccIMT in a community-based sample from the general population within 1 year, although this beneficial effect was modest (with all participants included). The results also confirm that beneficial effects on ccIMT appear to be more easily demonstrable in groups of individuals at higher CVD risk, as indicated by higher baseline mean ccIMT values. Furthermore, the HLCP-2 study confirms that, at the group level, repeatability of ccIMT can be sufficiently good using standard equipment used by many family physicians. This confirms the recommendation by the Mannheim consensus (published one decade ago) that prospective ccIMT measurements (while not recommended for individuals) can be useful for groups (for example, in clinical trials). The HLCP-2 study appears to be the first controlled trial to demonstrate that a lifestyle programme with a strong focus on a healthy plant-based diet may result in significant favourable effects on ccIMT in a community-based sample from the general population, an effect which was observed within a relatively short period of time (1 year). In the subgroup analysis of participants with high baseline mean ccIMT (≥0.800 mm), the observed between-group difference in 1-year trajectories of mean ccIMT was relatively large (-0.051 mm; favouring the intervention group), indicating that a highly clinically relevant decrease in CVD risk appears to have been achieved in this subgroup. However, non-randomization and the time-delayed start of the control group strongly limit the interpretation of the results.

Furthermore, the HLCP-2 study successfully reduced body weight, BMI, waist circumference, REM-C, and RHR at 1 year. However, effectiveness of the HLCP-2 intervention in reducing other risk markers (TC, LDL-C, non-HDL-C, TAG, glucose, HbA1c, insulin, BP, or pulse pressure) could not be shown. The uncontrolled trial confirmed the results of the controlled trial that after the intensive phase of the programme (i.e. after 10 weeks) significant reductions in TC, calculated LDL-C, non-HDL-C, HDL-C, glucose, and insulin were observed but that these reductions were not maintained in the medium term. In addition, our results contribute the important finding that the lifestyle programme, which included plant-based diet recommendations as well as information about vitamin B12, was not associated with adverse effects on Hcy within 1 year. Furthermore, the HLCP-2 and HLCP-3 studies showed a decrease in Apn and oxLDL, respectively, with 10 weeks. Future studies should test whether the Apn decrease may have been a chance finding or whether it may be a true effect associated with a change towards a plant-based diet (potentially similar to the reduction in HDL-C). As the HLCP-3 study was uncontrolled, the observed oxLDL decrease requires confirmation in future studies.

Although the HLCP-2 study showed a successful decrease in hs-CRP, this finding could not be replicated in the uncontrolled trial (HLCP-3 study). Thus, the results cannot clearly confirm the effectiveness of the intervention in reducing hs-CRP. Similarly, the studies found conflicting results regarding REM-C, and the role of REM-C as a CVD risk marker and the ideal way to determine REM-C require further investigation. Furthermore, the effectiveness of the intervention in lowering BP or pulse pressure could not be clearly demonstrated. Both studies indicate a decrease in RHR, but this was not statistically significant in the HLCP-3 study. Future studies should assess which lifestyle components (such as diet, physical activity, and mental relaxation) are most relevant to RHR reduction. The significant associations of RHR change with improved dietary scores in the HLCP-2 study indicate that the dietary changes appear to have contributed to decreasing RHR. As very little evidence from controlled trials exists regarding the effectiveness of a healthy plant-based diet in lowering RHR, this potential effect should be a focus of future investigations.

Our study samples consisted of participants recruited from the general population in rural northwest Germany, and the consistent findings regarding reductions in body weight, BMI, and waist circumference are likely applicable to similar populations. Future studies should consider comparing potential effects of different more specific plant-based dietary approaches, for example, by including certain specific foods such as particular spices, plant oils, or soya products (comparable to the traditional Mediterranean diet which includes specific food components) or by putting more emphasis on personalized recommendations (for example, salt reduction for those with hypertension).

5. References

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Appendix: Table 1

Table 1. Controlled trials (n = 6) with multimodal lifestyle interventions assessing the effect on mean ccIMT					
Population (Country)	**Design**	**IN**	**CON**	**ccIMT change from baseline: IN vs. CON**	**Reference**
Adults with hypertension (Greece)	RCT; 6 months	Intensive lifestyle treatment: diet plus exercise with monthly visits; recommendations included increased fruit and vegetable intake, reduced salt intake, weight loss, and the need for regular physical activity; n = 38	Usual care; n = 38	IN: ~ -0.020 mm; CON: +0.010 mm pBG: **p = 0.037** [See table 2 in [160]]	Vamvakis et al. 2020 [160]
Patients with clinically confirmed coronary artery disease (United States)	RCT; 6 and 12 months	Ornish Program: vegetarian, very low-fat diet; recommendations: a plant-based diet with 75% of total calories from complex carbohydrates, ≥15% of calories from protein, and <10% of calories from fat; liberal consumption of fruit, vegetables, whole grains, and legumes; daily servings of soya food; multivitamin supplement; linseed (flaxseed) as a source of omega-3-fatty acids; moderate sugar intake; n = 41 (6 months); n = 40 (12 months)	Usual care: traditional cardiac rehabilitation; n = 42 (6 months); n = 37 (12 months)	*6 months:* IN: -0.016 mm; CON: +0.004 mm *12 months:* IN: -0.028 mm; CON: -0.025 mm; pBG (both time points): not significant (no explicit p-values reported) [See table 2 in [50]]	Aldana et al. 2007 [50]
Patients with type 2 diabetes (South Korea)	RCT; 6 months	Lifestyle modification: basic dietary education (unspecified); a 16-lesson curriculum covering diet, exercise, and behaviour modification; n = 32	Usual care: usual medical care and 1 session of dietary; counselling at baseline; n = 26	IN: -0.040 mm; CON: + 0.083 mm pBG: **p = 0.007** [See table 2 in [162]]	Kim et al. 2006 [162]
Suburban population (Japan)	N-RCT; 2 years [both groups not similar]	Lifestyle modification: dietary recommendations: <30% total fat, <7% saturated fat, <200 mg/d cholesterol; n = 437 [IN: participants with TC ≥220 mg/dl]	No intervention; n = 794 [CON: participants with TC <220 mg/dl; defined as normo-cholesteraemic controls]	*Men:* IN: -0.060 mm; CON: -0.040 mm; pBG: ≥0.05 *Women:* IN: -0.040 mm; CON: - 0.010 mm; pBG: ≥0.05 [See tables 2 and 3 and figure 2 in [161]]	Okada et al. 2004 [161]
Perimenopausal women (United States)	RCT; different follow-up times over the course of 4 and 6.5 years	Dietary and exercise intervention; recommendations: to reduce total fat and saturated fatty acid intake, to prevent weight gain, and to increase physical activity; 25% of calories as fat, 7% as saturated fatty acids; 100 mg/d cholesterol; reduction in caloric intake to 1,300 kcal/day; n = 166	No intervention: assessment only; n = 188 [n for both groups combined reported as: n = 353]	Annual change: IN: +0.006 mm/year; CON: +0.005 mm/year pBG: p = 0.738 [See table 2 and figure 2 in [163]]	Wildman et al. 2004 [163]
Hypertensive men at high CVD risk and on antihypertensive medication (Sweden)	RCT; different follow-up times (mean follow-up time: 6.2 years)	Dietary recommendations "similar to established guidelines"; comprehensive risk factor modification programme; one information meeting followed by 5 weekly sessions with participation of patients and spouses; n = 52	Usual care: recommendation to stop smoking and to lower the intake of "fat and glucose"; n = 45	IN: +0.053 mm; CON: +0.044 mm BG 95% CI: -0.080 mm to +0.096 mm [See table 4 and figure 3 in [164]]	Agewall et al. 2001 [164]
ccIMT: common carotid intima-media thickness; IN: intervention group; CON: control group; RCT: randomized controlled trial; N-RCT: non-randomized controlled trial; pBG: p-value for between-group difference; BG 95% CI: 95% confidence interval for between-group difference; CVD: cardiovascular disease; TC: total cholesterol					

Appendix: Table 2

Table 2. Controlled trials (n = 10) with interventions based on dietary patterns/foods assessing the effect on mean ccIMT					
Population (Country)	**Design**	**IN**	**CON**	**ccIMT change from baseline: IN vs. CON**	**Reference**
Coronary heart disease patients (Spain)	RCT; 5 and 7 years	Traditional Mediterranean diet: ≥35% of calories as fat, 22% MUFA, 6% PUFA, <10% SFA, 15% protein, ≤50% carbohydrates, <300 mg/d cholesterol; high intakes of vegetables, fruit, legumes, whole grains, oily fish, nuts, and extra virgin olive oil; low intakes of red/processed meat and pastries/commercial bakery products; n = 432 (5 years); n = 396 (7 years)	Low-fat diet, rich in complex carbohydrates; 28% of calories as fat, 12% MUFA, 15% protein, >55% carbohydrates (as recommended by the National Cholesterol Education Program), <300 mg/d cholesterol, high intakes of vegetables, fruit, legumes, and whole grains; n = 377 (5 years); n = 335 (7 years)	*5 years:* IN: -0.027 mm; CON: no significant change (no value reported) pBG: **p <0.05** *7 years:* IN: -0.031 mm; CON: no significant change (no value reported) pBG: **p <0.05** [See table 2 in [170]]	Jimenez-Torres et al. 2021 [170]
Patients with poorly controlled type 2 diabetes (Taiwan)	RCT; 18 months	Moderate low-carbohydrate diet (90 g/d); n = 43	Traditional diabetes diet; n = 42	IN: 0.000 mm; CON: +0.070 mm pBG: p = 0.080 [The ccIMT values appear to have been allocated to the wrong groups in table 3 in [165].]	Chen et al. 2020 [165]
Patients with type 2 diabetes (United States)	N-RCT; 1 year	Carbohydrate-restricted diet, including nutritional ketosis; treatment from a health coach and medical provider; n = 144	Usual care; n = 68	IN: +0.002 mm; CON: +0.004 mm pBG: p = 0.74 [See table 2 in [166]]	Bhanpuri et al. 2018 [166]

Newly diagnosed patients with type 2 diabetes (Italy)	RCT; 2 years, 4 years, and end of trial (~8.1 years)	Traditional Mediterranean diet; n = 85 (2 years); n = 50 (4 years); n = 102 (end of trial)	Low-fat diet, rich in whole grains, low in added fats, sweets and high-fat snacks; goal: ≤30% of calories from fat and ≤10% from SFA;
			n = 64 (2 years);
			n = 50 (4 years);
			n = 99 (end of trial)
				2 years:
				IN: -0.032 mm; CON: -0.010 mm;
				pBG: p = 0.050
				4 years:
				IN: -0.022 mm; CON: -0.003 mm
				pBG: **p = 0.031**
				End of trial:
				IN: -0.026 mm; CON: -0.001 mm
				pBG: **p = 0.024** [See table 3 in [171]]
					Maiorino et al. 2017 [171]

Participants with prediabetes (Italy)	RCT; 24 weeks	Low-advanced glycation end product diet; n = 29	Standard diet: usual cooking habits; n = 28	IN: -0.040 mm CON: -0.020 mm pBG: p = 0.25 [See table 3 in [167]]	Di Pino et al. 2016 [167]
Patients with type 1 or type 2 diabetes (Australia)	RCT; 1 year	Recommendation to increase consumption of fruit (+1 serving/d; not juice), vegetables (+2 servings/d; not juice), and dairy (+1 serving/d; not cheese), regardless of usual intake; counselling from a dietitian at baseline and 1, 3, 6, and 9 months; n = 58	Usual diet; n = 60	IN: -0.030 mm CON: -0.004 mm pBG: **p = 0.009** [See table 2 in [51]]	Petersen et al. 2015 [51]
Subjects at high CVD risk but no CVD at enrolment (Spain)	RCT; 3 groups; mean of 2.4 years	Traditional Mediterranean diet, supplemented with either extra virgin olive oil or 30 g/d of mixed nuts; n = 57 (oil); n = 46 (nuts)	Low-fat diet; n = 61	IN-oil: -0.028 mm; IN-nuts: -0.021 mm CON: -0.004 mm pBG: p = 0.488 [See table 3 in [169]]	Sala-Vila et al. 2014 [169]

Table 2 continued					
Population (Country)	**Design**	**IN**	**CON**	**ccIMT change from baseline: IN vs. CON**	**Reference**
Subjects at high CVD risk (because of the presence of type 2 diabetes or at least 3 major CVD risk factors) but no CVD at enrolment (Spain)	RCT; 1 year	Traditional Mediterranean diet, supplemented (provided free of charge) with virgin olive oil or nuts (walnuts, almonds, hazelnuts); dietary behavioural counselling and personal education sessions; energy restriction was not advised nor physical activity promoted; n = 66 (oil); n = 59 (nuts)	Low-fat diet; recommendation to reduce all types of fat; energy restriction was not advised nor physical activity promoted; n = 62	*Whole group:* IN-oil: -0.016 mm; IN-nuts: -0.033 mm; CON: -0.010 mm; pBG: p = 0.38 *Subgroup (baseline ccIMT ≥0.9 mm):* IN-oil: -0.093 mm; IN-nuts: -0.086 mm; CON: -0.014 mm pBG: **p = 0.01** [See tables 2 and 3 in [174]]	Murie-Fernandez et al. 2011 [174]
Mildly to moderately hypercholesterolaemic subjects: TC ≥201 mg/dl (Finland)	RCT; 3 groups; 1 year	Habitual home diet but replacement of 25 g/d of regular fat intake with the respective test spreads: 1) plant stanol ester spread (2 g/d stanols); n = 93 2) plant sterol ester spreads (2 g/d sterols); n = 93	Habitual home diet but replacement of 25 g/d of regular fat intake with the control spread: 3) control spread (no added plant stanols or sterols, but it contained a small amount of natural plant sterols: 0.06 g/d); n = 96	1) +0.020 mm 2) +0.040 mm 3) +0.030 mm pBG: p = 0.312 [See table 4 in [168]]	Gylling et al. 2009 [168]
Elderly men (Norway) [Same trial as below]	RCT; the intervention group is a combined intervention group of diet change + EPA/DHA supplements and diet change + placebo; 3 years	Mediterranean-like diet; recommendations: ≤30% of calories from fat, 12–15% protein, ≥55% carbohydrates, SFA <1/3 of total fat, cholesterol <300 mg/day, ≤2–3% of calories from alcohol; recommendation to increase the intake of fresh fruit (including berries), fruit juice, fresh salads, and vegetables; to consume a glass of orange juice every morning; to consume bread and cereals; to consume fish ≥3 times/week plus to use various fish products; red meat limited to once/week; poultry as preferred meat; low-fat cheese, skimmed milk; avoidance of full-fat cream; cold-pressed rapeseed oil and margarine with rapeseed oil provided free of charge; wine limited to 1 glass/day; n = 233	No dietary advice + placebo; n = 231	IN: +0.044 mm CON: +0.062 mm pBG: **p = 0.047** [See table 4 in [172]]	Ellingsen et al. 2009 [172]
Elderly men with hypercholesterolaemia (Norway) [Same trial as above]	RCT, 4 groups; data from EPA/DHA supplement group not shown here; 3 years	1) Diet change (see study above) + placebo (corn oil); n = 117 2) Diet change (see study above) + EPA/DHA supplements; n = 120; dietary counselling individually was adapted; recommendation to increase the use of vegetable oils and soft margarines (rapeseed oil, olive oil and sunflower oil), vegetables, fruit, and fish and to decrease the use of meat and animal fat; participants with overweight were encouraged to adopt a calorie-restricted diet.	3) No dietary counselling + placebo (corn oil); n = 114	1) IN-diet: +0.038 mm 2) IN-diet+EPA/DHA: +0.050 mm 3) CON: +0.068 mm pBG(1vs3): p = **0.018** pBG(2vs3): p >0.05 [See table 4 [173]]	Hjerkinn et al. 2006 [173]

ccIMT: common carotid intima-media thickness; IN: intervention group; CON: control group; RCT: randomized controlled trial; N-RCT: non-randomized controlled trial; pBG: p-value for between-group difference; CVD: cardiovascular disease; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; TC: total cholesterol; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid

Appendix: Table 3

Table 3. Controlled trials (n = 5) with exercise interventions assessing the effect on mean ccIMT					
Population (Country)	**Design**	**IN**	**CON**	**ccIMT change from baseline: IN vs. CON**	**Reference**
Middle-aged women with overweight and low physical activity, BMI >27 kg/m^2 (Iran)	RCT, 3 groups; 12 weeks	1) High-intensity interval training; n = 10 2) Moderate-intensity continuous training; n = 11	3) No intervention; n = 9	1) IN-high: -0.020 mm 2) IN-mod: -0.040 mm 3) CON: +0.020 mm pBG: p = 0.310 [See table 3 in [488]]	Farahati et al. 2020 [488]
Individuals with type 2 diabetes, with no major micro- and macrovascular complications; age: 30–75 years (Portugal) [Same trial as below]	RCT; 3 groups; only right ccIMT; 1 year	1) Moderate continuous training combined + resistance training 2) High-intensity interval training + resistance training; n = 41 (both intervention groups combined)	3) Non-exercise control group, n = 22	1) and 2) combined IN: ~ -0.019 mm 3) CON: +0.040 mm pBG: **p <0.05** [See table 2 in [487]]	Hetherington-Rauth et al. 2020 [487]
Patients with type 2 diabetes (Portugal) [Same trial as above]	RCT, 3 groups, only right ccIMT; 1 year	Supervised exercise sessions 3 times/week: 1) combined high-intensity interval training with resistance training; n = 13 2) combined moderate continuous training with resistance training; n = 16	Non-exercise group: initial orientation session with standard counselling and information regarding general physical activity guidelines. Once every 4 weeks the control group met alongside the intervention groups for sessions with broad topics such as nutrition, physical activity, and clinical complications from diabetes; n = 22	1) IN-high: -0.010 mm 2) IN-mod: -0.026 mm 3) CON: +0.036 mm pBG: **p <0.05** [See table 2 in [486]]	Magalhães et al. 2019 [486]
Women with breast cancer undergoing anthracycline-based chemotherapy (United States)	RCT; 8 weeks	High-intensity interval training 3 times/week on a cycle ergometer; n= 15	Non-exercise control ; n= 15	IN: -0.003 mm; CON: +0.009 mm pBG: p = 0.23 [See Results (cIMT) and table 2 in [485]]	Lee et al. 2019 [485]
Adults with high normal blood pressure (systolic: 130–139 mmgHg, and/or diastolic: 85–89 mmHg) (Poland)	N-RCT; intervention group compared to healthy controls; 12 weeks [both groups not similar]	Aerobic training with a cycle ergometer; n = 31	Healthy adults not subjected to any intervention; n = 14	IN: -0.040 mm CON: +0.010 mm pBG: not reported [See tables 2 and 3 in [484]]	Glodzik et al. 2018 [484]
Patients with both type 2 diabetes and coronary artery disease (Norway)	RCT; 1 year	Exercise programme with 150 min/week of combined aerobic and resistance training; n = 61	Standard follow-up; n = 62	*Whole group:* IN: -0.016 mm CON: -0.007 mm pBG: p = 0.57 [Slightly different ccIMT changes reported in table 2 in [483]] *Subgroup (patients without identified carotid plaques):* IN: -0.034 mm; CON: +0.013 mm pBG: **p = 0.010** [See figure 1 in [483]]	Byrkjeland et al. 2016 [483]
ccIMT: common carotid intima-media thickness; IN: intervention group; CON: control group; RCT: randomized controlled trial; N-RCT: non-randomized controlled trial; pBG: p-value for between-group difference; BMI: body mass index					

Appendix: Supplementary material Paper 1

Supplementary table 1. Additional baseline characteristics of evaluable participants			
Variable	**Intervention group (n = 82)**	**Control group (n = 61)**	**p-value #**
Diagnosed hypertension, n (%)	31 (37.8) [n = 81]	23 (37.7) [n = 60]	1.000
Hypertension based on baseline values, n (%) §	54 (65.9)	38 (62.3)	0.725
Diagnosed dyslipidaemia, n (%)	4 (4.9) [n = 81]	1 (1.6) [n = 60]	0.394
Total cholesterol ≥200 mg/dl at baseline, n (%)	45 (54.9) [n = 81]	36 (59.0)	0.734
High LDL cholesterol (measured) ≥130 mg/dl at baseline, n (%)	42 (51.2) [n = 81]	36 (59.0)	0.496
LDL cholesterol (calculated) ≥130 mg/dl at baseline, n (%)	32 (39.0) [n = 81]	27 (44.3) [n = 60]	0.605
HDL cholesterol <40 mg/dl at baseline, n (%)	4 (4.9) [n = 81]	5 (8.2)	0.498
Triglycerides ≥150 mg/dl at baseline, n (%)	15 (18.3) [n = 81]	10 (16.4)	0.826
Family history of heart attack or stroke (siblings), n (%)	6 (7.3) [n = 81]	5 (8.2) [n = 59]	1.000
Family history of heart attack or stroke (parents), n (%)	44 (53.7) [n = 81]	24 (39.3) [n = 60]	0.125
Family history of heart attack or stroke (grandparents), n (%)	20 (24.4) [n = 77]	8 (13.1) [n = 56]	0.132
Diagnosed heart disease, n (%)	7 (8.5) [n = 81]	7 (11.5) [n = 60]	0.580
History of stroke, n (%)	1 (1.2) [n = 81]	3 (4.9) [n = 60]	0.312
Diagnosed peripheral artery disease, n (%)	1 (1.2) [n = 81]	0 [n = 60]	1.000
Diagnosed diabetes, n (%)	5 (6.1) [n = 81]	4 (6.6) [n = 60]	1.000
Diagnosed retinopathy, n (%)	3 (3.7) [n = 81]	3 (4.9) [n = 60]	0.699
Diagnosed peripheral neuropathy, n (%)	5 (6.1) [n = 81]	3 (4.9) [n = 60]	1.000
Diagnosed diabetic foot, n (%)	1 (1.2) [n = 81]	1 (1.6) [n = 60]	1.000
HbA1c ≥6.5% at baseline, n (%)	0 [n = 81]	4 (6.6)	**0.032**
Diagnosed kidney disease, n	0 [n = 81]	0 [n = 60]	-
Diagnosed allergy, n (%)	2 (2.4) [n = 81]	1 (1.6) [n = 60]	1.000
Diagnosed gastrointestinal disease, n (%)	3 (3.7) [n = 81]	2 (3.3) [n = 60]	1.000

Diagnosed thyroid disease, n (%)	10 (12.2) [n = 81]	3 (4.9) [n = 60]	0.155
Diagnosed depression, n (%)	2 (2.4) [n = 81]	3 (4.9) [n = 60]	0.651
History of cancer, n (%)	7 (8.5) [n = 81]	1 (1.6) [n = 60]	0.138
Diagnosed rheumatoid arthritis, n (%)	4 (4.9) [n = 81]	7 (11.5) [n = 60]	0.204
Diagnosed chronic pain, n (%)	9 (11.0) [n = 81]	7 (11.5) [n = 60]	1.000
Diagnosed lung disease, n (%)	9 (11.0) [n = 81]	7 (11.5) [n = 60]	1.000
Diagnosed bone disease, n (%)	15 (18.3) [n = 81]	16 (26.2) [n = 60]	0.305
Other diagnosed disease, n (%)	17 (20.7) [n = 81]	12 (19.7) [n = 60]	1.000
Free of diagnosed disease, n (%)	18 (29.0) [n = 81]	16 (26.2) [n = 60]	0.557
Alcohol intake frequency, categories (questionnaire), n (%)	• Never: 1 (1.2) • Once per month or less often: 16 (19.5) • 2–4 times/month: 33 (40.2) • 2–3 times/week: 20 (24.4) • ≥4 times/week: 12 (14.6)	• Never: 0 • Once per month or less often: 13 (21.3) • 2–4 times/month: 28 (45.9) • 2–3 times/week: 15 (24.6) • ≥4 times/week: 4 (6.6) [n = 60]	0.566
Values are numbers of individuals (percentage) in the study population. § Hypertension: systolic blood pressure ≥130 and/or diastolic blood pressure ≥80 mmHg; # p-value for comparisons between groups by Fisher's exact test (two-sided)			

Appendix: Supplementary material Paper 2

Supplementary material

Supplementary table 1

Supplementary table 1. Baseline and follow-up measurements in evaluable participants (baseline to 1½ years) **Intervention group: n = 86; control group: n = 45**										
Parameters	**Group**	**Baseline**	**10 wks**	**6 mo**	**1 yr**	**1½ yrs**	**Changes (baseline to 1½ yrs)**	**p WG** §	**p BG #**	**p BG #** (multivar.-adjusted)
Body weight, kg	IN	81.1 ± 2.0	77.7 ± 1.9	76.6 ± 1.7	77.1 ± 1.8	77.6 ± 1.8	-3.5 (-4.6, -2.5)	**<0.001**[a]	**<0.001**[c]	**<0.001**[d]
	CON	84.4 ± 2.9	84.1 ± 2.8	83.5 ± 2.7	83.8 ± 2.6	83.7 ± 2.5	-0.7 (-2.5, 1.1)	0.458[b]		
BMI, kg/m^2	IN	27.4 ± 0.6	26.3 ± 0.5	25.9 ± 0.5	26.1 ± 0.5	26.2 ± 0.5	-1.2 (-1.5, -0.8)	**<0.001**[a]	**<0.001**[c]	**<0.001**[d]
	CON	28.3 ± 0.9	28.2 ± 0.9	28.0 ± 0.9	28.1 ± 0.9	28.1 ± 0.9	-0.2 (-0.7, 0.4)	0.513[a]		
TC, mg/dl	IN	207.2 ± 4.2	183.1 ± 3.7	196.5 ± 4.4	206.7 ± 4.4	198.5 ± 4.2	-8.7 (-14.1, -3.3)	**0.002**[b]	0.169[c]	**0.049**[d]
	CON	204.8 ± 6.9	200.3 ± 6.2	193.9 ± 6.9	201.7 ± 7.0	199.6 ± 7.1	-5.3 (-13.9, 3.3)	0.222[b]		
Measured LDL-C, mg/dl	IN	132.9 ± 4.0	117.3 ± 3.5	127.2 ± 4.1	135.9 ± 4.2	123.8 ± 3.8	-9.1 (-13.7, -4.4)	**<0.001**[b]	0.246[c]	0.104[d]
	CON	135.7 ± 6.4	130.6 ± 5.5	134.1 ± 6.7	132.2 ± 6.0	130.1 ± 6.5	-5.6 (-14.4, -8.3)	0.205[b]		
Calculated LDL-C, mg/dl	IN	120.6 ± 4.0	102.4 ± 3.3	108.6 ± 4.0	119.6 ± 4.1	114.0 ± 3.6	-6.6 (-11.1, -2.2)	**0.004**[b]	**0.032**[c]	**0.008**[d]
	CON	120.0 ± 6.0	115.8 ± 5.1	111.2 ± 6.0	121.1 ± 5.8	118.5 ± 6.3	-1.5 (-10.2, 7.2)	0.735[b]		
non-HDL-C, mg/dl	IN	141.4 ± 4.7	122.5 ± 3.8	129.4 ± 4.5	141.1 ± 4.7	135.5 ± 4.2	-5.9 (-10.8, -1.0)	**0.019**[b]	**0.029**[c]	**0.005**[d]
	CON	141.7 ± 6.3	138.7 ± 5.6	133.7 ± 6.5	143.5 ± 6.4	140.3 ± 6.7	-1.5 (-10.5, 7.6)	0.744[b]		
REM-C, mg/dl	IN	8.5 ± 1.2	5.1 ± 1.0	2.1 ± 1.2	5.3 ± 1.1	11.7 ± 1.2	3.2 (1.2, 5.2)	**0.003**[a]	**0.031**[c]	**0.018**[d]
	CON	6.1 ± 2.2	8.1 ± 1.9	-0.4 ± 2.2	11.3 ± 1.6	10.2 ± 1.4	4.1 (-0.1, 8.4)	**<0.001**[a]		
HDL-C, mg/dl	IN	65.8 ± 2.0	60.6 ± 1.9	67.2 ± 2.1	65.5 ± 2.0	63.0 ± 1.8	-2.8 (-4.8, -0.9)	**0.007**[a]	0.092[c]	0.105[d]
	CON	63.1 ± 2.9	61.6 ± 3.0	60.2 ± 2.5	58.2 ± 2.3	59.3 ± 2.4	-3.8 (-6.3, -1.3)	**0.003**[a]		
TAG, mg/dl	IN	103.8 ± 5.6	100.4 ± 4.5	103.7 ± 5.5	107.5 ± 5.4	107.3 ± 5.3	3.6 (-3.5, 10.6)	0.194[a]	0.588[c]	0.373[d]
	CON	118.6 ± 13.3	118.8 ± 10.2	116.8 ± 9.3	115.2 ± 9.8	111.6 ± 8.3	-7.0 (-30.8, 16.8)	0.806[a]		
Glucose, mg/dl	IN	98.7 ± 1.3	93.8 ± 1.2	95.6 ± 1.1	98.7 ± 1.1	97.4 ± 1.2	-1.3 (-3.2, 0.6)	0.078[a]	0.057[c]	0.050[d]
	CON	101.0 ± 1.8	100.1 ± 2.2	101.6 ± 1.9	100.0 ± 1.6	98.7 ± 2.0	-2.3 (-4.7, 0.0)	**0.045**[a]		
HbA1c, %	IN	5.4 ± 0.1	5.4 ± 0.0	5.4 ± 0.0	5.4 ± 0.0	5.4 ± 0.0	0.0 (-0.1, 0.1)	0.318[a]	**0.007**[c]	<**0.001**[d]
	CON	5.5 ± 0.1	5.5 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	0.1 (0.0, 0.2)	**0.001**[a]		
Insulin, µU/ml	IN	12.8 ± 2.3	10.2 ± 0.9	13.0 ± 3.8	10.9 ± 0.9	10.2 ± 0.8	-2.6 (-6.0, 0.8)	0.329[a]	0.298[c]	0.236[d]

	CON	13.1 ± 1.2	13.2 ± 1.5	12.3 ± 1.5	12.3 ± 1.4	10.6 ± 1.3	-2.5 (-4.0, -1.0)	**0.001**[a]		
Systolic BP, mmHg	IN	133.3 ± 1.6	126.8 ± 1.6	127.8 ± 1.6	125.6 ± 1.6	128.7 ± 1.6	-4.6 (-7.7, -1.6)	**0.004**[b]	0.764[c]	0.987[d]
	CON	135.1 ± 2.2	129.3 ± 2.1	128.9 ± 2.5	127.8 ± 2.2	125.5 ± 2.1	-9.6 (-13.1, -6.1)	**<0.001**[b]		
Diastolic BP, mmHg	IN	81.2 ± 0.9	77.1 ± 0.9	78.8 ± 0.9	77.2 ± 1.1	76.7 ± 1.0	-4.5 (-6.4, -2.5)	**<0.001**[a]	0.922[c]	0.707[d]
	CON	81.1 ± 1.4	79.1 ± 1.5	78.2 ± 1.3	77.0 ± 1.5	75.6 ± 1.2	-5.5 (-7.5, -3.5)	**<0.001**[b]		
Pulse pressure, mmHg	IN	52.1 ± 1.2	49.7 ± 1.2	49.0 ± 1.3	48.4 ± 1.3	52.0 ± 1.1	-0.2 (-2.7, 2.4)	0.797[a]	0.764[c]	0.382[d]
	CON	54.0 ± 1.9	50.2 ± 1.6	50.7 ± 2.0	50.8 ± 1.9	50.0 ± 1.8	-4.1 (-7.4, -0.8)	**0.019**[a]		
RHR, bpm	IN	68.3 ± 1.2	62.6 ± 1.1	65.7 ± 1.2	63.9 ± 1.2	64.9 ± 1.2	-3.4 (-5.4, -1.5)	**0.001**[b]	0.095[c]	0.123[d]
	CON	69.6 ± 1.3	68.8 ± 1.7	67.9 ± 1.8	67.4 ± 1.6	65.0 ± 1.4	-4.6 (-7.2, -2.0)	**0.001**[b]		

Values are means ± SEM, except for qualitative variables, expressed as n (%). Changes are expressed as means and 95% CI. Waist circumference was not assessed at 1½ years in the control group. SEM: standard error of the mean; CI: confidence interval; p WG: p-values for within-group changes from baseline to 1½ years; p BG: p-values for between-group differences in 1½-year trajectories; IN: intervention; CON: control; BMI: body mass index; WC: waist circumference; TC: total cholesterol; LDL-C: LDL cholesterol; non-HDL-C: non-HDL cholesterol; REM-C: remnant cholesterol; HDL-C: HDL cholesterol; TAG: triglycerides; BP: blood pressure; RHR: resting heart rate; bpm: beats per minute;

§ p-value for within-group comparisons by:

[a] Wilcoxon test (two-sided)

[b] paired t-test (two-sided)

p-value for between-group comparisons by:

[c] repeated measures ANCOVA, adjusted for the baseline values of the respective parameters

[d] repeated measures ANCOVA, adjusted for the baseline values of the respective parameters, age, and sex

TC, measured LDL-C, non-HDL-C, REM-C, HDL-C, TAG, glucose: n = 85 (IN), n = 44 (CON); calculated LDL-C: n = 85 (IN), n = 43 (CON); HbA1c: n = 84 (IN), n = 44 (CON); insulin: n = 74 (IN), n = 44 (CON); systolic/diastolic BP, pulse pressure, RHR: n = 43 (CON);

Supplementary table 2

Supplementary table 2. Assessment methods and laboratory assays used			
Parameter	**Medium**	**Method**	**Equipment**
Body weight (kg)	-	-	Calibrated body scales
Body height (m)	-	Self-reported at baseline	-
Waist circumference (cm)	-	Measured at resting expiratory position between the highest point of the iliac crest and the lowest rib (approximately at the height of the lumbar spinal segment L4/L5)	No-stretch fabric measuring tape
TC, LDL-C, HDL-C, TAG, glucose (mg/dl)	Serum	Spectrometry: UV/VIS photometry	Roche Cobas c702
HbA1c (%)	EDTA whole blood	Ligand assay: turbidimetric immunoassay (TIA)	Roche Cobas c502
Insulin (mg/dl)	Serum	Ligand assay: electro-chemiluminescence immunoassay (ECLIA)	Roche Cobas e801
Systolic and diastolic BP (mmHg), RHR (bpm)	-	Assessed in duplicate; the reading in which diastolic BP was lower was used.	Calibrated BP gauge
All measurements and blood sampling were done in the morning and in the fasted state. Calculated LDL-C was calculated with the Friedewald formula [32]. Non-HDL-C was calculated by deducting HDL-C from TC. REM-C was calculated by deducting measured LDL-C and HDL-C from TC. BMI: body mass index; TC: total cholesterol; LDL-C: LDL cholesterol; HDL-C: HDL cholesterol; TAG: triglycerides; BP: blood pressure; RHR: resting heart rate; bpm: beats per minute;			

Supplementary table 3

Supplementary table 3. Changes from baseline to 10 weeks (CCA) **Intervention group: n = 106; control group: n = 63**								
Parameters	**Group**	**Baseline**	**10 wks**	**Changes (baseline to 10 weeks)**	**p-value** (within-group changes) §	**p-value** (between-group differences) #		
Body weight, kg	IN	81.5 ± 1.8	78.2 ± 1.6	-3.2 (-3.7, -2.8)	**<0.001**[a]	**<0.001**[c]	**<0.001**[d]	**<0.001**[e]
	CON	84.5 ± 2.5	84.4 ± 2.4	-0.1 (-0.5, 0.3)	0.668[b]			
BMI, kg/m^2	IN	27.6 ± 0.5	26.5 ± 0.5	-1.1 (-1.2, -0.9)	**<0.001**[a]	**<0.001**[c]	**<0.001**[d]	**<0.001**[e]
	CON	28.2 ± 0.7	28.2 ± 0.7	0.0 (-0.2, 0.1)	0.832[a]			
Waist circumference, cm	IN	98.7 ± 1.4	95.3 ± 1.4	-3.5 (-4.1, -2.8)	**<0.001**[b]	**<0.001**[c]	**<0.001**[d]	**<0.001**[e]
	CON	96.7 ± 2.0	97.7 ± 1.9	1.0 (-0.2, 2.1)	0.096[b]			
TC, mg/dl	IN (n = 105)	206.0 ± 3.7	183.7 ± 3.5	-22.4 (-27.4, -17.3)	**<0.001**[b]	**<0.001**[c]	**<0.001**[d]	**<0.001**[e]
	CON (n = 62)	207.2 ± 5.6	205.1 ± 5.4	-2.1 (-7.7, 3.5)	0.453[b]			
LDL-C (measured), mg/dl	IN (n = 105)	132.0 ± 3.4	118.0 ± 3.2	-14.0 (-18.1, -9.9)	**<0.001**[b]	**0.001**[c]	**<0.001**[d]	**<0.001**[e]
	CON (n = 62)	138.0 ± 5.5	135.0 ± 5.1	-3.0 (-8.0, 2.0)	0.106[a]			
LDL-C (calculated), mg/dl	IN (n = 105)	119.7 ± 3.4	102.9 ± 3.0	-16.9 (-20.9, -12.9)	**<0.001**[a]	**<0.001**[c]	**<0.001**[d]	**<0.001**[e]
	CON (n = 61)	123.4 ± 5.2	121.2 ± 4.9	-2.1 (-6.8, 2.5)	0.178[a]			
non-HDL-C, mg/dl	IN (n = 105)	141.0 ± 4.0	123.7 ± 3.5	-17.3 (-21.7, -12.8)	**<0.001**[b]	**<0.001**[c]	**<0.001**[d]	**<0.001**[e]
	CON (n = 62)	144.7 ± 5.5	144.0 ± 5.3	-0.7 (-6.0, 4.6)	0.594[a]			
REM-C (calculated), mg/dl	IN (n = 105)	9.0 ± 1.1	5.7 ± 0.9	-3.2 (-4.7, -1.7)	**<0.001**[a]	**<0.001**[c]	**<0.001**[d]	**<0.001**[e]
	CON (n = 62)	6.7 ± 1.9	9.0 ± 1.7	2.3 (-0.6, 5.2)	**0.003**[a]			
HDL-C, mg/dl	IN (n = 105)	65.1 ± 1.8	59.9 ± 1.6	-5.1 (-7.1, -3.1)	**<0.001**[a]	**0.012**[c]	**0.019**[d]	**0.008**[e]
	CON (n = 62)	62.6 ± 2.3	61.1 ± 2.4	-1.4 (-3.1, 0.3)	0.110[b]			
TAG, mg/dl	IN (n = 105)	106.2 ± 5.3	104.1 ± 4.4	-2.0 (-9.2, 5.1)	0.867[a]	0.520[c]	0.157[d]	0.076[e]
	CON (n = 62)	114.1 ± 10.1	117.5 ± 7.8	3.4 (-14.8, 21.5)	0.128[a]			
Glucose, mg/dl	IN (n = 105)	99.4 ± 1.5	94.2 ± 1.1	-5.1 (-7.5, -2.8)	**<0.001**[a]	0.691[c]	**0.025** [d]	**0.008**[e]
	CON (n = 62)	103.9 ± 3.3	99.7 ± 1.9	-4.1 (-9.2, 0.9)	**0.028**[a]			
HbA1c, %	IN (n = 105)	5.4 ± 0.0	5.5 ± 0.0	0.0 (-0.1, 0.1)	0.237[a]	0.520[c]	0.333[d]	0.050[e]
	CON (n = 62)	5.5 ± 0.1	5.6 ± 0.1	0.1 (0.0, 0.1)	**0.001**[a]			

Insulin, µU/ml	IN (n = 105)	12.7 ± 1.7	10.0 ± 0.7	-2.7 (-5.7, 0.3)	**0.013**[a]	0.168[c]	**0.014**[d]	**0.007**[e]
	CON (n = 62)	12.6 ± 1.1	12.7 ± 1.2	0.2 (-1.3, 1.7)	0.656[a]			
Systolic BP, mmHg	IN	134.9 ± 1.5	128.8 ± 1.6	-6.1 (-8.4, -3.8)	**<0.001**[b]	0.638[c]	0.924[d]	0.939[e]
	CON (n = 62)	131.0 ± 2.2	125.8 ± 2.0	-5.2 (-8.2, -2.1)	**0.001**[b]			
Diastolic BP, mmHg	IN	81.5 ± 0.8	78.2 ± 0.8	-3.3 (-4.8, -1.9)	**<0.001**[a]	0.463[c]	0.884[d]	0.926[e]
	CON (n = 62)	79.2 ± 1.3	76.8 ± 1.3	-2.5 (-4.2, -0.8)	**0.006**[b]			
Pulse pressure, mmHg	IN	53.4 ± 1.2	50.7 ± 1.2	-2.7 (-4.6, -0.8)	**0.005**[b]	0.980[c]	0.633[d]	0.871[e]
	CON (n = 62)	51.7 ± 1.6	49.0 ± 1.3	-2.7 (-5.6, 0.2)	0.080[a]			
RHR, bpm	IN	68.5 ± 1.0	62.9 ± 1.0	-5.7 (-7.4, -4.0)	**<0.001**[b]	**0.001**[c]	**<0.001**[d]	**<0.001**[e]
	CON (n = 62)	69.5 ± 1.3	68.6 ± 1.5	-0.9 (-3.1, 1.3)	0.216[a]			

Values are means ± SEM, except for qualitative variables, expressed as n (%). Changes are expressed as means and 95% CI; CCA: complete case analysis; IN: intervention group; CON: control group; BMI: body mass index; TC: total cholesterol; LDL-C: LDL cholesterol; non-HDL-C: non-HDL cholesterol; REM-C: remnant cholesterol; HDL-C: HDL cholesterol; TAG: triglycerides; BP: blood pressure; RHR: resting heart rate; bpm: beats per minute; SEM: standard error of the mean; CI: confidence interval;

§ p-value for within-group comparisons by:

[a] Wilcoxon (two-sided)

[b] paired t-test (two-sided)

p-value for between-group comparisons by:

[c] one-way ANOVA

[d] one-way ANCOVA, adjusted for the baseline value of the respective parameters

[e] one-way ANCOVA, adjusted for the baseline value of the respective parameters, age, and sex

Supplementary table 4

Supplementary table 4. Bivariate correlations of diet score changes and risk marker changes (baseline to 10 weeks)

Parameters	Correlations with PDI change			Correlations with hPDI change			Correlations with uPDI change		
	r §	p-value	n	r §	p-value	n	r §	p-value	n
Body weight	-0.224	**0.005**	155	-0.400	**<0.001**	155	0.245	**0.002**	155
BMI	-0.239	**0.003**	155	-0.412	**<0.001**	155	0.253	**0.002**	155
WC	-0.225	**0.005**	155	-0.331	**<0.001**	155	0.246	**0.002**	155
TC	-0.228	**0.005**	153	-0.318	**<0.001**	153	0.085	0.298	153
LDL-C (measured)	-0.174	**0.032**	153	-0.260	**0.001**	153	0.051	0.531	153
LDL-C (calculated)	-0.199	**0.014**	152	-0.288	**<0.001**	152	0.074	0.365	152
non-HDL-C	-0.209	**0.009**	153	-0.296	**<0.001**	153	0.111	0.171	153
REM-C	-0.200	**0.013**	153	-0.162	**0.046**	153	0.090	0.270	153
HDL-C	-0.165	**0.042**	153	-0.179	**0.027**	153	0.010	0.902	153
TAG	-0.085	0.297	153	-0.021	0.795	153	0.039	0.635	153
Glucose	0.006	0.944	153	-0.077	0.344	153	0.175	**0.031**	153
HbA1c	-0.087	0.287	153	-0.080	0.324	153	0.022	0.788	153
Insulin	-0.136	0.095	153	-0.175	**0.031**	153	0.121	0.135	153
Systolic BP	-0.058	0.474	154	-0.061	0.449	154	-0.021	0.796	154
Diastolic BP	-0.096	0.234	154	-0.096	0.237	154	0.069	0.394	154
Pulse pressure	0.012	0.881	154	-0.022	0.783	154	-0.075	0.355	154
RHR	-0.086	0.288	154	-0.189	**0.019**	154	0.221	**0.006**	154

Participants of both the intervention and control groups are combined. § Spearman correlation coefficients; PDI: plant-based diet index; hPDI: healthful PDI; uPDI: unhealthful PDI; BMI: body mass index; WC: waist circumference; TC: total cholesterol; LDL-C: LDL cholesterol; non-HDL-C: non-HDL cholesterol; REM-C: remnant cholesterol; HDL-C: HDL cholesterol; TAG: triglycerides; BP: blood pressure; RHR: resting heart rate

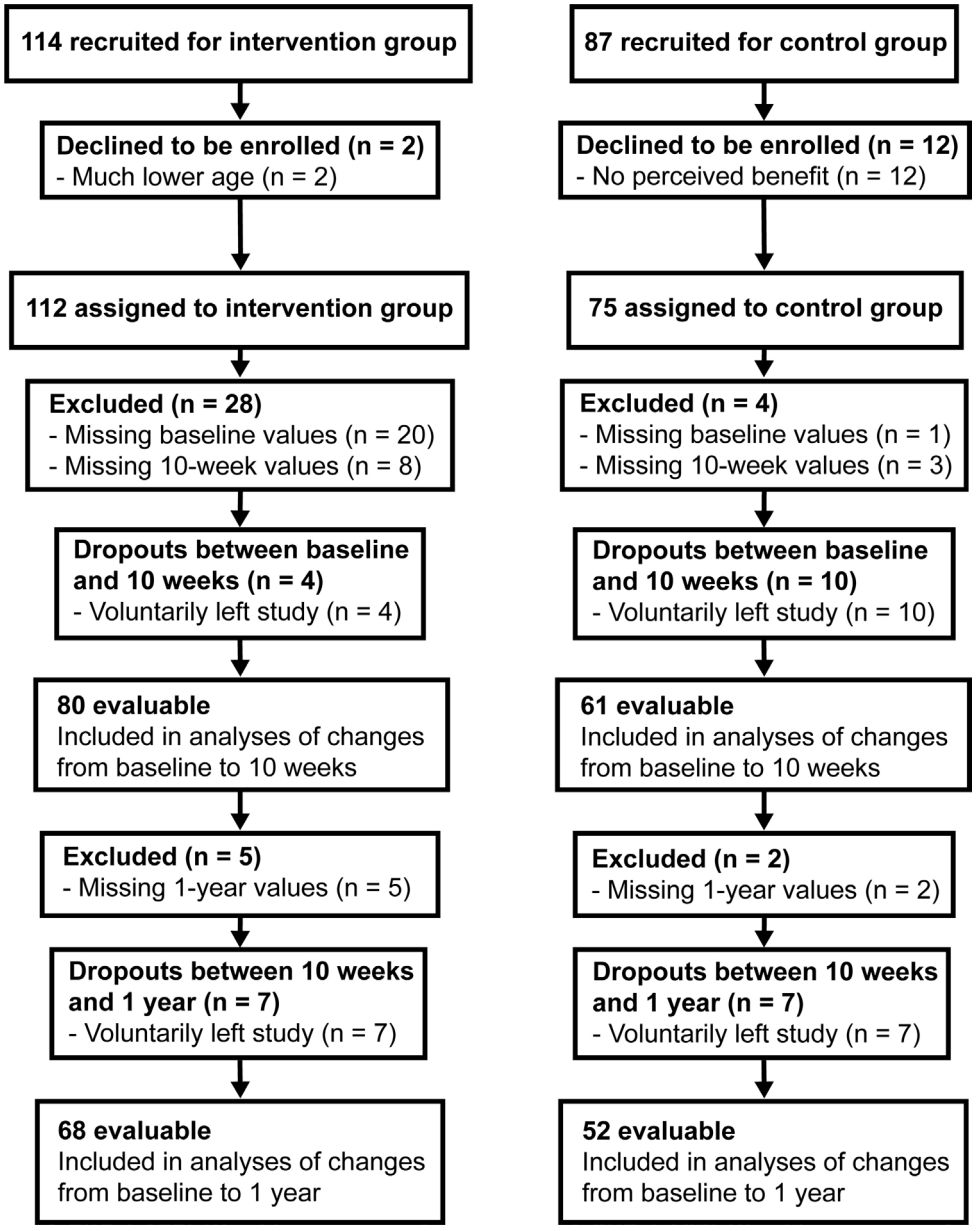
Appendix: Supplementary material Paper 3

Additional file 1

Supplementary table 1

Supplementary table 1: Assessment methods and laboratory assays			
Parameters	**Serum/Plasma**	**Methods**	**Equipment**
hs-CRP	Serum	Spectrometry: immunonephelometry	Siemens BN 2
Homocysteine	EDTA plasma	Competitive immunoassay	Siemens Immulite 1000
Adiponectin	EDTA plasma	Enzyme-linked immunosorbent assay (Human Total Adiponectin/Acrp30 Quantikine ELISA; R&D Systems)	Grifols Diagnostic Triturus
All blood samples were taken in the morning and in the fasted state and were analysed at the University Hospital of Münster (Germany). The same standard operating procedures were followed for blood collection, processing, and storage during all study phases. Venous fasting (overnight) blood samples were taken by nurses. After waiting for ~15 min, samples were centrifuged, after which serum/EDTA plasma was separated from the centrifugate. Samples were stored at -80°C. All assays were performed in the same laboratory. hs-CRP: high-sensitivity C-reactive protein			

Supplementary figure 1



Supplementary figure 1. Flow chart of participants through the study (homocysteine and adiponectin analysis)

Supplementary table 2

Supplementary table 2. hs-CRP in evaluable participants at baseline and 10 weeks (CCA)				
Parameter	**hs-CRP, mg/l**			
Group	**IN** (n = 98)		**CON** (n = 46)	
	Mean	SEM or 95% CI	Mean	SEM or 95% CI
Baseline	1.7	0.3	2.3	0.5
10 weeks	1.2	0.2	2.6	0.6
Δ(baseline, 10 weeks)	-0.5	-0.9, -0.1	0.3	-0.6, 1.2
p WG *	**<0.001** [a]		0.956 [a]	
p BG §	**0.006** [b]			
p BG § (multivariable-adjusted)	**0.002** [c]			
	0.004 [d]			

hs-CRP: high-sensitivity C-reactive protein; CCA: complete case analysis; IN: intervention; CON: control; SEM: standard error of the mean; CI: confidence interval; p WG: p-values for within-group changes from baseline to 10 weeks; p BG: p-values for between-group differences in changes from baseline to 10 weeks; BMI: body mass index; REM-C: remnant cholesterol; HDL-C: HDL cholesterol; BP: blood pressure; RHR: resting heart rate;
* p-value for within-group comparisons by:
[a] Wilcoxon test (two-sided)
§ p-value for between-group comparisons by:
[b] one-way ANCOVA, adjusted for the baseline hs-CRP
[c] one-way ANCOVA, adjusted for the baseline hs-CRP, age, sex, education level, marital status, alcohol intake, smoker status, BMI, REM-C, HDL-C, HbA1c, systolic BP, and RHR
[d] one-way ANCOVA, adjusted for the baseline hs-CRP, age, sex, education level, marital status, and changes in alcohol intake, smoker status, BMI, REM-C, HDL-C, HbA1c, systolic BP, and RHR

Supplementary table 3

Supplementary table 3. Hcy and Apn at baseline and 10 weeks in evaluable participants (CCA)								
Parameters	**Hcy, µmol/l**				**Apn, µg/ml**			
Group	**IN** (n = 80)		**CON** (n = 61)		**IN** (n = 80)		**CON** (n = 61)	
	Mean	SEM or 95% CI	Mean	SEM or 95% CI	Mean	SEM or 95% CI	Mean	SEM or 95% CI
Baseline	12.6	0.4	11.9	0.4	10.5	0.6	7.7	0.6
10 weeks	12.1	0.4	12.2	0.4	8.0	0.4	7.9	0.7
Δ(baseline, 10 weeks)	-0.4	-1.3, 0.5	0.2	-0.6, 1.1	-2.5	-3.5, -1.5	0.2	-0.5, 0.8
p WG *	0.366 a		0.736 a		**<0.001** [a]		0.595 [a]	
p BG §	0.656 b				**0.004** [b]			
p BG § (multivariable-adjusted)	0.592 c				**0.001** [c]			
	0.450 f				**0.002** [d]			
	0.259 g				**0.003** [e]			

Hcy: homocysteine; Apn: adiponectin; CCA: complete case analysis; IN: intervention; CON: control; SEM: standard error of the mean; CI: confidence interval; p WG: p-values for within-group changes from baseline to 10 weeks;
p BG: p-values for between-group differences in changes from baseline to 10 weeks; BMI: body mass index; TC: total cholesterol; HDL-C: HDL cholesterol; BP: blood pressure; RHR: resting heart rate;
* p-value for within-group comparisons by:
[a] Wilcoxon test (two-sided)
§ p-value for between-group comparisons by:
[b] one-way ANCOVA, adjusted for the baseline values of the respective parameters
[c] one-way ANCOVA, adjusted for the baseline values of the respective parameters, age, and sex
[d] one-way ANCOVA, adjusted for the baseline Apn, age, sex, education level, marital status, alcohol intake, smoker status, BMI, TC, HDL-C, insulin, diastolic BP, and RHR
[e] one-way ANCOVA, adjusted for the baseline Apn, age, sex, education level, marital status, and changes in alcohol intake, smoker status, BMI, TC, HDL-C, insulin, diastolic BP, and RHR
[f] one-way ANCOVA, adjusted for the baseline Hcy, age, sex, education level, marital status, alcohol intake, smoker status, and HbA1c
[g] one-way ANCOVA, adjusted for the baseline Hcy, age, sex, education level, marital status, and changes in alcohol intake, smoker status, and HbA1c

Appendix: Supplementary material Paper 4

Supplementary material

Additional information on the ccIMT measurement protocol

All assessments were made by the same technician and with the same ultrasound device (Mindray DC-N3, Mindray, Shenzhen, China). Two ccIMT measurements were taken on each side, resulting in four mean ccIMT and four maximal (max) ccIMT values (per person, per measurement time point. Each participant was examined in the supine position, with their head at a 45° angle turned away from the examiner. The left and right common carotid arteries were scanned longitudinally, scanning a 1 cm-long, plaque-free artery section, located 1 cm proximal to the carotid bulb. Plaque was defined as a focal thickening in the intima-media complex of >1.5 mm. Measurements were taken with the artery displayed clearly horizontally on the screen and with the carotid bifurcation visible in the image as a landmark. All measurements were made in B-mode and greyscale (the standard method of measurement).

Supplementary table 1

Supplementary table 1. Mean and max ccIMT changes within 1 year (CCA)

Parameters	**Mean ccIMT, mm**				**Max ccIMT, mm**			
Group	**IN** (n = 71)		**CON** (n = 55)		**IN** (n = 71)		**CON** (n = 55)	
	Mean	SEM or 95% CI	Mean	SEM or 95% CI	Mean	SEM or 95% CI	Mean	SEM or 95% CI
Baseline	0.695	0.016	0.674	0.019	0.856	0.019	0.825	0.023
6 months	0.704	0.014	0.704	0.019	0.855	0.016	0.846	0.023
1 year	0.721	0.015	0.718	0.020	0.862	0.018	0.859	0.025
Δ(baseline, 1 year)	0.026	0.012, 0.039	0.045	0.033, 0.056	0.006	-0.015, 0.028	0.034	0.015, 0.053
p WG *	**0.001** a		**<0.001** a		0.165 a		**0.001** a	
p BG †	**0.022** b				0.117 b			
p BG † (multivariable-adjusted)	**0.038** c				0.098 c			

Values are means and SEM, except changes which are expressed as means and 95% CI.
ccIMT: common carotid intima-media thickness; CCA: complete case analysis; IN: intervention; CON: control; p WG: p-values for within-group changes from baseline to 1 year; p BG: p-values for between-group differences in 1-year trajectories; SEM: standard error of the mean; CI: confidence interval;
* p-value for within-group comparisons by:
[a] Wilcoxon test (two-sided)
† p-value for between-group comparisons by:
[b] repeated measures ANCOVA, adjusted for the baseline values
[c] repeated measures ANCOVA, adjusted for the baseline values, age, and sex

Supplementary table 2

Supplementary table 2. Mean and max ccIMT changes within 1½ years (CCA)								
Parameters	**Mean ccIMT, mm**				**Max ccIMT, mm**			
Group	**IN** (n = 63)		**CON** (n = 47)		**IN** (n = 63)		**CON** (n = 47)	
	Mean	SEM or 95% CI	Mean	SEM or 95% CI	Mean	SEM or 95% CI	Mean	SEM or 95% CI
Baseline	0.692	0.017	0.685	0.020	0.853	0.020	0.838	0.026
6 months	0.704	0.014	0.712	0.021	0.854	0.017	0.855	0.026
1 year	0.717	0.015	0.729	0.023	0.858	0.018	0.871	0.028
1½ years	0.723	0.015	0.722	0.022	0.862	0.017	0.869	0.027
Δ(baseline, 1½ years)	0.031	0.014, 0.048	0.037	0.025, 0.050	0.009	-0.017, 0.036	0.031	0.011, 0.051
p WG *	**<0.001** a		**<0.001** a		0.077 a		**0.004** a	
p BG †	0.119 b				0.164 b			
p BG † (multivariable-adjusted)	0.217 c				0.166 c			

Values are means and SEM, except changes which are expressed as means and 95% CI.
ccIMT: common carotid intima-media thickness; CCA: complete case analysis; IN: intervention; CON: control; p WG: p-values for within-group changes from baseline to 1½ years; p BG: p-values for between-group differences in 1½-year trajectories; SEM: standard error of the mean; CI: confidence interval;
* p-value for within-group comparisons by:
[a] Wilcoxon test (two-sided)
† p-value for between-group comparisons by:
[b] repeated measures ANCOVA, adjusted for baseline values
[c] repeated measures ANCOVA, adjusted for baseline values, age, and sex

Supplementary table 3

Supplementary table 3. Mean and max ccIMT changes within 1 year (LOCF)								
Parameters	**Mean ccIMT, mm**				**Max ccIMT, mm**			
Group	**IN** (n = 101)		**CON** (n = 75)		**IN** (n = 101)		**CON** (n = 75)	
	Mean	SEM or 95% CI	Mean	SEM or 95% CI	Mean	SEM or 95% CI	Mean	SEM or 95% CI
Baseline	0.688	0.013	0.671	0.016	0.851	0.016	0.820	0.020
6 months	0.702	0.012	0.691	0.017	0.856	0.015	0.831	0.021
1 year	0.719	0.012	0.702	0.018	0.865	0.016	0.842	0.021
Δ(baseline, 1 year)	0.031	0.018, 0.043	0.031	0.021, 0.042	0.014	-0.005, 0.033	0.022	0.006, 0.038
p WG *	**<0.001** a		**<0.001** a		**0.041** a		**0.003** a	
p BG †	0.815 b				0.756 b			
p BG † (multivariable-adjusted)	0.497 c				0.354 c			

Values are means and SEM, except changes which are expressed as means and 95% CI.
ccIMT: common carotid intima-media thickness; LOCF: last observation carried forward (imputed data); IN: intervention; CON: control; p WG: p-values for within-group changes from baseline to 1 year; p BG: p-values for between-group differences in 1-year trajectories; SEM: standard error of the mean; CI: confidence interval;
* p-value for within-group comparisons by:
[a] Wilcoxon test (two-sided)
† p-value for between-group comparisons by:
[b] repeated measures ANCOVA, adjusted for the baseline values
[c] repeated measures ANCOVA, adjusted for the baseline values, age, and sex

Supplementary table 4

Supplementary table 4. Subgroup analysis: mean and max ccIMT changes within 1 year (CCA)								
Parameters	**Mean ccIMT, mm**				**Max ccIMT, mm**			
Group	**IN** (n = 18)		**CON** (n = 12)		**IN** (n = 18)		**CON** (n = 12)	
	Mean	SEM or 95% CI	Mean	SEM or 95% CI	Mean	SEM or 95% CI	Mean	SEM or 95% CI
Baseline	0.886	0.015	0.876	0.023	1.062	0.018	1.089	0.028
6 months	0.855	0.017	0.917	0.025	1.018	0.019	1.112	0.035
1 year	0.870	0.023	0.941	0.031	1.039	0.029	1.130	0.042
Δ(baseline, 1 year)	-0.016	-0.050, 0.017	0.065	0.033, 0.096	-0.023	-0.071, 0.025	0.041	-0.020, 0.102
p WG *	0.311 a		**0.001** a		0.327 a		0.168 a	
p BG †	**<0.001** b				**0.023** b			
p BG † (multivariable-adjusted)	**<0.001** c				**0.003** c			

Values are means and SEM, except changes which are expressed as means and 95% CI. Subgroup: participants with baseline mean ccIMT ≥0.800 mm.
ccIMT: common carotid intima-media thickness; CCA: complete case analysis; IN: intervention; CON: control; p WG: p-values for within-group changes from baseline to 1 year; p BG: p-values for between-group differences in 1-year trajectories; SEM: standard error of the mean; CI: confidence interval;
* p-value for within-group comparisons by:
[a] paired t-test (two-sided)
† p-value for between-group comparisons by:
[b] repeated measures ANCOVA, adjusted for baseline values
[c] repeated measures ANCOVA, adjusted for baseline values, age, and sex

Supplementary table 5

Supplementary table 5. Subgroup analysis: mean and max ccIMT changes within 1½ years (CCA)								
Parameters	**Mean ccIMT, mm**				**Max ccIMT, mm**			
Group	**IN** (n = 15)		**CON** (n = 11)		**IN** (n = 15)		**CON** (n = 11)	
	Mean	SEM or 95% CI	Mean	SEM or 95% CI	Mean	SEM or 95% CI	Mean	SEM or 95% CI
Baseline	0.883	0.016	0.883	0.024	1.057	0.020	1.103	0.026
6 months	0.857	0.019	0.924	0.026	1.020	0.021	1.123	0.037
1 year	0.860	0.023	0.948	0.033	1.033	0.031	1.142	0.044
1½ years	0.876	0.020	0.937	0.034	1.042	0.022	1.134	0.046
Δ(baseline, 1½ years)	-0.007	-0.055, 0.041	0.054	0.028, 0.080	-0.016	-0.079, 0.048	0.031	-0.022, 0.084
p WG *	0.757 a		**0.001** a		0.610 a		0.221 a	
p BG †	**0.001** b				0.061 b			
p BG † (multivariable-adjusted)	**0.001** c				**0.011** c			

Values are means and SEM, except changes which are expressed as means and 95% CI. Subgroup: participants with baseline mean ccIMT ≥0.800 mm.
ccIMT: common carotid intima-media thickness; CCA: complete case analysis; IN: intervention; CON: control; p WG: p-values for within-group changes from baseline to 1½ years; p BG: p-values for between-group differences in 1½-year trajectories; SEM: standard error of the mean; CI: confidence interval;
* p-value for within-group comparisons by:
[a] paired t-test (two-sided)
† p-value for between-group comparisons by:
[b] Repeated measures ANCOVA, adjusted for the baseline values
[c] Repeated measures ANCOVA, adjusted for the baseline values, age, and sex

Supplementary table 6

Supplementary table 6. Subgroup analysis: mean and max ccIMT changes within 1 year (LOCF)								
Parameters	**Mean ccIMT, mm**				**Max ccIMT, mm**			
Group	**IN** (n = 23)		**CON** (n = 15)		**IN** (n = 23)		**CON** (n = 15)	
	Mean	SEM or 95% CI	Mean	SEM or 95% CI	Mean	SEM or 95% CI	Mean	SEM or 95% CI
Baseline	0.883	0.014	0.883	0.019	1.061	0.016	1.089	0.022
6 months	0.861	0.014	0.918	0.020	1.029	0.016	1.111	0.028
1 year	0.873	0.018	0.937	0.025	1.043	0.023	1.125	0.033
Δ(baseline, 1 year)	-0.010	-0.038, 0.019	0.054	0.027, 0.082	-0.018	-0.056, 0.020	0.036	-0.012, 0.084
p WG *	0.429 a		**0.002** a		0.340 b		0.128 b	
p BG †	**<0.001** c				**0.017** c			
p BG † (multivariable-adjusted)	**<0.001** d				**0.002** d			

Values are means and SEM, except changes which are expressed as means and 95% CI. Subgroup: participants with baseline mean ccIMT ≥0.800 mm.
ccIMT: common carotid intima-media thickness; LOCF: last observation carried forward (imputed data); IN: intervention; CON: control; p WG: p-values for within-group changes from baseline to 1 year; p BG: p-values for between-group differences in 1-year trajectories; SEM: standard error of the mean; CI: confidence interval;
* p-value for within-group comparisons by:
[a] Wilcoxon test (two-sided)
[b] paired t-test (two-sided)
† p-value for between-group comparisons by:
[c] Repeated measures ANCOVA, adjusted for the baseline values
[d] Repeated measures ANCOVA, adjusted for the baseline values, age, and sex

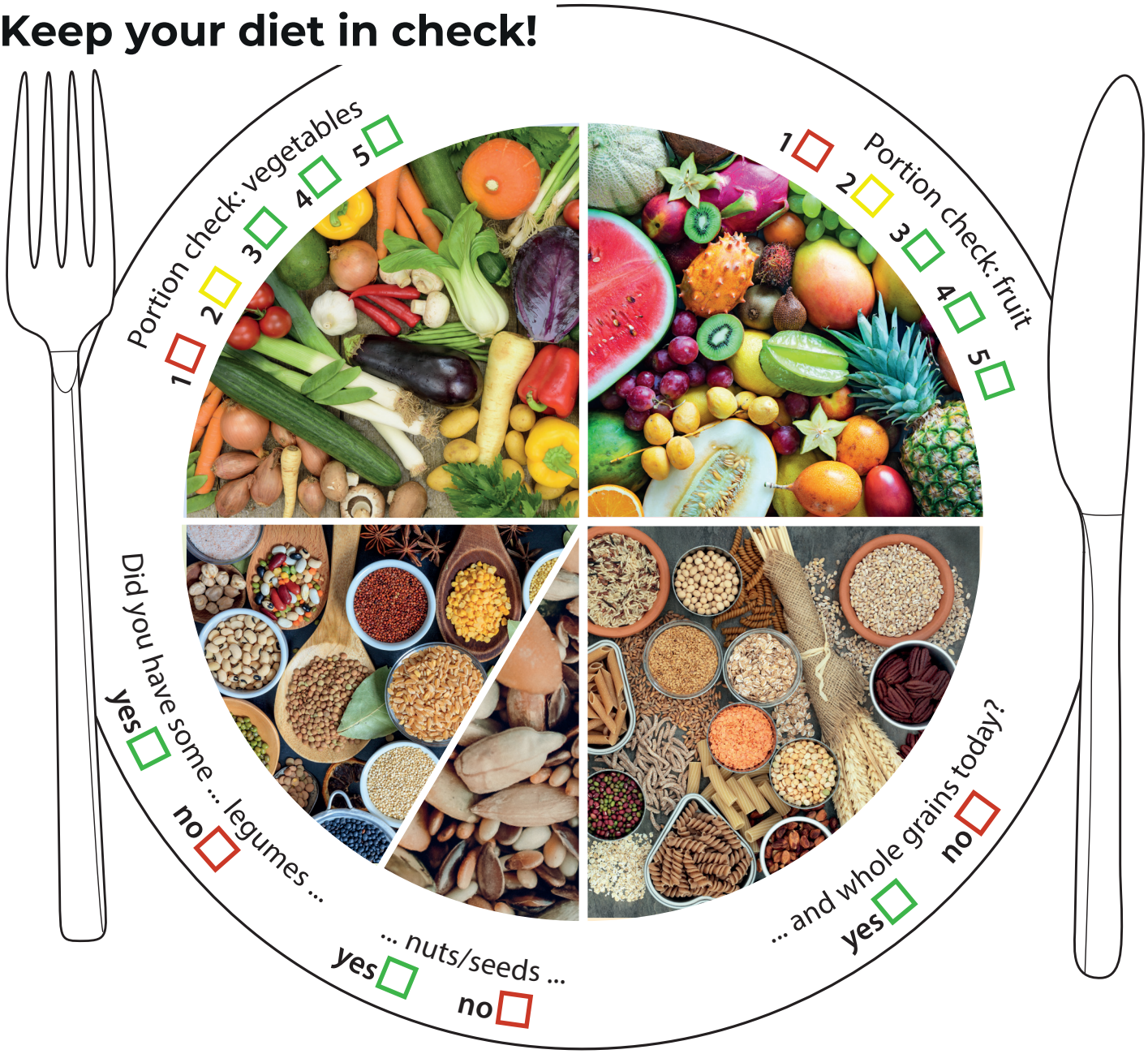
Additional information regarding the lifestyle recommendations

The healthy lifestyle handbook which the participants of the intervention group received contained chapters on the following topics: (1) opportunities and limitations of modern medicine, (2) a healthy lifestyle: the best medicine, (3) eat yourself healthy, (4) fibre: your good friend, (5) with fork and knife against overweight and type 2 diabetes, (6) concerns of the heart: fat & cholesterol, (7) blood pressure: the silent threat, (8) taking cancer prevention into your own hands, (9) physical activity: running away from illness, (10) manage stress – a life with more relaxation, (11) healthy and fit at any age, (12) an experiment: the contract between yourself and your health, (13) change is possible, (14) on the tracks of your personal strengths, (15) from “I” to “we”.

The recipe booklet contained only vegetarian recipes (most of which were vegan) based on whole grains, legumes, vegetables, fruit, nuts/seeds, and healthy oils. While the recommendation to adopt an entirely vegetarian/vegan diet was not stated, the idea was to improve the typical German diet rich in animal-source foods and highly processed foods by adding more healthy plant foods.

An English translation of the laminated information sheet can be found at the end of this Supplementary material document (the following two pages).

Keep your diet in check!



Keep your habits in check!

Water

Today I had ...

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐

6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 ☐

... glasses of water or unsweetened tea!

Satiety

Today after dinner I felt ...

☐ much too full ☐ a little too full ☐ comfortably full

Activity

I've done this today:

☐ unfortunately didn't exercise ☐ 30 min or 5000 steps ☐ 60 min or 10000 steps

Mindfulness

This many times today I took a minute off just for myself:

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 ☐

Today I did something to make myself feel relaxed! yes ☐ no ☐

Social life

☐ I received some support from others today.
☐ I took some time today to spend with friends or family.
☐ I did some socializing today.

Start every day with a kick-start!

Have more of these ...

Fruit and vegetables
Eat at least 2 portions of fresh fruit a day. Avoid canned fruit, and use dried fruit for sweetening. Enjoy a variety of different types of vegetables and eat 3 or more portions every day.

Legumes and potatoes
Enjoy legumes such as lentils, soya foods (for example, tofu), peas, and beans daily. Eat boiled or baked potatoes or sweet potatoes and avoid high-calorie sauces.

Whole grains and whole grain products
Whole grain cereals such as millet, barley, quinoa, rolled oats and wheat can be eaten in breakfast porridge or as side or main dishes. Also enjoy bread, rice, pasta, etc. in their whole-grain varieties.

Nuts, seeds, and oils
Preferably consume rapeseed and olive oil. Make nuts and seeds (unsalted!) a regular part of your diet. But don‘t eat more than a handful a day.

Water
Drink 10 glasses of water or unsweetened tea every day. Spice up your water with some fresh herbs, slices of lemon or ginger, or pieces of fresh fruit.

Have less of these ...

Animal fats and animal protein
Eat meat or offal 0 to 2 times a week. Avoid sausages and processed meats if possible.
Use low-fat milk and dairy products, and try out plant-based alternatives.

Sugar, sweets, and snacks
Consume sugar, honey, syrup, cakes, sweets, soft drinks, high-sugar desserts and spread in small quantities only. And save these for special occasions.

Salt
Use salt sparingly. Discover the large variety of fresh herbs and spices for seasoning your meals!
Do not consume salty foods such as crisps, salted nuts, crackers, soya sauce, or convenience foods (for example, pizza) or consume them in moderation.

Eggs
Eat 0 to 3 eggs a week. Also watch out for hidden eggs in cakes pasta, and cookies.

Alcohol
Do not drink alcohol more than once or twice a week. Reserve drinking alcohol for special occasions and have only small amounts.

Establish healthy habits!

Food habits

- ✓ After getting up, drink a glass of (lukewarm) water.
- ✓ Enjoy your meals in the compans of friendly people.
- ✓ Eat only as much as will leave your comfortably full.
- ✓ Allow your digestive system to rest for at least 4 hours between meals.
- ✓ Have dinner no later than 3 hours before bedtime.

Relaxation habits

- ✓ Take 5 minutes each morning to consciously start the day.
- ✓ Take enough time for relaxation and rest and incorporate breaks into your daily routine.
- ✓ Several times a day pause for a minute. Close your eyes. Take several deep breaths and let go.
- ✓ Go to bed and get up at the same times each day.

Physical activity habits

- ✓ Avoid sitting for long periods of time and exercise for at least 30 minutes every day - preferably outdoors.
- ✓ Choose a kind of physical activity that you enjoy and proceed at your own pace.

Socializing habits

- ✓ Take some time off several times a week for activities with friends and family.
- ✓ Do something good for others more often ...
- ✓ ... and allow yourself to receive support and enjoy nice gestures from others.

Appendix: Acknowledgements

I would like to extend my gratitude to my thesis supervisors Prof Dr Heike Englert and Prof Dr Andreas Hahn for putting their trust in me, for their many words of encouragement, their advice, and their ongoing support. I would like to sincerely thank Prof Dr Jan Philipp Schuchardt for acting as a co-referee and the chair of the examination board for the defence of this thesis. I would also like to express my deepest appreciation to the entire research group at the University of Applied Sciences Münster who were all involved in conducting our lifestyle intervention trials: apart from the research group's leader, Prof Dr Heike Englert, this includes Corinna Anand, Sarah Husain, Ragna-Marie-Kranz, Nora Schoch, Dima Alzughayyar, and Alwine Kraatz. I am also deeply indebted to Dr Norman Bitterlich for his continued feedback, detailed explanations, and suggestions in terms of statistical analysis. Furthermore, I extend my gratitude to all my friendly colleagues in the entire project of "münster.land.leben", as a part of which our studies were undertaken. I am very grateful to all the study participants, student assistants, and local supporters who facilitated the execution of our studies and want to particularly thank the physician Dominik Zeljko for conducting the ccIMT measurements and supporting the lifestyle intervention in many ways. I would also like to extend my sincere thanks to the wonderful library staff at the University of Applied Sciences Münster and the University of Münster as well as the University of Hannover (in particular, Jessica Michel for her unremitting and very friendly replies). I am also grateful to all my friendly colleagues (as well as the students) at the Department of Nutrition at the University of Applied Sciences Münster for their support and trust over the years. In addition, I wish to thank the friendly staff at the Münster University Hospital (Elke Börger, Dr Manfred Fobker, and Brigitte Wewel) for all their hard work and additional information and feedback.

For inspiration: I would like to express my gratitude to Dr Tsige-Yohannes Habte for pointing the way to a PhD, to Dr Armando Perez Cueto for brightening that way, and to Dr Daniel Dorado and Dr Oscar Horta for shining in and of themselves; to Dr Salim Hage for his quiet brilliance; to Ash Hooper for pointing out my nonsense (and pointing the way to vegan nutrition science); to Brenda Davis for pointing the way towards studying nutrition science; to Dr Helga D'Havé for the many words of wisdom (and support); to Shankar Narayan for his relentless spirit of satyagraha (and his relentless friendship); to José Miguel Facal for his wisdom (and Spanish grammar); to Barbro Nordling, Pete Amedeo, and Aikko Bonanati for their decades-long support and friendship and for her Nordic assistance, his rambunctiousness, and his historicocritical stuff, respectively. Ultimately yet importantly, I would also like to really thank my family, Hiasma, the Tempster, BB, skate pals, Joe Frank, and all my hitherto unmentioned friends and foes.

Appendix: Curriculum vitae

University education:

January 2018 to July 2022: Pursuing a doctoral degree in natural sciences, Leibniz University Hannover (Germany) in cooperation with the University of Applied Sciences Münster (Germany); defence of the dissertation: 12 July 2022 (Leibniz University Hannover, Germany)

May 2015 to June 2017: Master of Science in nutritional sciences, Justus Liebig University Gießen (Germany)

October 2012 to May 2015: Bachelor of Science in nutritional sciences, Justus Liebig University Gießen (Germany)

Book publications:

2020: Vegan baby, a guide to complementary feeding, for vegans between the ages of 4 and 12 months (also in German, Spanish, and Portuguese)

2017: *Nutrición vegana, separando la evidencia de la creencia* (Spanish; also as an Italian translation)

2014: *Veganismus* (German)

Professional experience:

January 2018 to April 2022: research assistant, Department of Nutrition, University of Applied Sciences Münster (Germany)

January 1999 to December 2017: various types of employment, including translations for a sustainable kitchen utensil company (Belgium; online), literature research for a correspondence course on vegan nutrition (Germany; online), working in customer service and administration at a vegan shoe and fashion company (London, England), supporting activities and events at the Indian Vegan Society (India), working as a chef and administrator in various vegetarian/vegan restaurants (London, England), and working at a methadone dispensary (Kingston-upon-Thames, England).

Volunteering (selection):

Various types of volunteering, including writing articles for *La Vegetariana* (Buenos Aires, Argentina); giving presentations about vegan nutrition at the International Animal Rights Conference (Luxembourg) and for Animal Ethics (Madrid and Barcelona, Spain); supervision at the local indoor skateboard park (Gießen, Germany); establishment of a small non-governmental organization providing evidence-based vegan nutrition information (Santiago, Chile); and a large number of English-German translations.

Appendix: Complete list of scientific publications

- **Koeder, C**., Hahn, A. & Englert, H. Healthy lifestyle changes can improve cardiovascular markers within 10 weeks. *Atherosclerosis* 355, P158; **DOI**: 10.1016/j.atherosclerosis.2022.06.682 (2022)
- **Koeder, C**. & Perez-Cueto F.J.A. Vegan nutrition: a preliminary guide for health professionals. *Crit Rev Food Sci Nutr*, 12 August 2022, online ahead of print, 1–38; **DOI**: 10.1080/10408398.2022.2107997 (2022)
- **Koeder, C**. *et al.* Healthy lifestyle changes favourably affect common carotid intima-media thickness: the Healthy Lifestyle Community Programme (cohort 2). *J Nutr Sci* 11, e47; **DOI**: 10.1017/jns.2022.46 (2022)
- Anand, C.; Kranz R.-M.; Husain S.; **Koeder C**. *et al.* Bridging the gap between science and society: long-term effects of the Healthy Lifestyle Community Programme (HLCP, cohort 1) on weight and the metabolic risk profile: a controlled study. *BMJ Nutr Prev Health* 5, 44–54; **DOI**: 10.1136/bmjnph-2021-000340 (2022)
- **Koeder, C**. *et al.* Effect of a 1-year controlled lifestyle intervention on body weight and other risk markers (the Healthy Lifestyle Community Programme, cohort 2). *Obes Facts* 15, 228–239; **DOI**: 10.1159/000521164 (2021)
- **Koeder, C**., Hahn, A. & Englert, H. Effect of a 6-month controlled lifestyle intervention on common carotid intima-media thickness. *J Nutr Health Aging* 25, 869–877; **DOI**: 10.1007/s12603-021-1628-0 (2021)
- **Koeder, C**., Hahn, A. & Englert, H. Is fruit intake associated with common carotid intima-media thickness? *Eur J Public Health* 31, ckab165.391; **DOI**: 10.1093/eurpub/ckab165.391 (2021)
- **Koeder, C**., Hahn, A. & Englert, H. A plant-based diet and healthy lifestyle lower C-reactive protein levels. In: The Future of Food and Healthcare. VegMed Web 2021. Scientific Congress for Plant-Based Nutrition and Medicine. February 28 to March 2, 2021. Abstracts. *Complement Med Res* 28, 6–7; **DOI**: 10.1159/000514476 (2021)
- **Koeder, C**., Englert, H. & Hahn, A. No clear association of sleep duration or bedtime with common carotid intima-media thickness. *Atherosclerosis* 331, e150-e151; **DOI**: 10.1016/j.atherosclerosis.2021.06.452 (2021)
- **Koeder, C**. Understanding the situation of vegans. *Eating Weight Disord* 26, 2807–2808; **DOI**: 10.1007/s40519-021-01127-2 (2021)
- Englert, H., Anand, C. & **Koeder, C**. Das Healthy-Lifestyle-Community-Programm: ein Community-basiertes, ganzheitliches Lebensstil-Interventionsprojekt zum gesunden Leben und Arbeiten. In: Weidmann, C. & Reime, B. *Gesundheitsförderung und Versorgung im ländlichen Raum*. S. 295–308; **DOI:** 10.1024/85979-000 (Hogrefe, 2021)

- **Koeder, C**. Nachhaltigkeit veganer Ernährungsweisen. In: Englert, H. & Siebert, S. *Vegane Ernährung*. Zweite Auflage, S. 267–293 (Haupt Verlag, Bern, 2020)
- **Koeder, C**. Nutrient recommendations for vegans – what should we recommend? In: Kessler, C.: VegMed: «VegMed - Scientific Congress for Plant-based Nutrition and Medicine», April, 20-22, 2018, Berlin: Abstracts. NR. 56. *Complement Med Res* 25, 14; **DOI**: 10.1159/000488417 (2018)