Exercise- and diet-induced metabolic and physiological adaptations

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Abstract

Background and aim: 71% of all deaths worldwide are caused by non-communicable diseases such as cardiovascular diseases or type-2 diabetes. In modulation of those diseases, exercise and a healthy diet are preventive measures. Additionally, both components favorably impact the process of aging. Although some studies have investigated potential synergistic effects of exercise and dietary modifications, most research has focused on participants with health conditions (e.g., obesity, pre-diabetes, type-2 diabetes), leaving out the untrained but otherwise healthy older population. Therefore, it remains unknown to what extent healthy elderly individuals may benefit from exercise combined with dietary modifications. As a consequence, the aim of this thesis was to study the health benefits and synergistic effects of exercise combined with dietary measures (healthy diet vs. *Calanus finmarchicus* oil intake) in untrained healthy elderly individuals. Beyond positive health effects of exercise and a healthy diet, there are also scenarios in which exercise and (inadequate) dietary intake may negatively impact health. Therefore, a second aim was to assess the health risks associated with low energy availability, commonly observed in athletes in a performance-oriented environment.

Methods: Altogether, 134 elderly participants (50-70 years, BMI 18-30 kg/m^2, no sports within the last two years) were included in a 12-week randomized, controlled interventional trial. Using stratified randomization according to sex, BMI, and age, participants were allocated to one of four study groups: 1) control group with no intervention (CON); 2) 2x/week aerobic and resistance exercise only (EX); 3) exercise routine as in 2) combined with dietary counseling in accordance with the guidelines of the German Nutrition Society (EXDC); 4) exercise routine as in 2) combined with intake of 2 g/day *Calanus finmarchicus* oil (EXCO). At the beginning and end of the study body composition was analyzed and fasted blood samples were taken to evaluate metabolic markers of glucose and lipid metabolism, sirtuins and immune cells. Additionally, at the beginning, after six weeks, and at the end of the study, physical activity outside of the intervention using the Freiburger questionnaire for physical activity and dietary patterns using 3-day dietary food logs and food frequency questionnaires (FFQs) were recorded. Lastly, a literature review was performed to assess the health risks related to low energy availability.

Results: Overall, exercise alone increased sirtuin activity but did not show any favorable changes in terms of body composition, glucose or lipid metabolism. In contrast, exercise combined with a healthy diet or *Calanus finmarchicus* oil intake led to a significant decrease in fat mass but did not affect markers of glucose metabolism or blood lipids. However, *Calanus finmarchicus* oil intake led to a significant increase of the omega-3 index. Regarding sirtuins, the effect of the exercise routine was further enhanced by the dietary modifications. Despite the impact of the intervention, an additional cross-sectional analysis of baseline data revealed that T-cell senescence may be a

mediator of insulin sensitivity. Ultimately, despite all positive health benefits, exercise may impair health if exercise energy expenditure and dietary intake are not matched.

Conclusion: Taken together, results from this thesis indicate that a combination of a low dose, moderate exercise training combined with a healthy diet or *Calanus finmarchicus* oil supplementation has a more favorable impact on health markers in elderly, untrained, overweight subjects when compared to exercise only. To further elucidate the additive effects of exercise combined with such dietary modifications, future studies should investigate potential effects of a healthy diet (rich in omega-3 polyunsaturated fatty acids) vs. *Calanus finmarchicus* oil supplementation. This would also be of interest in combination with a higher dose exercise program.

Trial registration: German Register of Clinical Trials DRKS00014322

Key words: exercise, nutrition, health

Zusammenfassung

Hintergrund und Ziel: 71% aller Todesfälle weltweit werden durch nicht übertragbare Krankheiten wie Herz-Kreislauf-Erkrankungen oder Typ-2-Diabetes verursacht. Sowohl bei der Prävention als auch bei der Therapie dieser Krankheiten erwiesen sich insbesondere Bewegung als auch eine gesunde Ernährung als wirksame Maßnahmen. Darüber hinaus wirken sich beide Komponenten günstig auf den Alterungsprozess aus. Obwohl einige Studien mögliche synergistische Effekte von Bewegung und Ernährungsmaßnahmen untersuchten, konzentrierten sich die meisten von ihnen auf Personen mit bereits bestehenden, chronischen Erkrankungen (z. B. Adipositas, Prä-Diabetes, Typ-2-Diabetes), wobei die untrainierte, gesunde ältere Bevölkerung bisher ausgelassen wurde. Daher ist unzureichend geklärt, inwieweit gesunde ältere Menschen von Bewegung in Kombination mit Ernährungsmaßnahmen profitieren können. Infolgedessen war das Ziel dieser Arbeit, die gesundheitlichen Vorteile und synergistischen Effekte von Bewegung in Kombination mit Ernährungsmaßnahmen (gesunde Ernährung vs. *Calanus finmarchicus* Supplementation) bei untrainierten, gesunden älteren Menschen zu untersuchen. Neben den gesundheitsfördernden Effekten von Bewegung und einer gesunden Ernährungsweise gibt es auch Szenarien, in denen viel Bewegung und eine unzureichende Nahrungsaufnahme die Gesundheit negativ beeinflussen können. Ein sekundäres Ziel war daher die Bewertung von Gesundheitsrisiken im Zusammenhang mit einer geringen Energieverfügbarkeit, wie sie vor allem bei leistungsorientierten Sportlern beobachtet wird.

Methoden: Insgesamt wurden 134 ältere Teilnehmer (50-70 Jahre, BMI 18-30 kg/m^2, keine regelmäßige sportliche Aktivität innerhalb der letzten zwei Jahre) in eine 12-wöchige randomisierte, kontrollierte Interventionsstudie eingeschlossen. Unter Verwendung einer stratifizierten Randomisierung nach Geschlecht, BMI und Alter wurden die Teilnehmer einer von vier Studiengruppen zugeordnet: 1) Kontrollgruppe ohne Intervention (CON); 2) 2x/Woche Ausdauer- und Krafttraining (EX); 3) Trainingsroutine kombiniert mit einer Ernährungsberatung nach den Richtlinien der Deutschen Gesellschaft für Ernährung (EXDC); 4) Trainingsroutine kombiniert mit einer Einnahme von 2 g/Tag *Calanus finmarchicus* Öl (EXCO). Zu Beginn und am Ende der Studie wurde die Körperzusammensetzung gemessen und Blutproben entnommen, um Marker des Glukose- und Lipidstoffwechsels, Sirtuine sowie Immunzellen zu analysieren. Zusätzlich wurden die körperliche Aktivität außerhalb der Intervention sowie das Ernährungsverhalten zu Beginn, nach sechs Wochen und am Ende der Studie unter Verwendung des Freiburger Fragebogens für körperliche Aktivität, 3-Tage Ernährungsprotokollen und Verzehrshäufigkeitsfragebögen (FFQs) aufgezeichnet. Darüber hinaus wurde eine Literaturrecherche durchgeführt, um die Gesundheitsrisiken im Zusammenhang mit einer geringen Energieverfügbarkeit zu bewerten.

Ergebnisse: Insgesamt führte das Training allein zu keinen günstigen Veränderungen der Körperzusammensetzung, des Glukose- oder Lipidstoffwechsels, zeigte jedoch einen positiven Effekt auf die Sirtuinaktivität. Im Gegensatz dazu führte das Training in Kombination mit einer

gesunden Ernährung oder der Aufnahme von *Calanus finmarchicus* Öl zu einer signifikanten Abnahme der Fettmasse; hatte jedoch ebenfalls keinen Einfluss auf den Glukosestoffwechsels oder die Blutfette. Die Aufnahme von *Calanus finmarchicus* Öl führte zudem zu einem signifikanten Anstieg des Omega-3-Index. Hinsichtlich der Sirtuinaktivität wurde der Effekt des Trainings durch die Ernährungsumstellung aber durch die Einnahme von *Calanus finmarchicus* Öl zusätzlich verstärkt. Zusätzlich zeigte eine Querschnittsanalyse der Basisdaten, dass T-Zellseneszenz ein Mediator der Insulinsensitivität sein kann. Zusätzlich konnte gezeigt werden, dass trotz aller gesundheitsfördernden und präventiven Effekte, intensiver Sport auch negative gesundheitliche Folgen haben kann, wenn der sportbedingte Energieverbrauch und die nahrungsbedingte Energiezufuhr nicht aufeinander abgestimmt sind.

Schlussfolgerung: Zusammenfassend zeigen die Ergebnisse dieser Arbeit, dass eine Kombination aus einem niedrig dosierten, mäßigen Training mit einer gesunden Ernährung oder einer *Calanus finmarchicus* Öl Supplementierung die Gesundheit bei älteren, untrainierten, übergewichtigen Personen günstiger beeinflussen kann als das Training allein. Um die gesundheitlichen Vorteile weiter zu untersuchen, sollten künftige Studien mögliche Auswirkungen einer gesunden Ernährung (reich an Omega-3 Fettsäuren) im Vergleich zur CO-Supplementierung untersuchen. Dies wäre auch in Kombination mit einem höher dosierten Training von Interesse.

Studienregistrierung: Deutsches Register Klinischer Studien DRKS00014322

Schlagworte: Bewegung, Ernährung, Gesundheit

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List of abbreviations

ACSM	American College of Sports Medicine
ALA	Alpha-linolenic acid
AMPK	AMP-activated protein kinase
ARA	Arachidonic acid
BMI	Body-Mass-Index
BMR	Basal metabolic rate
CMV	Cytomegalovirus
CO	*Calanus finmarchicus* oil
DGE	German Nutrition Society (Deutsche Gesellschaft für Ernährung)
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FA(s)	Fatty acid(s)
FFM	Fat free mass
FOXO	forkhead box O
FM	Fat mass
IL	Interleukin
IoM	Institute of Medicine
ISSFAL	International Society for the Study of Fatty Acids and Lipids
kcal(s)	Kilocalorie(s)
kJ(s)	Kilojoule(s)
LA	Linoleic acid
LBM	Lean body mass
LEA	Low energy availability
MET(s)	Metabolic equivalent(s)
MUFA(s)	Monounsaturated fatty acids(s)
n	Omega
NCD(s)	Non-communicable disease(s)
NF-κb	Nuclear factor-κB
NVS II	Nationale Verzehrsstudie II
O3I	Omega-3 index
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PL(s)	Phospholipids(s)
PPAR(s)	Peroxisome proliferator-activated receptor(s)
PUFA(s)	Polyunsaturated fatty acid(s)
RBC(s)	Red blood cell(s)

SD	Standard deviation
SDA	Stearidonic acid
SFA(s)	Saturated fatty acids
SIR	Silent information regulator
SIRT	Sirtuin
SIRT1	Sirtuin 1
SIRT3	Sirtuin 3
SIRT5	Sirtuin 5
TAG(s)	Triglyceride(s)
T_{CEM}	Central memory T-cells
T_{EM}	Effector memory T-cells
T_{EMRA}	Effector memory cells re-expressing CD45RA
TNF-α	Tumor necrosis factor alpha
WHO	World Health Organization

1. General introduction

1.1 Background and aim of this dissertation

Worldwide, 71% of deaths are caused by non-communicable diseases (NCDs) such as cardiovascular diseases, type-2 diabetes, and cancers [1,2]. Lifestyle represents one of the key components to modulation those diseases, as well as maintenance of optimal health throughout the lifespan [3]. In that regard, particularly exercise and a healthy diet are preventive measures against the development of NCDs. Further, both lifestyle factors can also favorably impact the process of aging, which is accompanied by a progressive decline in physiological and physical function and body composition changes, additionally increasing the risk for NCDs in older individuals.

Exercise prevents, mitigates, and even partially reverses age-related changes, such as a progressive decline in muscle and increase in fat mass [4]. Moreover, it helps to maintain and improve cardiovascular and muscular function and has broad beneficial effects on metabolic health by favorably impacting glucose and lipid metabolism [5–8]. Exercise is also a modulator of the immune system [9,10] and was described to influence health even on a molecular level (e.g. via modulating gene expression) [11,12]. At the same time, beneficial effects elicited from exercise can further be supported by dietary intake. In fact, all physiological processes require nutrients from the diet and can negatively be impacted by excessive or deficient intake. Despite the acknowledgement for the need of a nutrient dense, healthy diet, certain health benefits are also ascribed to specific dietary compounds. In that regard, omega-3 polyunsaturated fatty acids (n-3 PUFAs) are of interest, as they were shown to contribute to improved metabolic processes, body composition, and decreased cardiovascular risk [13–15] – all of which are of particular importance within an older population. One novel source of n-3 PUFAs, whose supplementation has promising effects on blood glucose control, atherosclerosis, and obesity is oil from the copepod *Calanus finmarchicus* [16,17]. However, evidence on health effects in humans is still sparse.

Although some studies investigated the effects of exercise combined with dietary interventions, most of them focused on participants with health conditions (e.g. obesity, pre-diabetes, type-2 diabetes, sarcopenia) [18–23]. Moreover, studies in older populations used either a healthy diet [21,24] or n-3 PUFA supplementation [25–27], but have never directly compared a healthy diet to supplementation. Therefore, it remains unknown to what extent healthy elderly individuals may benefit from aerobic and resistance exercise alone versus the same exercise routine combined with a healthy diet or *Calanus finmarchicus* oil intake. On the other hand, health effects of exercise and dietary intake can also be diminished when energy expended

through exercise is not adequately matched with dietary intake. This is particularly observed in performance orientated athletes.

Study objectives

To gain knowledge about the health benefits and synergistic effects of an aerobic and resistance exercise routine combined with dietary measures (healthy diet vs. intake of oil from the copepod *Calanus finmarchicus*) in untrained, healthy elderly adults, a 12-week randomized, controlled intervention was conducted. To assess the health benefits, body composition, metabolic markers (glucose and lipid metabolism) and enzyme activity (sirtuins) were analyzed. In addition, a cross-sectional analysis of immune cells (T-cells) was performed to assess the role of immunosenescence on insulin sensitivity. Lastly, a literature review was performed to assess the health risks related to exercise and dietary behaviors observed in athletes exercising in a performance oriented environment. This dissertation thesis is based on the following research questions, which are answered within the respective scientific publications:

1. Can exercise combined with dietary counseling in accordance with the German Nutrition Society or supplementation of oil from *Calanus finmarchicus* positively influence body composition, markers of glucose metabolism, or blood lipids? (Paper I, chapter 2.1)
2. Can *Calanus finmarchicus* oil supplementation effectively increase the omega-3-index as a biomarker of fatty acid supply and cardiovascular risk and is it influenced by exercise? (Paper II, chapter 2.2)
3. Can exercise combined with dietary counseling or supplementation of oil from *Calanus finmarchicus* positively influence sirtuin activity? (Paper III, chapter 2.3)
4. In addition to diet and exercise, is immune cell aging a modulator of insulin sensitivity? (Paper IV, chapter 2.4)
5. How can performance oriented exercise and dietary intake impact health in athletes? (Paper V, chapter 2.5)

1.2 Physical activity and health

1.2.1 Definition and categorization of physical activity

Physical activity is defined as "*any bodily movement produced by skeletal muscles that results in energy expenditure*" [28]. The amount of energy expended can be measured as kilocalories (kcal), kilojoules (kJ), oxygen consumption, or metabolic equivalents (METs), which is used to categorize physical activity as shown in **Table 1** [29].

Table 1: Categorization of physical activity based on the amount of energy expended expressed as METs.

Activity	METs*	Example activity
Sedentary activity	1.0-1.5	Lying or sitting
Light intensity activity	1.6-3.0	Standing, slow walk
Moderate-intensity activity	3.0-6.0	Brisk walk, activities such as vacuuming
Vigorous-intensity activity	6.0 or greater	Very fast walk, activities such as shoveling snow, running, aerobics class

* 1 MET expresses the amount of oxygen (O_2) consumed while sitting at rest, which is 3.5 ml O_2/kg body weight/min [30].
Table created in accordance with the 2018 Physical Activity Guidelines Advisory Committee Scientific Report [29]

Exercise represents a subcategory of physical activity and can be defined as "*physical activity that is planned, structured, repetitive, and designed to improve or maintain physical fitness, physical performance, or health*" [28]. Exercise itself can further be categorized as aerobic or resistance exercise [31], which represent "*two extremes on the exercise continuum*" [32]. While aerobic exercise usually consists of a high number of muscular contractions at low loads and primarily leads to improvements in endurance and the cardiovascular system, resistance exercise consists of a low number of muscular contractions at higher loads targeting the improvement of muscular strength and power [31,32]. As it becomes evident that training both modalities may be more beneficial than focusing on one of the two, the present work aimed to investigate the effects of a combined resistance and aerobic training program.

1.2.2 Response and adaptations to exercise

With regard to the responses and adaptations to exercise can be distinguished between short-term responses to acute exercise, meaning a single bout, and long-term adaptations as a result of chronic exercise, meaning repeated bouts.

Short-term responses to acute exercise include overall increased oxygen uptake, increased metabolism of the skeletal muscles and myocardium as well as the cardiovascular and respiratory system (e.g. increased blood flow, ventilation) [33,34]. As short-term responses are not part of this work, a more detailed discussion is not included.

Briefly, the long-term adaptation to chronic exercise is an improvement of physical fitness, which can be defined as a "*set of attributes that people have or achieve*" that determines the ability to perform daily tasks [28]. Those attributes encompass cardiorespiratory and muscular endurance, muscular strength, reaction speed, flexibility, balance, and body composition [28,31]. Aside from improved physical fitness, exercise also contributes to health on many other levels, such as improvements in cognitive and metabolic function (e.g. improved insulin sensitivity, mitochondrial biogenesis) [34–36]. Conversely, physical inactivity contributes to a deterioration of physical fitness and negatively impacts health (**Figure 1**). In fact, physical inactivity is one of the major factors facilitating the development of NCDs and lowering life expectancy [37].

Physically inactive   Physically active

Brain function
↑↓ Neurodegeneration
↓↑ Cognitive function

Muscle
↓↑ Strength, power, endurance
↓↑ Flexibility

Cardiovascular function
↑↓ Heart rate
↓↑ Blood volume
↑↓ Blood pressure
↓↑ Endothelial function

Body composition
- ↑ -↓ Weight
↓↑ Muscle mass
↑↓ Fat mass

Lung function
↓↑ Capacity

Metabolism
↓↑ Insulin sensitivity
↓↑ Fat oxidation
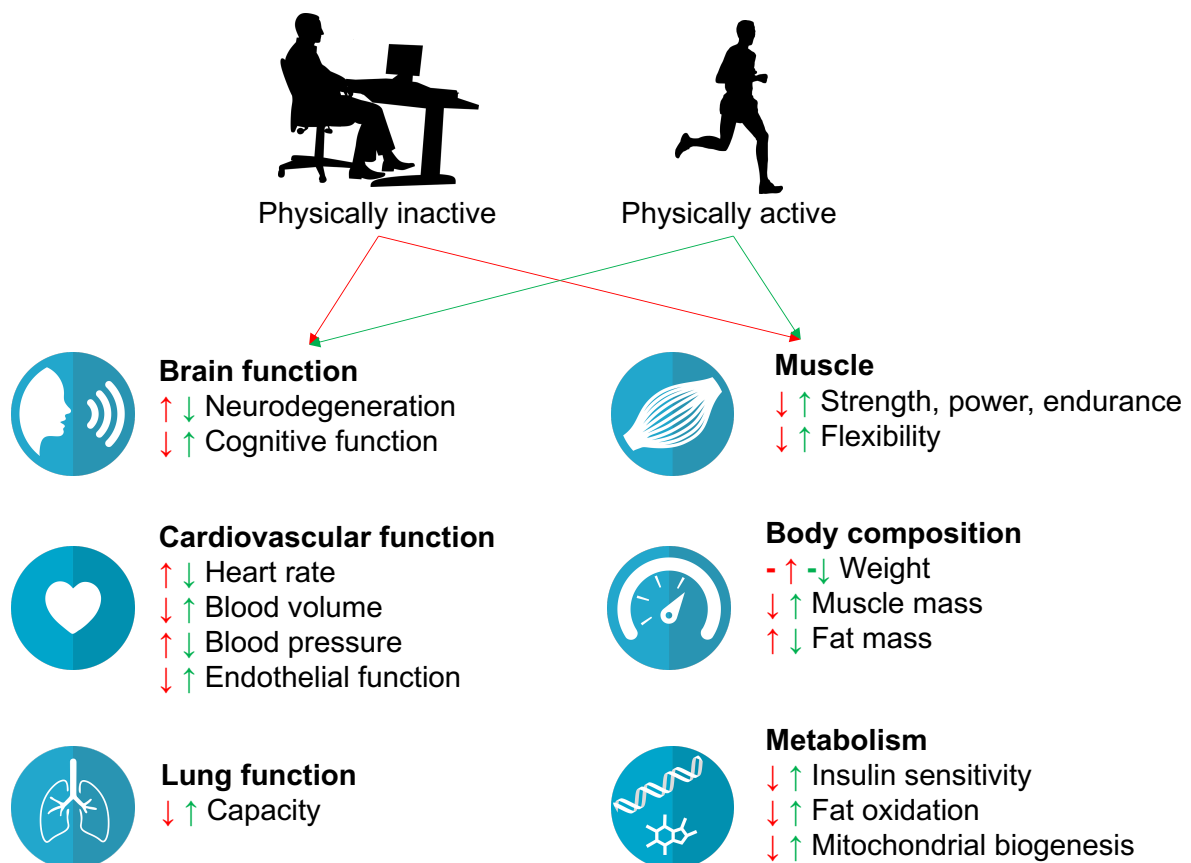↓↑ Mitochondrial biogenesis

Figure 1: The impact of physical inactivity and activity on physical function and health.
Arrows indicate an increase(↑), decrease (↓) or stagnation (-) of the respective health outcome.
Figure was created in accordance with [34–36,38–40].

1.2.3 Recommendations and status

To give guidance to the general population, organizations such as the American College of Sports Medicine (ACSM) and the World Health Organization (WHO) published recommendations on physical activity and exercise, which are both based on the 2018 physical activity guidelines from the U.S. Department of Health and Human Services [29,41,42]. These recommendations for adults are summarized in **Table 2**. The ACSM categorizes the type of physical activity as exercise, while the WHO refers to physical activity.

Table 2: Recommendations on physical activity and exercise from the ACSM and the WHO.

ACSM (18-65 years)	WHO (18-64 years)
• ≥30 min/day on ≥5 days/week for a total of ≥150 min/week of moderate-intensity cardiorespiratory exercise training	• at least 150 min of moderate-intensity aerobic physical activity throughout the week
or	**or**
• ≥ 20 min/day on ≥3 /days/week for a total of (≥75 min/week), vigorous-intensity cardiorespiratory exercise training	• at least 75 minutes of vigorous-intensity aerobic physical activity throughout the week
or	**or**
• combination of moderate- and vigorous-intensity exercise to achieve a total energy expenditure of ≥500-1000 MET/min/week on 2-3 days/week,	• an equivalent combination of moderate- and vigorous-intensity activity
	• aerobic activity should be performed in bouts of at least 10 min
• on 2 days/week add resistance exercise for major muscle groups (60 s per exercise) and neuromotor exercise involving balance, agility, and coordination, flexibility exercises	• ≥ 2 days/week muscle-strengthening activities involving major muscle groups
	• for additional health benefits, increase moderate-intensity physical activity to 300 min/week or 150 min vigorous-intensity aerobic physical activity

Table created in accordance with [29,41].

Based on these recommendations, the WHO reported that the region with highest proportion of insufficiently active people in 2016 was America (39%). The lowest prevalence was found in the Western Pacific (18%). With approximately 29%, Europe was placed as the fourth most inactive region out of seven [43].

Regarding the current situation in Germany, the latest data available was obtained from the study „Gesundheit in Deutschland aktuell" (GEDA 2014/2015 EHIS). This data shows only 42.6% of all women and 48.0% of all men achieve aerobic activity as recommended by the WHO. Regarding the recommended muscle strengthening exercises, only 27.5% of women and 31.2% of men reported adequate implementation. When combining the two modalities, only 20.5% of women and 24.7% of men achieved the recommendations [44]. When subdivided into age groups, older adults aged 45-64 years still showed similar outcomes (women 22.7%; men 21.1%) [44].

Those results not only highlight the overall need for increased physical activity, but also the need for promotion of regular aerobic and, particularly, resistance exercise. In older adults, the importance of exercise is further underlined by the age-related progressive decline of functional capacity (encompassing the cardiovascular system, aerobic capacity, muscular strength and function) [45] as well as changes in body composition such as progressive decline in muscle and increase in fat mass [46] – all of which can be modulated by exercise.

Although most adults are generally aware of the health benefits of regular exercise, inactive adults show a lower motivation to engage in exercise [47]. This can be due to a number of psychological or environmental reasons such as believing they are unable to perform the exercise (self-efficacy), lack of social support, as well as time requirement or location of the facilities. Regarding the exercise routine itself, older adults preferably engage in moderate intensity exercise routines that are deemed safe [48]. Therefore, for older adults, a circuit based combined aerobic and resistance training at moderate intensity may be a good and easy strategy to engage in regular exercise. To lower the barrier to start exercising and to develop sustainable habits fitting the respective lifestyle, a minimal dose approach of 2x a week could be a starting point. Particularly, as low dose approaches were still reported as health-beneficial [49,50].

1.3 Health-promoting diet

1.3.1 General aspects

Within the 20th century, the shift of an undersupply with food towards an oversupply together with an increasing prevalence of nutrition-related NCDs such as type-2 diabetes, hypertension and cardiovascular diseases [51,52], has led to a paradigm shift concerning the main function of a health-promoting diet. While its previous function was to primarily avoid nutritional deficiencies and deficiency symptoms, presently, a health-promoting diet has an extended dual function. This means, it is not only required to supply the organism with all of the nutrients needed for physiological functions and to avoid deficiencies but it is also needed to support long-term health, well-being, and prevent chronic diseases [53].

Generally, this is achieved through an adequate intake of energy and nutrients from the diet, which is neither deficient nor excessive in one of the two components. While macronutrients such as carbohydrates, fats, and proteins are primarily important for energy supply and as structural components, micronutrients, such as minerals and vitamins, and water are needed for the regulation and maintenance of physiological processes. However, there are also other dietary compounds, such as dietary fibers, phytochemicals, and vitaminoids, whose consumption can elicit additional health-promoting effects. For example, adequate fiber intake favorably impacts gut health, bowel movement, contributes to lower cholesterol levels, and increases satiety [53]. Phytochemicals can contribute to increased antioxidative capacity and vitaminoids play a role in metabolic processes, serve as structural components, or have antioxidative functions [54,55]. Although the absence of those dietary components will not result in clinically relevant deficiency symptoms, their health-supporting properties highlight their relevance in a health-promoting diet.

Although the requirements for different nutrients are dependent on various individual factors (e.g. age, gender, size, health status), societies such as the Institute of Medicine (IoM) [56–59] and the German, Austrian and Swiss Nutrition Societies (Deutsche, Österreichische und Schweizerische Gesellschaften für Ernährung; D-A-C-H) [60] give recommendations and reference values for their intake based on existing data from experimental and epidemiological studies. In line with the extended dual function of a health-promoting diet, these recommendations not only aim to prevent deficiencies but also promote both health and prevent diseases. In any case, the respective recommendations of the societies lay out the foundation for dietary recommendations for the general population.

1.3.3 Dietary guidelines and status

To ensure adequate nutrient intake and an overall health-promoting diet within the general population, expert societies and organizations across the globe use food-based dietary guidelines based on the respective scientific evidence. Generally, these guidelines use food groups (e.g., vegetables, fruits, dairy products) and give recommendations on the respective amounts that should be consumed. Additionally, the recommendations vary depending on the country-specific dietary patterns. In any case, all dietary guidelines across the globe recommend a predominant intake of plant-based foods such as vegetables, grain and whole-grain products, and fruit, which should be complemented with dairy products and smaller amounts of meats, fish, or seafood [61–63]. Additionally, limiting sodium and sugar intake as well as saturated and trans fatty acid is often recommended.

Regarding the preventive function of the diet, the importance of such guidelines is further underlined by results from studies that linked higher intakes of vegetables, fruits, and whole grains to decreased risks of cardiovascular diseases, various forms of cancer, type-2 diabetes, and all-cause mortality [64–68]. For example, increasing fruit and vegetable intake from <3 portions (235 g/day) to >5 portions (391 g/day) was reported to decrease the risk of coronary heart disease by 17% [69]. Similar results were also reported by the Prospective Urban Rural Epidemiology (PURE) study, which linked intakes of 3-4 portions (375-500 g/day) to a lower risk of non-cardiovascular mortality and total mortality [66]. At the same time, results from the European Prospective Investigation of Cancer and Nutrition (EPIC) study demonstrated that decreased intake of processed and red meat is associated with a lower risk of cardiovascular diseases, cancer, and type-2 diabetes [70–72]. Ultimately, the promotion of a health-promoting diet is underlined from by findings from the Global Burden of Disease study, which reported that a total of approximately 11 million deaths in 2017 are associated with dietary factors. Of the 15 examined risk factors, high sodium intake, low intake of fruit, and low intake of whole grains were the three leading risk factors [73].

To ensure a health-promoting diet within the German population, the German Nutrition Association (Deutsche Gesellschaft für Ernährung, DGE) recommends a "wholesome diet" to all adults between18 to 65 years of age. This is defined as a diet rich in vegetables and fruits (3 portions of vegetables and 2 portions of fruit per day), whole grain cereal products, daily intake of dairy products, moderate meat intake (<600 g per week) and 1-2 portions of fish per week [63].

Although results from the German National Consumption Study (Nationale Verzehrsstudie II, NVS II) reported an overall good nutrient supply, it also revealed unfavorable dietary patterns such as intake of vegetables, fruits, and fish below the DGE recommendations and ingestion of vitamins C, D, E, folic acid, and fiber below the D-A-C-H reference values [74]. Vegetable and fruit intake is particularly important for the supply of vitamins, minerals, fiber and phytochemicals. Consumption of fish is not only needed for supply with protein, iodine and selenium, but particularly fatty cold water fish is an important source of omega-3 polyunsaturated fatty acids.

1.3.4 Impact of omega-3 polyunsaturated fatty acids

General aspects

In general, fatty acids (FAs) are carboxyl acids with an aliphatic chain. Depending on the double bonds present in the chain, they can be classified as saturated FAs (SFAs) with no double bond, monounsaturated FAs (MUFAs) with one double bond, or polyunsaturated FAs (PUFAs) with two or more double bonds. According to the position of the first double bond counted from the methyl-group, they can further be classified as n-3, n-6 or n-9 FAs. While n-9 FAs belong to the category of MUFAs, n-3 and n-6 FAs belong to PUFAs [53].

Although the human body can synthesize most of the fatty acids needed, it cannot synthesize the n-6 PUFA linoleic acid (18:2n6, LA) or the n-3 PUFA alpha-linolenic acid (18:3n3, ALA), which is why these FAs are categorized as essential FAs and need to be consumed in the diet. Once these FAs have entered the body, their derivatives can be synthesized by desaturation and elongation (**Figure 2**).
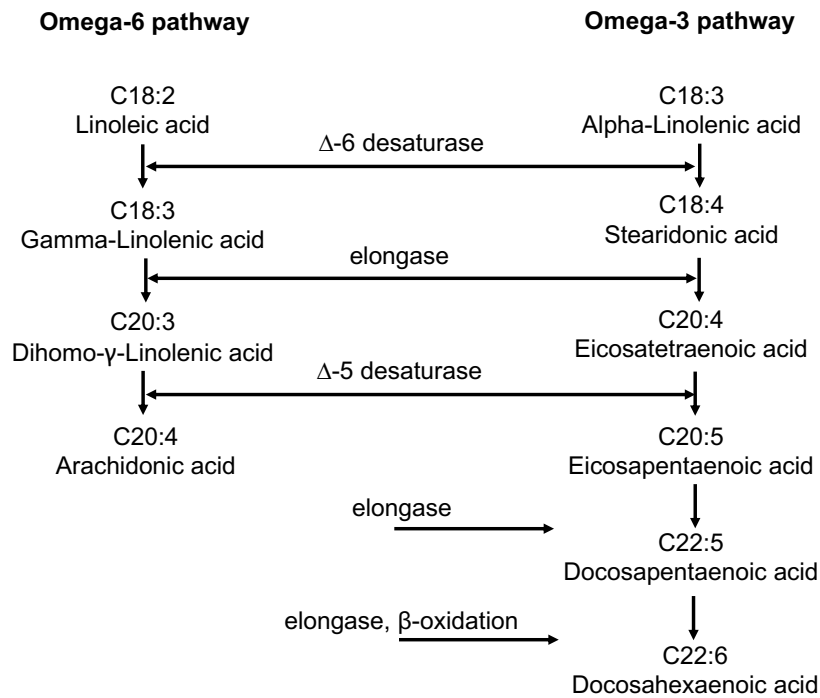
Omega-6 pathway   **Omega-3 pathway**

C18:2
Linoleic acid

C18:3
Alpha-Linolenic acid

Δ-6 desaturase

C18:3
Gamma-Linolenic acid

C18:4
Stearidonic acid

elongase

C20:3
Dihomo-γ-Linolenic acid

C20:4
Eicosatetraenoic acid

Δ-5 desaturase

C20:4
Arachidonic acid

C20:5
Eicosapentaenoic acid

elongase

C22:5
Docosapentaenoic acid

elongase, β-oxidation

C22:6
Docosahexaenoic acid

Figure 2 : Simplified overview of the n-6 und n-3 PUFA synthesis from their precursor FAs ALA and LA.

Abbreviations: ALA, alpha-linolenic acid, FA, fatty acid, LA, linoleic acid, n-3, omega-3; n-6 omega-6; PUFA, polyunsaturated fatty acid; Δ, delta.

Figure was created in accordance with [75,76].

Regarding the physiological effects of n-3 and n-6 PUFAs, the traditional view is that both have antagonistic effects in the regulation of physiological processes such as inflammation or vascular tone. Modulation of those processes occurs via oxidized metabolites of PUFAs, summarized as oxylipins [77]. Generally, the conversion of PUFAs to oxylipins is mediated through enzymatic activity of lipoxygenases (LOXs), cyclooxygenase (COXs), cytochrome P450 monooxygenases (CYPs) but also through non-enzymatic pathways [78]. In line with the above mentioned traditional view, most antagonistic health effects of PUFAs were led back to eicosanoids, which is a class of lipid mediators synthesized from the C20 PUFAs arachidonic acid (20:4n6, ARA) and eicosapentaenoic acid (20:5n3, EPA). While ARA metabolites predominantly exhibit pro-inflammatory and vasoconstrictive effects, EPA metabolites are less pro-inflammatory and vasodilatory. As both FAs are dependent on the same enzymes, health benefits of n-3 PUFAs were discussed to be mediated through suppression of ARA oxidation. However, the current perspective assumes, that several ARA metabolites also display anti-inflammatory properties and that not only EPA, but also docosapentaenoic acid (22:5n3, DPA) and docosahexaenoic acid (22:6n3, DHA) contribute to production of a novel class of potent pro-resolving lipid mediators such as resolvins, protectins and maresins [79,80]. As described

by Serhan (2014), these mediators are not only involved in resolution of inflammation but also host defense, pain, organ protection and tissue remodeling [81].

However, which metabolites are being produced is also dependent on substrate availability. This means, that dietary intake of fatty acids such as ARA, EPA and DHA is a crucial factor in modulation of inflammation and inflammatory disease. In fact, the composition of membrane lipids (phospholipids), which commonly serve as FA source for production, is largely modulated by dietary intake [53,82]. Regarding the dietary ratio of n-6 to n-3 PUFAs it has been reported that intakes of ≤ 5:1 may be health beneficial [82]. However, within the last decades a dietary shift towards a "Western diet", characterized by high intakes of animal products, processed foods and vegetable oils has led to higher intakes of SFAs, trans FAs and a n-6:n-3 ratio with an average of 15:1 [83]. Such dietary patterns can favor chronic systemic inflammation which can in turn contribute to development of NCDs such as type-2 diabetes, cardiovascular diseases or cancers [84,85]. Therefore, the need for a decreased intake of n-6 PUFAs with a concurrent increase in n-3 PUFAs becomes evident. However, adequate intake of the n-3 PUFAs EPA and DHA is not only of importance due to their regulation of inflammatory processes. Evidence shows that supply with these n-3 PUFAs is of additional importance for brain development, cognitive function and cardiovascular health [15,86]. In fact, higher supplies with EPA and DHA are linked to lower risks of cardiovascular events [87–89]. Underlying mechanisms are discussed to be driven through the incorporation of those FAs in the phospholipid membranes [90].

Sources, recommendations, status

The primary source for EPA and DHA is oily cold-water fish such as tuna, salmon, herring or mackerel. However, the respective amounts of the FAs are dependent on the type of fish. For example, 100 g of tuna provides 0.8 g of EPA+DHA (0.2 g EPA, 0.6 g DHA), while 100 g of mackerel provides 1.3 g EPA+DHA (0.6 g EPA, 0.7 g DHA) [91]. Further, other factors such as living or farming conditions and feed can influence the FA content. This is also underlined by the fact that fish themselves do not produce EPA and DHA, but obtain it from consumption of n-3 PUFA producing microalgae [92].

Aside from the direct consumption of EPA and DHA, it could also be argued that those FAs can also be synthesized from their precursor FA ALA. Although this is possible, conversion of ALA to EPA was found to have a low efficacy [93,94].

Regarding the optimal intake of the n-3 PUFAs EPA and DHA no consensus exists. In fact, organizations such as the IoM and the DGE give only recommendations on intakes of the essential FAs, LA and ALA [60,95]. On the other hand, organizations such as the European Authority for Food Safety (EFSA) International Society for the Study of Fatty Acids and Lipids (ISSFAL) extend their recommendations beyond LA and ALA and give recommendations on EPA and DHA intake [96,97] (**Table 3**).

Table 3: Recommendations and tolerable upper intake level (UL) on EPA+DHA intake for adults from selected organizations.

Organization	Recommendation	UL
ISSFAL	500 mg/day	not available
EFSA	250 mg/day[1]	5 g

[1] pregnant and breastfeeding women: additional intake of 100-200 mg DHA/day

Abbreviations: EFSA, European Authority for Food Safety; ISSFAL, International Society for the Study of Fatty Acids and Lipids.

Table was created in accordance with [96–98].

With respect to the dietary intakes a large-scale nutrition survey reported a generally low intake of seafood derived n-3 PUFAs across the world [99]. The study showed that out of the 187 countries surveyed (representing approximately 82% of the population) respondents in only 45 countries had intakes of ≥250 mg/day. Intakes of ≥500 mg/day were only reported for few countries, e.g. Greenland, Norway, Spain, Japan [99]. However, the estimation of dietary intake of n-3 PUFAs is not as reliable as assessing blood levels. In that regard, the omega-3 index (O3I) is a reliable biomarker. The O3I represents the relative amount of EPA+DHA measured in red blood cells (RBCs) and is expressed as percentage [100]. According to the percent, the supply of EPA+DHA can be evaluated as follows: <4% is considered as very low, 4-6% as low, 6-8% as moderate and >8% as optimal [101]. In line with results on dietary intake, a global analysis that used pooled, extrapolated data reported a very low or low EPA+DHA status across countries with western-style diets [102]. With regards to Germany, a cross-sectional study with 200 participants demonstrated a generally low supply situation with a mean O3I of 5.5% [103].

Beyond this background, it becomes evident that supplementation of EPA and DHA may be a good strategy to improve supply of these FAs and thereby contribute to general health and prevention of NCDs.

Sources of n-3 PUFA supplements

The most common source for n-3 PUFA containing supplements are marine oils, mainly retrieved from fish. Fish oil contains FAs, mainly in the form of triglycerides (TAGs) [104]. However, for production of purified and higher concentrated supplements, fish oil is first refined in a process including degumming, deacidification, bleaching, and deodorization [105]. Subsequently, concentration of the oils is often achieved via transesterification which results in synthetically produced FA ethyl esters (FA-EEs) or re-esterified TAGs (rTAGs). Data is controversial but suggests, that ethyl esters have a lower bioavailability when compared to TAGs or rTAGs [104,106]. In addition to fish oil, another source used for supplement production is krill oil. This oil contains FAs mainly bound as phospholipids (PLs) and only smaller amounts of TAGs. In terms of bioavailability, data is still conflicting but indicates comparable or better bioavailability when comparing PLs to TAGs [104,107]. Additionally, it also contains the antioxidant astaxanthin, giving the oil a red color.

Another novel source of n-3 PUFAs is oil obtained from the copepod *Calanus finmarchicus* (*C. finmarchicus*). Contrary to other marine oils, >80% of FAs present in this oil are bound as wax esters [108]. Additionally, PLs, TAGs, cholesterol, and free FAs are present. The respective amounts of the lipid classes present can vary depending on the life stage, location and harvest season of *C. finmarchicus*. Commonly, *C. finmarchicus* is harvested in summer. During this time, approximately 85.4% of total lipids are wax esters, 8.9% are TAGs, 4.2% are PLs, 1.2% are cholesterol, and 0.2% are free FAs [109]. The FA profile *C. finmarchicus* oil (CO) is shown in **Table 4**.

Table 4 : Lipid classes and fatty acid profile from *C. finmarchicus* oil (CO) as well as harvested *C. finmarchicus* females. Amounts are given in mass %.

Fatty acid (common name)	CO[1]	WE[2]	TAG[2]	PL[2]
		Lipid classes		
14:0 (Myristic acid)	6.4	26.3	12	3.3
16:0 (Palmitic acid)	4.5	9.8	30.4	25.8
18:0 (Stearic acid)	0.2	0.9	6.1	3.6
16:1n-7 (Palmitoleic acid)	1.7	6.7	3.6	1.1
18:1n-9 (Oleic acid)	1.6	5.3	10.4	2.5
20:1n-9 (Gondoic acid)	2.4	7.8	n.d.	0.2
22:1n-9 (Erucic acid)	0.3	0.2	n.d.	n.d.
22:1n-11 (cetoleic acid)	4.3	7.0	2.2	0.2
18:2n-6 (LA)	0.7	1.2	2.7	1.5
18:3n-3 (ALA)	1.4	1.5	2.3	0.6
18:4n-3 (SDA)	7.0	13.7	5.9	2.5
20:5n-3 (EPA)	5.5	11.4	8.7	19.2
22:5n-3 (DPA	0.3	n.d.	1.2	0.2
22:6n-3 (DHA)	3.9	2.2	5.8	37.4

[1] Fatty acid content of oil obtained from *C. finmarchicus* according to [110].

[2] Fatty acid content of *C. finmarchicus* females harvested in Fram Strait East according to [111].

Abbreviations: ALA, alpha-linolenic acid; CO, oil from *C. finmarchicus*; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; n.d., not detected; SDA, stearidonic acid; TAG, triglycerides; PL, phospholipids; WE, wax ester.

Table was modified in accordance with [112]

Irrespective of the lipid classes, n-3 PUFAs make up roughly 18% of the oil from *C. finmarchicus*. The most abundant one is 18:4n3 FA stearidonic acid (SDA) (7%), while a minor part is made up of EPA (6%) and DHA (4%). This is of interest as SDA is another precursor in EPA and DHA synthesis. In fact, SDA is the product of ALA desaturation by the Δ-6 desaturase (see **Figure 2**). As this is the rate limiting step in the conversion of ALA to EPA, it was assumed that conversion from SDA to EPA may be more efficient. Indeed, several studies reported that conversion from this SDA to EPA is efficient [113,114].

In addition to the n-3 PUFAs, CO also contains fatty alcohols and astaxanthin. In fact, the high content of astaxanthin (~1500 ppm) [108] is responsible for the deep red color of the oil. In addition to the already mentioned antioxidative function of astaxanthin, studies have also demonstrated anti-inflammatory effects [115,116].

Supplementation with n-3 PUFAs has already demonstrated beneficial effects on metabolic markers such as blood triglycerides [117,118] and insulin sensitivity [14]. Further, evidence also suggests that n-3 PUFAs may contribute to improved muscle protein synthesis [119,120] and therefore favorably impact lean body mass (LBM), as well as support weight (and fat) loss [121–124]. However, evidence about the potential beneficial health effects of CO is still sparse. Data from animal studies indicate that CO supplementation may positively impact glucose metabolism and inflammatory processes [16,17], atherosclerosis [125], and elicit cardioprotective [126] and anti-obesogenic effects [17].

1.4. Selected health markers

Regarding the assessment of the current health status, many different markers exist that can provide information about the absence, onset, or presence of disease. To evaluate the preventive impact of exercise and dietary measures on health in older adults, the present work focused on body composition, markers of glucose and lipid metabolism and sirtuin activity. Additionally, the impact of immune cell aging on insulin sensitivity was investigated in a cross-sectional analysis.

1.4.1 Body composition

Anthropometric measurements play a central role in evaluating an individual's health status [127]. Analysis of body weight, height, and calculation of the body-mass-index (BMI) allow a first categorization of a person's nutritional status. According to the BMI, a person is categorized as underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2) or overweight ($\geq$ 25 kg/m^2). Overweight can further be subdivided in pre-obese (25-29.5 kg/m^2) and obese ($\geq$ 30 kg/m^2) [53].

Further, bioelectrical impendence analysis allows a detailed analysis of body composition. This method is based on measuring the total resistance (impendence) of the body to a harmless alternating current conducted by placing electrodes on the body. The impendence comprises the resistance, which is the resistance of the electrolyte containing body water, and reactance, which is the resistance of the cell membranes. Due to the different conductivity of the body compartments and using calculations, the total body water (TBW), the LBM, and the fat mass (FM) can be determined. Devices using multifrequency current further allow further subdivision of the LBM to body cell mass (BCM) and extracellular mass (ECM). The BCM is of particular interest, as it represents the metabolically active cells including muscle cells. An additional marker for health is the phase angle, which gives information on the quality of the cells: the higher the phase angle, the better the condition and quality of the cell [128].

The assessment of body composition is of interest as it can directly impact an individual's health. In that regard, a higher fat mass is linked to an increased risk for cardiovascular diseases, metabolic dysfunctions (e.g. type-2 diabetes or dyslipidemia), and inflammatory processes [129,130]. This interrelation is particularly driven by adipose tissue, whose function extends far beyond energy storage. Adipose tissue exhibits an endocrine function and secretes signaling molecules, known as adipokines. Adipokines encompass the appetite regulating hormone leptin and also pro- and anti-inflammatory cytokines that are secreted by immune cells residing in the adipose tissue [131]. Expansion and remodeling of adipose tissue as a result of a hypercaloric dietary intake contributes to the production of pro-inflammatory

cytokines (i.e., tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, IL-8). Production of pro-inflammatory cytokines can contribute to systemic low grade inflammation and thereby increase the risk for insulin resistance or cardiovascular disease [132,133]. At the same time, metabolic diseases such as type-2 diabetes are also facilitated by decreased muscle mass, as skeletal muscles are largely involved in the postprandial insulin-mediated glucose uptake, glucose storage, and oxidation [134].

Maintaining lower body fat and higher muscle mass throughout the lifespan is of additional importance, as a decrease in muscle mass with a concurrent increase in fat mass are known to occur with aging [135,136].

Regarding the effect of exercise on body composition, it was demonstrated, that endurance training is particularly beneficial in reducing total body mass (meaning weight) and fat mass. However, this can also be accompanied by a low LBM. In contrast, resistance training contributes to an increase in LBM, therefore, total body mass is not necessarily reduced by resistance training [137]. Thus, a combination of aerobic and resistance training may be superior to only performing one of the two modalities. Additionally, body composition is also largely influenced by dietary intake. First, caloric intake will determine whether body mass stays the same, decreases, or increases [138]. Further, it can also be modulated by the overall quality of the diet (a higher quality diet being associated with lower body fat) [139–141], and macronutrient intake (e.g. adequate protein intake is required for maintenance of muscle mass) [142].

Overall, it becomes evident, that reducing body fat and maintaining or increasing muscle mass can favorably impact health.

1.4.2 Metabolic markers

As outlined in chapter 1.3, carbohydrates and lipids represent essential components of human nutrition. However, disturbances in their metabolism can drive the drive the development of metabolic and cardiovascular diseases. Therefore, assessment of markers of glucose and lipid metabolism can help to evaluate the current health status and, if present, identify risk factors.

Glucose metabolism

Although dietary carbohydrates have various functions in the human body, their main function is to supply energy in the form of glucose. In fact, the central nervous system, the kidney marrow and erythrocytes use glucose as their only source of energy supply. Generally, glucose is obtained from breakdown of dietary carbohydrates. After its absorption from the intestine, it can either be directly used for energy production (anaerobic or aerobic glycolysis) or stored as glycogen within the liver and muscles. While muscle glycogen is only used for on-site energy production, the liver can break down glycogen back to glucose (glycogenolysis) and, therefore, help to maintain blood glucose levels and ensure energy supply. Overall, blood glucose levels are monitored and tightly regulated by the pancreas via secretion of the antagonistic hormones insulin and glucagon (**Figure 3**) [143]. Briefly, insulin mediates insulin-dependent glucose uptake in the muscle and adipose tissue and induces glycogen production (glycogenesis) in the liver, while glucagon promotes glycogenolysis.
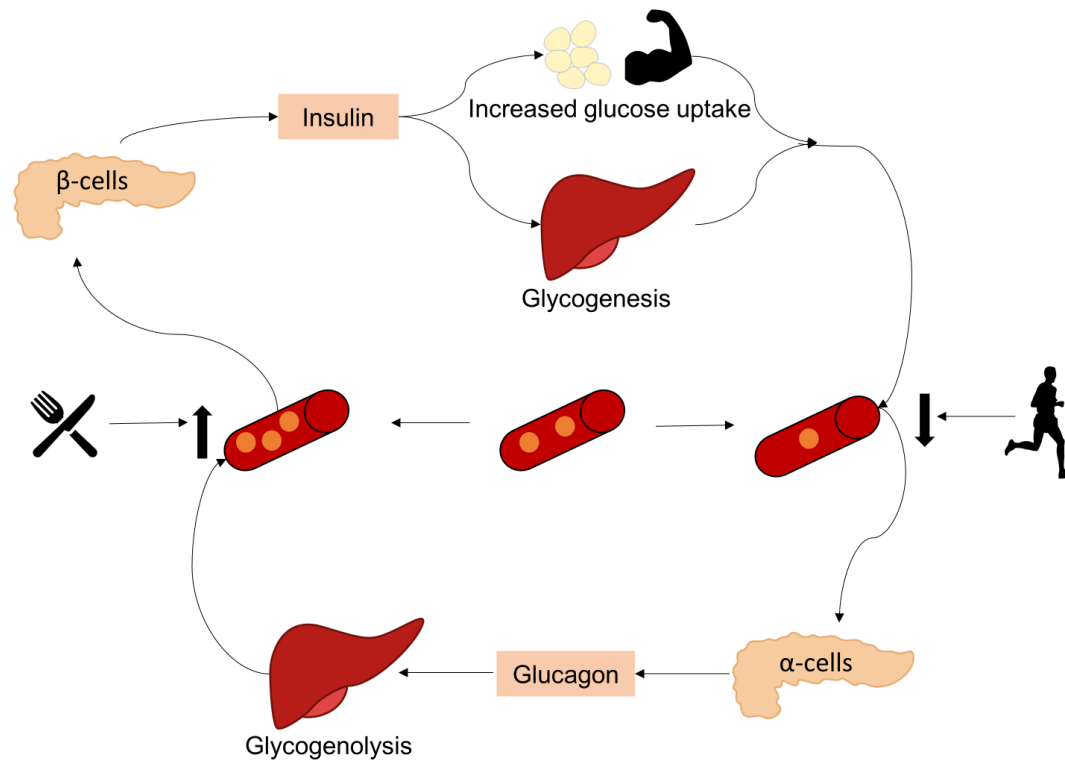
Figure 3: Regulation of blood glucose via the antagonistic hormones insulin and glucagon.

When blood glucose increases (e.g., after a meal), pancreatic β-cells secrete insulin into the blood stream. This leads to increased glucose uptake in muscle cells, adipose tissue, and the liver. The muscle cells and liver can store glucose as glycogen. Consequentially, the increased glucose uptake from the blood leads to a decreased blood glucose level. When blood glucose drops (e.g., during exercise), the α-cells of the pancreas secrete glucagon, which stimulates glycogenolysis within the liver and results in increased blood glucose.

This figure was modified and created in accordance with [143].

Disturbances of glucose metabolism are commonly characterized by chronically elevated blood glucose levels (hyperglycemia), which is a cause of absolute or relative insulin deficiency and summarized as diabetes mellitus. Depending on the cause of this disease, it can generally be subdivided into type-1 and type-2 diabetes (other forms such as gestational diabetes will not be considered in this work). Type-1 diabetes is caused by autoimmunological processes which ultimately destroy the insulin secreting β-cells, leading to absolute insulin deficiency. In this day and age, the most prevalent form is type-2 diabetes, which is characterized by insulin resistance and an accompanying compensatory hyperinsulinemia. In latter stages a decline in β-cells can be observed as well. Type-2 diabetes is assessed via the measurement of fasting glucose and glycated hemoglobin (HbA1c), which measures the glucose levels of the last 8-12 weeks [144]. Both markers are also used by the American Diabetes Association to classify

an individual as healthy, prediabetic or diabetic [145] (**Table 5**). Additionally, calculation of the homeostasis model assessment (HOMA) from fasting glucose and insulin gives information about insulin resistance. For non-diabetic individuals values of ≥ 2.0 can indicate insulin resistance [146], while values ≥ 2.5 are a stronger indicator [147].

Table 5: Classification of fasting blood glucose and HbA1c levels according to the American Diabetes Association.

	Fasting glucose (mg/dl)	HbA1c (%)
Normal	<100	<5.6
Prediabetic	100-125	5.7-6.4
Diabetic	$\geq$126	$\geq$6.5

Abbreviations: HbA1c, glycated hemoglobin.

Table was created in accordance with [145].

Although development of type-2 diabetes is linked to genetic predisposition, it is also largely modulated by lifestyle factors such as dietary intake and physical activity. As stated in 1.4.1, obesity as a result of a hypercaloric diet can facilitate insulin resistance and therefore represents one of the main factors driving the development of this disease. Next to a hypercaloric diet, ingestion of large amounts of sugar sweetened beverages and low amounts of fiber also contribute to the development of type-2 diabetes [148]. Similarly, physical inactivity not only favors development of obesity, but also results in decreased muscle mass, which plays a crucial role in glucose uptake. This also explains the high prevalence of type-2 diabetes among older individuals as a result of age-related loss of muscle mass.

A healthy diet that provides calories matching energy expenditure together with increased physical activity or exercise can be a preventive measure. The importance of exercise is further emphasized because the process of cellular glucose uptake during exercise is mediated through a contraction-induced, insulin independent manner [149]. Thereby, as a result of chronic exercise, insulin sensitivity can increase [6,150,151].

Altogether type-2 diabetes represents a multifactorial disease that develops silently as a result of various factors. As dietary intake and exercise are important regulators of glucose control and preventive measures for the manifestation of type-2 diabetes, evaluation of benefits of a combined exercise and diet intervention for adults without diagnosed chronic disease is still of interest.

Lipid metabolism

Lipoproteins play a central role in lipid metabolism as they transport lipids within the human body. Generally, lipoproteins are ball-shaped, have a hydrophilic outer layer consisting of proteins (apoproteins) and amphiphilic lipids (e.g., phospholipids), carrying the hydrophobic triglycerides and cholesterol esters on the inside. Depending on the density and composition of lipoproteins, they can be classified as chylomicron, chylomicron remnant, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) or high density lipoprotein (HDL) [152]. An overview of the pathways of lipoproteins within the human body is summarized in **Figure 4**.
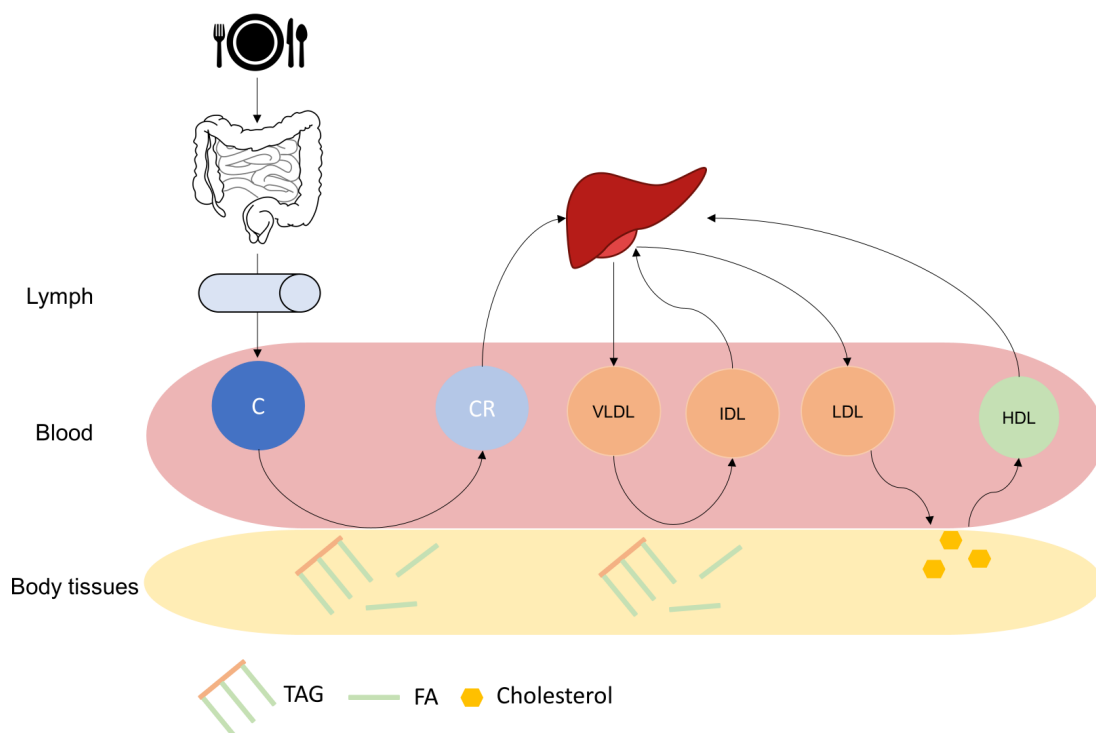

Figure 4: Exogenous and endogenous pathways of lipoproteins.

Exogenous pathway: Chylomicrons (C) transport TAGs and cholesterol from the intestine to muscle and adipose tissue where they are either used for energy production or stored. Thereafter remaining chylomicron remnants (CR) travel back to the liver where they are metabolized. Endogenous pathway: The liver itself produces VLDLs, which mainly consists of TAGs, phospholipids, and cholesterol and also ensures the supply of muscle and adipose tissues. The remaining IDL travels back to the liver where it is either degraded or metabolized to LDL, which is rich in cholesterol and cholesterol esters. HDL is produced by the liver and the intestine. Its function is the transport of cholesterol from the extrahepatic tissues back to the liver.

Abbreviations: C, chylomicron; CR, chylomicron remnant; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; TAG, triglyceride; FA, fatty acid.

The figure was created in accordance with [53].

The lipoproteins LDL and HDL are of particular interest in regards to their impact on health. This results from evidence that links higher levels of LDL to a higher risk for atherosclerotic cardiovascular disease [153,154]. Similarly, higher levels of HDL are associated with a decreased risk of cardiovascular disease [155], which increases the relevance of evaluation of the LDL/HDL ratio [156,157]. The amount of both lipoproteins is also commonly expressed as total cholesterol. According to the amount of the respective lipoprotein, total cholesterol and triglycerides, reference ranges indicating an increased risk for cardiovascular events can be established (**Table 6**).

Table 6: Categorization of respective lipid fractions according to their impact on cardiovascular risk. Values are given in mg/dl.

Fraction	Ideal	Borderline high	Increased risk
Total cholesterol	< 200	200-239	≥ 240
LDL	< 129	130-159	≥ 160
HDL	m: ≥ 60	m: ≤ 50	m: ≤ 50
	f: ≥ 60	f: ≤ 40	f: ≤ 40
Triglycerides	< 150	≥ 150	≥ 150

Abbreviations: m, male; f, female.

Table created in accordance with [158,159], obtained from [53].

Lipid metabolism and the amount of lipoproteins present is strongly modulated by dietary intake. However, it is not mainly mediated by total fat intake, but rather by ingestion of specific fatty acids [160]. Therefore, the cardiovascular risk can also be influenced by dietary fatty acids. In that regard, particularly SFAs and trans fatty acids were found to contribute to an increased cardiovascular risk [161]. Those FAs are commonly consumed as a result of the prevalent dietary patterns in Western countries (see chapter **1.3** for reference). Replacement of SFAs and trans fatty acids with MUFAs as well as PUFAs was shown to decrease total cholesterol and LDL and thereby contribute to a lower risk for cardiovascular events [162,163]. Moreover, ingestion of EPA+DHA was shown effectively lower blood triglycerides in individuals with hypertriglyceridemia [117,164].

Despite the profound effects of dietary intake, exercise can also favorably impact lipid metabolism. Evidence suggests that exercise can increase HDL levels, and help to maintain or lower levels of LDL [5,165]. However, as the effects on lipid metabolism are suggested to occur in a dose-dependent manner, meaning higher intensity yields better outcomes, potential synergistic effects of a moderate intensity training combined with dietary measures still need to be determined.

In addition to the assessment of lipoproteins, another marker that can help to evaluate the risk for cardiovascular diseases is the O3I, which was already introduced in chapter 1.3.4 [104,166]. In fact, a lower O3I is linked to an increased risk of cardiovascular events, which is why an O3I of <4% is considered as "high risk"- and 4-8% as "intermediate risk"-zones [88,166,167]. However, the O3I is not only influenced by the dietary intake, but also by other factors such as age, sex, weight [168]. Although the O3I is effectively increased by supplementation with fish or krill oil, no data on CO exists. Further, no information on the impact of an exercise intervention on the O3I is available to date.

1.4.3 Sirtuins

Other modulators of health and metabolism that have gained growing interest in recent years are enzymes from a family of nicotinamide adenine dinucleotide (NAD+)-dependent deacetylases called sirtuins (SIRT). First identified as silent information regulator (SIR) in *Saccharomyces cerevisiae*, interest in those enzymes emerged when overexpression of Sir2 led to an increase of *S. cerevisiae* lifespan [169]. Till now, seven homologues of SIR2 (SIRT1-SIRT7) have been identified in mammals. Of those, SIRT1, SIRT6, and SIRT7 are primarily localized in the nucleus, SIRT3-SIRT5 in the mitochondria, and SIRT2 in the cytosol [170]. Their main function is the modification of protein lysine residues via deacetylation but other reactions such as adenosine diphosphate (ADP)-ribosylation, demalonylation, and desuccinylation have been reported as well [171,172]. Via these posttranslational modifications, sirtuins are able to regulate a number of processes including gene expression and DNA repair [173], regulation of the cell cycle [174], mitochondrial biogenesis and metabolism [175], lipid and glucose metabolism [176], and many more. The enzymes elicit their function by either activation or inhibition of targets such as forkhead box O (FOXO) transcription factors, peroxisome proliferator-activated receptors (PPAR), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), AMP-activated protein kinase (AMPK), nuclear factor-κB (NF-κb) or p53, which are modulators of energy metabolism, cellular stress response, inflammation and/or regulation of cellular senescence (cell cycle arrest) and apoptosis (cell death) [177]. An overview of the sirtuins and their respective functions is shown in **Figure 5**.
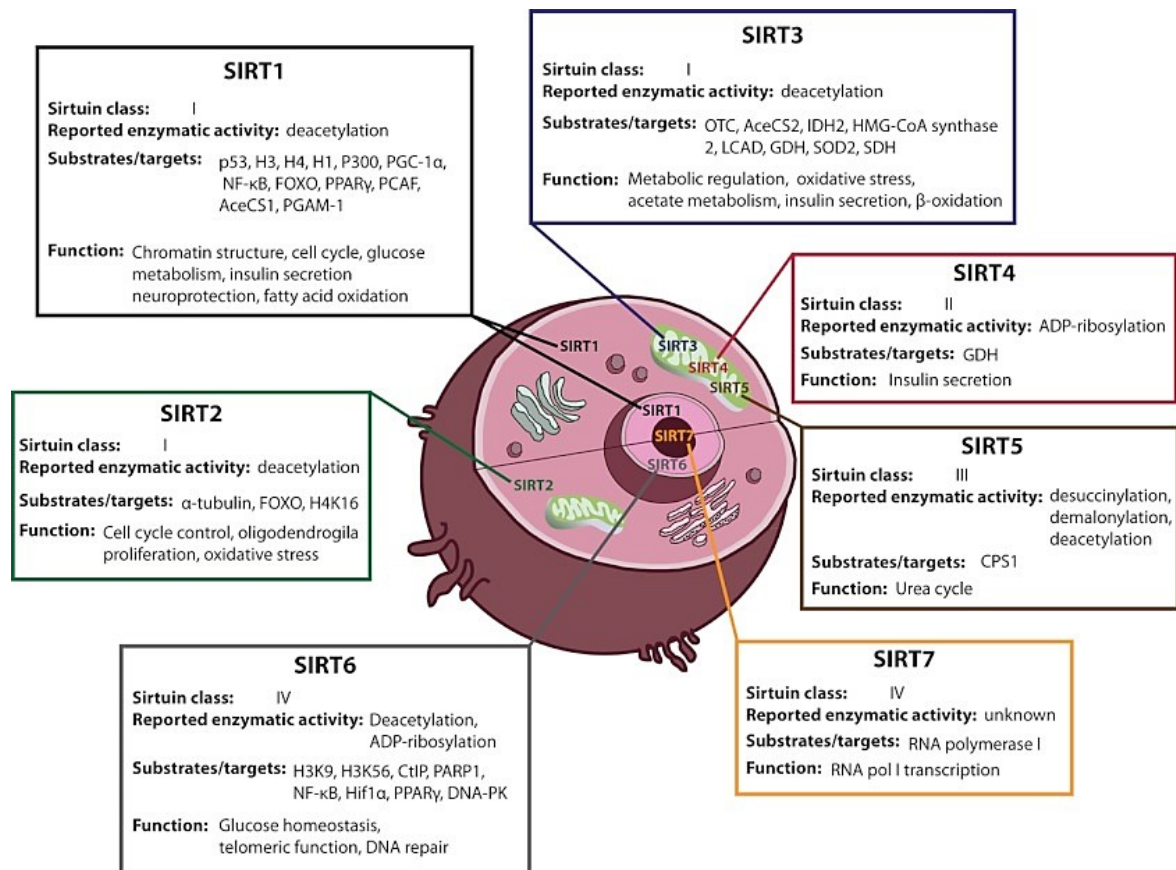
Figure 5: Sirtuins with their respective localization, enzymatic activity, substrates/targets, and functions.

Figure taken from [178].

Given the numerous functions within the human body, it is not surprising that studies have linked reduced sirtuin activity to diabetes mellitus type 1 and 2 [179,180], coronary artery diseases [181,182], and cancer [173]. Similarly, aging was associated with reduced sirtuin activity [177], which highlights their impact on the manifestation of age-related diseases and "pro-aging" pathways.

In line with their role in energy metabolism and nutrient sensing pathways, sirtuin activity is influenced by exercise and dietary measures. With regards to chronic exercise, particularly SIRT1 and SIRT3 are upregulated [183,184]. As both sirtuins target metabolic processes and mitochondrial function, an increase in those sirtuins might be beneficial in the prevention of metabolic dysfunctions. Similarly, caloric restriction was also shown to increase SIRT1, particularly in the context of aging [185,186]. Additionally, dietary compounds such as the polyphenol resveratrol also favorably impact sirtuins [187,188]. A few studies have also demonstrated the effects of the n-3 PUFAs EPA and DHA on SIRT1 [189,190] and EPA on SIRT3 regulated pathways [191].

Existing data on sirtuins are mainly based on *in vitro* or animal studies, with little evidence on their actual impact in humans *in vivo*. Further, no data on the effect of CO supplementation on sirtuins exists. Given their importance in a plethora of metabolic processes, obtaining more data on the modulation of sirtuins in humans can help to further inform on their role in maintaining better health throughout the lifespan.

1.4.5 Immune system

The immune system plays a vital role in human health, as it protects the organism from harmful external factors such as pathogens. Generally, there are two lines of defense to pathogens: the innate and the adaptive, or acquired, immune system. Innate immunity encompasses a rather unspecific, first line of defense mediated by basophils, eosinophils, neutrophils, mast cells, natural killer cells, monocytes, dendric cells and macrophages. Adaptive immunity is a more specialized, antigen-specific second line of defense that is mediated by B- and T-lymphocytes (also referred to as B- and T-cells) [192]. Throughout the life span, the immune cells are subject to cellular aging, which consequentially leads to a deterioration of the immune response and is summarized as immunosenescence. Profound changes are particularly observed after the age of 50 [193]. Although immunosenescence is noticeable in all components of the immune system, more pronounced effects are observed in T-cells [194]. Therefore, only this cell type will be described in more detail.

T-cells migrate from the bone narrow to the thymus where they mature into T-cells expressing either CD8 glycoprotein or CD4 glycoprotein on their surface. According to the respective surface proteins, T-cells can then be classified as CD8+ T-cells or CD4+ T-cells [195]. CD8+ T-cells are also named cytotoxic T-cells as they help to kill infected or tumor cells via secretion of cytotoxins. CD4+ T-cells primarily help to regulate the immune response, and are also called helper cells. In brief, subsets of CD4+ T-cells encompass Th1, Th2 and Th17 cells which exhibit pro-inflammatory activity, as well as regulatory T-cells which have anti-inflammatory properties [196,197].

Before T-cells undergo antigen contact, they are called naïve T-cells. After antigen contact, the cells will either go into apoptosis or differentiate into effector memory (T_{EM}) and central memory cells (T_{CEM}). This "memory" of the adaptive immune response enables a faster response to repetitive exposure to the same pathogen [198]. Lastly, effector memory cells re-expressing CD45RA (T_{EMRA}) represent the "terminally differentiated" or "exhausted" memory T-cells and are categorized as such by the re-expression of the surface marker CD45RA. Those cells are termed exhausted, as they exhibit a much lower proliferative capacity (**Figure 6**).
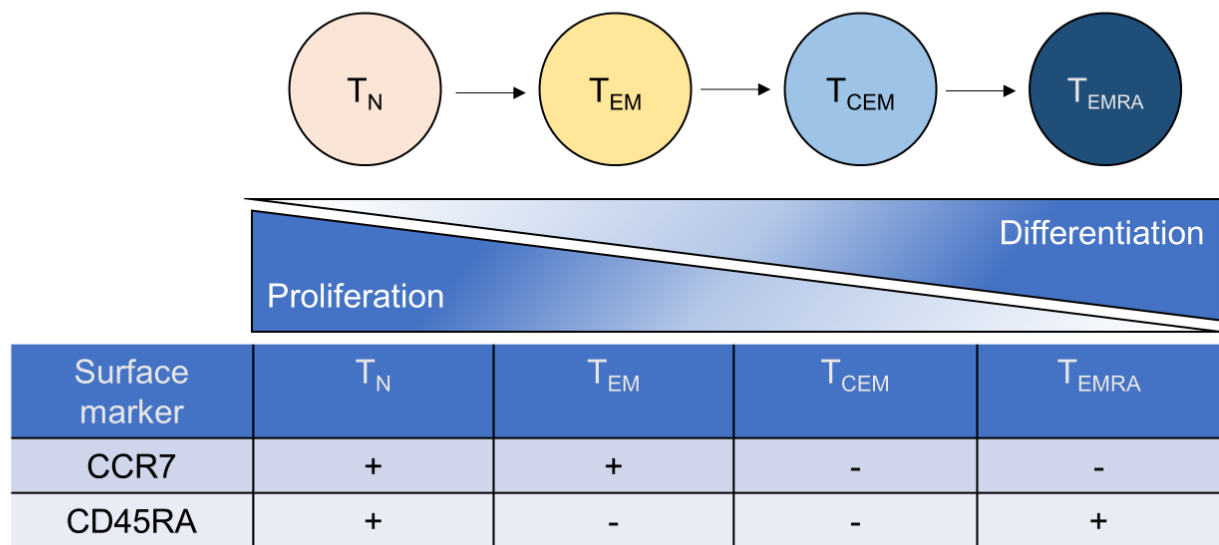

Surface marker	T_N	T_{EM}	T_{CEM}	T_{EMRA}
CCR7	+	+	-	-
CD45RA	+	-	-	+

Figure 6: Differentiation of T-cells and selected surface markers.

Naïve T-cells differentiate into either T_{EM} or T_{CEM} cells and can be characterized as such by expression of the respective surface markers (only selected surface markers are presented). Terminally differentiated effector memory cells, T_{EMRA} cells represent the final stage of differentiation. Throughout their differentiation the proliferation capacity of the immune cells declines.

Abbreviations: T_N, naïve cell; T_{EM}, effector memory cell; T_{CEM}, central memory cell; T_{EMRA}, terminally differentiated memory cell.

Figure was modified and created in accordance with [199].

Within the T-cell pool, immunosenescence manifests as an overall reduced number of naïve cells, which is a consequence of age-related thymic involution [192], a reduced CD4+/CD8+ ratio (decreased CD4+, increased CD8+) and an increased number of senescent cells within both cell types. Cellular senescence is a state of terminal differentiation and irreversible cell cycle arrest and is most commonly observed in T_{EMRA} cells [199]. This state is further accompanied by a shift to a pro-inflammatory secretome which is also summarized as senescent associated secretory phenotype [193,200]. Next to the influence of aging,

immunosenescene can also be driven by repeated exposure to latent viruses such as the cytomegalovirus (CMV) [201]. As the Center for Disease Control and Prevention reports that by the age of 40 50% of adults are CMV positive, determination of the CMV status is valuable information when examining the immune status [202].

Overall, immunosenescence is also associated with promotion of chronic low-grade inflammation that further contributes to immunosenescence by constant activation of the immune response. In older individuals the development of chronic-low grade inflammation is also referred to as "inflammaging" [203]. Increased levels of proinflammatory cytokines, such as TNF- α, IL-1β, IL-8 or IL-18, are also associated with atherosclerosis or metabolic dysfunctions, including type-2 diabetes [204]. Therefore, it is plausible, that the proportion of senescent cells may predict the risk of developing cardiovascular or metabolic diseases. Results from animal studies suggest a link between T-cell aging, glucose metabolism, and development of type-2 diabetes [205,206]. Further, Yi et al. reported that patients with prediabetes show a larger proportion of senescent CD8+ cells when compared to normoglycemic individuals [205]. However, more data on this interrelation is warranted.

1.5 Beyond prevention: exercise and diet in athletes

In contrast to the preventive aspect of exercise, there are also performance-oriented athletes who maximize their performance with rigorous exercise and diet modifications. In that regard, particularly athletes from weight sensitive or aesthetic sports (e.g., endurance, gymnastics, bodybuilding) or sports with high energy expenditure (e.g., rowing) are at risk of experiencing a state of low energy availability (LEA) [207,208]. Low energy availability is the result of a mismatch in dietary energy and energy expended through exercise. As a result the body does not have enough energy to maintain all physiological functions and therefore optimal health [207]. As both short- and long-term LEA can negatively impact an athlete's health the reasons and health consequences of LEA are discussed in a literature review as a part of this work.

2. Scientific publications

2.1 Paper I

Effects of Exercise Combined with a Healthy Diet or
Calanus finmarchicus Oil Supplementation on Body Composition
and Metabolic Markers - A Pilot Study

Authors: Wasserfurth P, Nebl J, Schuchardt JP, Müller M, Boßlau TK, Krüger K, Hahn A

nutrients

MDPI

Article

Effects of Exercise Combined with a Healthy Diet or *Calanus finmarchicus* Oil Supplementation on Body Composition and Metabolic Markers—A Pilot Study

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Abstract: Aging is accompanied by a progressive decline in muscle mass and an increase in fat mass, which are detrimental changes associated with the development of health conditions such as type-2 diabetes mellitus or chronic low-grade inflammation. Although both exercise as well as nutritional interventions are known to be beneficial in counteracting those age-related changes, data to which extent untrained elderly people may benefit is still sparse. Therefore, a randomized, controlled, 12-week interventional trial was conducted in which 134 healthy untrained participants (96 women and 38 men, age 59.4 ± 5.6 years, body mass index (BMI) 28.4 ± 5.8 kg/m^2) were allocated to one of four study groups: (1) control group with no intervention (CON); (2) 2×/week aerobic and resistance training only (EX); (3) exercise routine combined with dietary counseling in accordance with the guidelines of the German Nutrition Society (EXDC); (4) exercise routine combined with intake of 2 g/day oil from *Calanus finmarchicus* (EXCO). Body composition (bioelectrical impedance analysis), as well as markers of glucose metabolism and blood lipids, were analyzed at the beginning and the end of the study. The highest decreases in body fat were observed within the EXCO group (-1.70 ± 2.45 kg, $p < 0.001$), and the EXDC (-1.41 ± 2.13 kg, $p = 0.008$) group. Markers of glucose metabolism and blood lipids remained unchanged in all groups. Taken together results of this pilot study suggest that a combination of moderate exercise and intake of oil from *Calanus finmarchicus* or a healthy diet may promote fat loss in elderly untrained overweight participants.

Keywords: aging; exercise; obesity; omega-3 fatty acid; body composition; fat loss; glucose metabolism

1. Introduction

Aging is accompanied by changes in body composition, which include a progressive decline in muscle mass as well as an increase in body fat mass [1]. If not counteracted, those changes may have multiple health consequences such as development of obesity, type-2 diabetes, chronic low-grade inflammation, or cardiovascular diseases [1–3]. Regular exercise is known to prevent, attenuate and even reverse the described changes [4–6] as it helps to prevent muscle loss, build new muscle mass, and reduce adipocyte cell size and lipid content [7]. Moreover, it has broad beneficial effects on human energy metabolism and glucose control as it elicits contraction-induced, insulin-independent glucose uptake in skeletal muscle [8,9].

However, body composition and metabolic markers are also largely influenced by the diet. A diet that is high in energy and high glycemic foods may facilitate the development of obesity, cardiovascular diseases or diabetes [10,11]. Conversely, a nutrient-dense diet providing adequate intake of macro- and micronutrients, fiber, and phytochemicals can help to prevent those diseases and support the beneficial effects of exercise [12–16]. To promote such "healthy diet", the German Nutrition Society recommends a diet rich in vegetables, fruits, whole grain cereal products, daily intake of dairy products, moderate meat intake and 1–2 portions of fish per week [17].

On the other hand, there are also individual nutrients, which have been shown to positively influence body composition as well as metabolic markers. In that regard, results from several studies demonstrated that supplementation of omega-3 polyunsaturated fatty acids (n-3 PUFAs) potentially reduces fasting insulin levels in diabetic individuals and positively influences body composition by supporting body weight loss [18–21]. Moreover, n-3 PUFAs are also linked to increases in muscle mass and muscle function in healthy older adults [20]. One novel source of n-3 PUFAs that showed promising effects on the reduction of abdominal fat and glucose control in animal studies is the oil from the copepod *Calanus finmarchicus* [22,23]. Of all fatty acids present in Calanus oil (CO) >80% are bound as wax esters. In addition to providing n-3 PUFAs such as stearidonic acid (SDA, C18:4n3), eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, 22:6n3), it also contains plant sterols and astaxanthin, therefore differing from conventional marine oils. Carotenoids such as astaxanthin are also discussed to play a role in counteracting obesity [24].

Although synergistic effects of exercise combined with nutritional measures are presumed, only a few studies have investigated the effects of a combined exercise and nutritional intervention. Moreover, most of those studies were focused on the pre-diabetic, diabetic, or obese populations leaving out the elderly untrained but otherwise healthy population [25–27]. Further, no studies have examined whether and how exercise combined with either a healthy diet versus supplementation of CO may differ.

Given the information above, we hypothesized that moderate exercise combined with nutritional measures may result in more favorable changes in body composition and metabolic markers than exercise alone. Further, we aimed to identify whether one of the two nutritional interventions would result in greater changes than the other. Therefore, a pilot study was conducted to investigate to which extent elderly untrained, but otherwise healthy, subjects may benefit in terms of body composition, glucose control, and blood lipids when performing exercise only or exercise combined with either a general dietary recommendation or intake of CO.

2. Materials and Methods

2.1. Study Participants

In total, 134 men and women were recruited via advertisements in local newspapers and public notice boards from the general population in Hannover, Germany between August 2018 and March 2019. The main inclusion criteria for participation were: age ≥ 50 and ≤ 70 years, no exercise training aside from the daily activities for at least 2 years, a stable body weight (± 5 kg) for at least 6 months, ability to physically perform the exercise intervention (exercise capacity) and consumption of an omnivorous diet. Exclusion criteria were defined as suspicion and diagnosis of cardiovascular diseases (angina pectoris, myocardial infarction, stroke, peripheral arterial occlusive disease, heart failure, cardiac arrhythmia), type 1 and 2 diabetes, renal insufficiency and liver diseases, blood coagulation disorders, chronic gastrointestinal disorders (e.g., ulcers, Crohn's disease), pancreatic insufficiency, immunological diseases (e.g., autoimmune diseases), intake of immunosuppressive drugs or laxatives, intake of supplements containing n-3 PUFAs, alcohol, drug and/or medicine dependency, pregnancy or lactation, retraction of the consent by the subject, concurrent participation in another clinical study, and participation in a study in the last 30 days. Inclusion and exclusion criteria were assessed using a

structured screening questionnaire. Cardiovascular health was determined by resting and exercise electrocardiogram, implemented by trained professionals and a physician.

2.2. Study Design

This single-center, randomized controlled trial in parallel group design was conducted by trained professionals using standardized methods at the Institute of Food Science and Human Nutrition, Leibniz University Hannover, Germany. The study involved a screening phase and 12-week intervention phase with two examination days; one at the beginning (t_0) and one at the end of the 12-week intervention (t_{12}). Additionally, there was a questionnaire-based examination after six weeks (t_6).

Ethical approval was provided by the Ethics Commission of the Medical Chamber of Lower Saxony (Hannover, Germany). In accordance with the guidelines of the Declaration of Helsinki, written informed consent was obtained from all participants prior to their participation in the study. This study is registered in the German Clinical Trial Register (DRKS00014322). The participants were randomly assigned by an independent researcher using stratified randomization according to the covariates sex, BMI, age (in descending order) to one of the four study groups: (1) control group (CON), (2) exercise only group (EX), (3) exercise and dietary counseling group (EXDC), (4) exercise and CO supplementation group (EXCO). The CON group served as control and participants of this group were asked to maintain their habitual diet and physical activity level throughout the 12-week investigation period. The EX, EXDC, and EXCO groups were instructed to perform exercise training twice a week. Participants randomized to the EX group were asked to maintain their normal diet. Participants randomized to the EXDC group were asked to adapt their diet in accordance with the dietary guidelines of the German Nutrition Society [17]. Therefore, participants of this group received an individualized nutrition counseling session by a professional nutritionist at the first examination. In general, dietary recommendations included the following advice: intake of 3 portions of vegetables and 2 portions of fruits daily, consumption of cereal products with a focus on whole-grain products, daily consumption of dairy products such as milk or cheese, limited meat intake of 300–600 g per week, consumption of fish once or twice a week, limited intake of salt and sugar [17]. In addition to exercise, participants randomized to the EXCO group were instructed to consume four capsules containing oil from *Calanus finmarchicus* (Calanus AS, Tromsø, Norway) daily during the 12-week intervention period. Therefore, participants received a counted number of capsules and were advised to return leftover capsules at the end of the study. The leftover capsules were then counted to assess the compliance in taking the supplement (at least 90% of the capsules had to be consumed). Four capsules provided 2 g of CO. The composition of the oil is shown in Table 1. Further, participants were asked to maintain their normal diet. Compliance of participants and adherence to the respective instructions was monitored within all study groups via fortnightly phone calls.

2.3. Exercise Training

The exercise training was performed in cooperating fitness centers after thorough instructions from a professional trainer. Each training session consisted of an initial warm-up, followed by two passes of a strength-endurance circuit.

The strength training consisted of six machine-supported exercises that included all major muscle groups and were performed for one minute each. During the initial training session, a maximum force test with three attempts was performed. The best of the three tests was scored and used to define the exercise intensity at about 60% of the participant's maximum force for the first two weeks of training. For the subsequent six weeks, the load was increased by 10% and again by 5% for the last four weeks. The endurance exercise consisted of a four-minute bout performed on bicycle ergometers and cross-trainers at a perceived exertion corresponding to an intensity of about 15 on the Borg-Scale. In between each exercise, the participants had 30 s of rest. Including the warm-up and rest periods, the training session could be completed in approximately one hour. Compliance of the participants was assessed via a training log and a questionnaire at the end of the study.

Table 1. Composition of oil from *Calanus finmarchicus*.

Components			mg/100 g CO	mg/2 g CO
SFA			12,428	249
MUFA			11,452	229
	Omega-3		18,181	364
		ALA	1149	23
		SDA	6186	124
PUFA		EPA	5439	109
		DHA	4342	87
	Omega-6		981	20
		LA	552	11
		ARA	169	3
Fatty alcohols			33,017	660
Astaxanthin			180	3.6

CO = *Calanus finmarchicus* oil, SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; ALA = Alpha-Linolenic acid; SDA = Stearidonic acid; EPA = Eisosapentaenoic acid; DHA = Docosahexaenoic acid; LA = Linoleic acid; ARA = Arachidonic acid. Other minor compounds are triacylglycerides, free fatty acids, phospholipids and plant sterols.

2.4. Monitoring of Dietary Intake and Physical Activity

The level of regular physical activity outside of the intervention was assessed using the German Freiburger Questionnaire for Physical Activity at the beginning, after six weeks, and at the end of the study [28]. Dietary intake of the participants was monitored via 3-day dietary records at the beginning, after six weeks, and at the end of the study. The records were checked by nutritionists for completeness, readability, and plausibility. If necessary, ambiguities were clarified with the participants. Energy and nutrient intake were estimated using the software PRODI6.4 (Nutri-Science GmbH, Freiburg, Germany). Additionally, consumption of specific food groups was assessed with the food frequency questionnaire (FFQ) from the German Health Examination Survey for Adults (Studie zur Gesundheit Erwachsener in Deutschland, DEGS) of the Robert Koch Institute.

2.5. Bodyweight and Body Composition

All measurements were performed under standardized conditions on both days of examination at the beginning and end of the study. Participants arrived at the institute between 6:00–10 a.m., rested, and after an overnight fast (≥10 h). For the second examination, approximately the same time of day was scheduled as for the first day of examination. At first height was measured with a stadiometer (seca GmbH & Co. KG, Hamburg, Germany). Therefore, participants were advised to take off their shoes, stand with the back to the stadiometer and touch the bar with the back of their head, back and buttocks and back of their feet. Next, their bodyweight was measured lightly clothed and without shoes on a digital scale to the nearest of 0.1 kg (seca GmbH & Co. KG, Hamburg, Germany). Body composition was analyzed to a nearest of 0.1 kg using a bipolar bioelectrical impedance analyzer (BIA) (Nutriguard M, Data Input Company, Darmstadt, Germany) and the software NutriPlus 5.4.1 (Data Input Company, Darmstadt, Germany). For the measurements, the participants were instructed to lay down on a stretcher and rest for a few minutes (~5 min) to ensure a balanced distribution of body fluids before the measurement. During the measurement, participants were instructed to lay still and in a relaxed position with the limbs slightly bent from the torso. All measurements were carried out by trained nutritionists. To avoid biases due to changing personal, the same nutritionists conducted the measurements throughout the study after thorough instructions.

2.6. Blood Sampling and Biochemical Indices Measurement

Blood samples were drawn from the participants after an overnight fast (≥10 h) by venepuncture of an arm vein using EDTA, serum and NaF Glucose tubes (Sarstedt AG & Co. KG, Nümbrecht, Germany). All samples were stored at ~5 °C and transferred to an accredited and certified laboratory

(Laborärztliche Arbeitsgemeinschaft für Diagnostik und Rationalisierung e.V., Hannover, Germany), where all analyses were performed.

Triglycerides, low-density lipoprotein cholesterol (LDL) and high-density lipoprotein cholesterol (HDL) were analyzed by a photometric method (Beckman Coulter GmbH, Krefeld, Germany). Total cholesterol and LDL/HDL-ratio was calculated from LDL and HDL values.

Fasting glucose was determined by a photometric method (Beckman Coulter GmbH, Krefeld, Germany). HbA_{1c} was analyzed using high-pressure liquid chromatography (HPLC) (Bio-Rad Laboratories GmbH, Feldkirchen, Germany). The electrochemiluminescence immunoassay method (ECLIA) using cobas 801e (Roche Diagnostics GmbH, Mannheim, Germany) was applied to determine insulin concentrations. To evaluate insulin resistance, the homeostatic model assessment (HOMA) was calculated as follows: HOMA-Index = fasting insulin (μU/mL) $\times$ fasting blood glucose (mg/dL)/405 [29].

2.7. Statistical Analyses

The sample size of n = 25 per group was based on an alpha of 0.05 and 0.80 beta to detect between-group differences, assuming an effect size of more than 0.8. With an estimated dropout rate of 15%, a total of at least 30 participants per intervention study group were recruited. Distribution of all data was assessed using a Shapiro-Wilk Test and Gaussian distribution. To assess differences between groups at baseline, data from all participants was analyzed. Because all data was not normally distributed, intergroup comparisons were assessed with Kruskal-Wallis-test while nominal data was analyzed using Chi-square-test. For further analysis, data from all participants who took part in both examination days was used. If the assumption of normality was not met, data was transformed using log, square root, or reciprocal transformation. The intervention effect on all outcomes was analyzed using a two-way repeated measures analysis of variance (ANOVA) with time (t_0 and t_{12}) and intervention (CON, EX, EXCO, EXDC). If significant time*intervention effects were detected, a post hoc analysis with Bonferroni correction was performed. *p*-values of <0.05 were considered as significant. All statistical analyses were carried out using SPSS software (version 23.0; SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Baseline Characteristics

After screening for eligibility, 134 participants met the eligibility criteria and were randomly assigned to one of the four study groups (Figure 1). Of all participants randomized, 72% were female and 28% were male with a mean age of 59.2 $\pm$ 5.60 years. With a mean weight of 83.0 $\pm$ 20.2 kg and an average BMI of 28.4 $\pm$ 5.80 kg/m^2, the study population can be classified as overweight.

At baseline, there were no differences in age, weight or BMI among the study groups (Table 2).

Table 2. Baseline characteristics of all participants.

Parameter	CON (n = 28)	EX (n = 36)	EXDC (n = 34)	EXCO (n = 36)	p
Sex (f/m)	21/7	24/12	26/8	25/11	0.797
Age (years)	59.3 $\pm$ 5.07	59.7 $\pm$ 6.39	60.2 $\pm$ 5.11	58.5 $\pm$ 5.69	0.563
Height (m)	169.1 $\pm$ 8.05	170.9 $\pm$ 8.73	171.0 $\pm$ 8.37	171.3 $\pm$ 8.74	0.774
Body weight (kg)	84.8 $\pm$ 22.6	81.9 $\pm$ 18.6	85.0 $\pm$ 20.3	80.7 $\pm$ 20.3	0.771
BMI (kg/m^2)	29.5 $\pm$ 6.80	28.0 $\pm$ 5.51	28.95 $\pm$ 5.80	27.28 $\pm$ 5.26	0.427
SBP (mmHg)	138.0 $\pm$ 20.4	129.4 $\pm$ 14.7	133.8 $\pm$ 16.9	134.1 $\pm$ 20.1	0.332
DBP (mmHg)	81.1 $\pm$ 10.5	76.2 $\pm$ 6.16	79.2 $\pm$ 10.6	78.6 $\pm$ 7.91	0.327
Pulse	71.4 $\pm$ 8.57	74.6 $\pm$ 9.58	72.6 $\pm$ 9.23	73.8 $\pm$ 8.25	0.513

Values are given as mean $\pm$ SD. Distribution of sexes between groups was analyzed using Chi-square-test. All other group differences were assessed with Kruskal-Wallis-Test. f = females, m = male, BMI = Body-Mass-Index, SBP = systolic blood pressure, DBP = diastolic blood pressure.
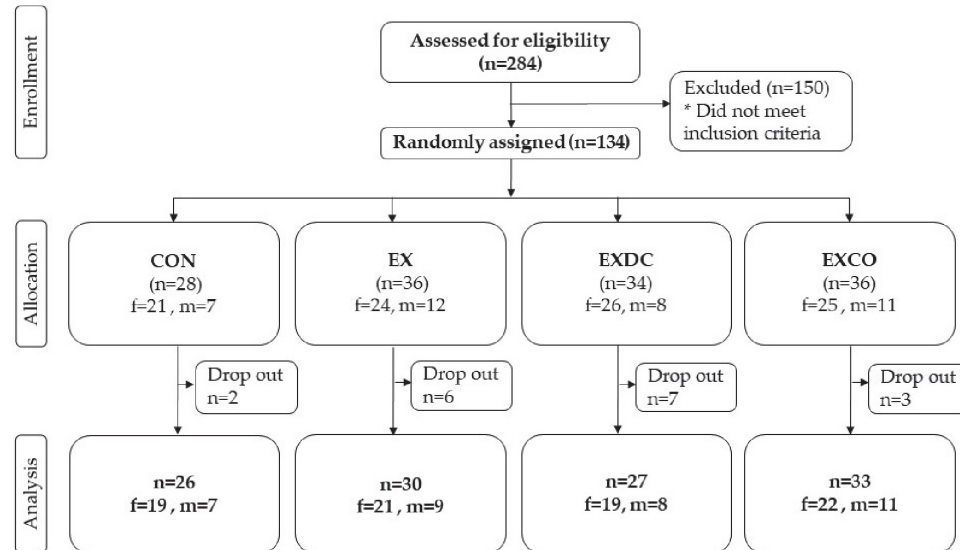
Figure 1. Flow chart of all participants screened, randomized, allocated and analyzed.

3.2. Physical Activity and Training Sessions

In compliance with the given instructions, there were no significant changes in physical activity outside the intervention among the four study groups during the study period (Table S1). However, an overall increase in basal activity could be observed in all groups, which was mostly due to increased seasonal activities such as gardening. Additionally, no significant difference in the number of completed training sessions was detected between the EX, EXCO and EXDC group (data not shown).

3.3. Dietary Intake

Intake of specific food groups assessed by FFQs of all study groups before and after the intervention is presented in Table S2. In compliance with the received dietary recommendations, participants in the EXDC group had a significant increase in fruit portions per day ($p = 0.001$) and vegetable portions per day ($p = 0.006$) as well as fish intake per week ($p < 0.001$) as compared to the other groups. Except for a significant decline of vegetable portions per day in the CON group ($p = 0.018$), no other significant changes were observed.

Dietary intake from the background diet calculated from 3-day dietary food logs is shown in Table 3. In all three exercise groups (EX, EXCO, and EXDC), average energy intake decreased about 200 kcal per day after the intervention period, yet this was not statistically different between groups. While protein intake stayed the same throughout the intervention and between groups, significant differences among all four study groups were found in fat ($p = 0.016$), carbohydrate ($p = 0.015$) and fiber intake ($p = 0.004$) after the intervention. Relative fat intake was significantly higher after 12 weeks in the CON group ($+4.32 \pm 8.17\%$E, $p = 0.008$), while no significant differences were found in the intervention groups. Similarly, CON showed a significant decrease in carbohydrate intake ($-5.11 \pm 6.65\%$E, $p = 0.002$). A significant decrease in carbohydrate consumption was also detected in the EX group ($-2.76 \pm 8.36\%$E, $p = 0.034$). Fiber intake increased significantly in the EXDC group ($+3.00 \pm 8.39$ g/day, $p = 0.021$) while it decreased in the EX group (-4.08 ± 6.79 g/day, $p = 0.004$). With regards to fatty acid intake, no differences in intake of saturated fatty acids (SFAs) or PUFAs could be found among the study groups. In contrast, intake of monounsaturated fatty acids (MUFAs) decreased significantly in the EXCO (-3.41 ± 12.0 g/day, $p = 0.040$) and EXDC group (-4.80 ± 9.17 g/day; $p = 0.011$).

Table 3. Daily dietary intake from the background diet calculated from 3-day dietary records and absolute changes (Δ) at the beginning (0) and at the end (12) of the study.

Parameter	t	CON	Δ	EX	Δ	EXDC	Δ	EXCO	Δ	p
Energy intake (kcal)	0	1873.7 ± 354.0		2023.5 ± 408.5		1887.4 ± 539.5		1923.5 ± 436.8		
	12	1852.7 ± 476.0	−21.0 ± 51.6	1806.1 ± 365.4	−217.3 ± 455.0	1659.9 ± 470.9	−227.5 ± 453.8	1776.8 ± 413.5	−146.8 ± 476.3	0.334
Protein (%E)	0	16.7 ± 2.5		16.1 ± 3.42		17.6 ± 3.66		15.6 ± 2.99		
	12	16.7 ± 2.0	−0.08 ± 2.40	16.9 ± 3.37	0.86 ± 3.84	17.6 ± 2.93	−0.02 ± 4.05	16.7 ± 3.26	1.05 ± 3.68	0.772
Fat (%E)	0	36.1 ± 7.39		36.2 ± 7.47		37.2 ± 7.01		38.1 ± 6.69		
	12	40.4 ± 6.24 [†]	4.32 ± 8.17	36.9 ± 6.00	0.69 ± 7.97	36.6 ± 7.93	−0.60 ± 7.04	35.6 ± 7.48	−2.51 ± 9.04	0.016
CHO (%E)	0	41.66 ± 6.23		42.6 ± 9.45		39.0 ± 6.16		41.92 ± 6.09		
	12	36.56 ± 5.93 [†]	−5.11 ± 6.65	39.8 ± 6.45 [*]	−2.76 ± 8.36	39.6 ± 7.76	0.66 ± 8.08	42.39 ± 7.86	0.47 ± 8.72	0.015
Fiber (g)	0	20.80 ± 7.44		23.4 ± 7.79		19.0 ± 5.97		22.0 ± 6.47		
	12	20.04 ± 9.51	−0.76 ± 7.11	19.3 ± 6.08 [†]	−4.08 ± 6.79	22.0 ± 6.43 [*]	3.00 ± 8.39	21.2 ± 8.80	−0.78 ± 8.03	0.004
SFA (g)	0	34.3 ± 14.2		35.7 ± 13.6		33.8 ± 14.1		36.9 ± 15.5		
	12	37.2 ± 13.6	2.90 ± 15.5	31.0 ± 11.2	−4.67 ± 15.7	30.2 ± 12.7	−3.61 ± 9.34	29.9 ± 11.1	−6.93 ± 15.2	0.060
MUFA (g)	0	25.1 ± 8.53		28.9 ± 12.5		27.3 ± 11.6		27.3 ± 9.61		
	12	28.6 ± 11.8	3.51 ± 13.2	26.0 ± 11.2	−2.99 ± 11.9	22.5 ± 11.5 [*]	−4.80 ± 9.17	23.9 ± 9.29 [*]	−3.41 ± 12.0	0.030
PUFA (g)	0	10.6 ± 5.16		11.9 ± 3.96		12.4 ± 7.30		11.8 ± 5.64		
	12	12.1 ± 7.69	1.58 ± 6.83	12.1 ± 5.75	0.21 ± 5.01	10.3 ± 5.35	−2.13 ± 6.86	10.5 ± 5.26	−1.26 ± 4.07	0.088
DHA (g)	0	0.19 ± 0.26		0.29 ± 0.34		0.26 ± 0.21		0.23 ± 0.30		
	12	0.18 ± 0.32	−0.02 ± 0.42	0.39 ± 0.47	0.11 ± 0.46	0.40 ± 0.43	0.15 ± 0.37	0.19 ± 0.19	−0.4 ± 0.35	0.728
EPA (g)	0	0.14 ± 0.42		0.27 ± 0.54		0.12 ± 0.17		0.18 ± 0.41		
	12	0.12 ± 0.30	−0.03 ± 0.56	0.30 ± 0.41	−0.01 ± 0.37	0.38 ± 0.42	0.25 ± 0.33	0.14 ± 0.19	0.09 ± 0.23	0.095

Values are given as mean ± SD. *p* values represent time*intervention interaction analyzed with two-way repeated measure ANOVA. In case of significance, asterisks indicate statistical differences within groups detected with Bonferroni post hoc test (* $p < 0.05$; † $p < 0.01$). t = time in weeks, %E = percent of total energy, CHO = carbohydrates.

Further, the intake of EPA and DHA from the background diet did not change significantly throughout the study between the four study groups. However, the highest increase in EPA and DHA intake could be observed in the EXDC group (+0.15 ± 0.37 g/day DHA; +0.25 ± 0.33 g/day EPA).

3.4. Body Composition

After the intervention, a significant difference in fat mass was detected among all four study groups ($p = 0.039$) (Table 4). Fat mass (FM) decreased significantly within the EXDC group (−1.41 ± 2.13 kg, $p = 0.008$) and the EXCO group (−1.70 ± 2.45 kg, $p < 0.001$). Total body water content and lean body mass (LBM) increased significantly in CON and EXCO (CON: +0.59 ± 1.28 L, $p = 0.015$; +0.76 ± 1.74 kg, $p = 0.018$; EXCO: +0.57 ± 2.03 L $p = 0.012$, +0.79 ± 2.79 kg, $p = 0.008$). Bodyweight and BMI did not differ significantly between all study groups after the intervention.

3.5. Markers of Glucose Metabolism

Overall, no significant differences in any markers of glucose metabolism were detected among the study groups (Table 5). Nonetheless, the highest changes in fasting insulin and HOMA-Index was observed within the EXDC group (−2.14 ± 4.59 µE/L and −0.56 ± 1.25), followed by the EXCO group (−1.42 ± 2.86 µE/L and −0.27 ± 0.72).

3.6. Blood Lipids

No significant differences in blood lipids among all four study groups were detected (Table 6). However, when compared with each other, EXCO showed the greatest decrease in triglycerides at the end of the intervention (−10.5 ± 23.6 mg/dL).

Table 4. Body composition and absolute changes (Δ) of the four groups at the beginning (0) and at the end (12) of the study of all four study groups.

Parameter	t	CON	Δ	EX	Δ	EXDC	Δ	EXCO	Δ	p
Weight (kg)	0	84.9 ± 23.0	−0.28 ± 1.97	81.0 ± 18.5	−1.12 ± 1.84	84.8 ± 20.8	−1.26 ± 2.34	82.2 ± 20.0	−1.16 ± 2.49	0.236
	12	84.6 ± 22.2		79.9 ± 18.0		83.6 ± 20.7		81.0 ± 19.0		
BMI (kg/m²)	0	29.5 ± 6.93	−0.09 ± 0.69	27.8 ± 5.39	−0.38 ± 0.64	28.7 ± 5.86	−0.43 ± 0.86	27.6 ± 5.25	−0.37 ± 0.81	0.229
	12	29.4 ± 6.66		27.4 ± 5.26		28.3 ± 5.78		27.2 ± 5.01		
Phase angle	0	5.44 ± 0.77	−0.04 ± 0.26	5.47 ± 0.87	0.16 ± 0.57	5.23 ± 0.50	0.10 ± 0.30	5.51 ± 0.81	0.11 ± 0.33	0.279
	12	5.39 ± 0.83		5.61 ± 0.65		5.33 ± 0.57		5.62 ± 0.79		
TBW (L)	0	39.1 ± 7.86	0.59 ± 1.28	40.2 ± 9.17	−0.72 ± 2.71	40.4 ± 9.54	0.07 ± 1.21	40.5 ± 9.91	0.57 ± 2.03	0.028
	12	39.7 ± 7.51 *		39.4 ± 7.61		40.5 ± 9.47		41.3 ± 9.36 *		
LBM (kg)	0	53.3 ± 10.7	0.76 ± 1.74	54.8 ± 11.9	−0.87 ± 3.53	55.3 ± 13.0	0.09 ± 1.67	55.3 ± 13.5	0.79 ± 2.79	0.031
	12	54.0 ± 10.1 *		53.7 ± 10.1		55.3 ± 12.9		56.4 ± 12.6 †		
BCM (kg)	0	26.3 ± 6.07	0.12 ± 1.32	27.0 ± 7.99	−0.03 ± 3.16	26.6 ± 7.24	0.35 ± 1.46	27.3 ± 7.63	0.71 ± 1.74	0.388
	12	26.4 ± 5.83		26.9 ± 6.08		27.0 ± 7.30		28.1 ± 7.18		
FM (kg)	0	31.6 ± 17.0	−0.35 ± 2.00	26.2 ± 11.2	−0.22 ± 3.03	29.6 ± 12.1	−1.41 ± 2.13	27.0 ± 11.3	−1.70 ± 2.45	0.039
	12	31.3 ± 16.5		26.2 ± 11.5		28.2 ± 12.1 †		24.7 ± 11.1 ‡		

Values are given as mean ± SD. p values represent time*intervention interaction analyzed with two-way repeated measure ANOVA. In case of significance, asterisks indicate statistical differences within groups detected with Bonferroni post hoc test (* $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$). t = time in weeks, BMI = body mass index, TBW = total body water, LBM = lean body mass, BCM = body cell mass, FM = fat mass.

Table 5. Markers of glucose metabolism and absolute changes (Δ) at the beginning (0) and at the end (12) of the intervention.

Parameter	t	CON	Δ	EX	Δ	EXDC	Δ	EXCO	Δ	p
Fasting Glucose (mg/dL)	0	92.9 ± 8.98	0.27 ± 12.46	94.0 ± 17.5	−1.40 ± 14.25	97.9 ± 26.5	+0.07 ± 10.63	88.6 ± 8.29	3.42 ± 15.97	0.668
	12	93.2 ± 14.3		92.6 ± 8.76		98.0 ± 25.5		92.1 ± 14.29		
HbA₁c (%)	0	5.47 ± 0.34	−0.08 ± 0.15	5.39 ± 0.28	−0.08 ± 0.17	5.58 ± 0.72	−0.05 ± 0.17	5.38 ± 0.36	−0.07 ± 0.22	0.954
	12	5.39 ± 0.33		5.30 ± 0.25		5.52 ± 0.72		5.31 ± 0.35		
HbA₁c (mol/molHB)	0	36.3 ± 3.75	−0.84 ± 1.65	35.4 ± 3.06	−0.90 ± 1.85	37.5 ± 7.88	−0.58 ± 1.84	35.3 ± 3.96	−0.80 ± 2.38	0.954
	12	35.4 ± 3.65		34.4 ± 2.76		36.8 ± 7.92		34.5 ± 3.79		
Insulin (µE/mL)	0	13.4 ± 11.1	−0.74 ± 4.52	9.80 ± 4.20	−0.48 ± 4.24	12.7 ± 8.48	−2.14 ± 4.59	10.2 ± 7.16	−1.42 ± 2.86	0.457
	12	12.7 ± 10.2		9.32 ± 3.88		10.5 ± 7.21		8.81 ± 5.45		
HOMA–Index	0	3.24 ± 3.19	−0.16 ± 1.19	2.40 ± 1.52	−0.23 ± 1.51	3.11 ± 2.36	−0.56 ± 1.25	2.31 ± 1.83	−0.27 ± 0.72	0.640
	12	3.08 ± 2.95		2.17 ± 1.00		2.56 ± 2.02		2.04 ± 1.41		

Values are given as mean ± SD. p values represent time*intervention interaction analyzed with two-way repeated measure ANOVA. In case of significance, asterisks indicate statistical differences within groups detected with Bonferroni post hoc test. t = time in weeks.

Table 6. Blood lipids and absolute changes (Δ) of the four groups at the beginning (0) and at the end (12) of the intervention.

Parameter	t	CON	Δ	EX	Δ	EXDC	Δ	EXCO	Δ	p
TG (mg/dL)	0	141.9 ± 104.1		115.0 ± 55.1		113.7 ± 44.3		107.3 ± 34.4		
	12	135.5 ± 89.2	−6.46 ± 43.2	111.6 ± 49.7	−3.40 ± 33.6	116.9 ± 68.9	3.22 ± 46.5	96.9 ± 28.5	−10.5 ± 23.6	0.470
TC (mg/dL)	0	258.3 ± 54.7		237.7 ± 39.2		244.3 ± 51.6		242.2 ± 37.2		
	12	256.2 ± 59.6	−2.08 ± 33.5	232.4 ± 37.9	−5.37 ± 20.9	242.7 ± 49.7	−1.59 ± 25.8	242.9 ± 38.2	0.70 ± 25.3	0.816
HDL (mg/dL)	0	62.8 ± 14.0		67.5 ± 15.4		61.1 ± 14.9		68.0 ± 18.2		
	12	62.7 ± 14.1	−0.08 ± 6.33	67.5 ± 15.2	0.03 ± 7.45	62.3 ± 13.8	1.22 ± 6.38	70.6 ± 19.3	2.61 ± 7.54	0.410
LDL (mg/dL)	0	167.8 ± 39.6		150.3 ± 31.7		157.7 ± 42.6		152.0 ± 29.2		
	12	168.8 ± 47.3	1.00 ± 25.2	147.6 ± 27.4	−2.67 ± 14.8	160.0 ± 40.6	2.30 ± 19.19	154.6 ± 29.1	2.55 ± 18.2	0.710
LDL/HDL Ratio (mg/dL)	0	2.77 ± 0.80		2.37 ± 0.88		2.75 ± 1.03		2.39 ± 0.71		
	12	2.81 ± 0.98	0.04 ± 0.45	2.31 ± 0.72	−0.07 ± 0.31	2.68 ± 0.88	−0.07 ± 0.32	2.32 ± 0.68	−0.08 ± 0.30	0.694

Values are given as mean ± SD. *p* values represent time*intervention interaction analyzed with two-way repeated measure ANOVA. In case of significance, asterisks indicate statistical differences within groups detected with Bonferroni post hoc test. t = time; weeks of the intervention, TC = total cholesterol, TG = triglycerides.

4. Discussion

In this study, we show that 12 weeks of resistance and aerobic training combined with adherence to a healthy diet or supplementation of 2 g *Calanus finmarchicus* oil may promote body fat loss, but did not affect markers of glucose or lipid metabolism in overweight but otherwise healthy (no diagnosed chronic diseases) elderly subjects.

With regards to the EXDC group, a significant increase in vegetables, fruit, and fish intake occurred, which makes it arguable that improved diet quality supported body fat loss in this group [30]. However, it is surprising that the healthy diet did not support further changes such as an increase in LBM. Comparable studies investigating the effect of a healthy diet with a n6/n3 ratio < 2 (dietary recommendations, uncontrolled) or a n-3 PUFA rich diet (≥500 g fatty fish and seafood per week) combined with 2×/week resistance training in elderly women showed an increase in leg lean mass and increased hypertrophy of type 2 muscle fibers after 24 weeks [31,32]. However, it should be noted that not only the fatty acid intake of the participants was modified, but also their energy intake, macronutrient ratio, and fiber intake in the previous study. Naturally, an improved energy and macronutrient intake will potentially improve body composition. In contrast, participants from our study received general dietary recommendations with no specifications on caloric intake or macronutrient distribution. Moreover, Strandberg et al., 2015 measured body composition only in the lower extremities, whereas we used whole body measurements. The differences in estimation of body composition make it difficult to directly compare the study outcomes. Furthermore, although an increase in fish consumption could be observed in our study, no information about the type of fish (lean or fatty fish) was collected. In any case, the intakes from the food logs indicated a slightly higher intake of EPA and DHA within the EXDC group, but this was not significantly different from the other study groups.

In contrast to the EXDC group that showed only changes in FM, the EXCO group showed several changes in body composition, which may be mediated by CO. As health benefits from marine oils are commonly attributed to long-chain n-3 PUFAs, this is by far the most studied component of CO. With regard to body composition, results from a meta-analysis showed that supplementation with n3-PUFAs as monotherapy can promote moderate weight loss, including a reduction in body fat [21]. When combined with exercise some [33,34], but not all studies [35], reported an additional beneficial effect of n-3 PUFAs on body fat loss in healthy active or sedentary overweight/obese participants. However, evidence about the combined effect of n-3 PUFA supplementation and exercise on body composition in the elderly population is limited. In elderly subjects, 12 weeks of 3× per week resistance training and intake of ~14 g alpha-linolenic acid (ALA, C18:3n3) from 30 mL flaxseed oil showed no beneficial effect on body composition when compared to placebo (corn oil) [36]. Even a high dose of 3 g long-chain n-3 PUFAs (1.98 g EPA and 0.99 g DHA) did not affect body composition when combined with 3× a week resistance training over 12 weeks in male participants [37].

Contrary to findings from a recent study investigating the effect of CO and exercise in elderly women (~70 years old with a BMI of ~26.7) that showed no effect of CO on FM but only visceral fat [38], results from our study showed the highest loss of FM in the EXCO group. Unfortunately, we did not measure visceral fat, but the different outcomes can be partially explained by a slightly higher BMI in our study population. Moreover, we also found a significant increase in LBM and consequentially body water in this group. This result would support findings from earlier studies, that found beneficial effects of n-3 PUFAs on muscle protein synthesis [20,39]. However, existing data on the effects of n-3 PUFAs on muscle mass and strength is limited and conflicting [40]. More importantly, in this study the CON group also showed a significant increase in LBM and body water. Although the control group was advised to abstain from any exercise and to maintain their normal physical activity level, it is still possible that participants of this group changed their behavior due to their participation in the study. Indeed, a trend to an increase in physical activity could be observed but this trend was visible in all four study groups. Even though a validated and reliable questionnaire to report physical activity was reported, additional monitoring of physical activity via an activity tracker may have been beneficial.

Importantly, when evaluating the changes in body composition it has to be kept in mind that the study population was untrained, but cannot be classified as sedentary. This may indicate, that the intensity of the moderate exercise routine as performed in this study may not have been enough to promote changes in body composition if not supported by nutritional measures.

Findings from this study indicate that moderate exercise combined with nutritional measures had beneficial effects in reduction of FM. While changes within the EXCD group may be explained by improved diet quality [30], the effects in the EXCO group are likely driven through n-3 PUFAs in CO. When discussing the effects of n-3 PUFAs it is important to consider the bioavailability of their binding form, as this is the basic requirement for a substance to enter the body and convey its effects [41]. In CO, most n-3 PUFAs are bound as wax esters. Although it was believed that digestion and absorption of wax esters may not be as effective in mammals [42], a study by Gorreta and colleagues showed that digestion of wax esters was comparable with triglycerides and ethyl esters in rats [43]. A similar result was also found in humans when plasma concentrations of EPA and DHA were compared over a 72-h window after intake of 4 g CO (providing 260 mg EPA and 156 mg DHA) and a fish oil supplement containing ethyl esters (providing 465 mg EPA and 375 mg DHA), revealing equal bioavailability [44].

Regarding body fat loss, several potential mechanisms of n-3 PUFAs and their metabolites have been proposed, including increased fat oxidation, improved adipocyte function (i.e., increased lipolysis and reduced lipogenesis) as well as reduction of pro-inflammatory cytokines and oxidative stress in the adipose tissue [45–47]. Increased fat oxidation is discussed as a consequence of improved β-oxidation and biogenesis of mitochondria, which Flachs and colleagues described as a "metabolic switch" [48]. Generally, the underlying mechanisms are likely mediated by the AMP-activated protein kinase (AMPK) and involve further activation of transcription factors. With regards to the latter, particularly transcription factors from the family of peroxisome proliferator-activated receptors (PPAR), namely PPARα, PPARγ, and PPARδ were shown to play a role in adipogenesis [47]. However, such mechanistic relationships were mainly investigated in animal models or in vitro [46,49]. Therefore, it remains unclear which dose of n-3 PUFAs exerts beneficial effects on body composition in humans. In any case, when compared to previous human studies using supplements with >2 g of EPA + DHA, the daily dose of n-3 PUFAs from CO was considerably lower with only ~200 mg EPA + DHA. This amount even falls below the recommendations of the International Society for the Study of Fatty Acids and Lipids of 0.5 g EPA + DHA per day [50]. Beyond this background, other potential mechanisms or a combination of several pathways needs to be considered.

One compound present in CO that may influence body composition is astaxanthin, a carotene that also serves as a natural antioxidant [51]. A recent review discussed the potential beneficial effects of carotenes in counteracting obesity [24]. Although data in humans is limited, some studies reported a significant decrease in adipose tissue in obese subjects after carotene supplementation [52,53]. In the two available interventional studies, the dosage and composition of the carotenes widely varied. While one study used a mixture containing ~24 mg of carotenes (500 μg α-carotene, 1200 μg β-carotene, 500 μg astaxanthin, 2000 μg zeaxanthin, 10 mg lutein, 10 mg lycopene) and 10 mg γ-tocopherol that was administered twice daily [52], another study administered 9.0 mg of xanthophyll per day [53]. As those doses highly exceed the amount of astaxanthin present in 2 g of CO used in this study, the theory of combined effects of the components present in CO is further supported.

In addition to influencing body composition, exercise was also shown to improve markers of glucose metabolism and blood lipids in older individuals [54,55]. Therefore, we sought to identify potential changes in such metabolic markers in the overweight study population. Although results from our study showed no significant improvements in any metabolic markers, a trend to reduced fasting insulin was observed in the EXDC and EXCO groups. Moreover, the highest, yet not significant, reduction of triglycerides was observed within the EXCO group, which falls in line with results showing a triglyceride lowering effect of n-3 PUFAs supplementation [56]. However, whether those effects indicate further potential benefits from exercise combined with a healthy diet or CO needs to be determined in larger scale studies.

5. Limitations

Although BIA is an accepted tool for estimation of body composition in longitudinal studies comparing group effects [57–59], this method also has its limitations as it was shown to be susceptible to confounders such as vigorous physical activity, dietary intake, or hydration status [57,60,61]. However, as all measurements were conducted at the same time in the morning after an overnight fast, acute effects of activity or dietary intakes were minimized. Another limitation of our study is the use of a FFQ, which did not allow checking for consumption of fatty fish versus lean fish. Next, although a validated questionnaire to monitor the physical activity outside of the intervention was used, an additional documentation of the physical activity level via activity trackers would have been an informative addition. Future investigations on the effect of CO should also include a placebo group. A placebo was not included in this setting, as we aimed to directly compare the healthy diet versus CO instead of effects of CO only.

6. Conclusions

Taken together, results from this study indicate that a combination of moderate exercise with a healthy diet or CO supplementation may promote body fat loss in elderly, untrained, overweight subjects when compared to exercise only. Metabolic markers such as blood lipids and markers of glucose metabolism did not change significantly. Future research should investigate potential effects of a healthy diet (rich in n-3 PUFAs) vs. CO supplementation with and without exercise, particularly in regards to body composition.

References

1. JafariNasabian, P.; Inglis, J.E.; Reilly, W.; Kelly, O.J.; Ilich, J.Z. Aging human body: Changes in bone, muscle and body fat with consequent changes in nutrient intake. *J. Endocrinol.* **2017**, *234*, R37–R51. [CrossRef] [PubMed]
2. Chia, C.W.; Egan, J.M.; Ferrucci, L. Age-Related Changes in Glucose Metabolism, Hyperglycemia, and Cardiovascular Risk. *Circ. Res.* **2018**, *123*, 886–904. [CrossRef] [PubMed]
3. Michaud, M.; Balardy, L.; Moulis, G.; Gaudin, C.; Peyrot, C.; Vellas, B.; Cesari, M.; Nourhashemi, F. Proinflammatory Cytokines, Aging, and Age-Related Diseases. *J. Am. Med. Dir. Assoc.* **2013**, *14*, 877–882. [CrossRef] [PubMed]
4. Stehr, M.D.; von Lengerke, T. Preventing weight gain through exercise and physical activity in the elderly: A systematic review. *Maturitas* **2012**, *72*, 13–22. [CrossRef]

5.   Garatachea, N.; Pareja-Galeano, H.; Sanchis-Gomar, F.; Santos-Lozano, A.; Fiuza-Luces, C.; Morán, M.; Emanuele, E.; Joyner, M.J.; Lucia, A. Exercise Attenuates the Major Hallmarks of Aging. *Rejuvenation Res.* **2015**, *18*, 57–89. [CrossRef]

6.   Nocon, M.; Hiemann, T.; Müller-Riemenschneider, F.; Thalau, F.; Roll, S.; Willich, S.N. Association of physical activity with all-cause and cardiovascular mortality: A systematic review and meta-analysis. *Eur. J. Cardiovasc. Prev. Rehabil.* **2008**, *15*, 239–246. [CrossRef]

7.   Stanford, K.I.; Goodyear, L.J. Exercise regulation of adipose tissue. *Adipocyte* **2016**, *5*, 153–162. [CrossRef]

8.   Richter, E.A.; Hargreaves, M. Exercise, GLUT4, and Skeletal Muscle Glucose Uptake. *Physiol. Rev.* **2013**, *93*, 993–1017. [CrossRef]

9.   Cartee, G.D.; Hepple, R.T.; Bamman, M.M.; Zierath, J.R. Exercise Promotes Healthy Aging of Skeletal Muscle. *Cell Metab.* **2016**, *23*, 1034–1047. [CrossRef]

10.   Reddy, K.S.; Katan, M.B. Diet, nutrition and the prevention of hypertension and cardiovascular diseases. *Public Health Nutr.* **2004**, *7*, 167–186. [CrossRef] [PubMed]

11.   Livesey, G.; Taylor, R.; Hulshof, T.; Howlett, J. Glycemic response and health—A systematic review and meta-analysis: Relations between dietary glycemic properties and health outcomes. *Am. J. Clin. Nutr.* **2008**, *87*, 258S–268S. [CrossRef] [PubMed]

12.   Polidori, M.C. Antioxidant micronutrients in the prevention of age-related diseases. *J. Postgrad. Med.* **2003**, *49*, 229–235. [PubMed]

13.   Foscolou, A.; D'Cunha, N.M.; Naumovski, N.; Tyrovolas, S.; Chrysohoou, C.; Rallidis, L.; Matalas, A.-L.; Sidossis, L.S.; Panagiotakos, D. The Association between Whole Grain Products Consumption and Successful Aging: A Combined Analysis of MEDIS and ATTICA Epidemiological Studies. *Nutrients* **2019**, *11*, 1221. [CrossRef] [PubMed]

14.   Everitt, A.V.; Hilmer, S.N.; Brand-Miller, J.C.; Jamieson, H.A.; Truswell, A.S.; Sharma, A.P.; Mason, R.S.; Morris, B.J.; Couteur, D.G.L. Dietary approaches that delay age-related diseases. *Clin. Interv. Aging* **2006**, *1*, 11–31. [CrossRef] [PubMed]

15.   Wahlqvist, M.; Savige, G. Interventions aimed at dietary and lifestyle changes to promote healthy aging. *Eur. J. Clin. Nutr.* **2000**, *54*, S148–S156. [CrossRef] [PubMed]

16.   Van Bussel, B.C.; Henry, R.M.; Ferreira, I.; van Greevenbroek, M.M.; van der Kallen, C.J.; Twisk, J.W.; Feskens, E.J.; Schalkwijk, C.G.; Stehouwer, C.D. A Healthy Diet Is Associated with Less Endothelial Dysfunction and Less Low-Grade Inflammation over a 7-Year Period in Adults at Risk of Cardiovascular Disease. *J. Nutr.* **2015**, *145*, 532–540. [CrossRef] [PubMed]

17.   Bechthold, A.; Breidenassel, C.; Egert, S.; Gahl, A.; Michel, M.; Oberritter, H.; Virmani, K. *Vollwertig Essen und Trinken Nach den 10 Regeln der DGE.*; Deutschen Gesellschaft für Ernährung: Bonn, Germany, 2018.

18.   Chen, C.; Yu, X.; Shao, S. Effects of Omega-3 Fatty Acid Supplementation on Glucose Control and Lipid Levels in Type 2 Diabetes: A Meta-Analysis. *PLoS ONE* **2015**, *10*, e0139565. [CrossRef]

19.   Hartweg, J.; Perera, R.; Montori, V.; Dinneen, S.; Neil, H.a.W.; Farmer, A. Omega-3 polyunsaturated fatty acids (PUFA) for type 2 diabetes mellitus. *Cochrane Database Syst. Rev.* **2008**, *1*, CD003205. [CrossRef]

20.   Smith, G.I.; Julliand, S.; Reeds, D.N.; Sinacore, D.R.; Klein, S.; Mittendorfer, B. Fish oil-derived n-3 PUFA therapy increases muscle mass and function in healthy older adults. *Am. J. Clin. Nutr.* **2015**, *102*, 115–122. [CrossRef]

21.   Bender, N.; Portmann, M.; Heg, Z.; Hofmann, K.; Zwahlen, M.; Egger, M. Fish or n3-PUFA intake and body composition: A systematic review and meta-analysis: Fish and body composition. *Obes. Rev.* **2014**, *15*, 657–665. [CrossRef]

22.   Höper, A.C.; Salma, W.; Khalid, A.M.; Hafstad, A.D.; Sollie, S.J.; Raa, J.; Larsen, T.S.; Aasum, E. Oil from the marine zooplankton *Calanus finmarchicus* improves the cardiometabolic phenotype of diet-induced obese mice. *Br. J. Nutr.* **2013**, *110*, 2186–2193. [CrossRef] [PubMed]

23.   Höper, A.C.; Salma, W.; Sollie, S.J.; Hafstad, A.D.; Lund, J.; Khalid, A.M.; Raa, J.; Aasum, E.; Larsen, T.S. Wax Esters from the Marine Copepod *Calanus finmarchicus* Reduce Diet-Induced Obesity and Obesity-Related Metabolic Disorders in Mice. *J. Nutr.* **2014**, *144*, 164–169. [CrossRef] [PubMed]

24.   Mounien, L.; Tourniaire, F.; Landrier, J.-F. Anti-Obesity Effect of Carotenoids: Direct Impact on Adipose Tissue and Adipose Tissue-Driven Indirect Effects. *Nutrients* **2019**, *11*, 1562. [CrossRef] [PubMed]

25. Aguiar, E.J.; Morgan, P.J.; Collins, C.E.; Plotnikoff, R.C.; Callister, R. Efficacy of interventions that include diet, aerobic and resistance training components for type 2 diabetes prevention: A systematic review with meta-analysis. *Int. J. Behav. Nutr. Phys. Act.* **2014**, *11*, 2. [CrossRef] [PubMed]

26. Zheng, L.; Wu, J.; Wang, G.; Persuitte, G.; Ma, Y.; Zou, L.; Zhang, L.; Zhao, M.; Wang, J.; Lan, Q.; et al. Comparison of control fasting plasma glucose of exercise-only versus exercise-diet among a pre-diabetic population: A meta-analysis. *Eur. J. Clin. Nutr.* **2016**, *70*, 424–430. [CrossRef] [PubMed]

27. Verreijen, A.M.; Engberink, M.F.; Memelink, R.G.; van der Plas, S.E.; Visser, M.; Weijs, P.J.M. Effect of a high protein diet and/or resistance exercise on the preservation of fat free mass during weight loss in overweight and obese older adults: A randomized controlled trial. *Nutr. J.* **2017**, *16*, 10. [CrossRef]

28. Frey, I.; Berg, A.; Grathwohl, D.; Keul, J. Freiburger Fragebogen zur körperlichen Aktivität-Entwicklung, Prüfung und Anwendung. *Soz. Präv. Soc. Prev. Med.* **1999**, *44*, 55–64. [CrossRef]

29. Matthews, D.R.; Hosker, J.R.; Rudenski, A.S.; Naylor, B.A.; Treacher, D.F.; Turner, R.C.; Research Laboratories, D. Homeostasis model assessment: Insulin resistance and fl-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **1985**, *28*, 412–419. [CrossRef]

30. Wong, J.E.; Parnell, W.R.; Howe, A.S.; Lubransky, A.C.; Black, K.E.; Skidmore, P.M. Diet quality is associated with measures of body fat in adolescents from Otago, New Zealand. *Public Health Nutr.* **2015**, *18*, 1453–1460. [CrossRef]

31. Strandberg, E.; Edholm, P.; Ponsot, E.; Wåhlin-Larsson, B.; Hellmén, E.; Nilsson, A.; Engfeldt, P.; Cederholm, T.; Risérus, U.; Kadi, F. Influence of combined resistance training and healthy diet on muscle mass in healthy elderly women: A randomized controlled trial. *J. Appl. Physiol.* **2015**, *119*, 918–925. [CrossRef]

32. Strandberg, E.; Ponsot, E.; Piehl-Aulin, K.; Falk, G.; Kadi, F. Resistance Training Alone or Combined With N-3 PUFA-Rich Diet in Older Women: Effects on Muscle Fiber Hypertrophy. *J. Gerontol. A. Biol. Sci. Med. Sci.* **2019**, *74*, 489–494. [CrossRef] [PubMed]

33. Hill, A.M.; Buckley, J.D.; Murphy, K.J.; Howe, P.R. Combining fish-oil supplements with regular aerobic exercise improves body composition and cardiovascular disease risk factors. *Am. J. Clin. Nutr.* **2007**, *85*, 1267–1274. [CrossRef]

34. Noreen, E.E.; Sass, M.J.; Crowe, M.L.; Pabon, V.A.; Brandauer, J.; Averill, L.K. Effects of supplemental fish oil on resting metabolic rate, body composition, and salivary cortisol in healthy adults. *J. Int. Soc. Sports Nutr.* **2010**, *7*, 31. [CrossRef] [PubMed]

35. DeFina, L.F.; Marcoux, L.G.; Devers, S.M.; Cleaver, J.P.; Willis, B.L. Effects of omega-3 supplementation in combination with diet and exercise on weight loss and body composition. *Am. J. Clin. Nutr.* **2011**, *93*, 455–462. [CrossRef] [PubMed]

36. Cornish, S.M.; Chilibeck, P.D. Alpha-linolenic acid supplementation and resistance training in older adults. *Appl. Physiol. Nutr. Metab.* **2009**, *34*, 49–59. [CrossRef]

37. Cornish, S.M.; Myrie, S.B.; Bugera, E.M.; Chase, J.E.; Turczyn, D.; Pinder, M. Omega-3 supplementation with resistance training does not improve body composition or lower biomarkers of inflammation more so than resistance training alone in older men. *Nutr. Res.* **2018**, *60*, 87–95. [CrossRef]

38. Daďová, K.; Petr, M.; Šteffl, M.; Sontáková, L.; Chlumský, M.; Matouš, M.; Štich, V.; Štěpán, M.; Šiklová, M. Effect of Calanus Oil Supplementation and 16 Week Exercise Program on Selected Fitness Parameters in Older Women. *Nutrients* **2020**, *12*, 481. [CrossRef]

39. Smith, G.I.; Atherton, P.; Reeds, D.N.; Mohammed, B.S.; Rankin, D.; Rennie, M.J.; Mittendorfer, B. Omega-3 polyunsaturated fatty acids augment the muscle protein anabolic response to hyperinsulinaemia-hyperaminoacidaemia in healthy young and middle-aged men and women. *Clin. Sci.* **2011**, *121*, 267–278. [CrossRef]

40. Rossato, L.T.; Schoenfeld, B.J.; de Oliveira, E.P. Is there sufficient evidence to supplement omega-3 fatty acids to increase muscle mass and strength in young and older adults? *Clin. Nutr.* **2020**, *39*, 23–32. [CrossRef]

41. Schuchardt, J.P.; Hahn, A. Bioavailability of long-chain omega-3 fatty acids. *Prostaglandins Essent. Fat. Acids* **2013**, *89*, 1–8. [CrossRef]

42. Place, A.R. Comparative aspects of lipid digestion and absorption: Physiological correlates of wax ester digestion. *Am. J. Physiol.* **1992**, *263*, R464–R471. [CrossRef] [PubMed]

43. Gorreta, F.; Bernasconi, R.; Galliani, G.; Salmona, M.; Tacconi, M.T.; Bianchi, R. Wax Esters of n-3 Polyunsaturated Fatty Acids: A New Stable Formulation as a Potential Food Supplement. 1—Digestion and Absorption in Rats. *LWT Food Sci. Technol.* **2002**, *35*, 458–465. [CrossRef]

44. Cook, C.M.; Larsen, T.S.; Derrig, L.D.; Kelly, K.M.; Tande, K.S. Wax Ester Rich Oil From The Marine Crustacean, *Calanus finmarchicus*, is a Bioavailable Source of EPA and DHA for Human Consumption. *Lipids* **2016**, *51*, 1137–1144. [CrossRef] [PubMed]

45. Albracht-Schulte, K.; Kalupahana, N.S.; Ramalingam, L.; Wang, S.; Rahman, S.M.; Robert-McComb, J.; Moustaid-Moussa, N. Omega-3 fatty acids in obesity and metabolic syndrome: A mechanistic update. *J. Nutr. Biochem.* **2018**, *58*, 1–16. [CrossRef] [PubMed]

46. Kopecky, J.; Rossmeisl, M.; Flachs, P.; Kuda, O.; Brauner, P.; Jilkova, Z.; Stankova, B.; Tvrzicka, E.; Bryhn, M. n-3 PUFA: Bioavailability and modulation of adipose tissue function: Symposium on "Frontiers in adipose tissue biology". *Proc. Nutr. Soc.* **2009**, *68*, 361–369. [CrossRef]

47. Flachs, P.; Rossmeisl, M.; Bryhn, M.; Kopecky, J. Cellular and molecular effects of n−3 polyunsaturated fatty acids on adipose tissue biology and metabolism. *Clin. Sci.* **2009**, *116*, 1–16. [CrossRef]

48. Flachs, P.; Horakova, O.; Brauner, P.; Rossmeisl, M.; Pecina, P.; Franssen-van Hal, N.; Ruzickova, J.; Sponarova, J.; Drahota, Z.; Vlcek, C.; et al. Polyunsaturated fatty acids of marine origin upregulate mitochondrial biogenesis and induce β-oxidation in white fat. *Diabetologia* **2005**, *48*, 2365–2375. [CrossRef]

49. Todorčević, M.; Hodson, L. The Effect of Marine Derived n-3 Fatty Acids on Adipose Tissue Metabolism and Function. *J. Clin. Med.* **2016**, *5*, 3. [CrossRef]

50. Cunnane, S.C.; Drevon, C.A.; Harris, B.; Sinclair, A.J.; Spector, A. Report of the Sub-Committee on Recommendations for Intake of Polyunsaturated Fatty Acids in Healthy Adults. *Int. Soc. Study Fatty Acids Lipids Retrieved Dec.* **2004**, *9*, 2015.

51. Ikeuchi, M.; Koyama, T.; Takahashi, J.; Yazawa, K. Effects of astaxanthin in obese mice fed a high-fat diet. *Biosci. Biotechnol. Biochem.* **2007**, *71*, 893–899. [CrossRef]

52. Canas, J.A.; Lochrie, A.; McGowan, A.G.; Hossain, J.; Schettino, C.; Balagopal, P.B. Effects of Mixed Carotenoids on Adipokines and Abdominal Adiposity in Children: A Pilot Study. *J. Clin. Endocrinol. Metab.* **2017**, *102*, 1983–1990. [CrossRef]

53. Kakutani, R.; Hokari, S.; Nishino, A.; Ichihara, T.; Sugimoto, K.; Takaha, T.; Kuriki, T.; Maoka, T. Effect of Oral Paprika Xanthophyll Intake on Abdominal Fat in Healthy Overweight Humans: A Randomized, Double-blind, Placebo-controlled Study. *J. Oleo Sci.* **2018**, *67*, 1149–1162. [CrossRef]

54. Kelley, G.A.; Kelley, K.S.; Tran, Z.V. Exercise, lipids, and lipoproteins in older adults: A meta-analysis. *Prev. Cardiol.* **2005**, *8*, 206–214. [CrossRef]

55. Forbes, S.C.; Little, J.P.; Candow, D.G. Exercise and nutritional interventions for improving aging muscle health. *Endocrine* **2012**, *42*, 29–38. [CrossRef]

56. Weber, P.; Raederstorff, D. Triglyceride-lowering effect of omega-3 LC-polyunsaturated fatty acids-a review. *Nutr. Metab. Cardiovasc. Dis.* **2000**, *10*, 28–37.

57. Kyle, U.G.; Bosaeus, I.; De Lorenzo, A.D.; Deurenberg, P.; Elia, M.; Manuel Gómez, J.; Lilienthal Heitmann, B.; Kent-Smith, L.; Melchior, J.-C.; Pirlich, M.; et al. Bioelectrical impedance analysis—part II: Utilization in clinical practice. *Clin. Nutr.* **2004**, *23*, 1430–1453. [CrossRef]

58. Verdich, C.; Barbe, P.; Petersen, M.; Grau, K.; Ward, L.; Macdonald, I.; Sørensen, T.I.; Oppert, J.-M. Changes in body composition during weight loss in obese subjects in the NUGENOB study: Comparison of bioelectrical impedance vs. dual-energy X-ray absorptiometry. *Diabetes Metab.* **2011**, *37*, 222–229. [CrossRef]

59. Thomson, R.; Brinkworth, G.D.; Buckley, J.D.; Noakes, M.; Clifton, P.M. Good agreement between bioelectrical impedance and dual-energy X-ray absorptiometry for estimating changes in body composition during weight loss in overweight young women. *Clin. Nutr.* **2007**, *26*, 771–777. [CrossRef]

60. Tinsley, G.M.; Morales, E.; Forsse, J.S.; Grandjean, P.W. Impact of Acute Dietary Manipulations on DXA and BIA Body Composition Estimates. *Med. Sci. Sports Exerc.* **2017**, *49*, 823–832. [CrossRef]

61. Thompson, D.L.; Thompson, W.R.; Prestridge, T.J.; Bailey, J.G.; Bean, M.H.; Brown, S.P.; McDaniel, J.B. Effects of hydration and dehydration on body composition analysis: A comparative study of bioelectric impedance analysis and hydrodensitometry. *J. Sports Med. Phys. Fit.* **1991**, *31*, 565–570.

2.2 Paper II

Intake of Calanus finmarchicus oil for 12 weeks improves omega-3 index in healthy older subjects engaging in an exercise programme

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Intake of *Calanus finmarchicus* oil for 12 weeks improves omega-3 index in healthy older subjects engaging in an exercise programme

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Abstract

The *n*-3 PUFA, EPA and DHA, play an important role in human health. As the intake of EPA and DHA from the diet is often inadequate, supplementation of those fatty acids is recommended. A novel source of *n*-3 PUFA is *Calanus finmarchicus* oil (CO) which contains fatty acids mainly bound in wax esters. To date, no data are available on the effects of long-term intake of this marine oil on *n*-3 PUFA blood levels. Therefore, the aim of this study was to evaluate the effect of CO on the *n*-3 PUFA blood levels using the omega-3 index (O3I). The data originate from a larger randomised controlled trial. For this analysis, samples from seventy-two participants (59·2 (sᴅ 6·2) years, BMI 27·7 (sᴅ 5·28) kg/m^2) were analysed. Of those, thirty-six performed 2×/week exercise and received 2 g of CO, which provided 124 mg stearidonic acid (SDA), 109 mg EPA and 87 mg DHA daily (EXCO group), while the other group performed exercise only (EX group) and served as a control for this analysis. The O3I increased from 6·07 (sᴅ 1·29) % at baseline to 7·37 (sᴅ 1·10) % after 12 weeks within the EXCO group ($P < 0·001$), while there were no significant changes in the EX group (6·01 (sᴅ 1·26)–6·15 (sᴅ 1·32) %, $P = 0·238$). These data provide first evidence that wax ester-bound *n*-3 PUFA from CO can significantly increase the O3I despite relatively low EPA + DHA amounts. Further, the effects of exercise could be excluded.

Key words: **PUFA: Marine oil: Wax esters: EPA: DHA**

n-3 PUFA are highly associated with human health, playing an important role in cardiovascular health, brain development and cognitive function as well as inflammation and inflammatory diseases[1,2].

Of all *n*-3 PUFA, most of those health benefits are attributed to the 20 : 5*n*-3 fatty acid EPA and the 22 : 6*n*-3 fatty acid DHA. Regarding their importance in human health and an unfavourable shift in the dietary fatty acid intake in favour of *n*-6 fatty acids, daily intake of at least 0·5 g of EPA and DHA is recommended by the International Society for the Study of Fatty Acids and Lipids (ISSFAL)[3]. The primary dietary source for EPA and DHA is coldwater fish such as tuna, salmon, herring or mackerel; however, intake of these types of fish is often low[4]. In Germany, this is also reflected by low median dietary intakes of 65–78 mg EPA and 107–135 mg DHA[5]. Therefore, supplementation of *n*-3 PUFA may be beneficial. Good *n*-3 PUFA sources that are commonly used for supplementation are marine oils, with fish oil being the most frequently used[6]. A novel *n*-3 PUFA source is the marine oil obtained from *Calanus finmarchicus*, a copepod found in the northern Atlantic sea. Unlike fish oil, where *n*-3 PUFA are bound in the form of TAG, >80 % of the fatty acids in *Calanus finmarchicus* oil (CO) are bound in the form of wax esters[7]. In any case, when compared with refined fish oil which commonly contains 300 mg EPA + DHA per g[8], the amount of EPA and DHA in CO is relatively low (about 100 mg EPA + DHA per g). A single-dose study has already shown that EPA and DHA from CO are equally bioavailable as from fish oil[9]. However, the long-term intake of *n*-3 PUFA from CO has not yet been studied. The omega-3 index (O3I) (relative content of EPA + DHA in erythrocytes) has been shown to be a good and reliable indicator in evaluating the fatty acid supply over a longer period of time[10,11].

In addition to being an indicator for the long-term fatty acid supply, an O3I of >8 % was also linked to a lower risk for

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cardiovascular events[10,12]. This is of particular interest for all age groups engaging in exercise, as exercise increases the demands of the cardiovascular system. Although it was already demonstrated that athletes show an insufficient supply with *n*-3 PUFA[13,14], no data about the effects of exercise on the O3I have been described to date.

Hence, the objective of the present study was to investigate the effect of CO providing physiological EPA + DHA doses on the O3I in a study collective of healthy elderly subjects who participated in an exercise programme. To account for potential effects of the exercise programme on the O3I, the group performing exercise only served as a control.

Materials and methods

Study design and participants

The present work is based on a single-centre, randomised controlled trial in parallel-group design which was conducted at the Institute of Food Science and Human Nutrition, Leibniz University Hannover, Germany. In brief, the study consisted of a screening and 12-week intervention phase with two examination days; one at the beginning (t_0) and one at the end of the 12-week intervention (t_{12}).

Participants for this study were recruited via advertisements in local newspapers and public notice boards from the general population in Hannover, Germany, between August 2018 and March 2019. The trial ended when the required sample size was achieved. The main inclusion criteria for participation were age ≥50 and ≤70 years, no exercise training aside the daily activities for at least 2 years, a stable body weight (±5 kg) for at least 6 months, being able to physically perform the exercise intervention (exercise capacity) and following an omnivorous diet. Exclusion criteria were defined as: suspicion and diagnosis of CVD (angina pectoris, myocardial infarction, stroke, peripheral arterial occlusive disease, heart failure and cardiac arrhythmia), type 1 and 2 diabetes, renal insufficiency and liver diseases, blood coagulation disorders, chronic gastrointestinal disorders (e.g. ulcers, Crohn's disease, pancreatic insufficiency, immunological diseases (e.g. autoimmune diseases)), intake of immunosuppressive drugs or laxatives, intake of supplements containing *n*-3 PUFA, alcohol, drug and/or medicine dependency, pregnancy or lactation, retraction of the consent by the subject, concurrent participation in another clinical study and participation in a study in the last 30 d. Inclusion and exclusion criteria were assessed using a structured screening questionnaire. Exercise capacity was determined during a resting and exercise electrocardiogram, implemented by trained professionals and a physician. This study was conducted according to the guidelines in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Commission of the Medical Chamber of Lower Saxony (Hannover, Germany) (Bo/07/2018, URL: https://www.drks.de/drks_web/setLocale_EN.do). This study is registered in the German Clinical Trial Register (DRKS00014322).

The participants were randomly assigned by an independent researcher using stratified randomisation according to the covariates (in descending order: sex, BMI and age) to one of four study groups: (1) control group (CON), (2) exercise only group (EX), (3) exercise and dietary counseling group (EXDC) and (4) exercise and CO supplementation group (EXCO). However, the present work focuses on the EX and EXCO groups only.

Both groups were instructed to perform exercise training twice a week and maintain their habitual diet. The exercise training was performed in fitness centres and consisted of a warm up followed by two passes of a strength-endurance circuit. The strength training consisted of six machine supported exercises that included all major muscle groups and were performed for 1 min each. During the initial training session, a maximum force test with three tries was performed. The best of the three tries was scored and used to set the machines to 60 % of the participants' maximum force for the first 2 weeks of training. For the subsequent 6 weeks, the load was increased by 10 % and again by 5 % for the last 4 weeks. The endurance exercise consisted of a 4-min bout performed on bicycle ergometers and cross-trainers at a perceived exertion that equaled a value of 15 on the Borg-Scale. In between each exercise, the participants had 30 s of rest. Including the warm-up and rest periods, the training session could be completed in approximately 1 hour. Compliance of the participants was assessed via a training log and a questionnaire at the end of the study.

In addition to the exercise training, participants from the EXCO group received capsules providing 2·0 g of oil from *Calanus finmarchicus* (Calanus AS) and were instructed to take them daily. The lipid profile of the capsules is shown in Table 1. More than 80 % of the fatty acids are bound as wax esters. The tolerability of the CO capsules was checked using questionnaires.

Compliance of participants and adherence to the instructions were monitored via fortnightly phone calls. Additionally, participants from the EXCO were instructed to return leftover capsules, which were then counted by study personal after delivery. Participants had to consume at least 90 % of the capsules to be considered as compliant.

Table 1. Fatty acid composition of the *Calanus finmarchicus* oil (CO) used in the study

Fatty acid*	Common name	mg/100 g CO	mg/2 g CO
14 : 0	Myristic acid	6232	125
15 : 0	Pentadeclic acid	323	6·5
16 : 0	Palmitic acid	5243	105
16 : 3		325	7
18 : 0	Stearic acid	399	8
18 : 1*n*-9	Oleic acid	1783	36
18 : 2*n*-6	Linoleic acid	552	11
18 : 3*n*-3	α-Linolenic acid	1149	23
18 : 3*n*-6	β-Linolenic acid	140	3
18 : 4*n*-3	Stearidonic acid	6186	124
20 : 1*n*-9	Gondoic acid	2148	43
20 : 4*n*-6	Arachidonic acid	169	3
20 : 5*n*-3	EPA	5439	109
22 : 1*n*-11	Cetoleic acid	3495	70
22 : 5*n*-3	Docosapentaenoic acid	254	8
22 : 6*n*-3	DHA	4342	87
24 : 1*n*-9		414	8

* >80 % of fatty acids are present as wax esters.

Dietary intake from background diet

Dietary intake of the participants was monitored via 3-d dietary food logs at the beginning, after 6 weeks and at the end of the intervention. The records were checked by nutritionists for completeness, readability and plausibility. If necessary, ambiguities were clarified with the participants. Energy and nutrient intake were estimated using the software PRODI6.4® (Nutri-Science GmbH).

Blood sampling and analysis

Blood samples were drawn from the participants after an overnight fast ($\geq$10 h) between 06.00 and 10.00 hours by venepuncture of an arm vein using EDTA tubes (Sarstedt AG & Co. KG). TAG, LDL-cholesterol and HDL-cholesterol were analysed by a photometric method (Beckman Coulter GmbH) by an accredited and certified laboratory (Laborärztliche Arbeitsgemeinschaft für Diagnostik und Rationalisierung e.V.). Total cholesterol and LDL:HDL ratio were calculated from LDL and HDL values.

For analysis of O3I, EDTA tubes were centrifuged for 10 min at 3000 rpm, buffy coat was removed and erythrocytes frozen at -80°C till analysis. The analysis was performed by the Omegametrix laboratory (Martinsried) according to the HS-n-3 Index methodology®[10,12] by GC. Accordingly, methyl esters of fatty acids were generated from erythrocytes by acid transesterification, and analysis performed using a GC2010 gas chromatograph (Shimadzu) equipped with a SP2560, 100 m column (Supelco). Hydrogen was used as the carrier gas. To identify fatty acids, a standard mixture characteristic for erythrocytes was used. The results obtained are given as percentage of total identified fatty acids after response factor correction. The O3I represents the sum of EPA + DHA in relation to total fatty acid content in red blood cell membranes. Quality was assured according to DIN ISO 15189.

Statistical analyses

Data for this analysis were derived from a larger interventional trial, which served as an explorative study. Based on an α of 0·05 and 0·80 β, assuming an effect size of more than 0·8, a sample size of n 25 was needed to detect between-group differences. Estimating a dropout rate of 15 % at least thirty participants per intervention group were recruited. For this analysis, a power calculation was performed using an online calculator[15]. As CO was demonstrated to be equally bioavailable as ethyl esters[9], the calculation was based on a study by Köhler *et al.*[16]. In the study by Köhler *et al.*[16], participants received 250 mg ethyl-ester bound EPA + DHA in the form of enriched sausages which resulted in an O3I increase from 4·18 (SD 0·54) to 5·72 (SD 0·66) % after 8 weeks. Therefore, we assumed that a change of the O3I of at least 1 % could be achieved after 12 weeks of CO supplementation providing about 200 mg EPA + DHA. Using the online calculator with alpha set to 0·05 and n 50 total participants, assuming a mean difference of 1·0 and a 0·7 % standard deviation (using 5·72 (SD 0·66) % from Köhler *et al.*[16] as the upper level for the standard deviation) a 99 % probability to detect a difference if the true difference between groups is 1 % was calculated.

Statistical analyses were performed for both the primary outcome O3I and the secondary outcomes (anthropometric data, dietary intake and other fatty acids in erythrocytes). Data are presented as mean values and standard deviations. Distribution of all data was assessed using the Shapiro–Wilk test and Gaussian distribution. Based on the distribution of data, differences in baseline characteristics were assessed using the Mann–Whitney U test or unpaired t test for continuous variables and the χ^2 test for nominal variables. Further analysis was performed with data obtained from all participants that arrived at the first and second day of examination (n_{EX} 6, n_{EXCO} 3). For analysis, not normally distributed data were log transformed, except C18 : 2n-6tt which was square root transformed, and differences were assessed using two-factor repeated measures ANOVA using time (t_0 and t_{12}) intervention (EX and EXCO). If statistically significant differences between groups were detected, a *post hoc* analysis with Bonferroni correction was performed within both groups. P values of <0·05 were considered as significant. All statistical analyses were carried out using SPSS software (version 23.0; SPSS Inc.).

Results

Baseline characteristics

Data analysed were obtained from a total of seventy-two participants (68 % female, 32 % male). Due to health and personal reasons, the study had a dropout of nine subjects (n_{EX} 6, n_{EXCO} 3). Baseline characteristics of the study population are shown in Table 2. The mean age was 59·2 (SD 6·2) years, and the average BMI was 27·7 (SD 5·28) kg/m². There were no differences in anthropometric parameters or blood lipids between both groups. The study population was slightly hypercholerosterolaemic.

Table 2. Baseline characteristics of all participants*
(Mean values and standard deviations)

Measure	Exercise (n 36) Mean	SD	Exercise + CO (n 36) Mean	SD	Group difference: P
Sex (f/m)	24/12	–	25/11	–	0·80
Age (years)	59·70	6·39	58·5	5·69	0·51
Height (m)	170·9	8·73	171·3	8·74	0·89
Body weight (kg)	81·9	18·6	80·7	20·3	0·61
BMI (kg/m²)	28·00	5·51	27·28	5·26	0·61
SBP (mmHg)	129·4	14·7	134·1	20·1	0·27
DBP (mmHg)	76·2	6·16	78·6	7·91	0·15
Pulse (bpm)	74·6	9·58	73·8	8·25	0·71
Total cholesterol (mmol/l)	6·15	1·01	6·26	0·96	0·97
HDL-cholesterol (mmol/l)	1·75	0·40	1·76	0·47	0·76
LDL-cholesterol (mmol/l)	3·89	0·82	3·93	0·76	0·83
TAG (mmol/l)	1·31	0·63	1·23	0·39	0·56

CO, *Calanus finmarchicus* oil; f, female; m, male; SBP, systolic blood pressure; DBP, diastolic blood pressure.
* Distribution of sexes between groups was analysed using the χ^2 test. All other group differences were assessed with the Mann–Whitney U test or unpaired t test.

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Dietary intake from background diet

The intake of PUFA and especially EPA and DHA showed high variability at both time points (Table 3). Throughout the study, the intake of individual and total fatty acids did not change significantly. Additionally, all other dietary variables did also not change significantly. However, there was a non-significant trend to a lower energetic intake of about 837 kJ (200 kcal) at the end of the intervention in both study groups.

Tolerability of the Calanus finmarchicus oil capsules

At the beginning of the study, one participant reported suffering from diarrhoea after taking the CO capsules. Despite the instructions to take the capsules with food, this participant took the capsules on an empty stomach. After starting taking the capsules as suggested, the symptoms disappeared. Otherwise, no adverse symptoms after the CO capsule intake were reported.

Fatty acid content of erythrocytes and omega-3 index

Comparison of baseline values between the two study groups showed no significant differences in fatty acid levels. In both groups, PUFA values declined in the following order ARA > LA > DHA > DPAn-3 = C22 : 4n-6 > C20 : 3n-6 > EPA > DPAn-6 > ALA = C20 : 2n-6 > C18 : 3n-6. Lowest quantities were observed for SDA. O3I was 6·01 (sd 1·26) % in EX and 6·07 (sd 1·29) % in EXCO (Table 4).

During the study, a significant increase in O3I occurred in the EXCO group from 6·07 (sd 1·29) to 7·37 (sd 1·10) % ($P < 0.001$). In line, EPA increased from 0·92 (sd 0·42) to 1·32 (sd 0·37) % ($P < 0.001$) and DHA from 5·15 (sd 1·03) to 6·04 (sd 0·94)

($P < 0.001$). Further, relevant increases occurred in SDA from 0·027 (sd 0·008) to 0·038 (sd 0·012) % ($P = 0.008$) and DPAn-3 from 2·67 (sd 0·41) to 2·86 (sd 0·41) % ($P < 0.001$). In contrast, levels of most n-6-PUFA slightly decreased in consequence of the intervention: ARA decreased from 15·3 (sd 1·07) to 14·9 (sd 0·94) % ($P < 0.001$), C22 : 4n-6 from 2·70 (sd 0·48) to 2·46 (sd 0·42) % ($P < 0.001$) and DPAn-6 from 0·63 (sd 0·18) to 0·56 (sd 0·18) % ($P < 0.001$).

The EX group showed no physiologically relevant changes in erythrocyte fatty acid levels throughout the intervention.

Discussion

Current evidence from preclinical studies indicates promising effects of CO on obesity and obesity-related inflammation as well as on blood glucose control and atherosclerosis[17–19]. However, the underlying physiological and molecular mechanisms of action are not yet fully understood. An important prerequisite when examining the physiological effects of marine oils is to ensure that active ingredients enter the body in sufficiently high amounts[11]. CO contains the n-3 PUFA SDA, EPA and DHA. Hence, the investigation of the effect of n-3 PUFA from CO on PUFA blood levels is necessary to understand the mode of action of this novel marine oil.

In evaluating the effect of fatty acids, several factors have to be considered with one of the first being the chemical binding form[11]. Commercially available marine n-3 PUFA supplements contain fatty acids in the form of TAG, ethyl esters or phospholipids. Contrary to that, >80 % of fatty acids in CO are bound to fatty alcohols, which classifies them as wax esters.

Table 3. Dietary energy and nutrient intake calculated from 3-d dietary records at the beginning (0) and at the end (12) of the intervention as well as absolute changes (Δ)*
(Mean values and standard deviations)

Parameters	t	Exercise (n 30) Mean	SD	Δ Exercise Mean	SD	Exercise + CO (n 33) Mean	SD	Δ Exercise + CO Mean	SD	Group difference: P
Energy intake (kcal/d)†	0	2024	409	−217	455	1924	437	−147	476	0·55
	12	1806	365			1777	415			
Protein (%/d)	0	16·1	3·42	0·86	3·84	15·6	2·99	1·05	3·68	0·84
	12	16·9	3·37			16·7	3·26			
Fat (%/d)	0	36·2	7·47	0·69	7·97	38·1	6·69	−2·51	9·04	0·14
	12	36·9	6·00			35·6	7·48			
Carbohydrates (%/d)	0	42·6	9·45	−2·76	8·36	41·92	6·09	0·47	8·72	0·08
	12	39·8	6·45			42·39	7·86			
Fibre (g/d)	0	23·4	7·79	−4·08	6·79	22·0	6·47	−0·78	8·03	0·15
	12	19·3	6·08			21·2	8·80			
SFA (g/d)	0	35·7	13·6	−4·67	15·7	36·9	15·5	−6·93	15·2	0·69
	12	31·0	11·2			29·9	11·1			
MUFA (g/d)	0	28·9	12·5	−2·99	11·9	27·3	9·61	−3·41	12·01	0·77
	12	26·0	11·2			23·9	9·29			
PUFA (g/d)	0	11·9	3·96	0·21	5·01	11·8	5·64	−1·26	4·07	0·21
	12	12·1	5·75			10·5	5·26			
DHA (g/d)	0	0·29	0·34	0·11	0·46	0·23	0·30	−0·4	0·35	0·41
	12	0·39	0·47			0·19	0·19			
EPA (g/d)	0	0·27	0·54	−0·01	0·37	0·18	0·41	0·09	0·23	0·17
	12	0·30	0·41			0·14	0·19			

CO, *Calanus finmarchicus* oil; t, times in weeks.
* P values represent time × intervention interaction analysed with two-way repeated-measures ANOVA.
† To convert kcal to kJ, multiply by 4·184.

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Table 4. Fatty acid content of erythrocytes (given as percentage of total fatty acids) at the beginning (0) and at the end (12) of the study and absolute changes study (Δ)*
(Mean values and standard deviations)

Fatty acid	t	Exercise (n 30) Mean	SD	P $t_0 \rightarrow t_{12}$	Δ Exercise Mean	SD	Exercise + CO (n 33) Mean	SD	P $t_0 \rightarrow t_{12}$	Δ Exercise + CO Mean	SD	Group difference: P
C14:0	0	0·37	0·10	–	−0·02	0·09	0·41	0·12	–	−0·02	0·07	0·90
	12	0·35	0·08				0·39	0·10				
C16:0	0	21·8	0·89	–	−0·03	0·48	21·5	0·85	–	0·09	0·37	0·23
	12	21·7	1·00				21·6	0·79				
C16:1n-7t	0	0·11	0·04	–	0·00	0·02	0·14	0·05	–	0·00	0·02	0·68
	12	0·11	0·04				0·14	0·05				
C16:1n-7	0	0·42	0·21	–	−0·05	0·13	0·41	0·18	–	−0·06	0·07	0·46
	12	0·38	0·18				0·35	0·16				
C18:0	0	16·5	0·94	–	0·11	0·60	16·7	0·92	–	0·05	0·49	0·68
	12	16·6	0·86				16·8	0·74				
C18:1t	0	0·46	0·09	0·09	−0·02	0·07	0·51	0·10	0·07	0·02	0·06	0·02
	12	0·44	0·08				0·53	0·10				
C18:1n-9	0	15·5	0·96	–	−0·11	0·55	15·4	1·12	–	−0·14	0·43	0·84
	12	15·4	1·00				15·2	1·11				
C18:2n-6tt	0	0·01	0·01	–	0·00	0·01	0·01	0·02	–	0·00	0·01	0·88
	12	0·01	0·02				0·01	0·02				
C18:2n-6ct	0	0·04	0·03	–	0·01	0·02	0·04	0·03	–	0·00	0·01	0·16
	12	0·05	0·04				0·04	0·03				
C18:2n-6tc	0	0·12	0·02	0·43	0·00	0·03	0·13	0·03	<0·001	0·02	0·02	0·01
	12	0·12	0·02				0·15	0·03				
C18:2n-6	0	11·3	1·54	–	−0·21	0·89	11·3	1·53	–	−0·52	0·99	0·22
	12	11·1	1·55				10·7	1·21				
C18:3n-3	0	0·19	0·09	–	−0·01	0·06	0·19	0·09	–	−0·02	0·07	0·63
	12	0·19	0·07				0·17	0·07				
C18:3n-6	0	0·09	0·05	–	0·00	0·04	0·09	0·03	–	−0·01	0·02	0·22
	12	0·09	0·02				0·08	0·02				
C18:4n-3	0	0·026	0·011	0·83	0·00	0·01	0·027	0·008	0·008	0·01	0·01	<0·001
	12	0·025	0·011				0·038	0·012				
C20:0	0	0·19	0·03	–	0·00	0·02	0·20	0·03	–	−0·01	0·01	0·29
	12	0·19	0·04				0·19	0·03				
C20:1n-9	0	0·28	0·05	–	0·01	0·04	0·26	0·05	–	0·02	0·03	0·18
	12	0·29	0·06				0·28	0·05				
C20:2n-6	0	0·23	0·04	–	0·00	0·02	0·22	0·03	–	0·00	0·02	0·15
	12	0·23	0·04				0·22	0·03				
C20:3n-6	0	1·72	0·36	0·02	−0·07	0·14	1·73	0·30	<0·001	−0·14	0·11	0·01
	12	1·66	0·31				1·59	0·28				
C20:4n-6	0	15·2	1·21	0·36	0·11	0·63	15·3	1·07	<0·001	−0·46	0·61	0·001
	12	15·3	1·35				14·9	0·94				
C20:5n-3	0	0·90	0·33	0·96	−0·01	0·18	0·92	0·42	<0·001	0·40	0·26	<0·001
	12	0·89	0·30				1·32	0·37				
C22:0	0	0·52	0·08	0·81	0·01	0·04	0·53	0·08	0·002	0·02	0·03	0·02
	12	0·52	0·09				0·51	0·08				
C22:4n-6	0	2·75	0·51	0·49	0·03	0·19	2·70	0·48	<0·001	−0·25	0·22	<0·001
	12	2·78	0·49				2·46	0·42				
C22:5n-3	0	2·58	0·38	0·07	0·06	0·20	2·67	0·41	<0·001	0·19	0·18	0·03
	12	2·64	0·32				2·86	0·41				
C22:5n-6	0	0·62	0·23	0·42	−0·01	0·13	0·63	0·18	<0·001	−0·07	0·06	0·02
	12	0·61	0·23				0·56	0·18				
C22:6n-3	0	5·12	1·03	0·09	0·14	0·43	5·15	1·03	<0·001	0·89	0·45	<0·001
	12	5·26	1·10				6·04	0·94				
C:24	0	1·37	0·15	–	0·01	0·12	1·36	0·20	–	−0·02	0·09	0·24
	12	1·38	0·16				1·34	0·17				
C24:1n-9	0	1·60	0·21	–	0·06	0·16	1·51	0·22	–	0·04	0·10	0·69
	12	1·66	0·27				1·55	0·23				
Omega-3 index	0	6·01	1·26	0·24	0·13	0·51	6·07	1·29	<0·001	1·29	0·66	<0·001
	12	6·15	1·32				7·37	1·10				

CO, *Calanus finmarchicus* oil; t, times in weeks.
* P values represent time × intervention interaction analysed with two-way repeated-measures ANOVA. In the case of significance, statistical differences within groups were detected with Bonferroni's *post hoc* test.

Overall wax esters have been discussed as both poorly digestible and bioavailable in mammals[20] and larger amounts are reported to cause gastrointestinal symptoms in humans[21]. In the present study, CO was well tolerated. Only one case reported gastrointestinal discomfort (diarrhoea) after ingesting the CO capsules on an empty stomach in the first week of intervention. Those symptoms disappeared immediately after the participant started following the instructions to take the

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capsules together with a meal. With regard to the bioavailability of wax esters, a single-dose study conducted by Cook *et al.* demonstrated that the bioavailability of wax esters from CO is comparable with ethyl esters from fish oil[9]. In the mentioned study, the bioavailability of 260 mg EPA and 156 mg DHA from 4 g of CO *v.* 465 mg EPA and 375 mg DHA from 1 g of ethyl esters from fish oil were compared over a 72 h. Although the dose of EPA + DHA from fish oil was almost twice as high as from CO (840 *v.* 416 mg), no significant differences in bioavailability were found. Moreover, plasma levels of EPA remained even higher in the CO group 24–72 h after intake, indicating that wax esters may be a highly available EPA/DHA source. However, the effect of a longer-term CO intake on EPA and DHA blood levels has not been studied.

In the present study, a 12-week intake of 2·0 g CO, providing about 200 mg of EPA + DHA, led to a significant increase of the O3I from 6·07 (sd 1·29) to 7·37 (sd 1·10) %, which is an increase of 1·29 (sd 0·66) %. In contrast, no difference was found in the control group that performed exercise only, indicating that engagement in a moderate exercise programme does not negatively influence the O3I.

With regard to the low EPA + DHA dose used in this study, there are only four studies using comparable doses[16,22–24]. Köhler *et al.*[16] investigated the effect of DHA + EPA from enriched sausages containing 250 mg/d EPA + DHA bound as ethyl esters and 250 mg/d ALA bound as TAG. A control group received sausages containing only 250 mg/d of ALA. After a study period of 8 weeks, a 1·5 % increase in O3I from 4·18 (sd 0·54) to 5·72 (sd 0·66) % was reported for the group consuming the enriched sausages, while no difference was observed for the control group. Another study reported significant improvements of the O3I after supplementing different doses of EPA + DHA using krill oil[24]. In krill oil, EPA + DHA are mainly bound in phospholipids. Two hundred and 400 mg/d EPA + DHA, respectively, led to an O3I increase from 3·56 (sd 0·82) to 4·19 (sd 0·79) % and 4·0 (sd 0·88) to 5·17 (sd 0·96) %, respectively[24]. Flock *et al.*[23] reported an 1·88 (sd 0·23) % O3I increase (4·29 (sd 0·22) to 6·19 (sd 0·23) %) after a 20-week supplementation of about 300 mg/d TAG-bound EPA + DHA, while Sarter *et al.* reported an increase of 1·7 % (3·9 (sd 1·0) to 4·8 (sd 0·8) %) after 16-week intake of 254 mg/d TAG-bound EPA + DHA[22]. Compared with the four previous studies, participants from this study showed an overall better EPA + DHA supply status with baseline values of about 6 %. This is of interest because it has been demonstrated that the response of O3I to *n*-3 PUFA supplementation is dependent on the baseline O3I, with lower O3I leading to greater O3I responses[25,26]. Nonetheless, the low dose of EPA + DHA from CO still successfully improved the O3I to an extent close to improvements observed in comparable studies with populations that had substantially lower baseline O3I values. Noteworthy, increases of only 1 % were reported to already decrease the risk for sudden cardiac death[27].

However, the effect of CO supplementation on the O3I is unlikely to be solely due to the gastrointestinal uptake of preformed EPA and DHA. It is more likely that SDA also contributes to the O3I increase as SDA is the most abundant *n*-3 PUFA in CO and one of the precursors in the metabolic pathway of EPA and

DHA synthesis. When compared among each other, 2·0 g of CO provided 124 mg SDA but only 109 mg EPA and 87 mg DHA. In a recent study, we investigated the short-term effect of a single-dose intake of 26 g echium oil containing 3 g of SDA and reported significant increases of EPA (47 %) and DHA (21 %) levels in plasma after 72 h[28]. Moreover, in a previous long-term study on SDA and O3I, a 16-week supplementation of SDA enriched soyabean oil providing 3·66 g SDA led to a 19·5 % increase of the O3I[29]. However, no significant changes in DHA were observed, while EPA and SDA levels increased in erythrocytes. In a recent study, we observed a similar outcome after a 12-week supplementation of 12·9 g ALA[30]. In comparison, the 12-week intake of the low *n*-3 PUFA dose in the present study led to increases of 41 % in SDA, 44 % in EPA and 17 % in DHA. Noteworthy, CO also contains a non-negligible amount (about 70 mg/2 g CO) of 22 : 1*n*-11 (cetoleic acid). A recent study demonstrated that cetoleic acid stimulated the conversion of ALA to EPA and DHA in human hepatocytes[31]. Therefore, it can be hypothesised that this fatty acid could have contributed to the observed elevated EPA + DHA levels in erythrocytes.

Furthermore, it is important to note that the participants of the present study can be classified as pre-obese according to the mean BMI. This is of importance as obesity is known to affect lipid metabolism[32,33]. The slightly elevated cholesterol levels in our study collective also reflect a pre-obese state. Moreover, body weight has been found to be a modulator of the response to supplementation of EPA + DHA where higher body weight is associated with a weaker response to a given amount of EPA + DHA[23]. Beyond this background, the present results are of interest as the low dose of EPA + DHA from CO significantly increased the O3I in this pre-obese study collective.

Finally, to fully elucidate the impact of CO supplementation on fatty acid metabolism and EPA + DHA status, further bioavailability studies are needed. Preferably, this should also be combined with a strictly controlled diet and PUFA intake.

Limitations

As data for this analysis were obtained from a larger interventional trial, there are also some methodical limitations. As mentioned above, when evaluating PUFA uptake, it is preferable to strictly control for dietary intake. That being said, one limitation of our study was the estimation of the EPA and DHA intake via 3-d food logs, as this data are self-reported and prone to over- or underestimation as well as different food selection of the participants. For example, if a participant eats a fish meal on 1 d during the 3-d food logs, this leads to massive fluctuations in the mean EPA + DHA intake levels of the background diet in each group. Moreover, EPA + DHA composition estimated from food logs should be evaluated cautiously as it may be incorrect due to errors in EPA + DHA compositions found in the food databases from nutrient intake calculation software[34]. This is also reflected by the variability of the estimated dietary PUFA intake levels at the beginning and at the end of the study. However, as no significant changes in dietary EPA and DHA intake from baseline until after the intervention could be detected among the study groups and, more importantly, no changes in erythrocyte PUFA levels in the EX group were seen, EPA and DHA intake

from the background diet can be excluded as a potential confounder in this study. Another limitation of this study is the lack of a placebo group due to the original study design, which encompassed four study groups and did not account for a placebo. Future studies should be conducted with a placebo group.

Conclusion

This is the first study to show that intake of 2 g CO over a period of 12 weeks significantly improves the O3I in elderly participants engaging in a moderate exercise intervention while exercise alone did not affect the O3I. These data provide the first indication that wax ester-bound n-3 PUFA from CO are well absorbed and are suited to cover the n-3 PUFA supply. Future studies should investigate the long-term bioavailability of n-3 PUFA from CO compared with TAG- or ethyl ester-bound n-3 PUFA from fish oil or phospholipid-bound n-3 PUFA from krill oil.

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References

1. Ruxton CHS, Reed SC, Simpson MJA, *et al.* (2004) The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *J Hum Nutr Diet* **17**, 449–459.
2. Calder PC & Yaqoob P (2009) Omega-3 polyunsaturated fatty acids and human health outcomes. *Biofactors* **35**, 266–272.
3. International Society for the Study of Fatty Acids and Lipids (2004) Report of the Sub-Committee on Recommendations for intake of polyunsaturated fatty acids in healthy adults. https://www.issfal.org/assets/issfal%2003%20pufaintakereccomd finalreport.pdf (accessed August 2020).
4. Micha R, Khatibzadeh S, Shi P, *et al.* (2014) Global, regional, and national consumption levels of dietary fats and oils in 1990 and 2010: a systematic analysis including 266 country-specific nutrition surveys. *BMJ* **348**, g2272.
5. Stehle P (2014) The Nutrition Report 2012 summary. *EJNFS* **4**, 14–62.
6. Lee JH, O'Keefe JH, Lavie CJ, *et al.* (2009) Omega-3 fatty acids: cardiovascular benefits, sources and sustainability. *Nat Rev Cardiol* **6**, 753–758.
7. Pedersen AM, Vang B & Olsen RL (2014) Oil from *Calanus finmarchicus* – composition and possible use: a review. *J Aquat Food Prod Technol* **23**, 633–646.
8. Calder PC (2015) Marine omega-3 fatty acids and inflammatory processes: effects, mechanisms and clinical relevance. *Biochim Biophys Acta Mol Cell Biol Lipids* **1851**, 469–484.
9. Cook CM, Larsen TS, Derrig LD, *et al.* (2016) Wax ester rich oil from the Marine Crustacean, *Calanus finmarchicus*, is a bioavailable source of EPA and DHA for human consumption. *Lipids* **51**, 1137–1144.
10. Harris WS (2008) The omega-3 index as a risk factor for coronary heart disease. *Am J Clin Nutr* **87**, 1997S-2002S.
11. Schuchardt JP & Hahn A (2013) Bioavailability of long-chain omega-3 fatty acids. *Prostaglandins Leukot Essent Fatty Acids* **89**, 1–8.
12. von Schacky C (2014) Omega-3 index and cardiovascular health. *Nutrients* **6**, 799–814.
13. von Schacky C, Kemper M, Haslbauer R, *et al.* (2014) Low omega-3 index in 106 German elite winter endurance athletes: a pilot study. *Int J Sport Nutr Exerc Metab* **24**, 559–564.
14. Wilson PB & Madrigal LA (2016) Associations between whole blood and dietary omega-3 polyunsaturated fatty acid levels in collegiate athletes. *Int J Sport Nutr Exerc Metab* **26**, 497–505.
15. Schoenfeld DA (2015) Statistical considerations for clinical trials and scientific experiments. http://hedwig.mgh.harvard.edu/sample_size/size.html (accessed May 2019).
16. Köhler A, Heinrich J & von Schacky C (2017) Bioavailability of dietary omega-3 fatty acids added to a variety of sausages in healthy individuals. *Nutrients* **9**, 629.
17. Höper AC, Salma W, Khalid AM, *et al.* (2013) Oil from the marine zooplankton *Calanus finmarchicus* improves the cardiometabolic phenotype of diet-induced obese mice. *Br J Nutr* **110**, 2186–2193.
18. Höper AC, Salma W, Sollie SJ, *et al.* (2014) Wax esters from the marine copepod *Calanus finmarchicus* reduce diet-induced obesity and obesity-related metabolic disorders in mice. *J Nutr* **144**, 164–169.
19. Eilertsen K-E, Mæhre HK, Jensen IJ, *et al.* (2012) A wax ester and astaxanthin-rich extract from the marine copepod *Calanus finmarchicus* attenuates atherogenesis in female apolipoprotein E – deficient mice. *J Nutr* **142**, 508–512.
20. Place AR (1992) Comparative aspects of lipid digestion and absorption: physiological correlates of wax ester digestion. *Am J Physiol* **263**, R464–R471.
21. Ho Ling K, Nichols PD & But PP (2009) Fish-induced keriorrhea. *Adv Food Nutr Res* **57**, 1–52.
22. Sarter B, Kelsey KS, Schwartz TA, *et al.* (2015) Blood docosahexaenoic acid and eicosapentaenoic acid in vegans: associations with age and gender and effects of an algal-derived omega-3 fatty acid supplement. *Clin Nutr* **34**, 212–218.
23. Flock MR, Skulas-Ray AC, Harris WS, *et al.* (2013) Determinants of erythrocyte omega-3 fatty acid content in response to fish oil supplementation: a dose–response randomized controlled trial. *J Am Heart Assoc* **2**, e000513.
24. Berge K, Musa-Veloso K, Harwood M, *et al.* (2014) Krill oil supplementation lowers serum triglycerides without increasing low-density lipoprotein cholesterol in adults with borderline high or high triglyceride levels. *Nutr Res* **34**, 126–133.
25. Cao J, Schwichtenberg KA, Hanson NQ, *et al.* (2006) Incorporation and clearance of omega-3 fatty acids in erythrocyte membranes and plasma phospholipids. *Clin Chem* **52**, 2265–2272.
26. Keenan AH, Pedersen TL, Fillaus K, *et al.* (2012) Basal omega-3 fatty acid status affects fatty acid and oxylipin responses to high-dose n-3-HUFA in healthy volunteers. *J Lipid Res* **53**, 1662–1669.

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27. Harris WS & von Schacky C (2008) Omega-3 fatty acids, acute coronary syndrome, and sudden death. *Curr Cardio Risk Rep* **2**, 161–166.

28. Greupner T, Koch E, Kutzner L, *et al.* (2019) Single-dose SDA-rich echium oil increases plasma EPA, DPA*n*-3, and DHA concentrations. *Nutrients* **11**, 2346.

29. Harris WS, Lemke SL, Hansen SN, *et al.* (2008) Stearidonic acid-enriched soybean oil increased the omega-3 index, an emerging cardiovascular risk marker. *Lipids* **43**, 805–811.

30. Greupner T, Kutzner L, Nolte F, *et al.* (2018) Effects of a 12-week high-α-linolenic acid intervention on EPA and DHA concentrations in red blood cells and plasma oxylipin pattern in subjects with a low EPA and DHA status. *Food Funct* **9**, 1587–1600.

31. Østbye T-KK, Berge GM, Nilsson A, *et al.* (2019) The long-chain monounsaturated cetoleic acid improves the efficiency of the *n*-3 fatty acid metabolic pathway in Atlantic salmon and human HepG2 cells. *Br J Nutr* **122**, 755–768.

32. Singla P (2010) Metabolic effects of obesity: a review. *World J Diabetes* **1**, 76–88.

33. Bays HE, Toth PP, Kris-Etherton PM, *et al.* (2013) Obesity, adiposity, and dyslipidemia: a consensus statement from the National Lipid Association. *J Clin Lipidol* **7**, 304–383.

34. Archer E, Hand GA & Blair SN (2013) Validity of U.S. Nutritional Surveillance: National Health and Nutrition Examination Survey Caloric Energy Intake Data, 1971–2010. *PLOS ONE* **8**, e76632.

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2.3 Paper III

Impact of resistance and aerobic training combined with a healthy diet or *Calanus finmarchicus* oil supplementation on sirtuins

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Article

Impact of resistance and aerobic training combined with a healthy diet or *Calanus finmarchicus* oil supplementation on sirtuins

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Abstract: Sirtuins are nicotinamide adenine dinucleotide (NAD+)-dependent deacetylases that regulate numerous pathways such as mitochondrial energy metabolism in the human body. Lower levels of those enzymes were linked to health conditions such as diabetes but also described as a result of aging. However, sirtuins are also under control of exercise and the diet. In this study we measured SIRT1, SIRT3 and SIRT5 in blood from a subpopulation of healthy elderly participants who performed a 12-week randomized, controlled trial during which they performed 2x/week aerobic and resistance training only (EX), exercise routine combined with dietary counseling in accordance with the guidelines of the German Nutrition Society (EXDC), exercise routine combined with intake of 2g/day oil from *Calanus finmarchicus* (EXCO) or received no treatment and served as control group (CON). The results show, that activity of SIRT1 and SIRT3 increased in response to the exercise intervention and that this increase was potentially enhanced by additional dietary modifications.

Keywords: aging; sirtuins; combined training; diet; exercise

1. Introduction

Physical exercise results in an increased energy demand, especially in skeletal and heart muscle. The energy demand may vary up to 10-fold in the heart [1] and up to 100-fold in skeletal muscle [2] and is mainly met by mitochondrial oxidative phosphorylation. Subsequently, the oxygen consumption varies > 4.5-fold in the heart, up to 17-fold in skeletal muscle and 25% in liver as well as kidneys [3]. Energy supply has to vary commensurate with energy demand, several mechanisms are operative to fine-tune energy metabolism. Passive regulation of the mitochondrial respiratory chain occurs via substrate (adenosine diphosphate [ADP])-saturation with enhanced physical exercise leading to increased ADP (substrate)-levels. Furthermore, active regulation of the mitochondrial ATPsynthase (complex V) has been demonstrated with mitochondrial electrochemical potential and calcium acting as regulatory elements [4]. Increasing calcium levels during exercise activate enzymes of the citric acid cycle [5–7]. Sirtuins, namely sirtuin 1 (SIRT1) and sirtuin 3 (SIRT 3), have been shown to increase during exercise which results in enhanced energy metabolism (for a recent review see: [8]). Recently, we have observed exercise-induced up-regulation of sirtuins in humans [9].

Sirtuins are a family of nicotinamide adenine dinucleotide (NAD+)-dependent deacetylases of which seven have been identified in mammals (SIRT1-SIRT7). While

SIRT1, SIRT6 and SIRT7 are primarily localized within the nucleus, SIRT3-SIRT5 are found in the mitochondria and SIRT2 in the cytosol [10]. The main function of sirtuins is the modification of proteins at their lysine residues [11]. Although the main enzymatic reaction is the NAD+-dependent deacetylation other reactions such as ADP-ribosylation are also catalyzed [12]. SIRT5 was shown to have a weaker deacetylation activity and predominantly functions as desuccinylase, demalonylase and deglutarylase [12–14].

In addition to exercise, sirtuins are also under control of the diet. The most studied dietary modification linked to an increased life span and upregulation of sirtuins is caloric restriction (CR). Particularly, SIRT1 was extensively studied and shown to respond to CR [15–17]. Moreover, other dietary compounds such as polyphenols were also demonstrated to promote SIRT1 activity [18]. Polyphenols are commonly found in fruits and vegetables but also seeds, whole-grain as well as coffee, green tea or wine [19]. The polyphenol resveratrol was reported to activate sirtuins, thus mimicking CR [20,21].

Other dietary compounds which have gained attention, are the omega-3 polyunsaturated fatty acids (n3-PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). While some studies linked them to improved mitochondrial function [22–24], another study showed decreased mitochondrial enzyme activity [25]. Regarding SIRT3, EPA is discussed to enhance SIRT3 expression and therefore positively impact mitochondrial oxidative capacity [26]. Moreover, n3-PUFAs are described to elicit anti-inflammatory effects through activation of SIRT1 pathways [27,28]. Furthermore, DHA was linked to SIRT1- dependent improvement in endothelial function [29].

One novel source of n-3 PUFAs is oil from *Calanus finmarchicus*, which contains fatty acids mainly bound as wax esters. This marine oil also contains the antioxidant astaxanthin which may have beneficial effects [30]. In a recent study, we could demonstrate, that an acute bout of exercise affected sirtuin activity in recreational runners consuming an omnivorous, lacto-ovo vegetarian or vegan diet differently [9].

Based on the existing literature and our previous findings we hypothesized, that dietary modifications may enhance exercise-induced upregulation of sirtuins. To test this hypothesis, we analyzed samples from an interventional trial, during which healthy elderly participants underwent a 12-week exercise program preceded by dietary counseling according to guidelines of the German Nutrition Society (healthy diet) or accompanied by the daily intake of 2 g of *Calanus finmarchicus* oil (CO) containing PUFAs.

2. Materials and Methods

2.1. Participants and study design

Data for this analysis were obtained from a larger single-center, randomized controlled trial in parallel group design. For the original study 134 participants between 50-70 years were recruited via advertisements in local newspapers and on public notice boards from the general population in Hannover, Germany.

Participants had to meet the following inclusion criteria: no exercise training aside from the daily activities for at least 2 years, a stable body weight (± 5 kg) for at least 6 months, ability to physically perform the exercise intervention (exercise capacity) and consumption of an omnivorous diet. Participants were excluded from the study if they met one of the following exclusion criteria: (suspected) diagnosis of cardiovascular diseases (angina pectoris, myocardial infarction, stroke, peripheral arterial occlusive disease, heart failure, cardiac arrhythmia), type 1 and 2 diabetes mellitus, renal insufficiency and liver diseases, blood coagulation disorders, chronic gastrointestinal disorders (e.g., ulcera, Crohn's disease, pancreatic insufficiency, immunological diseases (e.g. autoimmune diseases), intake of immunosuppressive drugs or laxatives, intake of supplements containing

n-3 PUFAs, alcohol, drug and/or pharmacological abuse, pregnancy or lactation, retraction of the informed consent by the subject, concurrent participation in another clinical study or participation in another study in the last 30 days. Eligibility was assessed using a screening questionnaire, while cardiovascular health was examined by resting and exercise electrocardiography, supervised by trained professionals and a physician.

Ethical approval was granted by the Ethics Commission of the Medical Chamber of Lower Saxony (Hannover, Germany). In accordance with the guidelines of the Declaration of Helsinki, written informed consent was obtained from all participants prior to their participation in the study. This study is registered in the German Clinical Trial Register (DRKS00014322).

In brief, the participants were randomly assigned by an independent researcher using stratified randomization according to the covariates (sex, BMI, age) to one of four study groups: 1) control group (CON), 2) exercise only group (EX), 3) exercise and dietary counseling group (EXDC), 4) exercise and CO supplementation group (EXCO).

Exercise training was performed for 12 weeks in fitness centers and consisted of a warm up and two passes of a strength-endurance circuit. The strength-training consisted of six machine-supported exercises that included all major muscle groups and were performed for one minute each. During the initial training session, a maximum force test with three tries respectively was performed. The best of the three tries was scored and used to set the machines to 60% of the participants maximum force for the first two weeks of training. For the subsequent six weeks, the load was increased by 10% and again by 5% for the last four weeks. The endurance exercise consisted of a four-minute bout performed on bicycle ergometers and cross-trainers at a perceived exertion that equaled a value of 15 on the Borg-Scale. During each exercise, the participants had 30 seconds of rest. Including the warm up and rest periods, the training session could be completed in approximately one hour. Compliance of the participants was assessed via a training log and a questionnaire at the end of the study.

While the EX group performed the exercise program only and was asked to maintain their habitual diet, the EXDC group received dietary counselling in accordance with the guidelines of the German Nutrition Society [31] prior to initiation of the exercise program. Participants from the EXCO were asked to maintain their habitual diet supplemented with capsules providing 2.0 g daily of oil from *Calanus finmarchicus* (Calanus AS, Tromsø, Norway). The capsules provided 109 mg EPA, 87 mg DHA, and 3.6 mg Astaxanthin (a detailed overview of the lipid profile was already reported elsewhere [32]).

Compliance of participants and adherence to the instructions was monitored via fortnightly phone calls. Additionally, participants from the EXCO group were instructed to return leftover capsules. Participants had to consume at least 90% of the capsules to be considered as compliant. In addition, compliance could be additionally verified by an increased omega-3-index detected in the EXCO group [32].

2.2 Dietary intake

The dietary behavior was monitored via 3-day dietary records at the beginning, after six weeks, and at the end of the study after 12 weeks. Nutritionists checked the records for completeness, readability, and plausibility. The software PRODI6.4® (Nutri-Science GmbH, Freiburg, Germany) was used to estimate the energy and nutrient intake. Food groups were analyzed with a food frequency questionnaire (FFQ) from the German Health Examination Survey for Adults (Studie zur Gesundheit Erwachsener in Deutschland, DEGS) of the Robert Koch Institute.

2.3 Sample collection and preparation

Venous blood samples were drawn from the participants after an overnight fast (≥ 10 h). For sirtuin analysis, 2 ml blood were collected using EDTA tubes (Sarstedt AG & Co.

KG, Nümbrecht, Germany). For RNA isolation 500 µl of EDTA-blood was transferred into RNAprotect Animal Blood Tubes (Qiagen, Hilden, Germany). The remaining blood was centrifuged at 3,300x g for 3 minutes and plasma used for analysis of sirtuins.

2.4 Sirtuin activity assay

Deacetylase activity of SIRT1 and SIRT3 as well as desuccinylase activity of SIRT5 were assayed with SIRT1, SIRT3 and SIRT5 fluorometric drug discovery assay kits (Enzo Life Science, Lörrach, Germany). The enzyme capacity was measured under substrate saturation which was achieved by addition of a surplus of NAD+ to the assays. Further, according to the manufacturers protocol, plasma samples were diluted 1:5 with HEPES buffer (110 mM NaCl, 2.6 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM MgSO4x7H2O, 1.0 mM CaCl2, 25 mM HEPES) and subsequently sonicated for 10 seconds at 20 kHz with an amplitude of 75% to break cell membranes. Total protein concentration of the analyzed samples was measured with the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, USA), for normalization of the detected SIRT-activity signals.

2.5. Data analysis and statistical methods

Sample size (n=7 per group) was calculated based on our previous study [9] assuming a two-sided level of significance of 5% and a power of 80%. Samples were chosen randomly from the participants in the respective subgroups.

Baseline data and data of dietary intake are presented as mean ± standard deviation (SD). To account for the variability of enzyme activity, sirtuin activity is presented as median (+max/-min). Due to the small sample size per group, we performed non-parametric analysis only. Within group differences were analyzed with the Wilcoxon test, differences between sexes using the Mann-Whitney-U test and group comparisons using the Kruskal-Wallis test. If significant group differences were detected, a post hoc analysis with Bonferroni correction was performed. Absolute differences (Δ) were calculated as t_{12-t0}. To determine correlations between changes in dietary intake and sirtuin activity Spearman correlations were performed. P-values of <0.05 were considered as significant. All statistical analyses were carried out using SPSS software (version 23.0; SPSS Inc., Chicago, IL, USA). Graphs were created with Prism 9 (GraphPad Software, La Jolla, CA).

3. Results

3.1 Baseline

Baseline characteristics of the probands from the respective subgroups analyzed are shown in Table 1. No statistically significant differences regarding distribution of gender, age, height, weight or BMI were found between the study groups. However, the EXDC group showed a higher BMI than the other study groups.

Table 1. Baseline characteristics of all participants.

	CON (n=9)	EX (n=14)	EXDC (n=8)	EXCO (n=9)	*p*
Sex [f/m]	7/2	11/3	5/3	7/2	0.836
Age [years]	60.6 ± 5.08	60.1 ± 6.27	58.9 ± 5.18	60.0 ± 3.42	0.825
Height [cm]	166 ± 5.63	168 ± 6.66	170 ± 8.32	174 ± 5.88	0.112
Body weight [kg]	75.1 ± 13.6	78.4 ± 19.0	86.9 ± 20.0	82.3 ± 16.8	0.503
BMI [kg/m²]	27.2 ± 4.07	27.7 ± 6.00	30.0 ± 5.73	27.4 ± 5.97	0.701

Data are shown as mean ± SD. Gender distribution between groups was analyzed using Chi-square-test. Differences among groups were assessed with Kruskal-Wallis-Test. f=females, m=male, BMI=Body-Mass-Index.

3.2 Dietary intake

Regarding the changes in dietary intakes estimated from 3-day dietary food logs, no significant differences neither within nor between the four study groups were detected (Table 2). However, a trend to a decreased energy intake was observed in the three intervention groups performing exercise. The highest decreased of approximately 16% was observed in the EXDC group (1,955 ± 527 to 1,634 ± 528 kcal, p=0.039). Further within group comparisons showed a significant increase in α-Tocopherol in CON (6.61 ± 2.03 /day to 8.85 ± 2.42 mg/day, p=0.004) as well as a decrease in fiber (24.05 ± 8.18 to 19.32 ± 7.37 g/day, p=0.030) and vitamin C (165.2 ± 102.2 to 106.0 ± 38.0 mg/day, p=0.035) in the EX group.

Table 2. Dietary intake from 3-day food logs.

	t	CON	EX	EXDC	EXCO	*p*
Energy intake [kcal]	0	1792 ± 322	2028 ± 483	1955 ± 527	1917 ± 541	0.501
	12	1906 ± 293	1814 ± 378	1634 ± 528*	1859 ± 417	0.548
	Δ	114 ± 435	-214 ± 436	-322 ± 422	-58.7 ± 674	0.215
Protein [%E]	0	17.7 ± 2.40	15.9 ± 4.08	16.6 ± 3.99	16.4 ± 3.73	0.629
	12	17.6 ± 1.59	16.6 ± 3.60	18.2 ± 3.69	16.3 ± 2.67	0.387
	Δ	-0.11 ± 2.42	0.66 ± 4.31	1.66 ± 4.14	-0.11 ± 4.95	0.722
Fat [%E]	0	36.2 ± 6.19	36.7 ± 9.44	40.8 ± 7.33	38.8 ± 6.70	0.340
	12	40.1 ± 7.12	37.9 ± 7.40	38.97 ± 9.47	36.0 ± 7.72	0.722
	Δ	3.89 ± 8.16	1.18 ± 8.31	-1.86 ± 7.10	-2.85 ± 7.49	0.283
CHO [%E]	0	41.3 ± 6.06	42.18 ± 11.23	37.3 ± 4.97	39.7 ± 6.91	0.125
	12	37.3 ± 7.20	39.7 ± 5.01	36.90 ± 6.79	41.1 ± 7.14	0.357
	Δ	-4.02 ± 6.91	-2.52 ± 8.79	-0.36 ± 7.32	1.33 ± 4.14	0.191
Fiber [g/d]	0	19.25 ± 7.75	24.1 ± 8.18	20.1 ± 9.26	22.5 ± 7.11	0.384
	12	19.4 ± 11.0	19.3 ± 7.37*	21.6 ± 5.31	25.9 ± 12.9	0.227
	Δ	0.11 ± 6.83	-4.73 ± 7.14	1.49 ± 8.84	3.37 ± 10.1	0.083
EPA [g/d]	0	0.24 ± 0.65	0.11 ± 0.17	0.03 ± 0.02	0.28 ± 0.64	0.841
	12	0.04 ± 0.03	0.16 ± 0.30	0.09 ± 0.07	0.07 ± 0.07	0.767
	Δ	-0.20 ± 0.64	0.05 ± 0.36	0.06 ± 0.08	-0.21 ± 0.66	0.241
DHA [g/d]	0	0.25 ± 0.41	0.21 ± 0.21	0.18 ± 0.11	0.28 ± 0.31	0.829
	12	0.12 ± 0.06	0.28 ± 0.46	0.21 ± 0.12	0.16 ± 0.17	0.538
	Δ	-0.13 ± 0.38	0.07 ± 0.51	0.03 ± 0.23	-0.12 ± 0.31	0.903
Retinol equivalent [μg/d]	0	1.252 ± 846	1.478 ± 731	1.486 ± 736	1.846 ± 2.217	0.627
	12	1.315 ± 861	1.299 ± 815	1.617 ± 1.064	1.466 ± 566	0.672
	Δ	62.7 ± 968	-179.2 ± 1.059	132.0 ± 1.495	-379.80± 2.227	0.892
Vitamin C [mg/d]	0	93.0 ± 59.5	165.2 ± 102.2	115.8 ± 59.	109.3 ± 43.7	0.138
	12	129.1 ± 115.4	106.0 ± 38.0*	152.6 ± 65.8	117.0 ± 76.6	0.277
	Δ	36.0 ± 80.83	-59.2 ± 108	36.9 ± 75.1	7.75 ± 61.4	0.058
α-Tocopherol [mg/d]	0	6.61 ± 2.03	10.2 ± 3.78	10.6 ± 7.32	10.9 ± 4.85	0.105
	12	8.85 ± 2.42**	9.85 ± 3.72	35.6 ± 74.1	11.0 ± 4.96	0.899
	Δ	2.24 ± 1.85	-0.37 ± 5.56	24.9 ± 76.2	0.09 ± 6.31	0.618

Data are shown as mean ± SD. Differences among groups were assessed with Kruskal-Wallis-Test, within groups detected with Wilcoxon test. Asterisks indicate statistical differences (* $p < 0.05$; ** $p < 0.01$; # $p < 0.001$).

Regarding the intake of food groups, a significant increase in fruit (1.49 ± 1.21 to 2.52 ± 1.26 portion/day, p=0.016) and an increased intake of vegetables (0.87 ± 0.28 to 1.44 ± 0.82 portion/day) were detected in EXDC which did not reach statistical significance (p=0.070). In the CON group, a significant decrease of vegetable intake (1.80 ± 3.15 to 0.72 ±0.58 portion/day, p=0.031) and cereal products (3.82 ± 2.41 to 2.44 ± 0.80 portion/day, p=0.039) occurred. Comparison of absolute intakes showed that intake of vegetables differed between

the four study groups (p=0.018), EXDC showed a significantly higher intake in daily vegetable intake than CON (0.57 ± 0.66 vs. -1.08 ± 2.67, p=0.019).

Table 3: Dietary intake from FFQs.

	t	**CON**	**EX**	**EXDC**	**EXCO**	*p*
Fruit intake	0	1.35 ± 1.10	1.69 ± 1.16	1.49 ± 1.21	1.82 ± 1.71	0.783
[portion/	12	1.35 ± 0.74	1.89 ± 2.28	2.52 ± 1.26*	1.38 ± 0.77	0.151
day]	Δ	0.00 ± 1.22	0.23 ± 1.33	1.03 ± 1.08	-0.44 ± 1.49	0.076
Vegetable in-	0	1.80 ± 3.15	1.17 ± 0.82	0.87 ± 0.28	0.89 ± 0.78	0.719
take [portion/	12	0.72 ±0.58*	1.02 ± 0.71	1.44 ± 0.82	1.01 ± 0.75	0.284
day]	Δ	-1.08 ± 2.67 ✝	-0.14 ± 0.52	0.57 ± 0.66 ✝	0.12 ± 0.96	**0.018**
Cereal intake	0	3.82 ± 2.41	3.01 ± 2.48	3.80 ± 2.88	3.37 ± 1.71	0.581
[portion/	12	2.44 ±0.80*	2.57 ± 1.35	2.89 ± 1.58	2.73 ± 1.44	0.983
day]	Δ	-1.38 ± 1.83	-0.45 ± 1.20	-0.91 ± 3.14	-0.64 ± 1.77	0.643
Green & black	0	0.32 ± 0.52	1.49 ± 2.20	0.47 ± 0.83	2.19 ± 3.07	0.582
tea [cups/	12	1.06 ± 1.54	0.90 ± 1.69	2.06 ± 2.98	0.57 ± 1.01	0.932
day]	Δ	0.74 ± 1.26	-0.59 ± 2.47	1.59 ± 3.40	-1.62 ± 3.27	0.232
Coffee	0	4.65 ± 7.94	2.57 ± 4.61	3.69 ± 2.09	2.84 ± 2.06	0.152
[cups/day]	12	1.43 ± 1.27	2.48 ± 1.74	2.81 ± 3.27	2.45 ± 1.31	0.490
	Δ	-3.22 ± 8.29	-0.10 ± 4.50	-0.88 ± 4.16	-0.39 ± 2.36	0.561
Wine & spar-	0	0.26 ± 0.23	0.39 ± 0.61	0.17 ± 0.20	0.45 ± 0.41	0.406
kling wine	12	0.22 ± 0.15	0.72 ± 1.07	0.23 ± 0.35	0.23 ± 0.16	0.192
[glases/ day]	Δ	-0.04 ± 0.22	0.33 ± 0.71	0.06 ± 0.42	-0.22 ± 0.35	0.097

Data are shown as mean ± SD. Differences among groups were assessed with Kruskal-Wallis-Test, within groups detected with Wilcoxon test. Asterisks indicate statistical differences (* p < 0.05; ** p < 0.01; # p < 0.001).

3.3 Sirtuin Activity

Sirtuin activities were analyzed in vitro (under substrate saturation) before and after the 12-week intervention. At baseline, no gender differences and no differences between groups for activities of SIRT1 and SIRT5 were found. For SIRT3 a significant difference among the groups could be detected (p=0.035). However, after Bonferroni correction for multiple comparisons, the differences were no longer significant (CON vs. EX, p=0.101; CON vs. EXDC, p=0.144; CON vs. EXCO, p=0.059).

Within group comparisons of sirtuin activities before and after the intervention showed, that activities of particularly SIRT1 and SIRT3 increased significantly within the three intervention groups performing exercise, while no significant changes occurred in the CON group. For SIRT1 activity increased in EX from 0.00087 U/μg (+0.00142/-0.00019) to 0.00099 U/μg (+0.00199/-0.00024) (p=0.002), in EXDC from 0.00086 U/μg (+0.001/-0.00064) to 0.00109 U/μg (+0.00152/-0.00083) (p=0.008) and in EXCO from 0.00096 (+0.00113/-0.00035) to 0.00113 (+0.00192/-0.00075) (p=0.012) (Figure 1 A). In a similar manner, SIRT3 increased in EX from 0.00244 U/μg (+0.00296/-0.00125) to 0.00309 U/μg (+0.00613/-0.00154) (p<0.001), in EXDC from 0.00236 U/μg (+0.00301/-0.00159) to 0.00323 U/μg (+0.00653/-0.00224) (p=0.008) and in EXCO 0.00252 U/μg (+0.00309/-0.00184) to

0.00402 U/µg (+0.00556/-0.00293) (p=0.004, Figure 1 B). SIRT5 only increased significantly in EX from 0.00024 U/µg (+0.00051/-0.00016) to 0.00028 U/µg (+0.00063/-0.00018) (p=0.001) and in EXCO from 0.00032 U/µg (+0.00056/-0.00012) to 0.00032 U/µg (+0.00061/-0.00016) (p=0.012, Figure 1 C).
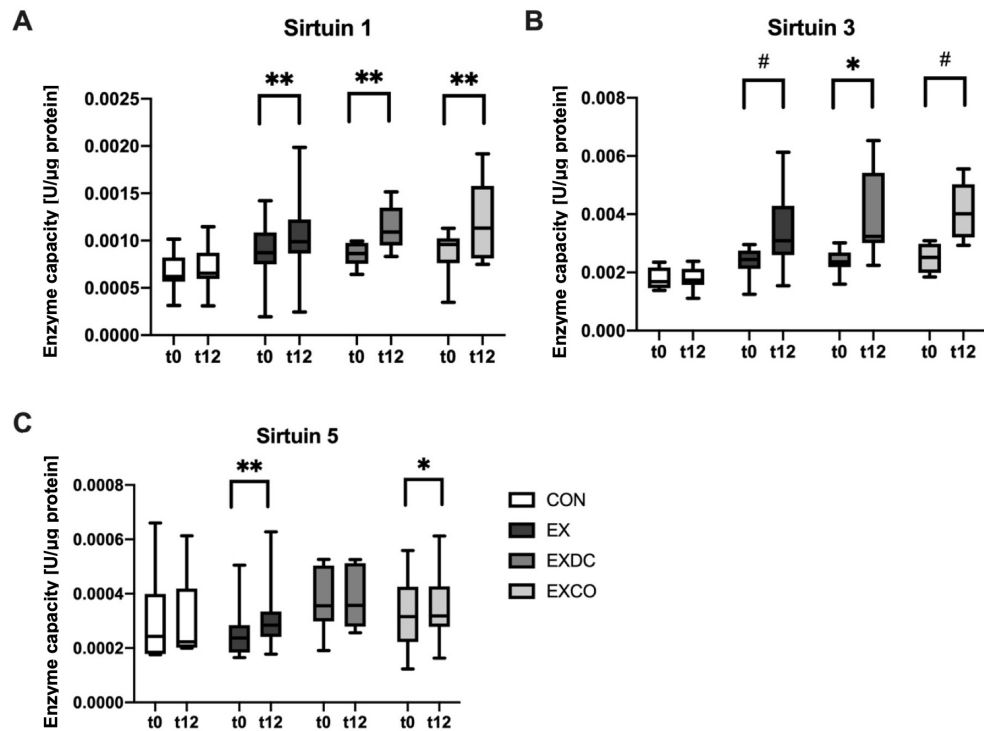

Figure 1: Absolute enzyme capacities (under substrate saturation) of SIRT1, SIRT3 and SIRT5 before (t0) and after (t12) the intervention, data shown as median ± quartiles and extremes. Asterisks: statistically different between groups as judged by Wilcoxon-test (* $p < 0.05$; ** $p < 0.01$; # $p < 0.001$).

Comparison of absolute differences in sirtuin capacity revealed significant differences among the study groups for all three sirtuins analyzed (Figure 2). As shown in Figure 2 A, SIRT1 activity increased by 0.00015 U/µg (+0.00056/- -0.00016) in EX, 0.00025 U/µg (+0.00052/-0.00006) in EXDC and 0.00040 U/µg (+0.00088/- -0.00012) in EXCO. Of those, EXCO and EXDC differed significantly from CON (CON vs. EXCO, p=0.003; CON vs. EXDC, p=0.010). Regarding the activity of SIRT3, EX showed an increase by 0.00080 U/µg (+0.00318/-0.00005), EXDC by 0.00095 U/µg (+0.00388/-0.00055) and EXCO by 0.00160 U/µg (+0.00285/-0.00070) (Figure 2 B). All increases were significantly different from CON (CON vs. EX, p=0.007; CON vs EXDC, p<0.001, CON vs. EXCO, p=0.004). Activity of SIRT5 was increased in all study groups (CON: 0.000003 U/µg (+0.00005/-0.00005), EX: 0.00005 U/µg (+0.0001/- -0.00001), EXDC: -0.000004 U/µg (+0.00007/- -0.00006), EXCO 0.00003 U/µg (+0.0001/- -0.000002) (Figure 2C). EX differed significantly from CON (p=0.032).
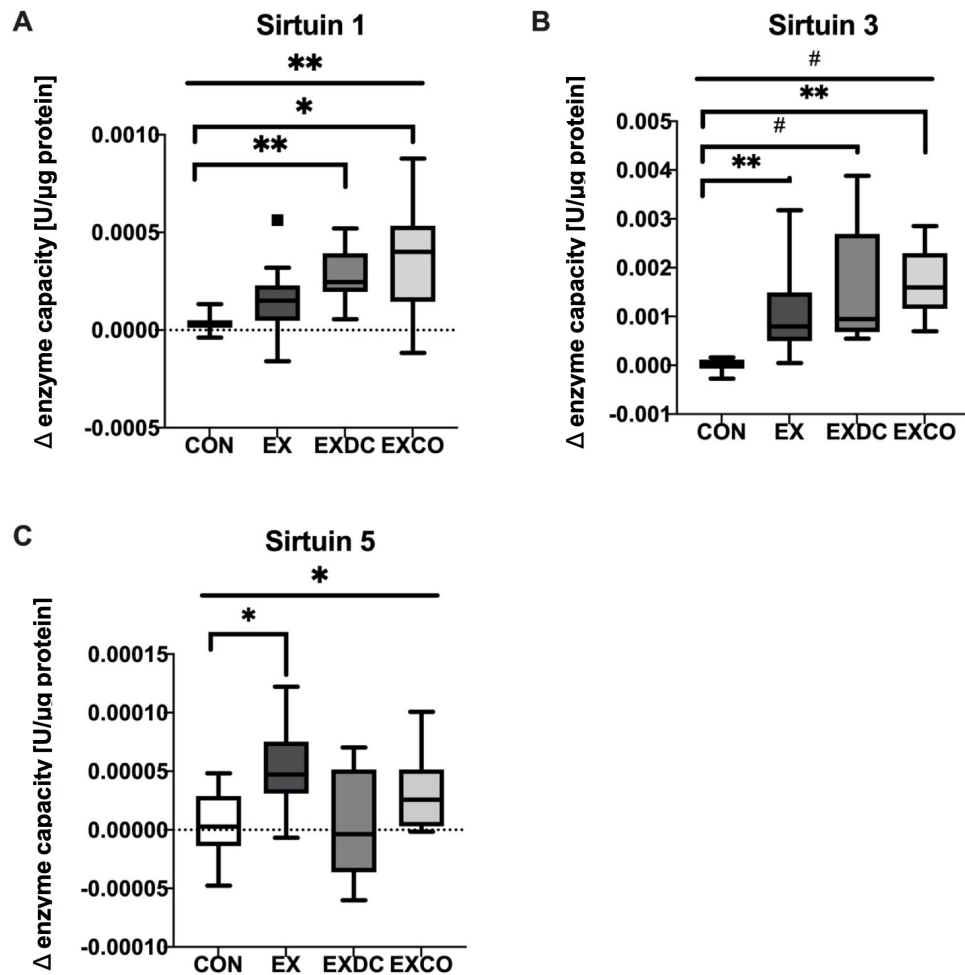
Figure 2: Absolute differences of enzyme capacity (under substrate saturation) of SIRT1, SIRT3 and SIRT5, shown as median ± quartiles and extrema. Asterisks: statistically different between groups judged by Kruskal- Wallis-test. (* $p < 0.05$; ** $p < 0.01$; # $p < 0.001$).

3.4 Correlation analysis

To account for potential effects of baseline differences in the capacity of SIRT1 and particularly SIRT3, correlation analyses of baseline values with absolute differences were performed for each group. No significant correlations were detected in the exercising study groups.

Although we could not detect any significant differences in dietary intake apart from increased vegetable intake in the EXDC group, we wanted to examine the potential impact of different dietary components on sirtuin activity. Therefore, we first examined correlations between sirtuin capacity and caloric intake as well as food groups containing polyphenols or flavonoids (fruits, vegetables, cereals, green and black tea, coffee, wine). This analysis did not reveal any significant correlations. As we could already observe a significant impact of intake of antioxidative substances on short-term exercise induced sirtuin capacity in recreational runners with different diets [9], we also examined the intake of

such substances in this analysis. However, sirtuin capacity seemed to be unaffected by dietary intake of tocopherol, vitamin C or vitamin A (expressed as retinol equivalent). Additionally, intake of EPA and DHA did not show any correlations. Lastly, no correlations between body weight, BMI or waist-to-hip ratio were observed. An overview of all correlated markers is shown in table 4.

Table 4: Overview of all baseline parameters correlated with baseline sirtuin activities.

Sirtuin	Correlation with	p-value
SIRT1, SIRT3, SIRT 5	Calories	n.s. for all sirtuins
	Fruits	n.s. for all sirtuins
	Vegetables	n.s. for all sirtuins
	Cereals	n.s. for all sirtuins
	Green & black tea	n.s. for all sirtuins
	Coffee	n.s. for all sirtuins
	Wine & sparkling wine	n.s. for all sirtuins
	Tocopherol	n.s. for all sirtuins
	Retinol equivalents	n.s. for all sirtuins
	Vitamin C	n.s. for all sirtuins
	EPA	n.s. for all sirtuins
	DHA	n.s. for all sirtuins
	Weight	n.s. for all sirtuins
	BMI	n.s. for all sirtuins

Correlations were performed using Spearman correlations.

4. Discussion

a) Exercise & Sirtuins:

To the best of our knowledge, this is the first investigation on the impact of chronic exercise versus chronic exercise combined with dietary modifications (healthy diet vs. CO supplementation), on sirtuins in human peripheral blood. Sirtuins were already reported to be impacted by chronic exercise, as well as dietary modifications such as caloric restriction or dietary components such as polyphenols [21]. With regards to exercise, SIRT1 and SIRT3 are particularly involved. SIRT1 was already shown to modulate the cellular stress response and energy metabolism via the activation of peroxisome proliferator-activated receptor gamma coactivator-1α (PGC1α) and forkhead box O (FOXO) transcription factors [33]. Upregulation will not only result in increased mitochondrial biogenesis and oxidative capacity but also affect lipid and glucose metabolism [34]. In any case, SIRT1 seems to be affected by acute [35,36] and chronic exercise [37,38]. On the contrary SIRT3 seems to be affected particularly by chronic exercise [39]. SIRT3 is involved in the regulation of mitochondrial activity through activation of complexes 1-5 of the respiratory chain [40] and protection against reactive oxygen species (ROS) which are commonly produced during exercise [41].

We hypothesized that chronic exercise over 12-weeks combined with dietary modifications (healthy diet vs. CO intake) may differentially impact sirtuin activity compared to exercise only. A previous study from our group found differences in short-term exercise-induced sirtuin activity in recreational athletes with different diets (omnivorous, lacto-ovo-vegetarian, vegan) [9]. In this study, sirtuin activity was also measured in peripheral blood, which gave first evidence that measurement of blood sirtuin levels may reflect sirtuin tissue levels.

Results from the present study suggest, that participation in a 12-week exercise program consisting of a combined resistance and aerobic training upregulates the activity of SIRT1 and SIRT3 in healthy elderly, overweight participants as an adaptation to meet increased energy demand which is regulated by these sirtuins. This is also supported by results from Villanova et al., who reported that sirtuin deacetylase activity is higher in athletic individuals when compared to non-trained individuals [42]. Generally, our results are in line with animal and human studies, that showed a positive impact on sirtuins by acute and chronic exercise ([8]) [43,44]. In that context, the most studied sirtuins are SIRT1 and SIRT3 which regulate many metabolic processes that are upregulated during acute exercise, namely energy metabolism, but also mediate systemic long-term adaptations to exercise [45,46]. SIRT3 and SIRT1 are involved in the regulation of fatty acid oxidation and mitochondrial biogenesis, while SIRT1 is also involved in the regulation of glucose homeostasis [47,48]. Up-regulation of SIRT5 by exercise was less pronounced and inconsistent. SIRT5 is known to regulate the citric acid cycle, fatty acid oxidation and the mitochondrial respiratory chain [49].

In general, we cannot be sure that sirtuins in plasma reflect sirtuin function in tissues. Animal studies investigating the effect of exercise on sirtuins commonly analyzed them in tissues such as the brain, skeletal muscles, heart, liver or adipose tissue [50–52]. When measured in human skeletal muscle, 2 weeks of high intensity training (HIIT) increased activity of SIRT1 while protein content stayed the same [38]. This is in line with findings from our study, which also showed an increased activity of SIRT1. Yet, we could not measure sirtuins at protein level as there was a sustained shortage of antibodies. Protein expression levels of SIRT3 in the vastus lateralis of young and older subjects that were classified as sedentary (defined as <30 min exercise a day, not more than twice per week) or trained (defined as 1h running or cycling, 6x/week for the last 4 years) showed that the sedentary subjects had lower levels than the trained subjects [53].

When we compare basal enzyme capacities of SRT1, SIRT3 and SIRT5 in the present study with our previous study [25–27][9], enzyme capacities were higher in the younger

participants (18-35 years) than in the older participants (50-70 years) in the present study. This is in line with findings of Villanova et al. [42] and Lalia et al. [23].

The underlying mechanisms for the exercise-induced increase of the enzyme activity are not fully elucidated, but may be mediated by exercise-induced changes of the NAD+/NADH- or AMP/ATP-ratio [54,55]. This explanation is supported by results from Lamb et al. 2020, who reported that 10 weeks of resistance training (2x/week) resulted in increased muscle NAD+-levels and higher global sirtuin activity in middle aged, untrained participants [56]. Although those results cannot be compared directly to our results due to the different methodologies in performing exercise and measuring sirtuin activities, the results are coherent. Furthermore, posttranslational modifications of sirtuins may play a role.

b) Diet & Sirtuins:

In the present study, diet had an additional impact on sirtuin up-regulation during exercise. The activities of SIRT1 and SIRT3 increased to a higher extent in EXDC and EXCO when compared to EX only.

Sirtuins are known to be regulated by polyphenols, which are abundant in vegetables, fruits, whole-grains, coffee, green tea and wine [19]. Indeed, there was a significant increase in fruit and vegetable intake in the EXDC group after dietary counseling. However, in case of SIRT1 the stability and metabolization of polyphenols are important [57] which has not been examined in our study. Therefore, the efficacy of increased vegetable and fruit intake is difficult to assess without specification of actual intake of polyphenolic compounds. In addition to an increased vegetable and fruit intake, the EXDC group also showed the highest reduction in caloric intake, which is another potential regulator of sirtuins.

In the EXCO group, the intake of PUFAs may be responsible for the increased response of sirtuin activities to exercise. Interestingly, supplementation of n-3 PUFAs has been reported to positively impact muscle strength and function in older adults [58,59]. 16-weeks of 3.6 g/day n-3 PUFA intake (2.7 g EPA+1.2 g DHA), increased myofibrillar and mitochondrial protein synthesis measured after a single bout of resistance exercise in older adults [23], which supports the idea that exercise combined with n-3 PUFA supplementation may also enhance long-term adaptations mediated by sirtuins. Generally, n-3 PUFAs are discussed to support muscle anabolism [60]. Yet, it has to be acknowledged that CO used in the present study provided only approximately 200 mg of EPA+DHA, which is far below the doses which reportedly have beneficial effects. It is already known, that n-3 PUFAs can modulate metabolic pathways via activation of transcription factors, namely the peroxisome proliferator-activated receptors (PPARs) [61]. This process may be mediated by sirtuins. Indeed the n-3 PUFAs EPA and DHA were linked to the activation of SIRT1 and SIRT3 dependent-pathways [26–29]. Another fatty acid, which was reported to impact SIRT1-associated pathways is the monounsaturated omega-9 fatty acid oleic acid [62], which is also present in CO (in this study 36 mg oleic acid). Lastly, CO contains the antioxidant astaxanthin. There is evidence from animal studies indicating that astaxanthin increases the expression of SIRT1 [63] and the mitochondrial sirtuins [64]. Both, the antioxidative properties and the potential impact of n-3 PUFAs on sirtuins may be responsible for the beneficial effects of CO on obesity-related disorders like insulin-resistance and atherosclerosis as previously reported from animal studies [65,66].

As increased body weight and metabolic disorders such as type 2 diabetes were already shown to correlate with sirtuin blood levels [67,68], we also investigated whether there were any links between body weight, BMI and metabolic markers using correlation analysis. As the study collective was very homogenous and metabolically healthy it seems not surprising, that no significant correlations were detected. To potentially establish the measurement of blood sirtuins as a marker for health, it would be of interest to perform analyses in different age groups and in patients with and without metabolic disorders.

Although we are aware, that blood sirtuins not directly reflect tissue levels, establishment of reference ranges in different study populations would be helpful to assess blood sirtuins as biomarkers of health status.

Overall, this study has also clear limitations. First of all, the evaluation of the impact of dietary modifications on sirtuin activity would have been best under strictly controlled dietary conditions. Although the 3-day dietary food logs and FFQs are commonly used instruments in nutritional assessments, they are subjective and self-reported. Future studies investigating the effect on dietary intakes should therefore use a better controlled setting and either provide standardized meals or a meal plan. Second, the measurement of blood sirtuins without measurement of tissue levels may also be considered as a limitation. Additional confirmation of sirtuin levels in muscle tissue would have been beneficial, but performing muscle biopsies was not possible in this study. Furthermore, sirtuins could not be analyzed at protein level, which would be interesting when considering post-translational modifications.

5. Conclusions

In summary, our study showed that 12 weeks of twice-weekly combined resistance and aerobic training up-regulates the capacity of SIRT1 and SIRT3 in untrained, elderly subjects, while the capacity of SIRT5 was less affected. Furthermore, we showed that the capacities of SIRT1, SIRT3 andSIRT5 decreased with age. A healthy diet or supplementation with CO further enhanced the effect of exercise-induced sirtuin activation. Further experimentation is needed to elucidate the precise mechanism of sirtuin activation and its effect on mitochondrial function.

References

1. Matthews, P.M.; Williams, S.R.; Seymour, A.-M.; Schwartz, A.; Dube, G.; Gadian, D.G.; Radda, G.K. A 31P-NMR Study of Some Metabolic and Functional Effects of the Inotropic Agents Epinephrine and Ouabain, and the Ionophore R02-2985 (X537A) in the Isolated, Perfused Rat Heart. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **1982**, *720*, 163–171, doi:10.1016/0167-4889(82)90008-8.

2. Wilson, D.F. Oxidative Phosphorylation: Unique Regulatory Mechanism and Role in Metabolic Homeostasis. *Journal of Applied Physiology* **2017**, *122*, 611–619, doi:10.1152/japplphysiol.00715.2016.

3. Boyman, L.; Karbowski, M.; Lederer, W.J. Regulation of Mitochondrial ATP Production: Ca2+ Signaling and Quality Control. *Trends in Molecular Medicine* **2020**, *26*, 21–39, doi:10.1016/j.molmed.2019.10.007.

4. Das, A.M.; Harris, D.A. Regulation of the Mitochondrial ATP Synthase in Intact Rat Cardiomyocytes. *Biochemical Journal* **1990**, *266*, 355–361, doi:10.1042/bj2660355.

5. McCormack, J.G.; Denton, R.M. The Role of Intramitochondrial Ca2+ in the Regulation of Oxidative Phosphorylation in Mammalian Tissues. *Biochemical Society Transactions* **1993**, *21*, 793–799, doi:10.1042/bst0210793.

6. McCormack, J.G. Characterization of the Effects of Ca2+ on the Intramitochondrial Ca2+-Sensitive Enzymes from Rat Liver and within Intact Rat Liver Mitochondria. *Biochemical Journal* **1985**, *231*, 581–595, doi:10.1042/bj2310581.

7. Bertero, E.; Maack, C. Calcium Signaling and Reactive Oxygen Species in Mitochondria. *Circ Res* **2018**, *122*, 1460–1478, doi:10.1161/CIRCRESAHA.118.310082.

8. *Sirtuin Biology in Medicine: Targeting New Avenues of Care in Development, Aging, and Disease*; Maiese, K., Ed.; 1st ed.; Elsevier: Waltham, 2021; ISBN 978-0-12-814118-2.

9. Potthast, A.B.; Nebl, J.; Wasserfurth, P.; Haufe, S.; Eigendorf, J.; Hahn, A.; Das, A. Impact of Nutrition on Short-Term Exercise-Induced Sirtuin Regulation: Vegans Differ from Omnivores and Lacto-Ovo Vegetarians. *Nutrients* **2020**, *12*, 1004, doi:10.3390/nu12041004.

10. Buler, M.; Andersson, U.; Hakkola, J. Who Watches the Watchmen? Regulation of the Expression and Activity of Sirtuins. *FASEB j.* **2016**, *30*, 3942–3960, doi:10.1096/fj.201600410RR.

11. Carrico, C.; Meyer, J.G.; He, W.; Gibson, B.W.; Verdin, E. The Mitochondrial Acylome Emerges: Proteomics, Regulation by Sirtuins, and Metabolic and Disease Implications. *Cell Metabolism* **2018**, *27*, 497–512, doi:10.1016/j.cmet.2018.01.016.

12. Kupis, W.; Pałyga, J.; Tomal, E.; Niewiadomska, E. The Role of Sirtuins in Cellular Homeostasis. *J Physiol Biochem* **2016**, *72*, 371–380, doi:10.1007/s13105-016-0492-6.

13. Tan, M.; Peng, C.; Anderson, K.A.; Chhoy, P.; Xie, Z.; Dai, L.; Park, J.; Chen, Y.; Huang, H.; Zhang, Y.; et al. Lysine Glutarylation Is a Protein Posttranslational Modification Regulated by SIRT5. *Cell Metab.* **2014**, *19*, 605–617, doi:10.1016/j.cmet.2014.03.014.

14. Du, J.; Zhou, Y.; Su, X.; Yu, J.J.; Khan, S.; Jiang, H.; Kim, J.; Woo, J.; Kim, J.H.; Choi, B.H.; et al. Sirt5 Is a NAD-Dependent Protein Lysine Demalonylase and Desuccinylase. *Science* **2011**, *334*, 806–809, doi:10.1126/science.1207861.

15. Cohen, H.Y.; Miller, C.; Bitterman, K.J.; Wall, N.R.; Hekking, B.; Kessler, B.; Howitz, K.T.; Gorospe, M.; de Cabo, R.; Sinclair, D.A. Calorie Restriction Promotes Mammalian Cell Survival by Inducing the SIRT1 Deacetylase. *Science* **2004**, *305*, 390–392, doi:10.1126/science.1099196.

16. Boily, G.; Seifert, E.L.; Bevilacqua, L.; He, X.H.; Sabourin, G.; Estey, C.; Moffat, C.; Crawford, S.; Saliba, S.; Jardine, K.; et al. Sirt1 Regulates Energy Metabolism and Response to Caloric Restriction in Mice. *PLoS ONE* **2008**, *3*, e1759, doi:10.1371/journal.pone.0001759.

17. Bordone, L.; Guarente, L. Calorie Restriction, SIRT1 and Metabolism: Understanding Longevity. *Nat Rev Mol Cell Biol* **2005**, *6*, 298–305, doi:10.1038/nrm1616.

18. Ajami, M.; Pazoki-Toroudi, H.; Amani, H.; Nabavi, S.F.; Braidy, N.; Vacca, R.A.; Atanasov, A.G.; Mocan, A.; Nabavi, S.M.

Therapeutic Role of Sirtuins in Neurodegenerative Disease and Their Modulation by Polyphenols. *Neuroscience & Biobehavioral Reviews* **2017**, *73*, 39–47, doi:10.1016/j.neubiorev.2016.11.022.

19. Pérez-Jiménez, J.; Neveu, V.; Vos, F.; Scalbert, A. Identification of the 100 Richest Dietary Sources of Polyphenols: An Application of the Phenol-Explorer Database. *Eur J Clin Nutr* **2010**, *64*, S112–S120, doi:10.1038/ejcn.2010.221.

20. Gertz, M.; Nguyen, G.T.T.; Fischer, F.; Suenkel, B.; Schlicker, C.; Fränzel, B.; Tomaschewski, J.; Aladini, F.; Becker, C.; Wolters, D.; et al. A Molecular Mechanism for Direct Sirtuin Activation by Resveratrol. *PLOS ONE* **2012**, *7*, e49761, doi:10.1371/journal.pone.0049761.

21. Villalba, J.M.; Alcaín, F.J. Sirtuin Activators and Inhibitors. *BioFactors* **2012**, *38*, 349–359, doi:10.1002/biof.1032.

22. Stanley, W.C.; Khairallah, R.J.; Dabkowski, E.R. Update on Lipids and Mitochondrial Function: Impact of Dietary n-3 Polyunsaturated Fatty Acids. *Current Opinion in Clinical Nutrition and Metabolic Care* **2012**, *15*, 122–126, doi:10.1097/MCO.0b013e32834fdaf7.

23. Lalia, A.Z.; Dasari, S.; Robinson, M.M.; Abid, H.; Morse, D.M.; Klaus, K.A.; Lanza, I.R. Influence of Omega-3 Fatty Acids on Skeletal Muscle Protein Metabolism and Mitochondrial Bioenergetics in Older Adults. *Aging* **2017**, *9*, 1096–1129, doi:10.18632/aging.101210.

24. Lepretti, M.; Martucciello, S.; Burgos Aceves, M.; Putti, R.; Lionetti, L. Omega-3 Fatty Acids and Insulin Resistance: Focus on the Regulation of Mitochondria and Endoplasmic Reticulum Stress. *Nutrients* **2018**, *10*, 350, doi:10.3390/nu10030350.

25. Sullivan, E.M.; Pennington, E.R.; Sparagna, G.C.; Torres, M.J.; Neufer, P.D.; Harris, M.; Washington, J.; Anderson, E.J.; Zeczycki, T.N.; Brown, D.A.; et al. Docosahexaenoic Acid Lowers Cardiac Mitochondrial Enzyme Activity by Replacing Linoleic Acid in the Phospholipidome. *Journal of Biological Chemistry* **2018**, *293*, 466–483, doi:10.1074/jbc.M117.812834.

26. Leger, T.; Azarnoush, K.; Traoré, A.; Cassagnes, L.; Rigaudière, J.-P.; Jouve, C.; Pagès, G.; Bouvier, D.; Sapin, V.; Pereira, B.; et al. Antioxidant and Cardioprotective Effects of EPA on Early Low-Severity Sepsis through UCP3 and SIRT3 Upholding of the Mitochondrial Redox Potential. *Oxidative Medicine and Cellular Longevity* **2019**, *2019*, 1–21, doi:10.1155/2019/9710352.

27. Inoue, T.; Tanaka, M.; Masuda, S.; Ohue-Kitano, R.; Yamakage, H.; Muranaka, K.; Wada, H.; Kusakabe, T.; Shimatsu, A.; Hasegawa, K.; et al. Omega-3 Polyunsaturated Fatty Acids Suppress the Inflammatory Responses of Lipopolysaccharide-Stimulated Mouse Microglia by Activating SIRT1 Pathways. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **2017**, *1862*, 552–560, doi:10.1016/j.bbalip.2017.02.010.

28. Xue, B.; Yang, Z.; Wang, X.; Shi, H. Omega-3 Polyunsaturated Fatty Acids Antagonize Macrophage Inflammation via Activation of AMPK/SIRT1 Pathway. *PLoS ONE* **2012**, *7*, e45990, doi:10.1371/journal.pone.0045990.

29. Jung, S.-B.; Kwon, S.K.; Kwon, M.; Nagar, H.; Jeon, B.H.; Irani, K.; Yoon, S.H.; Kim, C.S. Docosahexaenoic Acid Improves Vascular Function via Up-Regulation of SIRT1 Expression in Endothelial Cells. *Biochemical and Biophysical Research Communications* **2013**, *437*, 114–119, doi:10.1016/j.bbrc.2013.06.049.

30. Pedersen, A.M.; Vang, B.; Olsen, R.L. Oil from *Calanus Finmarchicus* —Composition and Possible Use: A Review. *J Aquat Food Prod Technol* **2014**, *23*, 633–646, doi:10.1080/10498850.2012.741662.

31. Bechthold, A.; Breidenassel, C.; Egert, S.; Gahl, A.; Michel, M.; Oberritter, H.; Virmani, K. *Vollwertig Essen Und Trinken Nach Den 10 Regeln Der DGE*; Deutschen Gesellschaft für Ernährung e. V.: Bonn, 2018;

32. Wasserfurth, P.; Nebl, J.; Boßlau, T.K.; Krüger, K.; Hahn, A.; Schuchardt, J.P. 12-Weeks of *Calanus finmarchicus* Oil Intake Improves Omega-3-Index in Healthy Older Subjects Engaging in an Exercise Program. *Br J Nutr* **2020**, 1–17, doi:10.1017/S0007114520002809.

33. Cantó, C.; Jiang, L.Q.; Deshmukh, A.S.; Mataki, C.; Coste, A.; Lagouge, M.; Zierath, J.R.; Auwerx, J. Interdependence of AMPK and SIRT1 for Metabolic Adaptation to Fasting and Exercise in Skeletal Muscle. *Cell Metabolism* **2010**, *11*, 213–219, doi:10.1016/j.cmet.2010.02.006.

34. Li, X. SIRT1 and Energy Metabolism. *Acta Biochimica et Biophysica Sinica* **2013**, *45*, 51–60, doi:10.1093/abbs/gms108.

35. Guerra, B.; Guadalupe-Grau, A.; Fuentes, T.; Ponce-González, J.G.; Morales-Alamo, D.; Olmedillas, H.; Guillén-Salgado, J.; Santana, A.; Calbet, J.A.L. SIRT1, AMP-Activated Protein Kinase Phosphorylation and Downstream Kinases in Response to a Single Bout of Sprint Exercise: Influence of Glucose Ingestion. *Eur J Appl Physiol* **2010**, *109*, 731–743, doi:10.1007/s00421-010-1413-y.

36. Radak, Z.; Bori, Z.; Koltai, E.; Fatouros, I.G.; Jamurtas, A.Z.; Douroudos, I.I.; Terzis, G.; Nikolaidis, M.G.; Chatzinikolaou, A.; Sovatzidis, A.; et al. Age-Dependent Changes in 8-Oxoguanine-DNA Glycosylase Activity Are Modulated by Adaptive Responses to Physical Exercise in Human Skeletal Muscle. *Free Radical Biology and Medicine* **2011**, *51*, 417–423, doi:10.1016/j.freeradbiomed.2011.04.018.

37. Little, J.P.; Safdar, A.; Wilkin, G.P.; Tarnopolsky, M.A.; Gibala, M.J. A Practical Model of Low-Volume High-Intensity Interval Training Induces Mitochondrial Biogenesis in Human Skeletal Muscle: Potential Mechanisms: Interval Training Adaptations. *The Journal of Physiology* **2010**, *588*, 1011–1022, doi:10.1113/jphysiol.2009.181743.

38. Gurd, B.J.; Yoshida, Y.; McFarlan, J.T.; Holloway, G.P.; Moyes, C.D.; Heigenhauser, G.J.F.; Spriet, L.; Bonen, A. Nuclear SIRT1 Activity, but Not Protein Content, Regulates Mitochondrial Biogenesis in Rat and Human Skeletal Muscle. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **2011**, *301*, R67–R75, doi:10.1152/ajpregu.00417.2010.

39. Vargas-Ortiz, K.; Pérez-Vázquez, V.; Macías-Cervantes, M.H. Exercise and Sirtuins: A Way to Mitochondrial Health in Skeletal Muscle. *IJMS* **2019**, *20*, 2717, doi:10.3390/ijms20112717.

40. Lombard, D.B.; Tishkoff, D.X.; Bao, J. Mitochondrial Sirtuins in the Regulation of Mitochondrial Activity and Metabolic Adaptation. *Handb Exp Pharmacol* **2011**, *206*, 163–188, doi:10.1007/978-3-642-21631-2_8.

41. He, F.; Li, J.; Liu, Z.; Chuang, C.-C.; Yang, W.; Zuo, L. Redox Mechanism of Reactive Oxygen Species in Exercise. *Front. Physiol.* **2016**, *7*, doi:10.3389/fphys.2016.00486.

42. Villanova, L.; Vernucci, E.; Pucci, B.; Pellegrini, L.; Nebbioso, M.; Mauri, C.; Marfe, G.; Spataro, A.; Fini, M.; Banfi, G.; et al. Influence of Age and Physical Exercise on Sirtuin Activity in Humans. *J. Biol. Regul. Homeost. Agents* **2013**, *27*, 497–507.

43. Suwa, M.; Sakuma, K. The Potential Role of Sirtuins Regarding the Effects of Exercise on Aging- Related Diseases. *CAS* **2013**, *6*, 178–188, doi:10.2174/18746098112059990035.

44. Covington, J.D.; Bajpeyi, S. The Sirtuins: Markers of Metabolic Health. *Mol. Nutr. Food Res.* **2016**, *60*, 79–91, doi:10.1002/mnfr.201500340.

45. Radak, Z.; Suzuki, K.; Posa, A.; Petrovszky, Z.; Koltai, E.; Boldogh, I. The Systemic Role of SIRT1 in Exercise Mediated Adaptation. *Redox Biology* **2020**, *35*, 101467, doi:10.1016/j.redox.2020.101467.

46. Kincaid, B.; Bossy-Wetzel, E. Forever Young: SIRT3 a Shield against Mitochondrial Meltdown, Aging, and Neurodegeneration. *Front. Aging Neurosci.* **2013**, *5*, doi:10.3389/fnagi.2013.00048.

47. Hirschey, M.D.; Shimazu, T.; Goetzman, E.; Jing, E.; Schwer, B.; Lombard, D.B.; Grueter, C.A.; Harris, C.; Biddinger, S.; Ilkayeva, O.R.; et al. SIRT3 Regulates Mitochondrial Fatty-Acid Oxidation by Reversible Enzyme Deacetylation. *Nature* **2010**, *464*, 121–125, doi:10.1038/nature08778.

48. Kong, X.; Wang, R.; Xue, Y.; Liu, X.; Zhang, H.; Chen, Y.; Fang, F.; Chang, Y. Sirtuin 3, a New Target of PGC-1α, Plays an Important Role in the Suppression of ROS and Mitochondrial Biogenesis. *PLoS ONE* **2010**, *5*, e11707, doi:10.1371/journal.pone.0011707.

49. Kumar, S.; Lombard, D.B. Functions of the Sirtuin Deacylase SIRT5 in Normal Physiology and Pathobiology. *Crit Rev Biochem Mol Biol* **2018**, *53*, 311–334, doi:10.1080/10409238.2018.1458071.

50. Ferrara, N.; Rinaldi, B.; Corbi, G.; Conti, V.; Stiuso, P.; Boccuti, S.; Rengo, G.; Rossi, F.; Filippelli, A. Exercise Training Promotes SIRT1 Activity in Aged Rats. *Rejuvenation Research* **2008**, *11*, 139–150, doi:10.1089/rej.2007.0576.

51. Steiner, J.L.; Murphy, E.A.; McClellan, J.L.; Carmichael, M.D.; Davis, J.M. Exercise Training Increases Mitochondrial Biogenesis in the Brain. *Journal of Applied Physiology* **2011**, *111*, 1066–1071, doi:10.1152/japplphysiol.00343.2011.

52. Huffman, D.M.; Moellering, D.R.; Grizzle, W.E.; Stockard, C.R.; Johnson, M.S.; Nagy, T.R. Effect of Exercise and Calorie Restriction on Biomarkers of Aging in Mice. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **2008**, *294*, R1618–R1627, doi:10.1152/ajpregu.00890.2007.

53. Lanza, I.R.; Short, D.K.; Short, K.R.; Raghavakaimal, S.; Basu, R.; Joyner, M.J.; McConnell, J.P.; Nair, K.S. Endurance Exercise as a Countermeasure for Aging. *Diabetes* **2008**, *57*, 2933–2942, doi:10.2337/db08-0349.

54. Richter, E.A.; Ruderman, N.B. AMPK and the Biochemistry of Exercise: Implications for Human Health and Disease. *Biochemical Journal* **2009**, *418*, 261–275, doi:10.1042/BJ20082055.

55. White, A.T.; Schenk, S. NAD$^+$/NADH and Skeletal Muscle Mitochondrial Adaptations to Exercise. *American Journal of Physiology-Endocrinology and Metabolism* **2012**, *303*, E308–E321, doi:10.1152/ajpendo.00054.2012.

56. Lamb, D.A.; Moore, J.H.; Mesquita, P.H.C.; Smith, M.A.; Vann, C.G.; Osburn, S.C.; Fox, C.D.; Lopez, H.L.; Ziegenfuss, T.N.; Huggins, K.W.; et al. Resistance Training Increases Muscle NAD+ and NADH Concentrations as Well as NAMPT Protein Levels and Global Sirtuin Activity in Middle-Aged, Overweight, Untrained Individuals. *Aging* **2020**, *12*, 9447–9460, doi:10.18632/aging.103218.

57. de Boer, V.C.J.; de Goffau, M.C.; Arts, I.C.W.; Hollman, P.C.H.; Keijer, J. SIRT1 Stimulation by Polyphenols Is Affected by Their Stability and Metabolism. *Mech Ageing Dev* **2006**, *127*, 618–627, doi:10.1016/j.mad.2006.02.007.

58. Smith, G.I.; Julliand, S.; Reeds, D.N.; Sinacore, D.R.; Klein, S.; Mittendorfer, B. Fish Oil-Derived n-3 PUFA Therapy Increases Muscle Mass and Function in Healthy Older Adults. *Am. J. Clin. Nutr.* **2015**, *102*, 115–122, doi:10.3945/ajcn.114.105833.

59. Rodacki, C.L.; Rodacki, A.L.; Pereira, G.; Naliwaiko, K.; Coelho, I.; Pequito, D.; Fernandes, L.C. Fish-Oil Supplementation Enhances the Effects of Strength Training in Elderly Women. *The American Journal of Clinical Nutrition* **2012**, *95*, 428–436, doi:10.3945/ajcn.111.021915.

60. McGlory, C.; Calder, P.C.; Nunes, E.A. The Influence of Omega-3 Fatty Acids on Skeletal Muscle Protein Turnover in Health, Disuse, and Disease. *Front. Nutr.* **2019**, *6*, 144, doi:10.3389/fnut.2019.00144.

61. Echeverría, F.; Ortiz, M.; Valenzuela, R.; Videla, L.A. Long-Chain Polyunsaturated Fatty Acids Regulation of PPARs, Signaling: Relationship to Tissue Development and Aging. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **2016**, *114*, 28–34, doi:10.1016/j.plefa.2016.10.001.

62. Lim, J.-H.; Gerhart-Hines, Z.; Dominy, J.E.; Lee, Y.; Kim, S.; Tabata, M.; Xiang, Y.K.; Puigserver, P. Oleic Acid Stimulates Complete Oxidation of Fatty Acids through Protein Kinase A-Dependent Activation of SIRT1-PGC1α Complex. *Journal of Biological Chemistry* **2013**, *288*, 7117–7126, doi:10.1074/jbc.M112.415729.

63. Gao, D.; Wang, H.; Xu, Y.; Zheng, D.; Zhang, Q.; Li, W. Protective Effect of Astaxanthin against Contrast-Induced Acute Kidney Injury via SIRT1-P53 Pathway in Rats. *Int Urol Nephrol* **2019**, *51*, 351–358, doi:10.1007/s11255-018-2027-2.

64. Nishida, Y.; Nawaz, A.; Kado, T.; Takikawa, A.; Igarashi, Y.; Onogi, Y.; Wada, T.; Sasaoka, T.; Yamamoto, S.; Sasahara, M.; et al. Astaxanthin Stimulates Mitochondrial Biogenesis in Insulin Resistant Muscle via Activation of AMPK Pathway. *Journal of Cachexia, Sarcopenia and Muscle* **2020**, *11*, 241–258, doi:10.1002/jcsm.12530.

65. Eilertsen, K.-E.; Mæhre, H.K.; Jensen, I.J.; Devold, H.; Olsen, J.O.; Lie, R.K.; Brox, J.; Berg, V.; Elvevoll, E.O.; Østerud, B. A Wax Ester and Astaxanthin-Rich Extract from the Marine Copepod Calanus Finmarchicus Attenuates Atherogenesis in Female Apolipoprotein E–Deficient Mice. *J Nutr* **2012**, *142*, 508–512, doi:10.3945/jn.111.145698.

66. Höper, A.C.; Salma, W.; Sollie, S.J.; Hafstad, A.D.; Lund, J.; Khalid, A.M.; Raa, J.; Aasum, E.; Larsen, T.S. Wax Esters from the Marine Copepod Calanus Finmarchicus Reduce Diet-Induced Obesity and Obesity-Related Metabolic Disorders in Mice. *J Nutr* **2014**, *144*, 164–169, doi:10.3945/jn.113.182501.

67. Mariani, S.; di Giorgio, M.R.; Martini, P.; Persichetti, A.; Barbaro, G.; Basciani, S.; Contini, S.; Poggiogalle, E.; Sarnicola, A.; Genco, A.; et al. Inverse Association of Circulating SIRT1 and Adiposity: A Study on Underweight, Normal Weight, and Obese Patients. *Front. Endocrinol.* **2018**, *9*, 449, doi:10.3389/fendo.2018.00449.

68. Al-Khaldi, A.; Sultan, S. The Expression of Sirtuins, Superoxide Dismutase, and Lipid Peroxidation Status in Peripheral Blood from Patients with Diabetes and Hypothyroidism. *BMC Endocr Disord* **2019**, *19*, 19, doi:10.1186/s12902-019-0350-y.

2.4 Paper IV

Abdominal Obesity-Related Disturbance of Insulin Sensitivity Is Associated with CD8+ EMRA Cells in the Elderly

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MDPI

Article

Abdominal Obesity-Related Disturbance of Insulin Sensitivity Is Associated with CD8+ EMRA Cells in the Elderly

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Abstract: Aging and overweight increase the risk of developing type 2 diabetes mellitus. In this cross-sectional study, we aimed to investigate the potential mediating role of T-EMRA cells and inflammatory markers in the development of a decreased insulin sensitivity. A total of 134 healthy older volunteers were recruited (age 59.2 ($_{SD}$ 5.6) years). T cell subpopulations were analyzed by flow cytometry. Furthermore, body composition, HOMA-IR, plasma tryptophan (Trp) metabolites, as well as cytokines and adipokines were determined. Using subgroup and covariance analyses, the influence of BMI on the parameters was evaluated. Moreover, correlation, multiple regression, and mediation analyses were performed. In the subgroup of participants with obesity, an increased proportion of CD8+EMRA cells and elevated concentrations of plasma kynurenine (KYN) were found compared to the lower-weight subgroups. Linear regression analysis revealed that an elevated HOMA-IR could be predicted by a higher proportion of CD8+EMRA cells and KYN levels. A mediation analysis showed a robust indirect effect of the Waist-to-hip ratio on HOMA-IR mediated by CD8+EMRA cells. Thus, the deleterious effects of abdominal obesity on glucose metabolism might be mediated by CD8+EMRA cells in the elderly. Longitudinal studies should validate this assumption and analyze the suitability of CD8+EMRA cells as early predictors of incipient prediabetes.

Keywords: elderly; obesity; T-EMRA cells; kynurenine pathway; insulin resistance

1. Introduction

During aging, the adaptive immune system undergoes a remodeling process characterized by altered T cell subtype composition and changes in major T cell functions. While the number of CD8+ cells generally increases, the proportion of CD4+ cells slightly decreases, resulting in a reduced CD4+/CD8+ T cell ratio [1]. Within both T cell populations, the relative proportion of cells with a naïve phenotype progressively decreases. Homeostatic proliferation of existing naive T cells in the periphery partially compensates for the reduced number of naive T cells [2]. Functionally, the T cell receptor (TCR) repertoire is diminished, resulting in a limited response against emerging pathogens [3]. Accompanying the exhaustion in the naïve T cell pool, an accumulation of terminally differentiated cells occurs. This

is caused by repeated T cell stimulation, e.g., by latent viruses, such as cytomegalovirus (CMV) infection, or by a state of chronic inflammation [4,5].

T effector memory re-expressing CD45RA cells (T-EMRA cells) represent a heterogeneous group of terminally differentiated cells characterized by their surface expression profile CCR7−/CD45RA+. These cells can be further subdivided regarding their expression of CD57 into a functionally viable "young" fraction (CD57−) with proliferative and antiviral capacity and a senescent fraction (CD57+) with extensive functional quiescence that is proliferation incompetent in response to antigen-specific stimulation and susceptible to apoptosis upon T cell activation [6,7]. With age, the number of T-EMRA cells increases, which can be interpreted as a hallmark of immunosenescence [8]. T-EMRA cells are suggested to contribute to various pathological processes by exacerbating inflammation and exhibiting atypical cytotoxic activity towards endogenous structures, such as the vascular endothelium [9,10]. Moreover, T-EMRA cells have been identified as predictors of cardiovascular mortality in the elderly and as risk factors of graft dysfunction in kidney transplant recipients [11,12].

Accelerated T cell differentiation is bi-directionally related to the development of a chronic low-grade inflammation, termed "inflammaging" [13,14]. On the one hand, chronic inflammation is a driver of T cell differentiation by constantly activating existing immune cells. On the other hand, highly differentiated T-EMRA cells represent a pro-inflammatory phenotype and a source of inflammatory factors [10,15]. The concentration of various inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α), progressively increases during aging, which contributes to age-associated morbidity and mortality [16]. Overweight superimposed on aging accelerates low-grade inflammation, which might play a leading role not only in the pathogenesis of cardiovascular but also of metabolic diseases, such as diabetes type 2. Accordingly, patients with prediabetes or diabetes show significantly lower insulin sensitivity, increased proportions of terminally differentiated T cells, and higher levels of inflammatory cytokines compared to metabolically healthy individuals [17]. Besides glucose metabolism, the kynurenine (KYN) pathway as the major route of tryptophan degradation represents another popular example of the interplay between metabolism and inflammation. The initial and rate-limiting enzyme indoleamine 2-3-dioxygenase (IDO1) strongly increases in response to elevated proinflammatory cytokine levels [18], leading to a stimulation of the KYN pathway that has been demonstrated in several inflammation-associated pathologies [19,20].

Based on these findings, we speculate that T-EMRA cells, as well as parameters of the kynurenine pathway, may be related to a disturbance of insulin sensitivity in obesity and that this is detectable even in clinically healthy elderly subjects. We explicitly addressed the question: Do increased proportions of T-EMRA cells or activation of the KYN pathway favor an early dysregulation of insulin sensitivity in subjects aged 50–70 without any previous chronic illness? Furthermore, we asked whether T-EMRA cells and changes in Trp metabolites are potential mediators between abdominal obesity and disturbed insulin sensitivity.

2. Materials and Methods

The current work is based on a recent study of our group [21]. Blood samples were obtained from the same subjects. Data regarding anthropometric and physiological characteristics have been incorporated from this publication.

2.1. Study Participants

We enrolled 134 men and women from the general population in Hannover, Germany, between August 2018 and March 2019. Recruitment was conducted widely distributed via advertisements in local newspapers and public notices. The inclusion and exclusion criteria for each participant were determined using a formalized questionnaire. Inclusion criteria for participation were age ≥50 and ≤70 years, no regular exercise training aside from the daily activities for at least 2 years, and stable body weight (±5 kg) for at least 6 months.

Exclusion criteria were defined as cardiovascular diseases (angina pectoris, myocardial infarction, stroke, peripheral arterial occlusive disease, heart failure, cardiac arrhythmia), type 1 and 2 diabetes, renal insufficiency and liver diseases, blood coagulation disorders, chronic gastrointestinal disorders (e.g., ulcers, Crohn's disease, pancreatic insufficiency), immunological diseases (e.g., autoimmune diseases), intake of immunosuppressive drugs or laxatives, intake of supplements containing n3-FAs, smoking, alcohol, drug and/or medicine dependency, pregnancy or lactation, retraction of the consent by the subject, concurrent participation in another clinical study, and participation in a study in the last 30 days. Ethical approval was provided by the Ethics Commission of the Medical Chamber of Lower Saxony (Hannover, Germany). Following the guidelines of the Declaration of Helsinki, written informed consent was obtained from all participants before participation in the study. Study design and the procedure for the different analyses are shown in Figure 1a.
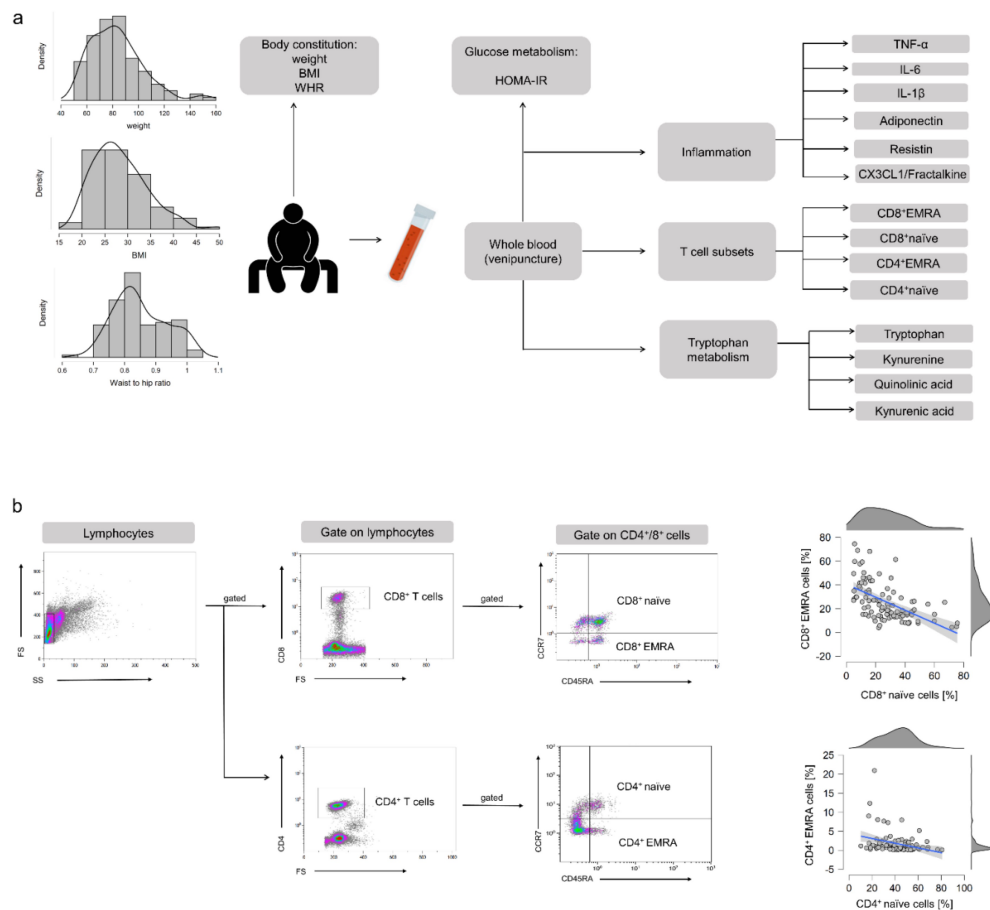
Figure 1. (**a**) Presentation of the study design and the course of analyses. (**b**) Flow cytometric analysis of T cell subpopulations. Lymphocytes were gated using the forward- and sideward scatter. Populations were subdivided into CD4+ T-helper cells and CD8+ cytotoxic T cells. Both cell types were further differentiated into naïve (CD45RA+/CCR7+), central memory (CD45RA−/CCR7+), effector memory (CD45RA−/CCR7−), and effector memory re-expressing CD45RA (CD45RA+/CCR7−) cells. Associations between naïve and EMRA T cells.

2.2. Body Weight and Body Composition

Waist and hip circumferences were measured using a measuring tape in a standing position. Waist-to-hip-ratio (WHR) was calculated. Height was measured with a stadiometer (seca GmbH & Co. KG, Hamburg, Germany) and body weight was measured on a digital scale to the nearest 0.1 kg (seca GmbH & Co. KG, Hamburg, Germany). BMI was calculated by the ratio of weight to the squared height. Body composition was analyzed to a nearest of 0.1 kg using a bioelectrical impedance analyzer (BIA) (Nutriguard M, Data Input Company, Darmstadt, Germany) and the software NutriPlus© 5.4.1 (Data Input Company, Darmstadt, Germany). For the measurements, the participants arrived rested and after an overnight fast ($\geq$10 h). They were instructed to lay down on a stretcher and rest for at least five minutes to ensure a balanced distribution of body fluids before the measurement. During the measurement, participants were instructed to lay still and in a relaxed position with the limbs slightly bend from the torso. All measurements were carried out by the same study personnel.

2.3. Blood Sampling

Blood samples were taken in the morning following a resting period and after an overnight fast ($\geq$10 h) using serum, EDTA, and NaF Glucose tubes (Sarstedt AG & Co. KG, Nümbrecht, Germany). Blood was either processed directly or stored at $-80\,^{\circ}$C in the form of serum and plasma for later analysis.

2.4. Analysis of Glucose Metabolism and Insulin Resistance

Analysis of markers of glucose metabolism was performed by a certified laboratory (Laborärztliche Arbeitsgemeinschaft für Diagnostik und Rationalisierung e.V., in Hannover, Germany). Fasting glucose was analyzed photometrically (Beckman Coulter GmbH, Krefeld, Germany). HbA1c was analyzed using high-performance liquid chromatography (HPLC) (Bio-Rad Laboratories GmbH, Feldkirchen, Germany). For the determination of insulin, the electrochemiluminescence immunoassay method (ECLIA) using cobas 801e (Roche Diagnostics GmbH, Mannheim, Germany) was applied. Insulin sensitivity was evaluated using the homeostatic model assessment (HOMA): HOMA-IR = fasting insulin (μU/mL) $\times$ fasting blood glucose (mg/dL)/405 [22].

2.5. Analysis of T Cell Subpopulations

Peripheral Blood Mononuclear Cells (PBMCs) were isolated from fresh EDTA whole blood by ficoll density gradient centrifugation. After PBMCs were washed, 1×10^6 cells in 100 μL PBS were stained for 20 min in the dark with 5 μL of different fluorescence-coupled antibodies, respectively (BioLegend Inc., San Diego, CA, USA & ImmunoTools GmbH, Hamburg, Germany). The antibody cocktails were composed as follows. Analysis of CD4+ subtypes: anti-CD4-FITC (clone MEM-241), anti-CD197(CCR7)-PE (clone G043H7), anti-CD45RA-PerCP (clone HI100); Analysis of CD8+ subtypes: anti-CD8-FITC (clone MEM-31), anti-CD197(CCR7)-PE (clone G043H7), anti-CD45RA-PerCP (clone HI100). Percentages of naïve (CD45RA+/CCR7+), central memory (CD45RA-/CCR7+), effector memory (CD45RA-/CCR7$-$), and EMRA (CD45RA+/CCR7$-$) CD4 and CD8+ T cells were quantified by flow cytometer FC 500 using the CXP software (Beckman Coulter, Irving, TX, USA). The gating strategy is shown in Figure 1b and was implemented according to Koch et al. [8]. Besides, CD4+/CD8+ T cell ratio was determined.

2.6. Analysis of Tryptophan Metabolites

Trp and its metabolites KYN, quinolinic acid (QA), and kynurenic acid (KA) were measured via high-performance liquid chromatography (HPLC) coupled to a mass spectrometer (MS). Serum was stored in 50 μL aliquots at $-80\,^{\circ}$C until analysis. The analysis was performed on a Waters ACQUITY UPLC® system equipped with an ACQUITY UPLC® HSS T3 analytical column coupled to a Xevo® TQ-XS triple quadrupole mass spectrometer (Waters, Eschborn, Germany) as described elsewhere [20].

2.7. Analysis of Plasma Cytokines

Plasma levels of IL-1β, IL1ra, IL-6, IL-15, TNF-α, CX3CL1/fractalkine, adiponectin, and resistin were determined using a human Magnetic Luminex Assay (Bio-Techne, Abingdon, Oxon, UK) and a Magpix Luminex instrument (Luminex Corp, Austin, TX, USA) according to the manufacturer's instructions.

2.8. Analysis of Cytomegalovirus (CMV) Serostatus

Serum anti-CMV immunoglobulin G (IgG) antibodies were detected using a semiquantitative sandwich enzyme-linked immunosorbent assay (ELISA-Viditest anti-CMV IgG, VIDIA, Czech Republic). The procedure followed the manufacturer's instructions. Endpoint optical density was measured by the Emax Plus ELISA reader (Molecular Devices, Sunnyvale, CA, USA).

2.9. Statistical Analysis

First, descriptive statistics were performed for all measured variables and results are given below as mean $\pm$ SD. In the next step, we tested all observed variables regarding their distribution features. As most of them did not meet the criterion of a normal distribution, we used Spearman's rank correlation to determine possible relationships between measurements of body composition (as indicated by BMI and WHR), metabolism (as indicated by HOMA-IR and Trp metabolites), CD4+ and CD8+EMRA cells, as well as pro-inflammatory cytokines (e.g., IL-6, TNF-α) and adipokines (adinopectine, resistin) for exploarory purposes. Missing data were addressed using pairwise deletion.

As a preliminary analysis, we further divided our study collective into three BMI subgroups: group 1: 18.5–24.9 kg/m^2 (normal weight); group 2: 25–29.9 kg/m^2 (overweight); group 3: >30 kg/m^2 (obesity). We calculated an ANCOVA for the dependent variable CD8+EMRA cells and Trp metabolite KYN and the independent variable BMI group using age as a covariate to assess the impact of a BMI classified within the obese range (>30) on the proportion of T-EMRA cells and enhanced Trp metabolites.

In the next step, we analyzed the impact of obesity, proportion of T-EMRA cells, and levels of Trp metabolites as potential predictors of abnormal glucose metabolism (indicated by HOMA-IR) using multiple regression analyses. We added several product terms to the model to test for an interaction between abdominal obesity, immune aging, and Trp metabolites. Before multiple regression analysis, we z-standardized all variables. To identify a possible mechanism that underlies the observed relationship between abdominal obesity and disturbed glucose metabolism, we tested the mediation of this relationship by assessing the factors of immune aging, as well as the concentration of tryptophan metabolites. More precisely, we calculated a mediation analysis using bootstrapping with 5000 bootstraps with the predictor variable WHR, the mediators CD8+EMRA cells as well as KYN, and HOMA-IR as the outcome variable. For all statistical analyses as well as data visualization, we used JASP, version 14.0. p values < 0.05 are considered significant.

3. Results

3.1. Baseline Characteristics

After screening for eligibility, 134 participants met the eligibly criteria (Figure 2). Their data are specified as mean $\pm$ SD. Of all participants, 72% were female and 28% were male with a mean age of 59.2 $\pm$ 5.6 years. Participants were further characterized by a weight of 83.0 $\pm$ 20.3 kg, BMI of 28.3 $\pm$ 5.8 kg/m^2, a WHR of 0.85 $\pm$ 0.09, and a HOMA-IR of 2.72 $\pm$ 2.26. The study population can be classified as healthy, but pre-obese. All measured baseline characteristics are summarized in Table 1.
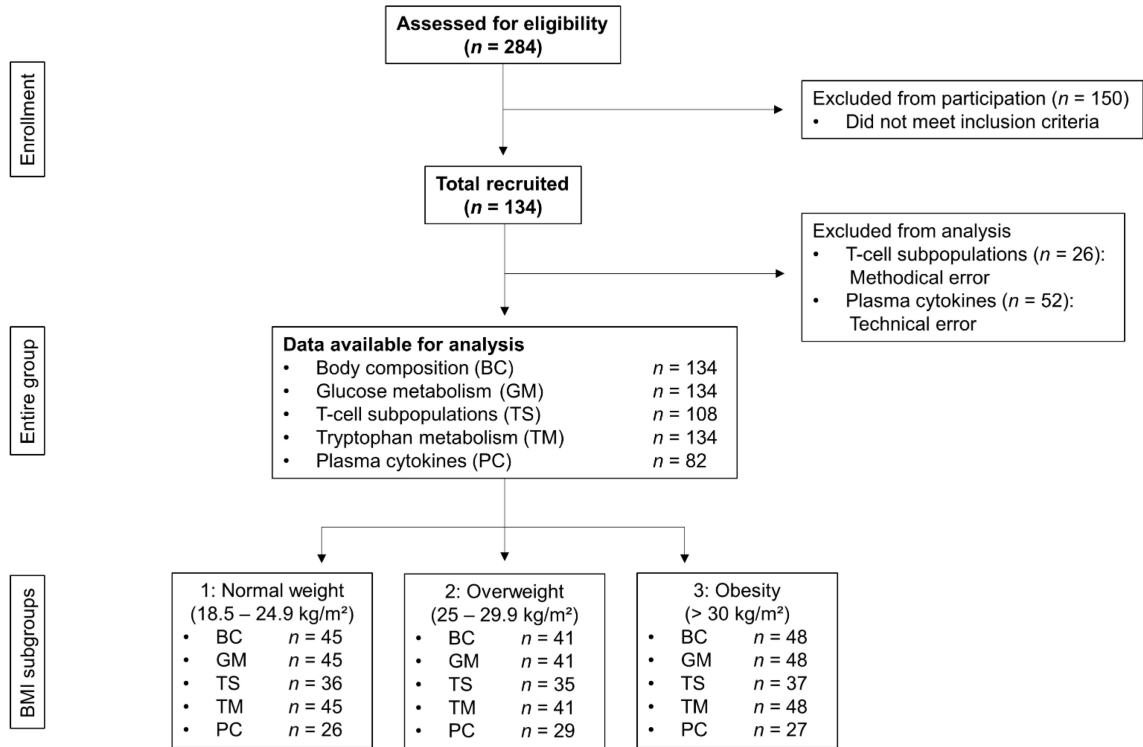
Figure 2. Flow chart of all participants screened and analyzed.

Table 1. Baseline characteristics (mean ± SD) of all participants.

Baseline Characteristics	
Sex [f/m]	[96/38]
Age [years]	59.2 ± 5.6
Height [m]	170.9 ± 8.7
Body weight [kg]	83.0 ± 20.3
BMI [kg/m^2]	28.3 ± 5.8
Waist circumference [cm]	93.30 ± 14.53
Hip circumference [cm]	108.32 ± 12.15
WHR	0.85 ± 0.09
Fasting Glucose [mg/dL]	93.13 ± 16.78
HbA1c [%]	5.44 ± 0.45
Insulin [µU/mL]	11.39 ± 7.96
HOMA-Index	2.72 ± 2.26
CMV positive [Yes/no]	[72/63]

3.2. Associations between Body Composition, Glucose Metabolism, T-EMRA Cells, Trp Metabolites, and Cytokine Status

Proportions of T cell subpopulations and levels of measured cytokines, adipokines, and Trp metabolites of all participants can be found in Supplementary Tables S1–S3.

We examined the Spearman rank correlation between either BMI, WHR, HOMA-IR, CD8+EMRA cells, IL-6, resistin, and KYN separately for each participant. Note that for all

upcoming results, we used the z-standardized variables. The correlation analysis revealed correlations between WHR and CD8+EMRA cells (r = 0.351, p < 0.001), IL-6 (r = 0.249, p = 0.021), resistin (r = 0.221, p = 0.046), KYN (r = 0.207, p = 0.041), as well as HOMA-IR (r = 0.447, p < 0.001). CD8+EMRA cells were correlated with KYN (r = 0.242, p < 0.05), BMI (r = 0.235, p < 0.05), and HOMA-IR (r = 0.250, p < 0.05). IL-6 was associated with BMI (r = 0.352, p < 0.001). Resistin was positively correlated with BMI (r = 0.349, p < 0.01). Furthermore, we found a correlation between KYN and BMI (r = 0.355, p < 0.001) and HOMA-IR (r = 0.224, p < 0.05). All results of the correlation analysis are depicted in Figure 3.
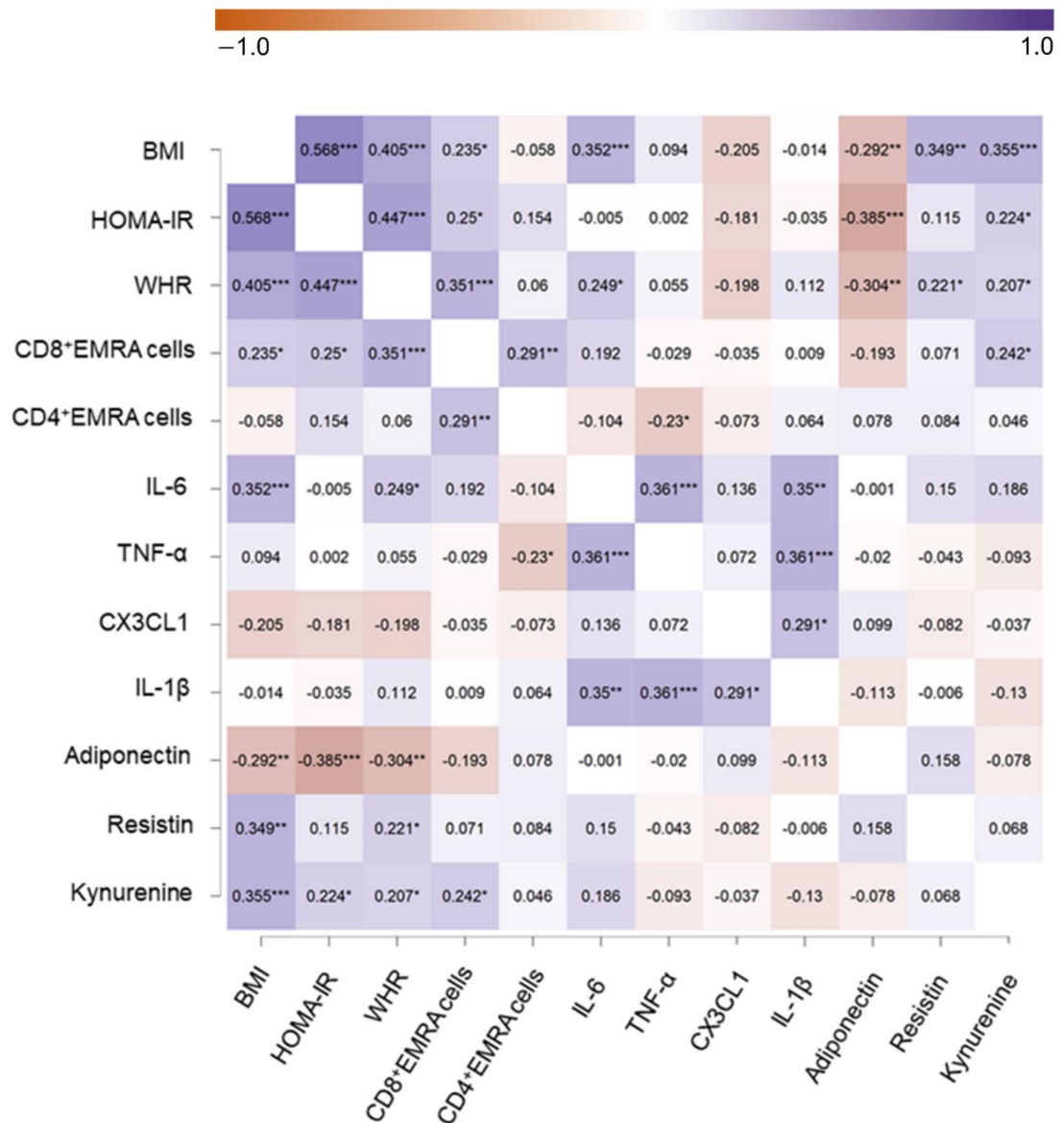

Figure 3. Correlation heat map including BMI, HOMA-IR, WHR, CD8+ and CD4+EMRA cells, IL-6, TNF-α, CX3CL1, adinopectin, resistin, and KYN. Raw p values of Spearman's rank s correlations are presented. * means p < 0.05, ** means p < 0.01, *** means p < 0.001 not corrected for multiple tests.

3.3. Effect of BMI on T-EMRA Cells and Trp Metabolism

As a pre-analysis, we tested whether a BMI classified within the obese range (>30) in particular leads to an increased proportion of T-EMRA cells as well as to enhanced levels of Trp metabolites. The calculated ANCOVAs including age as a covariate revealed a significant main effect of BMI group on the number of CD8+EMRA cells, $F_{(2, 89)} = 3.897$, $p = 0.024$, $\eta p2 = 0.081$, as well as on the level of the Trp metabolite KYN, $F_{(2, 94)} = 6.625$, $p = 0.002$, $\eta p2 = 0.124$. Post-hoc analyses using Bootstrapping indicated that, overall, a BMI > 30 is likely to lead to an increased proportion of CD8+EMRA cells (BMI group 2 vs. 3: $p < 0.05$) as well as to enhanced levels of KYN (BMI group 1 vs. 3 and BMI group 2 vs. 3: $p < 0.05$). Results are depicted in Figure 4a.
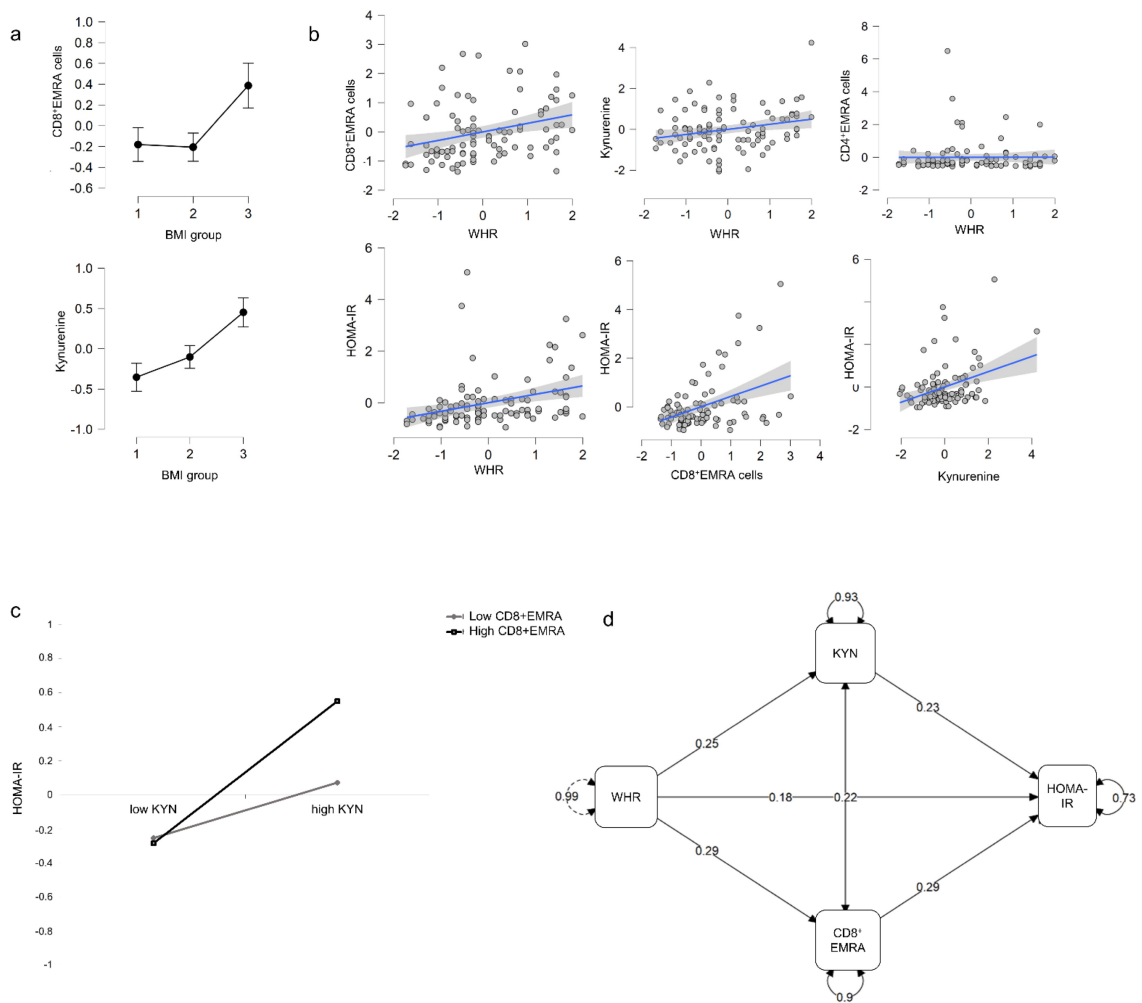
Figure 4. (**a**) Proportion of CD8+EMRA cells and KYN in BMI group 1 (BMI 18.5–24.9 kg/m^2), group 2 (BMI 25–29.9 kg/m^2), and group 3 (BMI > 30 kg/m^2). (**b**) Correlations between WHR and the proportion of CD8+EMRA cells, WHR and KYN, WHR and proportion of CD4+EMRA cells, WHR and HOMA-IR, CD8+EMRA and HOMA-IR, as well as KYN and HOMA-IR. (**c**) Associations between low/high kynurenine levels, levels of CD8+EMRA cells, and HOMA-IR. (**d**) Interaction of WHR on HOMA-IR mediated by CD8+EMRA cells as well as by KYN. Furthermore, a significant covariation between KYN and CD8+EMRA is demonstrated.

Further analyses revealed no main effect of sex, muscle mass, or weekly physical activity level on CD8+EMRA cells and KYN levels. Moreover, no significant interaction effects, for example, between sex and BMI, were found (data not shown).

3.4. Moderation of the Glucose Metabolism by Abdominal Obesity, the Proportion of T-EMRA Cells, and Trp Metabolites

We performed a multiple regression analysis investigating whether WHR, the proportion of T-EMRA cells, and changes of Trp metabolites are predictors of an altered glucose metabolism. Here, the WHR was used because it represents a better indicator of abdominal obesity and related health risks than BMI [23]. Furthermore, we tested whether the proportion of T-EMRA cells and KYN levels moderate the effect of abdominal obesity on the disturbances of glucose metabolism. The performed linear regression analysis revealed that the HOMA-IR could be predicted by the proportion of CD8+EMRA cells and KYN. Results showed that the HOMA-IR increases significantly when CD8+EMRA cells increase ($\beta = 0.302$, $p < 0.01$). Moreover, increased KYN levels correlated significantly with increases in HOMA-IR ($\beta = 0.212$, $p < 0.05$) (Figure 4b). The total variance explained by the model as a whole was adjusted $R2 = 0.232$, $F(3, 89) = 10.271$, $p < 0.001$. The model that included the product terms did explain little additional variance in the HOMA-IR score, adjusted $R2 = 0.276$, $F(7, 85) = 6.001$, $p < 0.001$. Results revealed a significant interaction between the proportion of CD8+EMRA cells and KYN ($\beta = 0.277$, $p < 0.01$) reflecting that higher CD8+EMRA cells accompanied by higher KYN levels lead to a stronger increase of HOMA-IR (Figure 4c).

3.5. Abdominal Obesity Affects T-EMRA Cells and Trp Metabolites, Which in Turn Influences HOMA-IR

We further analyzed whether the effect of abdominal obesity on glucose metabolism is mediated by immune senescence and Trp metabolites. A mediation analysis using bootstrapping showed a robust indirect effect of WHR on HOMA-IR mediated by CD8+EMRA cells ($\beta = 0.084$, $p < 0.05$). Additionally, a mediating tendency for KYN ($\beta = 0.058$, $p = 0.077$) was observed. Furthermore, the analysis revealed a significant covariation between KYN and CD8+EMRA cells ($\beta = 0.22$, $p < 0.05$) (Figure 4d).

4. Discussion

Effects of an individual's body composition, namely an increased BMI or abdominal obesity, are well-documented drivers of a dysregulated glucose metabolism [24]. However, potential mediators of this effect remain largely unclear, especially in a population of healthy aged subjects. Our findings show statistical associations between glucose metabolism, levels of Trp metabolites, and proportions of circulating T-EMRA cells. In particular, increased numbers of CD8+EMRA cells and increased levels of KYN could be potential predictors of a dysregulated glucose metabolism, even in clinically as yet unremarkable individuals. The present data also indicated that both the proportion of CD8+EMRA cells and KYN levels might play a mediating role between the increase in abdominal fat and an elevated HOMA-IR. This was supported by the fact that, in particular, the group of subjects with BMI classified as obese had increased KYN levels and CD8+EMRA concentrations.

A methodological limitation of our study in the context of flow cytometric evaluation was that we gated the CD4+ and CD8+ cells separately and not on one plot. CD4+ CD8+ double-positive cells could thus have been captured twice [25]. Although there usually is only a small proportion of double-positive cells, this aspect should be considered in future work. Due to the cross-sectional design, the present study can provide only initial insights into possible relationships but no causal conclusions can yet be drawn with absolute certainty. Nevertheless, the strength of the present work is the large representative human sample in an age-cohort of interest concerning the first occurrence of lifestyle-related diseases. Thus, high generalizability of our results can be assumed. Besides, our preliminary considerations were based on knowledge of biological correlations and

mechanisms in experimental (animal) studies, as described below. This legitimizes our statistical approach, supports our tentative conclusions, and reinforces the utility of follow-up studies in a longitudinal design to validate the assumptions we made here.

4.1. The Role of T-EMRA Cells in Obesity-Related Disturbance of Glucose Homeostasis

Overweight and obesity facilitate the incidence of many internal diseases and various data suggest that this occurs in parallel to the age-related remodeling of the adaptive immune system, particularly due to the accumulation of terminally differentiated T cells [26]. An important driver of these changes are lifestyle factors, such as inactivity and malnutrition, which lead to an increase in visceral adipose tissue (VAT). On one hand, the expansion of VAT favors a dysfunctional metabolic environment, but on the other hand, it accelerates T cell differentiation [27,28]. In this regard, it was previously shown that obesity accelerates thymic involution resulting in a lower naïve T cell production, leading to a proportional increase of terminally differentiated T cells [29]. In parallel, the expansion of visceral adipose tissue accelerates T cell differentiation by the progressive release of pro-inflammatory adipokines and cytokines [30]. A self-reinforcing cycle of progressive T cell differentiation and inflammatory cytokine production occurs because T-EMRA cells themselves secrete a variety of pro-inflammatory factors [10,15].

Our results concretize these data by showing an increased accumulation of T-EMRA cells in aged subjects with obesity and the association between abdominal fat and proportions of CD8+EMRA cells. As described in the introduction, T-EMRA cells are a heterogeneous subset of cells consisting of functional (CD57−) and senescent (CD57+) cells which occur in approximately equal proportions [6]. This further subdivision was not performed in the context of our study. However, results of mouse experiments suggest that mainly the exhausted, senescent T-EMRA cells may be crucial mediators of obesity-associated dysregulated glucose homeostasis.

Yi et al. [31] adoptively transferred senescent CD8+ T cells isolated from the spleens of mice fed a high-fat diet into young mice. Subsequently, the recipients showed aggravation of systemic glucose tolerance and insulin sensitivity compared to control mice. Another causal relationship between T cell aging and metabolic dysfunction was indicated in a study with obese mice. Here, the deletion of senescent cells alleviates the high-fat diet-induced metabolic dysfunction [32]. Cell culture experiments demonstrated a direct interaction between aged senescent CD8+ cells and hepatic insulin sensitivity by suppressing the activity of glycolytic enzymes [31]. However, the detailed molecular interactions between CD8+EMRA cells and metabolic pathways are still unknown. We can only speculate that cellular interactions or secreted molecules reduce tissue insulin sensitivity and possibly disrupt β-cell function. In this context, several inflammatory cytokines, such as IL-6, TNF-α, adiponectin, and resistin, are directly or indirectly involved in the regulation of insulin sensitivity [33,34]. Moreover, IL-6 and resistin have been associated with abdominal obesity on the one hand and an increased number of senescent T cells on the other hand [35]. For T-EMRA cells in general, secretion of TNF-alpha has also been demonstrated [10]. However, in our study, we did not observe a mediating role of any of these cytokines at the systemic level. Thus, follow-up studies should erode this potential mechanism at the tissue or cellular level.

Our finding that CD8+EMRA cells might represent a physiological mediator of disturbed glucose homeostasis is important from two perspectives. First, it would be important to investigate the potential direct molecular interactions, how these immune cells interact with beta cells or insulin-sensing organs, to uncover conceivable preventive and therapeutic targets. On the other hand, analyses of CD8+EMRA cells could represent an early predictive marker of a metabolic shift before subjects show clinically relevant symptoms of metabolic diseases. These assumptions need to be addressed in future (longitudinal) studies. In this context, a more specific subdivision of the T-EMRA cell population concerning additional surface markers, e.g., senescence marker CD57, or functional proper-

ties would be beneficial. This could generate further mechanistic associations and possibly increase the uniqueness of the observed effects.

4.2. KYN as a Mediator between Abdominal Fat and a Disturbed Glucose Metabolism

Our results show associations between Trp metabolite KYN and disturbed glucose metabolism. This finding corresponds with previous studies which found the involvement of tryptophan metabolites in the control of pancreatic hormonal secretion and hepatic glucose production [36]. Increased KYN levels suggest increased activity of IDO1. The turnover rate of this enzyme is dependent on inflammatory signaling. In our work, we could not find a strong correlation between KYN levels and any of the detected cytokines at the systemic level. However, based on our data, we cannot draw any conclusion about possible molecular interactions at the tissue level, for example, in the abdominal adipose store. Enhanced KYN levels in the subgroup with obesity and the association with WHR suggests that there is a mechanistic link to the amount of abdominal fat. Previous studies on this topic found that IDO1 expression and activity are enhanced in adipose tissue during conditions of inflammation [37]. Since chronic inflammation is also favoring T cell differentiation and metabolic disturbances related to overweight, the inflammatory shift in adipose tissue seems to be a precursor for early pathological processes [38]. The positive association between KYN and HOMA-IR corresponds with previous data which found an association between Trp metabolites and insulin resistance. Mechanistically, Trp metabolites have been shown to affect the biosynthesis, release, and activity of insulin [39].

5. Conclusions

Overweight facilitates the incidence of many internal diseases, specifically in those subjects at the end of the BMI spectrum. Especially metabolic pathologies develop slowly and gradually, and such changes can be detected even in individuals clinically assessed as healthy, like in the present study [40].

The increase in body fat in elderly subjects is related to increased CD8+EMRA populations as well as elevated plasma KYN levels and both are in turn associated with pre-clinical metabolic dysregulation. These findings integrate previous data of patients in different disease groups and are more remarkable because we found it in a population of clinically healthy older subjects. It is assumed, that the WHR dependent metabolic changes represent a preliminary stage of incipient pathological processes, which announce themselves especially in the subjects who develop obesity. The study once again highlights the importance of maintaining reliable control over the development of abdominal fat through specific lifestyle factors, especially in older age. At the same time, the data suggest that changes in Trp metabolites and an increase in CD8+EMRA cells may represent early predictors of prediabetes. This needs to be confirmed in longitudinal studies. Furthermore, from a basic science perspective, the interaction of CD8+EMRA cells with metabolic changes related to glucose regulation and Trp metabolites is of particular interest.

References

1. Makinodan, T. Studies on the influence of age on immune response to understand the biology of immunosenescence. *Exp. Gerontol.* **1998**, *33*, 27–38. [CrossRef]
2. Goronzy, J.J.; Fang, F.; Cavanagh, M.M.; Qi, Q.; Weyand, C.M. Naive T cell maintenance and function in human aging. *J. Immunol.* **2015**, *194*, 4073–4080. [CrossRef]
3. Jacomet, F.; Cayssials, E.; Basbous, S.; Levescot, A.; Piccirilli, N.; Desmier, D.; Robin, A.; Barra, A.; Giraud, C.; Guilhot, F.; et al. Evidence for eomesodermin-expressing innate-like CD8(+) KIR/NKG2A(+) T cells in human adults and cord blood samples. *Eur. J. Immunol.* **2015**, *45*, 1926–1933. [CrossRef]
4. Goronzy, J.J.; Weyand, C.M. Successful and Maladaptive T Cell Aging. *Immunity* **2017**, *46*, 364–378. [CrossRef] [PubMed]
5. Wertheimer, A.M.; Bennett, M.S.; Park, B.; Uhrlaub, J.L.; Martinez, C.; Pulko, V.; Currier, N.L.; Nikolich-Žugich, D.; Kaye, J.; Nikolich-Žugich, J. Aging and cytomegalovirus infection differentially and jointly affect distinct circulating T cell subsets in humans. *J. Immunol.* **2014**, *192*, 2143–2155. [CrossRef] [PubMed]
6. Verma, K.; Ogonek, J.; Varanasi, P.R.; Luther, S.; Bünting, I.; Thomay, K.; Behrens, Y.L.; Mischak-Weissinger, E.; Hambach, L. Human CD8+ CD57- TEMRA cells: Too young to be called "old". *PLoS ONE* **2017**, *12*, e0177405. [CrossRef]
7. Brenchley, J.M.; Karandikar, N.J.; Betts, M.R.; Ambrozak, D.R.; Hill, B.J.; Crotty, L.E.; Casazza, J.P.; Kuruppu, J.; Migueles, S.A.; Connors, M.; et al. Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. *Blood* **2003**, *101*, 2711–2720. [CrossRef] [PubMed]
8. Koch, S.; Larbi, A.; Derhovanessian, E.; Ozcelik, D.; Naumova, E.; Pawelec, G. Multiparameter flow cytometric analysis of CD4 and CD8 T cell subsets in young and old people. *Immun. Ageing* **2008**, *25*, 5–6. [CrossRef] [PubMed]
9. Nakajima, T.; Schulte, S.; Warrington, K.J.; Kopecky, S.L.; Frye, R.L.; Goronzy, J.J.; Weyand, C.M. T-cell-mediated lysis of endothelial cells in acute coronary syndromes. *Circulation* **2002**, *105*, 570–575. [CrossRef]
10. Tilly, G.; Doan-Ngoc, T.M.; Yap, M.; Caristan, A.; Jacquemont, L.; Danger, R.; Cadoux, M.; Bruneau, S.; Giral, M.; Guerif, P.; et al. IL-15 Harnesses Pro-inflammatory Function of TEMRA CD8 in Kidney-Transplant Recipients. *Front. Immunol.* **2017**, *8*, 778. [CrossRef]
11. Spyridopoulos, I.; Martin-Ruiz, C.; Hilkens, C.; Yadegarfar, M.E.; Isaacs, J.; Jagger, C.; Kirkwood, T.; von Zglinicki, T. CMV seropositivity and T-cell senescence predict increased cardiovascular mortality in octogenarians: Results from the Newcastle 85+ study. *Aging Cell* **2016**, *15*, 389–392. [CrossRef]
12. Yap, M.; Boeffard, F.; Clave, E.; Pallier, A.; Danger, R.; Giral, M.; Dantal, J.; Foucher, Y.; Guillot-Gueguen, C.; Toubert, A.; et al. Expansion of highly differentiated cytotoxic terminally differentiated effector memory CD8+ T cells in a subset of clinically stable kidney transplant recipients: A potential marker for late graft dysfunction. *J. Am. Soc. Nephrol.* **2014**, *25*, 1856–1868. [CrossRef] [PubMed]
13. Boren, E.; Gershwin, M.E. Inflamm-aging: Autoimmunity, and the immune-risk phenotype. *Autoimmun. Rev.* **2004**, *3*, 401–406. [CrossRef] [PubMed]
14. Franceschi, C. Inflammaging as a major characteristic of old people: Can it be prevented or cured? *Nutr. Rev.* **2007**, *65*, 173–176. [CrossRef]
15. Broadley, I.; Pera, A.; Morrow, G.; Davies, K.A.; Kern, F. Expansions of Cytotoxic CD4+CD28- T Cells Drive Excess Cardiovascular Mortality in Rheumatoid Arthritis and Other Chronic Inflammatory Conditions and Are Triggered by CMV Infection. *Front. Immunol.* **2017**, *8*, 195. [CrossRef] [PubMed]
16. Wikby, A.; Månsson, I.A.; Johansson, B.; Strindhall, J.; Nilsson, S.E. The immune risk profile is associated with age and gender: Findings from three Swedish population studies of individuals 20–100 years of age. *Biogerontology* **2008**, *9*, 299–308. [CrossRef] [PubMed]
17. Bardini, G.; Dicembrini, I.; Cresci, B.; Rotella, C.M. Inflammation markers and metabolic characteristics of subjects with 1-h plasma glucose levels. *Diabetes Care* **2010**, *33*, 411–413. [CrossRef] [PubMed]
18. Baumgartner, R.; Forteza, M.J.; Ketelhuth, D.F.J. The interplay between cytokines and the Kynurenine pathway in inflammation and atherosclerosis. *Cytokine* **2019**, *122*, 154148. [CrossRef]
19. Song, P.; Ramprasath, T.; Wang, H.; Zou, M.H. Abnormal kynurenine pathway of tryptophan catabolism in cardiovascular diseases. *Cell Mol. Life Sci.* **2017**, *74*, 2899–2916. [CrossRef] [PubMed]

20. Joisten, N.; Kummerhoff, F.; Koliamitra, C.; Schenk, A.; Walzik, D.; Hardt, L.; Knoop, A.; Thevis, M.; Kiesl, D.; Metcalfe, A.J.; et al. Exercise and the Kynurenine pathway: Current state of knowledge and results from a randomized cross-over study comparing acute effects of endurance and resistance training. *Exerc. Immunol. Rev.* **2020**, *26*, 24–42.

21. Wasserfurth, P.; Nebl, J.; Boßlau, T.K.; Krüger, K.; Hahn, A.; Schuchardt, J.P. Intake of Calanus finmarchicus oil for 12 weeks improves omega-3 index in healthy older subjects engaging in an exercise programme. *Br. J. Nutr.* **2020**, *125*, 432–439. [CrossRef] [PubMed]

22. Matthews, D.R.; Hosker, J.P.; Rudenski, A.S.; Naylor, B.A.; Treacher, D.F.; Turner, R.C. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **1985**, *28*, 412–419. [CrossRef]

23. Noble, R.E. Waist-to-hip ratio versus BMI as predictors of cardiac risk in obese adult women. *West. J. Med.* **2001**, *174*, 240–241. [CrossRef] [PubMed]

24. Ryan, A.S. Insulin resistance with aging: Effects of diet and exercise. *Sports Med.* **2000**, *30*, 327–346. [CrossRef] [PubMed]

25. Parel, Y.; Chizzolini, C. CD4+ CD8+ double positive (DP) T cells in health and disease. *Autoimmun. Rev.* **2004**, *3*, 215–220. [CrossRef] [PubMed]

26. Foster, A.D.; Sivarapatna, A.; Gress, R.E. The aging immune system and its relationship with cancer. *Aging Health* **2011**, *7*, 707–718. [CrossRef]

27. Duggal, N.A. Reversing the immune ageing clock: Lifestyle modifications and pharmacological interventions. *Biogerontology* **2018**, *19*, 481–496. [CrossRef] [PubMed]

28. Aguilar, E.G.; Murphy, W.J. Obesity induced T cell dysfunction and implications for cancer immunotherapy. *Curr. Opin. Immunol.* **2018**, *51*, 181–186. [CrossRef]

29. Yang, H.; Youm, Y.H.; Vandanmagsar, B.; Rood, J.; Kumar, K.G.; Butler, A.A.; Dixit, V.D. Obesity accelerates thymic aging. *Blood* **2009**, *114*, 3803–3812. [CrossRef]

30. Shirakawa, K.; Yan, X.; Shinmura, K.; Endo, J.; Kataoka, M.; Katsumata, Y.; Yamamoto, T.; Anzai, A.; Isobe, S.; Yoshida, N.; et al. Obesity accelerates T cell senescence in murine visceral adipose tissue. *J. Clin. Investig.* **2016**, *126*, 4626–4639. [CrossRef]

31. Yi, H.S.; Kim, S.Y.; Kim, J.T.; Lee, Y.S.; Moon, J.S.; Kim, M.; Kang, Y.E.; Joung, K.H.; Lee, J.H.; Kim, H.J.; et al. T-cell senescence contributes to abnormal glucose homeostasis in humans and mice. *Cell Death Dis.* **2019**, *10*, 249. [CrossRef] [PubMed]

32. Palmer, A.K.; Xu, M.; Zhu, Y.; Pirtskhalava, T.; Weivoda, M.M.; Hachfeld, C.M.; Prata, L.G.; van Dijk, T.H.; Verkade, E.; Casaclang-Verzosa, G.; et al. Targeting senescent cells alleviates obesity-induced metabolic dysfunction. *Aging Cell* **2019**, *18*, e12950. [CrossRef] [PubMed]

33. Nieto-Vazquez, I.; Fernández-Veledo, S.; Krämer, D.K.; Vila-Bedmar, R.; Garcia-Guerra, L.; Lorenzo, M. Insulin resistance associated to obesity: The link TNF-alpha. *Arch. Physiol. Biochem.* **2008**, *114*, 183–194, Erratum in **2009**, *115*, 117. [CrossRef]

34. Kim, J.H.; Bachmann, R.A.; Chen, J. Interleukin-6 and insulin resistance. *Vitam. Horm.* **2009**, *80*, 613–633. [CrossRef]

35. Dienz, O.; Rincon, M. The effects of IL-6 on CD4 T cell responses. *Clin. Immunol.* **2009**, *130*, 27–33. [CrossRef] [PubMed]

36. Liu, J.J.; Raynal, S.; Bailbé, D.; Gausseres, B.; Carbonne, C.; Autier, V.; Movassat, J.; Kergoat, M.; Portha, B. Expression of the kynurenine pathway enzymes in the pancreatic islet cells. Activation by cytokines and glucolipotoxicity. *Biochim. Biophys. Acta* **2015**, *1852*, 980–991. [CrossRef]

37. Wolowczuk, I.; Hennart, B.; Leloire, A.; Bessede, A.; Soichot, M.; Taront, S.; Caiazzo, R.; Raverdy, V.; Pigeyre, M.; ABOS Consortium; et al. Tryptophan metabolism activation by indoleamine 2,3-dioxygenase in adipose tissue of obese women: An attempt to maintain immune homeostasis and vascular tone. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2012**, *303*, 135–143. [CrossRef] [PubMed]

38. Batatinha, H.A.P.; Rosa Neto, J.C.; Krüger, K. Inflammatory features of obesity and smoke exposure and the immunologic effects of exercise. *Exerc. Immunol. Rev.* **2019**, *25*, 96–111. [PubMed]

39. Oxenkrug, G. Insulin resistance and dysregulation of tryptophan-kynurenine and kynurenine-nicotinamide adenine dinucleotide metabolic pathways. *Mol. Neurobiol.* **2013**, *48*, 294–301. [CrossRef] [PubMed]

40. McKee, A.; Morley, J.E. Obesity in the Elderly. Available online: https://www.ncbi.nlm.nih.gov/books/NBK532533/ (accessed on 11 December 2020).

2.5 Paper V

Reasons and consequences of low energy availability in female and male athletes: social environment, adaptations, and prevention

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REVIEW ARTICLE   **Open Access**

Reasons for and Consequences of Low Energy Availability in Female and Male Athletes: Social Environment, Adaptations, and Prevention

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Abstract

Low energy availability (LEA) represents a state in which the body does not have enough energy left to support all physiological functions needed to maintain optimal health. When compared to the normal population, athletes are particularly at risk to experience LEA and the reasons for this are manifold. LEA may result from altered dietary behaviours that are caused by body dissatisfaction, the belief that a lower body weight will result in greater performance, or social pressure to look a certain way. Pressure can also be experienced from the coach, teammates, and in this day and age through social media platforms. While LEA has been extensively described in females and female athletes have started fighting against the pressure to be thin using their social media platforms, evidence shows that male athletes are at risk as well. Besides those obvious reasons for LEA, athletes engaging in sports with high energy expenditure (e.g. rowing or cycling) can unintentionally experience LEA; particularly, when the athletes' caloric intake is not matched with exercise intensity. Whether unintentional or not, LEA may have detrimental consequences on health and performance, because both short-term and long-term LEA induces a variety of maladaptations such as endocrine alterations, suppression of the reproductive axis, mental disorders, thyroid suppression, and altered metabolic responses. Therefore, the aim of this review is to increase the understanding of LEA, including the role of an athlete's social environment and the performance effects related to LEA.

Key Points

- Reasons for low energy availability (LEA) are manifold and may range from unintentional undereating to severe eating disorders. The dietary behaviour of an athlete can be affected by the exercise practice environment. In addition, new challenges from the use of social media have arisen.
- Adaptations associated with LEA are known to negatively influence muscular adaptations in both

endurance and strength and power athletes. Endurance athletes, because of a negative impact on mitochondrial protein synthesis and strength, and power athletes, because of a negative impact on muscle protein synthesis.

- Underperformance due to LEA may not always be noticeable as it can be masked by the positive influence of lower body weight in some sports. Athletes experiencing LEA either increase, stagnate, or decrease performance, depending on the intensity of LEA adaptation and importance of body weight on their performance. If not recognised, LEA can lead to severe health issues that can affect the ability to practice and compete.

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Introduction

It is undeniable that a lower body weight has beneficial effects on athletic performance in distance running velocity, jump height, or aesthetics. However, depending on the sport, the respective body shape and also perceived "ideal" body image varies: While athletes from endurance-based sports commonly seek low body fat and an overall thinner appearance, athletes from strength-orientated and aesthetic sports aim for low body fat and high muscle mass [1]. To achieve the desired outcome, athletes more often go on a variety of diets in an attempt to lose weight [2, 3]. Consequentially, athletes were shown to have a high relative weight variability, body dissatisfaction, and a higher frequency of eating disorders [1, 4, 5].

The pursuit of a certain body image or lower body weight to increase performance may result in low energy availability (LEA)—a state in which the body does not have enough energy to support all physiological functions needed to maintain optimal health. However, athletes may also unintentionally run into LEA during periods with increased training volume or when engaging in sports with high energy expenditure (e.g. rowing or cycling). In the context of exercise-related health risks, LEA has been extensively described in female athletes as a part of the female athlete triad in relation to bone health and menstrual function [6]. Recently, the (IOC) has expanded the triad concept with the term relative energy deficiency in sports (RED-S) to address the accompanying consequences with LEA on health and performance in both sexes [7]. Despite gaining more attention in research, many athletes and their coaches are still not aware of the health consequences of LEA related to the RED-S syndrome or are not aware of the syndrome at all [8, 9]. The need for a better understanding of LEA and RED-S is also reflected by the low priority this topic was found to have within sports federations on the international level [10]. Further, with regard to the female athlete triad, research has shown that coaches often care much more for high performance rather than preserving the long-term health of the athlete [11–13]. Beyond this background and the direct impact of the coach's behaviour on the athletes' health, including their dietary behaviour, coaches need more knowledge about how to act more responsibly and to think beyond "performance only". This is evidenced by female elite runners who now fight against grievances in female sports using the hashtag #fixgirlssports. However, aside from the coach, an athlete's health can also be largely influenced by his or her social environment (e.g. teammates) and, in this day and age, also by social media platforms.

LEA—whether unintentional or not—may have detrimental consequences on health and performance, because both short-term and long-term LEA induces a variety of maladaptations such as endocrine alterations, suppression of the reproductive axis, mental disorders, thyroid suppression, and altered metabolic responses. As the reasons for and consequences of LEA across both sexes are manifold, the aim of this review is to increase the understanding of LEA, including the role of an athlete's social environment and the performance effects related to LEA. We discuss the LEA risk factors influenced by the athletes' direct training environment and explain the physiologic factors and their impact on the athletes' performance. Our suggestions could help to create a better training environment that supports long-term health and exercise participation.

Energy Balance and Energy Availability

The foundation of an appropriate diet with sufficient intake of macro- and micronutrients that will cover an athlete's needs is formed by adequate energy intake. Energy balance is achieved when dietary energy intake matches total energy expenditure. Furthermore, energy availability (EA) is defined as:

$$EA = \frac{\text{Dietary energy intake (kcal)} - \text{Exercise energy expenditure (kcal)}}{\text{Fat Free Mass (kg)}}$$

which equals the dietary energy left after exercise [7]. Low energy availability occurs when either dietary energy intake is too low or energy expanded through exercise is too high, leading to an insufficient amount of energy left to maintain normal physiological functions such as metabolic and immune function, bone health, and the menstrual cycle in female athletes [14, 15]. Although, to date, there are no guidelines prescribing an optimal EA for high performance athletes, studies on habitually sedentary, normal-weight women defined 45 kcal/kg fat-free mass (FFM) as a threshold at which optimal energy balance can be achieved [16, 17]. In contrast, a study on exercising men by Koehler et al. used 40 kcal/kg FFM as a threshold for a balanced EA and showed that this was still enough to support energy balance [18]. However, an EA of 30–45 kcal/kg FFM is already considered a reduced EA, and athletes should only stay within this range for a short period of time, e.g. when aiming to reduce body weight [19]. In any case, clinical studies showed that an EA of < 30 kcal/kg FFM appears to be a threshold at which severe health implications can be observed after only 5 days in healthy young women [17, 20, 21]. Therefore, low EA is commonly defined as EA < 30 kcal/kg FFM. For males, Fagerberg described a prolonged EA < 25 kcal/kg FFM as critical [22]. Nonetheless, despite those attempts to describe precise cut-offs at which symptoms low EA can be observed, individual factors influencing energy availability in an athlete should be considered [23].

Reason for LEA and Prevalence in Different Sports across Sexes

Reasons for the development of LEA are manifold [21]. Changes in dietary intake are often a result from some form of body dissatisfaction and the desire to change the body composition. Body image satisfaction and dissatisfaction were studied in athletes compared to non-athletes and led to inconsistent findings. While some studies reported a generally greater body satisfaction in athletes compared to non-athletes, some reported the opposite, particularly when athletes compared to non-athletes came from a leanness-focused or weight class sport [24, 25]. However, it was found that elite athletes or athletes from a high-performance environment are at higher risk to experience body dissatisfaction which in turn may impact an athlete's dieting behaviour and puts them at risk for eating disorders (ED) and consequentially LEA [1, 26, 27]. In this regard, it is important to understand that LEA can occur with or without an eating disorder [19]. Interestingly, most studies concerning body image, low energy intake, and ED primarily focused on female athletes, as it was thought that females are more susceptible to ED and LEA than males. This is also reflected by the intensively studied female triad—a condition describing the interrelation of LEA and its consequences on the menstrual cycle and bone health in female athletes [28, 29]. Although it may be true to some extent that females are more likely to experience LEA than males, growing evidence indicates that body image issues and unhealthy dietary behaviours are common in male athletes, as well [30]. In any case, restrictive dieting, ED, and the consequential LEA may not only result from body dissatisfaction but also from the belief that changes in body composition will improve performance or the social pressure to look a certain way [1].

Dietary Behaviour and Disordered Eating

Chronic low caloric intake may result from harmless reasons, such as lack of knowledge about appropriate nutrition and the need for optimal energy balance, lack of time to prepare meals, inadequate cooking skills, and financial or even physiological reasons, i.e. loss of appetite after a training session [31]. However, the boundaries between unintentional LEA and the development of an ED are marginal and fluid, e.g. small dietary changes that were started for weight loss can become compulsive [32]. Further, additional factors, including the development of body dissatisfaction or the belief that the athlete needs to be "thin to win", can also manifest in disordered eating [2]. Although knowledge about nutrition is more accessible than ever, and athletes were shown to generally have a better understanding of nutrition than non-athletes, many

misbeliefs, such as "carbohydrates will make you gain weight" or "food intake should only occur within certain time windows", are still common [33–35]. Overall, insufficient knowledge of general sports nutrition in athletes is still evident [31, 36, 37]. Believing and trying some of those misconceptions, e.g. avoiding specific foods, could potentially lead to a lower energy intake than is required to maintain optimal health and performance. This is also reflected by inadequate nutrient intake, particularly with regard to carbohydrates [38, 39]. Overall, the risk to experience any form of ED was increased in athletes when compared to non-athletic controls (13.5% vs. 4.6%) [5]. Particularly athletes engaging in aesthetic, leanness-focused, or weight-sensitive sports were at a higher risk to develop disordered eating patterns than athletes from sports where body weight or shape is secondary (e.g. ball sports) [5, 26, 40]. This was also shown in a study conducted by Torstveit et al., which reported a higher prevalence of EDs in female athletes from leanness-focused sports (46.7%) in comparison to athletes from non-leanness-focused sports (19.8%) [41]. In male athletes, roughly 25% of athletes from aesthetic, leanness-focused, or weight-sensitive sports showed disordered dietary patterns. This was also closely associated with greater body fat percentages and body dissatisfaction [42]. To prevent the development of disordered eating while maintaining optimal health and increasing performance of athletes, needs-based nutritional strategies for athletes in their competitive season, as well as in their off-season, are warranted. We propose to teach athletes a flexible eating behaviour. A flexible eating behaviour that acknowledges the importance of a nutrient-dense diet, while not putting labels such as "good" or "bad" on single food groups or macronutrients (e.g. low carb diet) will support psychological and physiological health in the long run [43]. Further, staff working with athletes should be educated on how to screen and detect signs of disordered eating. If an athlete shows signs of disordered eating, he or she should be guided towards seeking psychological support from a professional. Overall, ensuring psychological support for athletes can be beneficial not only for prevention and treatment of disordered eating behaviours, but also the athlete's overall mental health [44]. Therefore, physiological counselling should be considered an inherent part of working with athletes.

In sum, it becomes obvious that food choice and dietary behaviour of an athlete are influenced by their body image and body satisfaction. However, these are also largely influenced by their social environment. Therefore, the role of coaches, teammates, and the new challenges arising from exposure and use of social media platforms need further attention.

Coaches' Role

Regardless of whether working with an individual athlete or in team sports, coaches play a crucial role in an

athlete's physiological and psychological health [45–47]. Although some coaches work together with different health practitioners to ensure optimal health and performance of their athlete(s), there are still coaches that do not acknowledge the importance of their athletes' nutrition. In fact, evidence shows that not only knowledge about the existence and symptoms of RED-S and the female athlete triad, but also general sports nutrition is poor among coaches (and athletes, as described above) [11–13, 37, 48]. Such lack of knowledge directly affects an athlete's health. Incorrect nutritional beliefs will arise by sharing general knowledge about nutrition, by exposure to unconventional methods to lose weight, or by making weight for a certain weight class [40]. Another difficulty far beyond nutrition is inappropriate comments about an athlete's body, e.g. when he or she has gained some weight or when the coach generally thinks that the athlete would benefit from a lighter body weight [49]. The case of the elite runner Mary Cain is one well-known example that showed how such beliefs can massively influence an athlete's physical and psychological health. In 2019, Cain broke her silence to reveal how her former coach Alberto Salazar excessively pressured her to continue to lose weight. In addition to negative comments about her appearance and body shaming in front of her teammates, Cain also reported about extreme methods to keep her thin (e.g. intake of diuretics). Consequentially, she further reported that she suffered from amenorrhea and osteoporosis—both of which are consequences of prolonged LEA [50, 51]. Kong and Harris showed that more than 60% of the elite athletes from leanness- and non-leanness-focused sports reported feeling pressure from their coaches with regard to their body [52]. Depending on the psychological health of an athlete, exposure to negative comments or drastic methods to lose weight may lay the foundation for body dissatisfaction, low energy intake, and eventually disordered eating in the long run [53]. Moreover, coaches should aim to think beyond "performance only" and keep a critical eye on an athlete's body weight and dietary behaviour, in particular in sports where athletes think they could perform better with a lower body weight, e.g. running.

A healthy coach-athlete relationship is neither athlete- nor coach-centred but is viewed as the type of relationship that leads to mutual benefit for both parties and can ultimately also lead to optimal performance [54]. However, this should also include appropriate nutrition and nutritional strategies for the competitive- and the off-season. If needed, coaches should collaborate with dietitians to help their athletes reach their full potential without sacrificing their health.

Teammates' Role

Another factor that should be considered is the mutual influence among teammates—regardless of team sports or among individual athletes that are trained by the same coach. Although some relationships among teammates may be based on friendship, some may also be based on competitiveness and the urge to be "better than the other". Especially in weight-sensitive sports, weight loss of one athlete may influence the dieting behaviour of the others. In the desire to be "better than her/him", athletes can be influenced by the thought that "If she/he is losing more weight, then I need to lose some too!" or the perception that they are "bigger" than their teammates [55]. Furthermore, teammates can share more drastic methods to lose weight [56]. They can begin with the simple recommendation to skip meals, up to encouraging one another that it is okay to "throw up" when one has eaten too much. Reel and colleagues showed that among female college athletes, teammates had a slightly higher impact on perceived pressure concerning their weight than coaches (36.5% vs. 33.8%) [57]. Therefore, the relationship between teammates should not be overlooked when trying to identify the root cause for a lower energy intake.

The Role of Social Media

Far beyond potential pressure from the immediate environment, athletes in this day and age are largely influenced by media and social media platforms. Particularly in regard to body image, growing evidence has shed light on the harmful effects of media exposure on both sexes [58, 59]. However, despite the promotion of "thinspiration" or "fitspiration" content, new challenges from the use of social media platforms may arise.

Athletes will compare their current size and shape to what they see on social media. Many athletes tend to post favourable pictures [60, 61]. If athletes choose to predominately post pictures in flattering poses or their "top form", it may lead to the impression that the respective athlete can keep the same shape all year round. Unintentionally, this may be putting pressure on other athletes to keep their top form as well without acknowledging that changes in body composition between the competitive and the off-season are normal and, in some cases, (e.g. in bodybuilding) necessary. Additionally, athletes are likely to compare meals, training volume, and load to their opponents' social media posts. In line with the findings from Vogel et al., athletes exposed to profiles of other athletes that seem to have superior positive characteristics may experience a negative influence on their self-esteem [62].

Lastly, athletes are often victims of body shaming and cyber bullying [63]. The smallest changes in an athlete's body shape, such as weight gain, will engender many comments by the social media crowd. Well known cases of body shaming occurred after the 2016 Olympic Games, when the Mexican gymnast Alexa Moreno and

the Ethiopian swimmer Robel Kiros Habt were publicly body shamed for not meeting the "lean body image" that was expected from Olympic athletes [63, 64]. Taken together, negative comments on appearance can negatively impact body image, body satisfaction, and ultimately also influence dietary behaviour, and support the development of LEA, which in severe cases can be accompanied by an eating disorder [65, 66].

On the other side of the spectrum, social media can also be used by athletes themselves to draw attention to grievances within the sporting community. After elite runner Mary Cain shared her story, other female athletes raised their voices and shared their stories. Using their social media platforms, the female runners wanted to draw attention to grievances in female sport and advocate that coaches should think far beyond "body weight only" and put an athlete's health first [67].

Adaptations to Short-Term and Long-Term LEA

Short-term LEA causes a disturbance of metabolic homeostasis in athletes [68]. Early human and animal studies found that fuel is spared at the cost of growth and reproduction to maintain cell survival in times of energy deprivation, [69]. Thus, metabolic mechanisms to conserve energy are evident in male and female athletes in response to prolonged LEA.

Energy Expenditure Adaptations

To maintain basic vital functions at rest, the human body needs a certain amount of energy known as the basal metabolic rate (BMR) or the resting energy expenditure (REE). Together with the non-resting energy expenditure (NREE), REE makes up the total daily energy expenditure (TDEE). While the REE makes up the largest portion of TDEE (roughly 60–70%), the NREE makes up a much smaller portion and can be further subdivided into the non-exercise activity thermogenesis (NEAT), the thermic effect of food (TEF), and exercise activity thermogenesis (EAT) [70].

In male endurance athletes, LEA was associated with a lower REE when compared to athletes with an adequate energy supply [71]. Moreover, as energy homeostasis is also controlled by the secretion of leptin from adipose tissue via feedback to the hypothalamus (e.g. reduce REE when fat stores are low), it is unsurprising that short-term LEA, at 15 kcal/kg FFM/day, decreased basal leptin and insulin levels of exercising men concurrently with fat loss [18]. Lower leptin levels depend solely on energy availability, as shown in male rowers [72] and exercising healthy females [16], but are also a response to chronic exercise training [73]. Hilton and Loucks found lower 24-h leptin and a lower amplitude of the diurnal rhythm of leptin when EA fell below 30 kcal/kg FFM/day [16]. In addition to reducing the BMR, lower leptin levels also

suppress the thyroid, the reproductive and growth hormone axes, and the inflammatory response [74, 75].

A reduction of TDEE is also mediated by thyroid suppression. Four days of LEA, at a threshold between 19 and 25 kcal/kg LBM/day, induced low triiodothyronine (T3) in exercising women who were previously inactive [76]. In contrast, males are more robust to short-term LEA as indicated by no significant alteration of free T3 concentration after five days of LEA at 15 kcal/kg FFM/day [18]. However, 3 weeks of caloric restriction attenuates T3 and NEAT in lean healthy non-exercising males [77]. In general, chronic exercise training induces a light physiologic rise of thyroid hormones in elite strength athletes and female endurance runners [78, 79], which may, to a certain degree, counteract the reduction of T3 and NEAT.

Taken together data suggest, that as energy availability declines, whether intentional through caloric restriction or unintentional through increased exercise energy expenditure, metabolic adaptions will occur (Fig. 1). Although those alterations are normal and negligible if athletes return to an appropriate energy intake, e.g. after a structured dieting phase, it may be problematic in individuals that have a constant drive towards getting thinner and/or leaner. While body weight will drop at the beginning of a dieting phase, a plateau in weight loss will inevitably occur after prolonged low energy intake [68]. Although this is a normal physiological adaption, some athletes may start to further decrease energy intake to continue to lose weight. This behaviour will lead to a downward spiral of caloric restriction, losing weight, and plateauing followed by another cycle—all of which will ultimately result in LEA and likely in the development of an ED.

Changes in Blood Substrate Levels

Metabolic changes following LEA are also evident on the blood substrate level. Following short-term LEA lasting 5 days, fasting blood glucose and insulin levels decrease, while free fatty acids and glycerol increase in male athletes [18]. In a similar fashion, insulin levels decrease, while the ketone β-hydroxybutyrate (BHB) increases in females [20]. Additionally, in female athletes positive for the female triad, hypoglycaemia and hypercholesterolemia are common [19]. In opposition to the cardio-protective function of exercise, the altered cholesterol substrate levels may be unfavourable for cardiovascular health in the long term [80]. The results indicate that decreased glycolytic activity and increased lipolytic activity during LEA in athletes of both sexes occur in order to save mainly one fuel: carbohydrates. Conceivably, this is due to limited glycogen stores [81]. Reasonably, the higher lipolytic activity in very lean athletes may be a challenge for the physiologic system since high-performance athletes' fat stores were often

Fig. 1 Components of total daily energy expenditure and adaptions to low energy availability are shown. **a** Total daily energy expenditure (TDEE) consists of the resting energy expenditure (REE) and the non-resting energy expenditure (NREE). NREE can be further subdivided into the thermic effect of food (TEF), non-exercise activity thermogenesis (NEAT), and exercise activity thermogenesis (EAT). Of those components, NEAT and EAT describe energy expended through physical activity (PA). **b** When energy availability is low, either by restricted dietary energy intake or increased energy exercise expenditure (EEE), metabolic adaptions to conserve energy occur. Those encompass a decline in basal metabolic rate (BMR), NEAT, and, if caloric intake is restricted, also in TEF. Generally, EAT will decrease as well but may be elevated in individuals increasing their training volume. Therefore, adaptations in this component are variable. Figure modified according to MacLean et al. [70]

reported to be close to the lower limit of 5% for men and 12% for women, particularly in athletes engaging in endurance or aesthetic sports [22, 82, 83]. In one study, only 3 days of LEA reduced muscular glycogen stores and conserved energy in the form of adipose tissue in male endurance athletes [84]. Moreover, in female athletes with severe eating disorders, such as anorexia nervosa, the restriction of food and fluid intake can lead to imbalanced electrolytes, anaemia, and hypotension [19, 85]. As the impact of LEA slightly differs across sex, we further discuss reproductive and bone adaptations in separate sections.

Sex-Specific Endocrine and Bone Adaptations
Suppression of the female athlete's reproductive system by LEA has been described extensively within the triad research [86]. Reproductive health in females is sensitive to short-term LEA, in terms of disrupting luteinising hormone pulsatility during waking and sleeping hours when EA falls below 30 kcal/kg FFM/day [20]. LEA below 30 kcal/kg/FFM indicates a clinical menstrual status and clearly differentiates amenorrhea, defined as no menses for 90 days, from eumenorrhea in exercising female athletes [87]. Female runners with functional hypothalamic amenorrhea (FHA) express lower oestrogen levels [88]. Accordingly, rapid bone loss due to low oestrogen is associated with menstrual disorders [29]. For instance, the bone fracture risk of amenorrhoeic

female elite runners was nine times higher than their healthy counterparts [89].

Furthermore, during LEA, oestradiol and progesterone are reduced in female athletes with RED-S [90]. In females, oestradiol levels are extremely sensitive to and are attenuated by within-day LEA [91]. Oestradiol preserves bone mass density (BMD) by increasing osteoclasts and decreasing osteoblast apoptosis [92]. Adequate oral administration of oestrogens can prevent a reduction in bone mass after menopause [92]. Before menopause, a lack of successful oral oestrogen therapy is likely due to the downregulation of insulin-like growth factor-1 (IGF-1) [93]. Therefore, in the young female athletes, improving LEA may have a stronger effect than oestrogen therapy. In contrast to oral oestrogens, transdermal oestrogen treatment has been effective in increasing bone mass density in amenorrhoeic female athletes [94].

The influence of LEA on BMD, apart from reproductive hormones, is highlighted by analysis of bone turnover markers [95]. Ihle and Loucks found changes in three bone turnover markers in response to short-term LEA in exercising females, namely a reduction of the bone formation markers plasma osteocalcin and serum type I procollagen carboxy-terminal propeptide, and an increase in the bone resorption marker urinary N-terminal telopeptide [96]. Extreme LEA (10 kcal/kg FFM/day) increased bone resorption markers, while formation

markers declined at minor levels of energy restriction between 20-30 kcal [96]. In contrast, a more recent study found that 3 days of LEA at 15 kcal/kg LBM did not significantly alter the common bone resorption markers, such as β-carboxyl-terminal cross-linked telopeptide of type I collagen amino-terminal propeptide of type 1 procollagen, though it also lowered the bone formation marker carboxyl-terminal propeptide of procollagen type 1 [97]. The decrease in the same bone formation marker during 5 days of LEA at 15 kcal/kg LBM was verified in another study by the same group. In the same study, no significant changes in these newer bone turnover markers were found in males [95].

Exercise hypogonadal male condition (EHMC) is the syndrome affecting reproductive function of males, akin to the triad in females. During EHMC, the hypothalamic-pituitary-gonadal axis is disturbed along with reduced serum testosterone levels (TES) as a response to LEA [98]. Although TES values remain in the low end of the normal clinical range [89], symptoms of hypogonadism—fatigue, sexual dysfunction, and low bone mineral density—are present. The syndrome was first diagnosed in endurance-trained males; however, it is also seen in power athletes [98] and energy-restricted bodybuilders [99]. Of note, protein intake does not mediate TES reduction in exercising males [100]. However, current information on LEA and endocrine changes in males is based on case reports and studies with small sample sizes [101]; therefore, further research is needed.

Similar to female athletes, progesterone and oestradiol levels are reduced in response to LEA in male athletes [90]. Consistently, architecture and turnover markers of bone were significantly reduced in endurance runners [89]. For male athletes other than runners, evidence on bone turnover markers is less clear. However, there is also some evidence of lower bone health in male athletes with LEA who participate in race horse riding or cycling events [102]. In addition, young males with a low BMI sharing the belief that leanness improves performance are more likely to have low BMD [103].

Overall, the altered endocrine profile caused by LEA, including decreased anabolic hormones (e.g. leptin, oestradiol, TES) in both male and female endurance athletes, is harmful to BMD [89]. The sensitivity of bone turnover makers seems to be sex-specific and higher in female than in males when experiencing short-term LEA. Of course, there are other factors affecting BMD. The interested reader may refer to a more comprehensive review on the athletes' bone health (see Sale and Elliot-Sale, 2019 [90]).
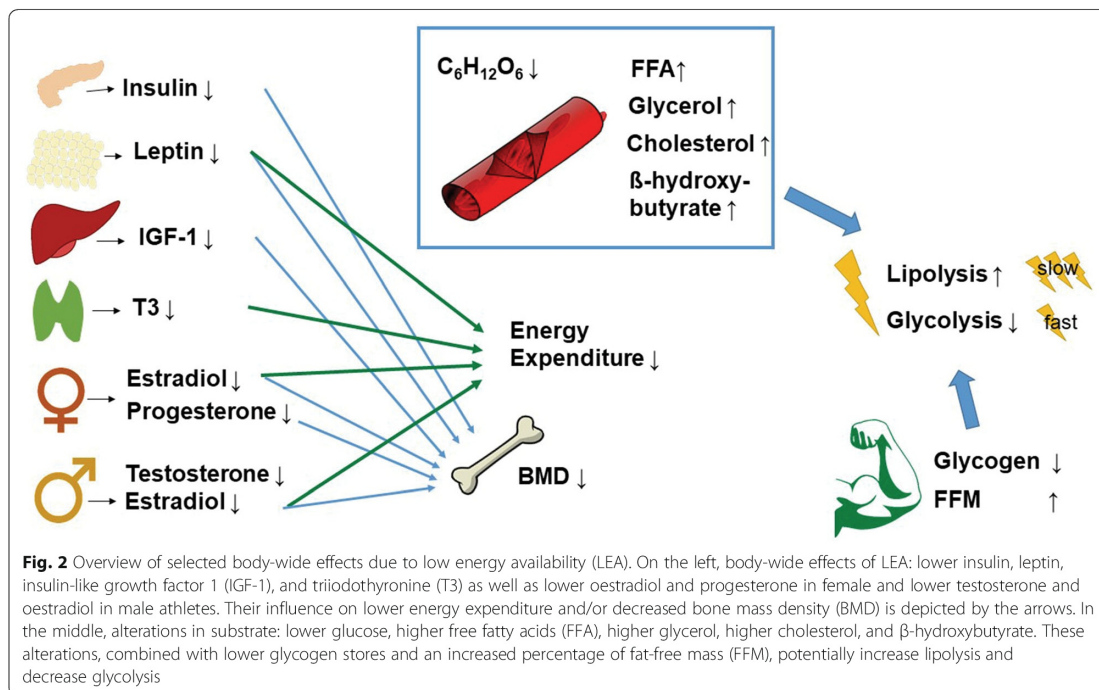
Suppressed Growth Hormone Axis
Other key metabolic hormones have been discussed to mediate TDEE adaptations by lowering REE such as those decreasing anabolic pathways [68]. For example, insulin-like growth factor 1 (IGF-1) mediating muscular and bone growth [104] is commonly higher in well-trained, lean subjects [105]. On the contrary, it is significantly lower when EA is reduced to 10–20 kcal/kg FFM/day in exercising women who were previously sedentary [20]. In line with the lower IGF-1, its carrier insulin-like growth factor-binding protein 3 is also decreased in females when they have LEA [20]. Overall, the concentration of IGF-1 is linked to BMD in pre- and post-menopausal women [92]. In contrast, males are more robust to short-term LEA, as IGF-1 was not significantly altered after five days of LEA at 15 kcal/kg FFM/day. However, a trend for a reduction was present [18]. Long-term caloric restriction in male lean body builders lasting 11 weeks reduced IGF-1 in the period prior to competition [99]. In contrast, growth hormone (GH) levels mediating IGF-1 are greater in male and female athletes positive for RED-S [90] and may indicate the development of a GH resistance [101]. For instance, in male power athletes with low body fat, growth hormone treatment administered in a double-blind controlled trial neither influenced body composition nor muscle strength [106]. Of note, protein intake does not mediate IGF-1 levels in exercising athletes—independent of their sex [100]. An overview of body-wide effects of LEA that lead to changes within the athlete's body and also have an impact on performance is provided in Fig. 2.

Immune Homeostasis
An association between LEA, impaired immunity, and infection is likely, as nutrients are also important for the immunometabolism of leukocytes [107]. However, there is currently no evidence supporting this hypothesis [108]. Athletes face multiple challenges other than LEA that suppress immune function, ranging from psychological stress to sleep deprivation [109]. Supporting of the RED-S definition, which includes impairments of immunity, is the association between LEA and upper respiratory tract infection (URTI) risk in female athletes [110]. In addition, Sarin et al. described immunosuppression after energy restriction in immune cells such as T cells and B cells [111]. Furthermore, interleukin-6 (IL-6) expression mediating inflammation is reduced by sufficient energy intake before exercise in order to spare muscular glycogen [112]. Pasiakos et al. found that increased IL-6 levels after endurance exercise are negatively correlated with energy balance and glycogen stores [113]. This change was also accompanied by increased hepcidin levels regulating iron metabolism. In this context, Badenhorst et al. assume that an increase in baseline levels of hepcidin arises when either LEA occurs for several days during high energy expenditure or when inflammation as indicated by increased baseline IL-6 levels

Fig. 2 Overview of selected body-wide effects due to low energy availability (LEA). On the left, body-wide effects of LEA: lower insulin, leptin, insulin-like growth factor 1 (IGF-1), and triiodothyronine (T3) as well as lower oestradiol and progesterone in female and lower testosterone and oestradiol in male athletes. Their influence on lower energy expenditure and/or decreased bone mass density (BMD) is depicted by the arrows. In the middle, alterations in substrate: lower glucose, higher free fatty acids (FFA), higher glycerol, higher cholesterol, and β-hydroxybutyrate. These alterations, combined with lower glycogen stores and an increased percentage of fat-free mass (FFM), potentially increase lipolysis and decrease glycolysis

is present [114]. Similarly, haematological constraints, such as abnormal bruising, anaemia, low haemoglobin, iron, or ferritin, are 1.6 times more likely in female athletes at risk for LEA [115]. A dysfunctional haematopoiesis may be present in female athletes with LEA, since prolonged energy restriction and intense exercise are associated with lower erythrocyte and platelet counts, while the number of white blood cells increases [111].

Mental Aspects and Predisposition
With regard to psychological stress, anxiety and depression have a significant effect on immunity, and an attenuated resistance to infections is well described [108]. Accordingly, male endurance athletes with symptoms of LEA more often achieve high exercise dependence scores known to correlate with ED [116]. An increased drive for thinness in female endurance runners with LEA and FHA compared to athletes with normal menses has also been found [19]. Apparently, increased psychological stress on the athlete, if present, will lead to an attenuated resistance to infections. Noticeably, a mental predisposition for an increased risk of LEA or for mental consequences of LEA has to be investigated in the future.

Chronic stress increases cortisol levels, which may increase the risk of anxiety and depression [117]. Elevated cortisol levels were also found in females with triad risk

factors [19], as these are closely related to augmented psychological stress during training and fasting. The cortisol change does not happen unequivocally due to severe energy restriction [118]. Furthermore, cortisol levels are highly variable throughout the day due to circadian rhythm [119]. Moreover, results on cortisol levels as evidence of associations between LEA and cortisol levels are inconsistent [90]. Again, cortisol levels in females may be more sensitive to LEA, since after mere hours of within-day LEA, levels significantly increased [91]. We need highly standardised research to clarify if changes in cortisol are a primary or secondary consequence of prolonged LEA in the future.

Consequences for Performance
As RED-S in athletes was defined recently, there is little research on performance in regard to this specific syndrome. One study by Ackerman et al. investigated the body-wide influence of LEA in female athletes through several questionnaires [115]. Performance effects of LEA were "decreased training response, impaired judgement, decreased coordination, decreased concentration, irritability, depression, and decreased endurance performance" [115], and lower bone health. The authors did not find any evidence of immunologically harmful adaptations due to LEA. As no evidence of an attenuated immune response exists (see the "Mental aspects and

predisposition" section), it is hypothesised that the immune system may be the last system to shut down. However, performance decrements are not exclusive to female athletes. In male cyclists, prolonged EA, despite higher training loads, resulted in underperformance, while there was no association between body fat and performance for this sport [120].

Even though there is a dearth of direct research on performance effects of RED-S, optimal energy supply is essential to optimise athletic performance [121]. We have outlined energy-conserving mechanisms by endocrine hormones and reduced glycogen stores as some of the homeostatic adaptations to LEA in the previous section. It is hypothesised that a deficient energy homeostasis is the main cause underlying the development of overtraining [121]. Skeletal muscle, which controls locomotion, is a key regulator of metabolic homeostasis. Repetitive exercise bouts increase metabolic enzymes and protein content in the long term, whereby variations exist, depending on the placement of the exercise on the continuum between endurance and resistance exercise. Endurance exercise has a pronounced effect on mitochondrial protein content and resistance exercise has a pronounced effect on myofibrillar protein content to enable performance enhancement [122]. T3 mediates the elevated mitochondrial content in endurance athletes as it stimulates ATPase activity and increases heat production [123]. Therefore, low T3 associated with LEA reduces ATPase activity, leading to reduced energy production by mitochondria and has a negative effect on aerobic energy production and vice versa. This is why increasing NEAT and reducing body weight by T3 supplementation is a promising strategy to enhance performance [124]. On the other edge of the exercise continuum is the pronounced effect on muscle protein content. On the one hand, stimulation of muscular protein synthesis is promoted by anabolic hormones, such as insulin, IGF-1, and TES. On the other hand, catabolic glucocorticoids, such as cortisol, increase protein turnover and initiate skeletal muscle protein breakdown [125]. A negative effect on muscular protein synthesis due to LEA is implied by reduced anabolic hormones and a potential increase of cortisol in more severe or prolonged LEA. Thus, it is unsurprising that female runners with secondary FHA demonstrated a lower neuromuscular performance reflected by longer manual reaction time and significant lower knee muscular strength and endurance compared to eumenorrheic athletes [88]. In judo athletes, caloric restriction was associated with poor performance while increasing fatigue and tension and decreasing vigour, as well [126]. In addition, a decreased performance in other power athletes seems likely due to decreases in glycogen stores [81]. To summarise, muscular adaptations important to both
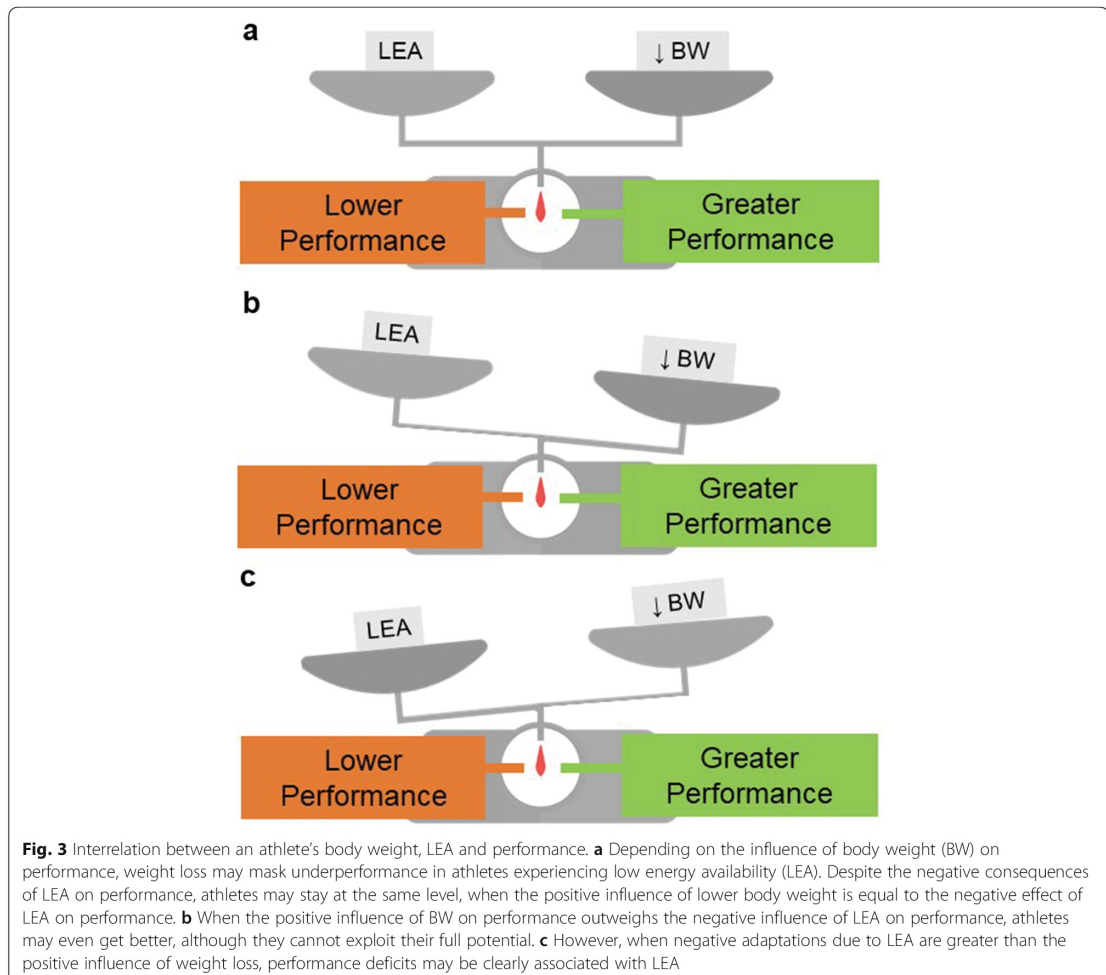
endurance and resistance athletes are disturbed by LEA alterations. The influence of LEA on performance may be masked by the tremendous effect of body weight on performance or may even result in slight performance enhancement or stagnation (Fig. 3). A clear decrease in muscular performance may not be obvious despite the athlete underperforming.

Next to optimal energy supply, continuous training is key for high-performance athletes to maintain high training volumes in order to reach the limits of physical performance. Of course, the more days athletes report as sick days, the more training hours are reduced [127]. There is some evidence that training absence due to illness is three times higher in athletes with a risk of LEA. These athletes miss more than 22 days of training within a year, which is 3 times more than athletes at no risk [128]. However, as already mentioned, illness data is scarce. As a reduced EA negatively influenced bone health in physically active individuals [129], more evidence is found on training absence due to bone injuries of male athletes with low TES and amenorrhoeic females in runners. Injury risk was 4.5 times higher as compared to healthy counterparts [89]. With increasing risk for the female triad, the risk for bone stress injuries in a large cohort of 259 females increased significantly [130]. However, while LEA in female athletes has a negative effect on BMD, there is a concurrent performance-enhancing outcome of endocrine alterations concerning oestrogen. The associated low oestrogen increases the stiffness of connective tissue such as ligaments and tendons [128]. A higher stiffness of the connective tissue is associated with performance parameters such as jump height [131]. Therefore, the risk of ligament injuries and the power performance of women with low oestrogen potentially increases [132]. As we pointed out in the Adaptations to Short-Term and Long-Term LEA section, for male athletes other than runners, evidence on BMD is less clear. Overall, the risk of training absence may increase when exercising with LEA and thus leads to underperformance—noticeable or not.

Conclusion
Taken together, the evidence shows that LEA causes body-wide effects paving the way for the recognition of RED-S as a multifactorial condition in athletes. Causes for LEA range from harmless reasons, such as lack of motivation to prepare meals, up to deliberate chronic undereating with severe Eds. The consequences on health and performance outlined by the female triad and the hypogonadal male condition are self-evident, though better-controlled, highly standardised trials are needed.

Given the information above, this article should highly encourage coaches to support a healthy environment

Fig. 3 Interrelation between an athlete's body weight, LEA and performance. **a** Depending on the influence of body weight (BW) on performance, weight loss may mask underperformance in athletes experiencing low energy availability (LEA). Despite the negative consequences of LEA on performance, athletes may stay at the same level, when the positive influence of lower body weight is equal to the negative effect of LEA on performance. **b** When the positive influence of BW on performance outweighs the negative influence of LEA on performance, athletes may even get better, although they cannot exploit their full potential. **c** However, when negative adaptations due to LEA are greater than the positive influence of weight loss, performance deficits may be clearly associated with LEA

during daily practice. Staff involved in the supervision of athletes should be sensitised for signs of LEA and openly talk to athletes. With regard to performance, professionals should keep in mind that performance reductions due to LEA might not necessarily come with LEA. In weight-sensitive sports, athletes may even enhance performance being at LEA due to lower body weight and a higher tissue stiffness. Beyond that, the sociocultural pressure and influence of media on athletes should not be overlooked. Yet again, more studies investigating their impact on athletes in this day and age are necessary. As evidence shows the high prevalence of feeling pressure from coaches or teammates, particularly coaches should acknowledge their impact on athletes and act responsibly.

Abbreviations
BMD: Bone mass density; BMR: Basal metabolic rate; EA: Energy availability; EAT: Exercise activity thermogenesis; ED: Eating disorders; EHMC: Exercise hypogonadal male condition; FFM: Fat-free mass; FHA: Functional hypothalamic amenorrhea; GH: Growth hormone; IGF-1: Insulin-like growth factor 1; IL-6: Interleukin-6; IOC: International Olympic Committee; LEA: Low energy availability; NEAT: Non-exercise activity thermogenesis; NREE: Non-resting energy expenditure; RED-S: Relative Energy Deficiency Syndrome; REE: Resting energy expenditure; T3: Triiodothyronine; TDEE: Total daily energy expenditure; TEF: Thermic effect of food; TES: Testosterone; URTI: Upper respiratory tract infection

Authors' Contributions
Jana Palmowski and Paulina Wasserfurth had the idea for the review, performed the literature search, and drafted the original manuscript. Andreas Hahn and Karsten Krüger critically revised the work. The authors read and approved the final manuscript.

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References
1. Sundgot-Borgen J, Torstveit MK. Aspects of disordered eating continuum in elite high-intensity sports: disordered eating in elite athletes. Scand J Med Sci Sports. 2010;20:112–21. https://doi.org/10.1111/j.1600-0838.2010.01190.x.
2. Martinsen M, Bratland-Sanda S, Eriksson AK, Sundgot-Borgen J. Dieting to win or to be thin? A study of dieting and disordered eating among adolescent elite athletes and non-athlete controls. Br J Sports Med. 2010;44: 70–6. https://doi.org/10.1136/bjsm.2009.068668.
3. de Bruin AP (Karin), Oudejans RRD, Bakker FC. Dieting and body image in aesthetic sports: a comparison of Dutch female gymnasts and non-aesthetic sport participants. Psychol Sport Exerc 2007;8:507–520. doi.https://doi.org/10.1016/j.psychsport.2006.10.002.
4. Hagmar M, Hirschberg AL, Berglund L, Berglund B. Special attention to the weight-control strategies employed by olympic athletes striving for leanness is required. Clin J Sport Med. 2008;18:5–9. https://doi.org/10.1097/JSM.0b013e31804c77bd.
5. Sundgot-Borgen J, Torstveit MK. Prevalence of eating disorders in elite athletes is higher than in the general population. Clin J Sport Med Off J Can Acad Sport Med. 2004;14:25–32. https://doi.org/10.1097/00042752-200401000-00005.
6. Slater J, Brown R, McLay-Cooke R, Black K. Low energy availability in exercising women: historical perspectives and future directions. Sports Med Auckl NZ. 2017;47:207–20. https://doi.org/10.1007/s40279-016-0583-0.
7. Mountjoy M, Sundgot-Borgen JK, Burke LM, Ackerman KE, Blauwet C, Constantini N, et al. IOC consensus statement on relative energy deficiency in sport (RED-S): 2018 update. Br J Sports Med. 2018;52:687–97. https://doi.org/10.1136/bjsports-2018-099193.
8. Tenforde A, Beauchesne A, Borg-Stein J, Hollander K, McInnis K, Kotler D, et al. Awareness and comfort treating the female athlete triad and relative energy deficency in sport among healthcare p-roviders. Dtsch Z Für Sportmed. 2020;71:76–80. https://doi.org/10.5960/dzsm.2020.422.
9. Brunet P, Ambresin A-E, Gojanovic B. What do you know of RED-S ? A field study on adolescent coaches' knowledge. Rev Med Suisse. 2019;15:1334–8.
10. Mountjoy M, Costa A, Budgett R, Dvorak J, Engebretsen L, Miller S, et al. Health promotion through sport: international sport federations' priorities, actions and opportunities. Br J Sports Med. 2018;52:54–60. https://doi.org/10.1136/bjsports-2017-097900.
11. Mukherjee S, Chand V, Wong XX, Choong PP, Lau VS, Wang SC, et al. Perceptions, awareness and knowledge of the female athlete triad amongst coaches – are we meeting the expectations for athlete safety? Int J Sports Sci Coach. 2016;11:545–51. https://doi.org/10.1177/1747954116654781.
12. Brown KN, Wengreen HJ, Beals KA. Knowledge of the female athlete triad, and prevalence of triad risk factors among female high school athletes and their coaches. J Pediatr Adolesc Gynecol. 2014;27:278–82. https://doi.org/10.1016/j.jpag.2013.11.014.
13. Pantano KJ. Knowledge, attitude, and skill of high school coaches with regard to the female athlete triad. J Pediatr Adolesc Gynecol. 2017;30:540–5. https://doi.org/10.1016/j.jpag.2016.09.013.
14. Shetty P. Adaptation to low energy intakes: the responses and limits to low intakes in infants, children and adults. Eur J Clin Nutr. 1999;53:s14–33. https://doi.org/10.1038/sj.ejcn.1600741.
15. Mountjoy M, Sundgot-Borgen J, Burke L, Carter S, Constantini N, Lebrun C, et al. The IOC consensus statement: beyond the female athlete triad—relative energy deficiency in sport (RED-S). Br J Sports Med. 2014;48: 491–7. https://doi.org/10.1136/bjsports-2014-093502.
16. Hilton LK, Loucks AB. Low energy availability, not exercise stress, suppresses the diurnal rhythm of leptin in healthy young women. Am J Physiol-Endocrinol Metab. 2000;278:E43–9. https://doi.org/10.1152/ajpendo.2000.278.1.E43.
17. Loucks AB, Verdun M, Heath EM, (With the Technical Assistance of T. Law, Sr. and J. R. Thuma). Low energy availability, not stress of exercise, alters LH pulsatility in exercising women. J Appl Physiol 1998;84:37–46. doi https://doi.org/10.1152/jappl.1998.84.1.37.
18. Koehler K, Hoerner NR, Gibbs JC, Zinner C, Braun H, De Souza MJ, et al. Low energy availability in exercising men is associated with reduced leptin and insulin but not with changes in other metabolic hormones. J Sports Sci. 2016;34:1921–9. https://doi.org/10.1080/02640414.2016.1142109.
19. Melin A, Tornberg ÅB, Skouby S, Møller SS, Sundgot-Borgen J, Faber J, et al. Energy availability and the female athlete triad in elite endurance athletes: energy availability in female athletes. Scand J Med Sci Sports. 2015;25:610–22. https://doi.org/10.1111/sms.12261.
20. Loucks AB, Thuma JR. Luteinizing hormone pulsatility is disrupted at a threshold of energy availability in regularly menstruating women. J Clin Endocrinol Metab. 2003;88:297–311. https://doi.org/10.1210/jc.2002-020369.
21. Melin AK, Heikura IA, Tenforde A, Mountjoy M. Energy availability in athletics: health, performance, and physique. Int J Sport Nutr Exerc Metab. 2019;29:152–64. https://doi.org/10.1123/ijsnem.2018-0201.
22. Fagerberg P. Negative consequences of low energy availability in natural male bodybuilding: a review. Int J Sport Nutr Exerc Metab. 2018;28:385–402. https://doi.org/10.1123/ijsnem.2016-0332.
23. De Souza MJ, Koltun KJ, Strock NC, Williams NI. Rethinking the concept of an energy availability threshold and its role in the female athlete triad. Curr Opin Physiol. 2019;10:35–42. https://doi.org/10.1016/j.cophys.2019.04.001.
24. Hausenblas HA, Downs DS. Comparison of body image between athletes and nonathletes: a meta-analytic review. J Appl Sport Psychol. 2001;13:323–39. https://doi.org/10.1080/104132001753144437.
25. Swami V, Steadman L, Tovée MJ. A comparison of body size ideals, body dissatisfaction, and media influence between female track athletes, martial artists, and non-athletes. Psychol Sport Exerc. 2009;10:609–14. https://doi.org/10.1016/j.psychsport.2009.03.003.
26. Byrne S, McLean N. Elite athletes: effects of the pressure to be thin. J Sci Med Sport. 2002;5:80–94. https://doi.org/10.1016/S1440-2440(02)80029-9.
27. Varnes JR, Stellefson ML, Janelle CM, Dorman SM, Dodd V, Miller MD. A systematic review of studies comparing body image concerns among female college athletes and non-athletes, 1997-2012. Body Image. 2013;10: 421–32. https://doi.org/10.1016/j.bodyim.2013.06.001.
28. De Souza MJ, Nattiv A, Joy E, Misra M, Williams NI, Mallinson RJ, et al. 2014 Female athlete triad coalition consensus statement on treatment and return to play of the female athlete triad: 1st International Conference held in San Francisco, California, May 2012 and 2nd International Conference held in Indianapolis, Indiana, May 2013. Br J Sports Med. 2014;48:289. https://doi.org/10.1136/bjsports-2013-093218.
29. Nattiv A, Loucks AB, Manore MM, Sanborn CF, Sundgot-Borgen J, Warren MP, et al. American College of Sports Medicine position stand. The female athlete triad. Med Sci Sports Exerc. 2007;39:1867–82. https://doi.org/10.1249/mss.0b013e318149f111.
30. Burke LM, Close GL, Lundy B, Mooses M, Morton JP, Tenforde AS. Relative energy deficiency in sport in male athletes: a commentary on its presentation among selected groups of male athletes. Int J Sport Nutr Exerc Metab. 2018;28:364–74. https://doi.org/10.1123/ijsnem.2018-0182.
31. Heaney S, O'Connor H, Naughton G, Gifford J. Towards an understanding of the barriers to good nutrition for elite athletes. Int J Sports Sci Coach. 2008; 3:391–401. https://doi.org/10.1260/174795408786238542.
32. Giel KE, Hermann-Werner A, Mayer J, Diehl K, Schneider S, Thiel A, et al. Eating disorder pathology in elite adolescent athletes. Int J Eat Disord. 2016; 49:553–62. https://doi.org/10.1002/eat.22511.
33. Condon EM, Dube KA, Herbold NH. The influence of the low-carbohydrate trend on collegiate athletes' knowledge, attitudes, and dietary intake of carbohydrates. Top Clin Nutr. 2007;22:175–84. https://doi.org/10.1097/01.TIN.0000270136.22969.d7.

34. Rosenbloom C. Popular diets and athletes: premises, promises, pros, and pitfalls of diets and what athletes should know about diets and sports performance. Nutr Today. 2014;49:244–8. https://doi.org/10.1097/NT.0000000000000043.

35. Shriver LH, Betts NM, Wollenberg G. Dietary intakes and eating habits of college athletes: are female college athletes following the current sports nutrition standards? J Am Coll Heal. 2013;61:10–6. https://doi.org/10.1080/07448481.2012.747526.

36. Cockburn E, Fortune A, Briggs M, Rumbold P. Nutritional knowledge of UK coaches. Nutrients. 2014;6:1442–53. https://doi.org/10.3390/nu6041442.

37. Torres-McGehee TM, Pritchett KL, Zippel D, Minton DM, Cellamare A, Sibilia M. Sports nutrition knowledge among collegiate athletes, coaches, athletic trainers, and strength and conditioning specialists. J Athl Train. 2012;47:205–11. https://doi.org/10.4085/1062-6050-47.2.205.

38. Condo D, Lohman R, Kelly M, Carr A. Nutritional intake, sports nutrition knowledge and energy availability in female Australian rules football players. Nutrients. 2019;11:971. https://doi.org/10.3390/nu11050971.

39. Baranauskas M, Stukas R, Tubelis L, Žagminas K, Šurkienė G, Švedas E, et al. Nutritional habits among high-performance endurance athletes. Medicina (Mex). 2015;51:351–62. https://doi.org/10.1016/j.medici.2015.11.004.

40. Sundgot-Borgen J, Garthe I. Elite athletes in aesthetic and Olympic weight-class sports and the challenge of body weight and body compositions. J Sports Sci. 2011;29:S101–14. https://doi.org/10.1080/02640414.2011.565783.

41. Torstveit MK, Rosenvinge JH, Sundgot-Borgen J. Prevalence of eating disorders and the predictive power of risk models in female elite athletes: a controlled study: eating disorders in athletes and controls. Scand J Med Sci Sports. 2007;18:108–18. https://doi.org/10.1111/j.1600-0838.2007.00657.x.

42. Goltz FR, Stenzel LM, Schneider CD. Disordered eating behaviors and body image in male athletes. Rev Bras Psiquiatr. 2013;35:237–42. https://doi.org/10.1590/1516-4446-2012-0840.

43. Helms ER, Prnjak K, Linardon J. Towards a sustainable nutrition paradigm in physique sport: a narrative review. Sports. 2019;7:172. https://doi.org/10.3390/sports7070172.

44. Henriksen K, Schinke R, Moesch K, McCann S, Parham WD, Larsen CH, et al. Consensus statement on improving the mental health of high performance athletes. Int J Sport Exerc Psychol. 2019:1–8. https://doi.org/10.1080/1612197X.2019.1570473.

45. Appleton PR, Duda JL. Examining the interactive effects of coach-created empowering and disempowering climate dimensions on athletes' health and functioning. Psychol Sport Exerc. 2016;26:61–70. https://doi.org/10.1016/j.psychsport.2016.06.007.

46. Beckner BN, Record RA. Navigating the thin-ideal in an athletic world: influence of coach communication on female athletes' body image and health choices. Health Commun. 2016;31:364–73. https://doi.org/10.1080/10410236.2014.957998.

47. Stirling AE, Kerr GA. The perceived effects of elite athletes' experiences of emotional abuse in the coach–athlete relationship. Int J Sport Exerc Psychol. 2013;11:87–100. https://doi.org/10.1080/1612197X.2013.752173.

48. Trakman G, Forsyth A, Devlin B, Belski R. A systematic review of athletes' and coaches' nutrition knowledge and reflections on the quality of current nutrition knowledge measures. Nutrients. 2016;8:570. https://doi.org/10.3390/nu8090570.

49. Heffner JL, Ogles BM, Gold E, Marsden K, Johnson M. Nutrition and eating in female college athletes: a survey of coaches. Eat Disord. 2003;11:209–20. https://doi.org/10.1080/10640260390218666.

50. I Was the Fastest Girl in America, Until I Joined Nike 2019. https://www.nytimes.com/2019/11/07/opinion/nike-running-mary-cain.html.

51. Ackerman KE, Stellingwerff T, Elliott-Sale KJ, Baltzell A, Cain M, Goucher K, et al. #REDS (Relative Energy Deficiency in Sport): time for a revolution in sports culture and systems to improve athlete health and performance. Br J Sports Med. 2020;54:369–70. https://doi.org/10.1136/bjsports-2019-101926.

52. Kong P, Harris LM. The sporting body: body image and eating disorder symptomatology among female athletes from leanness focused and nonleanness focused sports. Aust J Psychol. 2015;149:141–60. https://doi.org/10.1080/00223980.2013.846291.

53. Jones RL, Glintmeyer N, McKenzie A. Slim bodies, eating disorders and the coach-athlete relationship: a tale of identity creation and disruption. Int Rev Sociol Sport. 2005;40:377–91. https://doi.org/10.1177/1012690205060231.

54. Jowett S, Shanmugam V. Relational coaching in sport: its psychological underpinnings and practical effectiveness. Routledge Int. Handb. Sport Psychol. Abingdon, Oxon: Routledge; 2016. p. 471–84.

55. Arthur-Cameselle JN, Quatromoni PA. Factors related to the onset of eating disorders reported by female collegiate athletes. Sport Psychol. 2011;25:1–17. https://doi.org/10.1123/tsp.25.1.1.

56. Engel SG, Johnson C, Powers PS, Crosby RD, Wonderlich SA, Wittrock DA, et al. Predictors of disordered eating in a sample of elite Division I college athletes. Eat Behav. 2003;4:333–43. https://doi.org/10.1016/S1471-0153(03)00031-X.

57. Reel JJ, Petrie TA, SooHoo S, Anderson CM. Weight pressures in sport: examining the factor structure and incremental validity of the weight pressures in sport — Females. Eat Behav. 2013;14:137–44. https://doi.org/10.1016/j.eatbeh.2013.01.003.

58. Barlett CP, Vowels CL, Saucier DA. Meta-analyses of the effects of media images on men's body-image concerns. J Soc Clin Psychol. 2008;27:279–310. https://doi.org/10.1521/jscp.2008.27.3.279.

59. Grabe S, Ward LM, Hyde JS. The role of the media in body image concerns among women: a meta-analysis of experimental and correlational studies. Psychol Bull. 2008;134:460–76. https://doi.org/10.1037/0033-2909.134.3.460.

60. Geurin-Eagleman AN, Burch LM. Communicating via photographs: a gendered analysis of Olympic athletes' visual self-presentation on Instagram. Sport Manag Rev. 2016;19:133–45. https://doi.org/10.1016/j.smr.2015.03.002.

61. Smith LR, Sanderson J. I'm going to Instagram it! An analysis of athlete self-presentation on Instagram. J Broadcast Electron Media. 2015;59:342–58. https://doi.org/10.1080/08838151.2015.1029125.

62. Vogel EA, Rose JP, Roberts LR, Eckles K. Social comparison, social media, and self-esteem. Psychol Pop Media Cult. 2014;3:206–22. https://doi.org/10.1037/ppm0000047.

63. Villalon C, Weiller-Abels K. NBC's televised media portrayal of female athletes in the 2016 Rio Summer Olympic Games: a critical feminist view. Sport Soc. 2018;21:1137–57. https://doi.org/10.1080/17430437.2018.1442206.

64. Avena KA. Gender equality in the Olympic movement: not a simple question, not a simple answer. J Philos Sport. 2017;44:329–41. https://doi.org/10.1080/00948705.2017.1359616.

65. Bailey SD, Ricciardelli LA. Social comparisons, appearance related comments, contingent self-esteem and their relationships with body dissatisfaction and eating disturbance among women. Eat Behav. 2010;11:107–12. https://doi.org/10.1016/j.eatbeh.2009.12.001.

66. Nowell C, Ricciardelli LA. Appearance-based comments, body dissatisfaction and drive for muscularity in males. Body Image. 2008;5:337–45. https://doi.org/10.1016/j.bodyim.2008.06.002.

67. O'Malley K. The rhetoric surrounding Mary Cain. Pepperdine J Comm Res: 35–42.

68. Trexler ET, Smith-Ryan AE, Norton LE. Metabolic adaptation to weight loss: implications for the athlete. J Int Soc Sports Nutr. 2014;11:7. https://doi.org/10.1186/1550-2783-11-7.

69. Laughlin GA, Yen SSC. Hypoleptinemia in women athletes: absence of a diurnal rhythm with amenorrhea. J Clin Endocrinol Metab. 1997;82:318–21. https://doi.org/10.1210/jcem.82.1.3840.

70. MacLean PS, Bergouignan A, Cornier M-A, Jackman MR. Biology's response to dieting: the impetus for weight regain. Am J Physiol-Regul Integr Comp Physiol. 2011;301:R581–600. https://doi.org/10.1152/ajpregu.00755.2010.

71. Thompson J, Manore MM, Skinner JS. Resting metabolic rate and thermic effect of a meal in low- and adequate-energy intake male endurance athletes. Int J Sport Nutr. 1993;3:194–206. https://doi.org/10.1123/ijsn.3.2.194.

72. Desgorces F, Chennaoui M, Gomez-Merino D, Drogou C, Guezennec C. Leptin response to acute prolonged exercise after training in rowers. Eur J Appl Physiol. 2004;91. https://doi.org/10.1007/s00421-003-1030-0.

73. Fedewa MV, Hathaway ED, Ward-Ritacco CL, Williams TD, Dobbs WC. The effect of chronic exercise training on leptin: a systematic review and meta-analysis of randomized controlled trials. Sports Med. 2018;48:1437–50. https://doi.org/10.1007/s40279-018-0897-1.

74. Ahima RS, Saper CB, Flier JS, Elmquist JK. Leptin regulation of neuroendocrine systems. Front Neuroendocrinol. 2000;21:263–307. https://doi.org/10.1006/frne.2000.0197.

75. Bi X, Loo YT, Henry CJ. Does circulating leptin play a role in energy expenditure? Nutrition. 2019;60:6–10. https://doi.org/10.1016/j.nut.2018.08.015.

76. Loucks AB, Heath EM. Induction of low-T3 syndrome in exercising women occurs at a threshold of energy availability. Am J Phys. 1994;266:R817–23. https://doi.org/10.1152/ajpregu.1994.266.3.R817.

77. Müller MJ, Enderle J, Pourhassan M, Braun W, Eggeling B, Lagerpusch M, et al. Metabolic adaptation to caloric restriction and subsequent refeeding: the Minnesota Starvation Experiment revisited. Am J Clin Nutr. 2015;102: 807–19. https://doi.org/10.3945/ajcn.115.109173.

78. Alén M, Pakarinen A, Häkkinen K. Effects of prolonged training on serum thyrotropin and thyroid hormones in elite strength athletes. J Sports Sci. 1993;11:493–7. https://doi.org/10.1080/02640419308730018.

79. Hohtari H, Pakarinen A, Kauppila A. Serum concentrations of thyrotropin, thyroxine, triiodothyronine and thyroxine binding globulin in female endurance runners and joggers. Acta Endocrinol. 1987;114:41–6. https://doi.org/10.1530/acta.0.1140041.

80. Logue D, Madigan SM, Delahunt E, Heinen M, Mc Donnell S-J, Corish CA. Low energy availability in athletes: a review of prevalence, dietary patterns, physiological health, and sports performance. Sports Med. 2018;48:73–96. https://doi.org/10.1007/s40279-017-0790-3.

81. Stellingwerff T, Maughan RJ, Burke LM. Nutrition for power sports: middle-distance running, track cycling, rowing, canoeing/kayaking, and swimming. J Sports Sci. 2011;29:S79–89. https://doi.org/10.1080/02640414.2011.589469.

82. Silva M-RG, Paiva T. Low energy availability and low body fat of female gymnasts before an international competition. Eur J Sport Sci. 2015;15:591–9. https://doi.org/10.1080/17461391.2014.969323.

83. Sundgot-Borgen J, Meyer NL, Lohman TG, Ackland TR, Maughan RJ, Stewart AD, et al. How to minimise the health risks to athletes who compete in weight-sensitive sports review and position statement on behalf of the Ad Hoc Research Working Group on Body Composition, Health and Performance, under the auspices of the IOC Medical Commission. Br J Sports Med. 2013;47:1012–22. https://doi.org/10.1136/bjsports-2013-092966.

84. Kojima C, Ishibashi A, Tanabe Y, Iwayama K, Kamei A, Takahashi H, et al. Muscle glycogen content during endurance training under low energy availability. Med Sci Sports Exerc. 2020;52:187–95. https://doi.org/10.1249/MSS.0000000000002098.

85. Walsh JME, Wheat ME, Freund K. Detection, evaluation, and treatment of eating disorders: the role of the primary care physician. J Gen Intern Med. 2000;15:577–90. https://doi.org/10.1046/j.1525-1497.2000.02439.x.

86. De Souza MJ, Koltun KJ, Etter CV, Southmayd EA. Current status of the female athlete triad: update and future directions. Curr Osteoporos Rep. 2017;15:577–87. https://doi.org/10.1007/s11914-017-0412-x.

87. Reed JL, De Souza M, Mallinson RJ, Scheid JL, Williams NI. Energy availability discriminates clinical menstrual status in exercising women. J Int Soc Sports Nutr. 2015;12:11. https://doi.org/10.1186/s12970-015-0072-0.

88. Tornberg ÅB, Melin A, Koivula FM, Johansson A, Skouby S, Faber J, et al. Reduced neuromuscular performance in amenorrheic elite endurance athletes. Med Sci Sports Exerc. 2017;49:2478–85. https://doi.org/10.1249/MSS.0000000000001383.

89. Heikura IA, Uusitalo ALT, Stellingwerff T, Bergland D, Mero AA, Burke LM. Low energy availability is difficult to assess but outcomes have large impact on bone injury rates in elite distance athletes. Int J Sport Nutr Exerc Metab. 2018;28:403–11. https://doi.org/10.1123/ijsnem.2017-0313.

90. Sale C, Elliott-Sale KJ. Nutrition and athlete bone health. Sports Med. 2019; 49:139–51. https://doi.org/10.1007/s40279-019-01161-2.

91. Fahrenholtz IL, Sjödin A, Benardot D, Tornberg ÅB, Skouby S, Faber J, et al. Within-day energy deficiency and reproductive function in female endurance athletes. Scand J Med Sci Sports. 2018;28:1139–46. https://doi.org/10.1111/sms.13030.

92. Southmayd EA, De Souza MJ. A summary of the influence of exogenous estrogen administration across the lifespan on the GH/IGF-1 axis and implications for bone health. Growth Hormon IGF Res. 2017;32:2–13. https://doi.org/10.1016/j.ghir.2016.09.001.

93. Ackermann KE, Singhal V, Baskaran C, Slattery M, Campoverde Reyes KJ, Toth A, et al. Oestrogen replacement improves bone mineral density in oligo-amenorrhoeic athletes: a randomised clinical trial. Br J Sports Med. 2019;53: 229–36. https://doi.org/10.1136/bjsports-2018-099723.

94. Nose-Ogura S, Yoshino O, Kanatani M, Dohi M, Tabei K, Harada M, et al. Effect of transdermal estradiol therapy on bone mineral density of amenorrheic female athletes. Scand J Med Sci Sports. 2020;sms.13679. https://doi.org/10.1111/sms.13679.

95. Papageorgiou M, Elliott-Sale KJ, Parsons A, Tang JCY, Greeves JP, Fraser WD, et al. Effects of reduced energy availability on bone metabolism in women and men. Bone. 2017;105:191–9. https://doi.org/10.1016/j.bone.2017.08.019.

96. Ihle R, Loucks AB. Dose-response relationships between energy availability and bone turnover in young exercising women. J Bone Miner Res Off J Am Soc Bone Miner Res. 2004;19:1231–40. https://doi.org/10.1359/JBMR.040410.

97. Papageorgiou M, Martin D, Colgan H, Cooper S, Greeves JP, Tang JCY, et al. Bone metabolic responses to low energy availability achieved by diet or exercise in active eumenorrheic women. Bone. 2018;114:181–8. https://doi.org/10.1016/j.bone.2018.06.016.

98. Hooper DR, Tenforde AS, Hackney AC. Treating exercise-associated low testosterone and its related symptoms. Phys Sportsmed. 2018;46:427–34. https://doi.org/10.1080/00913847.2018.1507234.

99. Mäestu J, Eliakim A, Jürimäe J, Valter I, Jürimäe T. Anabolic and catabolic hormones and energy balance of the male bodybuilders during the preparation for the competition. J Strength Cond Res. 2010;24:1074–81. https://doi.org/10.1519/JSC.0b013e3181cb6fd3.

100. Henning PC, Margolis LM, McClung JP, Young AJ, Pasiakos SM. High protein diets do not attenuate decrements in testosterone and IGF-I during energy deficit. Metabolism. 2014;63:628–32. https://doi.org/10.1016/j.metabol.2014.02.007.

101. Wong HK, Hoermann R, Grossmann M. Reversible male hypogonadotropic hypogonadism due to energy deficit. Clin Endocrinol. 2019:cen.13973. https://doi.org/10.1111/cen.13973.

102. Tenforde AS, Barrack MT, Nattiv A, Fredericson M. Parallels with the female athlete triad in male athletes. Sports Med. 2016;46:171–82. https://doi.org/10.1007/s40279-015-0411-y.

103. Tenforde AS, Sayres LC, Sainani KL, Fredericson M. Evaluating the relationship of calcium and vitamin D in the prevention of stress fracture injuries in the young athlete: a review of the literature. PM R. 2010;2:945–9. https://doi.org/10.1016/j.pmrj.2010.05.006.

104. Elloumi M, El Elj N, Zaouali M, Maso F, Filaire E, Tabka Z, et al. IGFBP-3, a sensitive marker of physical training and overtraining. Br J Sports Med. 2005; 39:604–10. https://doi.org/10.1136/bjsm.2004.014183.

105. Nemet D, Connolly PH, Pontello-Pescatello AM, Rose-Gottron C, Larson JK, Galassetti P, et al. Negative energy balance plays a major role in the IGF-I response to exercise training. J Appl Physiol. 2004;96:276–82. https://doi.org/10.1152/japplphysiol.00654.2003.

106. Deyssig R, Frisch H, Blum WF, Waldhör T. Effect of growth hormone treatment on hormonal parameters, body composition and strength in athletes. Acta Endocrinol. 1993;128:313–8. https://doi.org/10.1530/acta.0.1280313.

107. Calder PC. Feeding the immune system. Proc Nutr Soc. 2013;72:299–309. https://doi.org/10.1017/S0029665113001286.

108. Walsh NP. Nutrition and athlete immune health: new perspectives on an old paradigm. Sports Med. 2019;49:153–68. https://doi.org/10.1007/s40279-019-01160-3.

109. Keaney LC, Kilding AE, Merien F, Dulson DK. The impact of sport related stressors on immunity and illness risk in team-sport athletes. J Sci Med Sport. 2018;21:1192–9. https://doi.org/10.1016/j.jsams.2018.05.014.

110. Drew M, Vlahovich N, Hughes D, Appaneal R, Burke LM, Lundy B, et al. Prevalence of illness, poor mental health and sleep quality and low energy availability prior to the 2016 Summer Olympic Games. Br J Sports Med. 2018;52:47–53. https://doi.org/10.1136/bjsports-2017-098208.

111. Sarin HV, Gudelj I, Honkanen J, Ihalainen JK, Vuorela A, Lee JH, et al. Molecular pathways mediating immunosuppression in response to prolonged intensive physical training, low-energy availability, and intensive weight loss. Front Immunol. 2019;10:907. https://doi.org/10.3389/fimmu.2019.00907.

112. Hennigar SR, McClung JP, Pasiakos SM. Nutritional interventions and the IL-6 response to exercise. FASEB J Off Publ Fed Am Soc Exp Biol. 2017;31:3719–28. https://doi.org/10.1096/fj.201700080R.

113. Pasiakos SM, Margolis LM, Murphy NE, McClung HL, Martini S, Gundersen Y, et al. Effects of exercise mode, energy, and macronutrient interventions on inflammation during military training. Phys Rep. 2016;4. https://doi.org/10.14814/phy2.12820.

114. Badenhorst CE, Black KE, O'Brien WJ. Hepcidin as a prospective individualized biomarker for individuals at risk of low energy availability. Int J Sport Nutr Exerc Metab. 2019;29:671–81. https://doi.org/10.1123/ijsnem.2019-0006.

115. Ackerman KE, Holtzman B, Cooper KM, Flynn EF, Bruinvels G, Tenforde AS, et al. Low energy availability surrogates correlate with health and

performance consequences of Relative Energy Deficiency in Sport. Br J Sports Med. 2019;53:628–33. https://doi.org/10.1136/bjsports-2017-098958.

116. Torstveit MK, Fahrenholtz IL, Lichtenstein MB, Stenqvist TB, Melin AK. Exercise dependence, eating disorder symptoms and biomarkers of relative energy deficiency in sports (RED-S) among male endurance athletes. BMJ Open Sport Exerc Med. 2019;5:e000439. https://doi.org/10.1136/bmjsem-2018-000439.

117. Leonard BE. HPA and immune axes in stress: involvement of the serotonergic system. Neuroimmunomodulation. 2006;13:268–76. https://doi.org/10.1159/000104854.

118. Nakamura Y, Walker BR, Ikuta T. Systematic review and meta-analysis reveals acutely elevated plasma cortisol following fasting but not less severe calorie restriction. Stress Amst Neth. 2016;19:151–7. https://doi.org/10.3109/10253890.2015.1121984.

119. Bellastella G, De Bellis A, Maiorino MI, Paglionico VA, Esposito K, Bellastella A. Endocrine rhythms and sport: it is time to take time into account. J Endocrinol Investig. 2019;42:1137–47. https://doi.org/10.1007/s40618-019-01038-1.

120. Keay N, Francis G, Hind K. Low energy availability assessed by a sport-specific questionnaire and clinical interview indicative of bone health, endocrine profile and cycling performance in competitive male cyclists. BMJ Open Sport Exerc Med. 2018;4:e000424. https://doi.org/10.1136/bmjsem-2018-000424.

121. Saris WH. The concept of energy homeostasis for optimal health during training. Can J Appl Physiol Rev Can Physiol Appl. 2001;26(Suppl):S167–75. https://doi.org/10.1139/h2001-051.

122. Egan B, Zierath JR. Exercise metabolism and the molecular regulation of skeletal muscle adaptation. Cell Metab. 2013;17:162–84. https://doi.org/10.1016/j.cmet.2012.12.012.

123. Steinacker JM, Brkic M, Simsch C, Nething K, Kresz A, Prokopchuk O, et al. Thyroid hormones, cytokines, physical training and metabolic control. Horm Metab Res. 2005;37:538–44. https://doi.org/10.1055/s-2005-870419.

124. Hildebrandt T, Lai JK, Langenbucher JW, Schneider M, Yehuda R, Pfaff DW. The diagnostic dilemma of pathological appearance and performance enhancing drug use. Drug Alcohol Depend. 2011;114:1–11. https://doi.org/10.1016/j.drugalcdep.2010.09.018.

125. Sheffield-Moore M, Urban RJ. An overview of the endocrinology of skeletal muscle. Trends Endocrinol Metab. 2004;15:110–5. https://doi.org/10.1016/j.tem.2004.02.009.

126. Degoutte F, Jouanel P, Bègue RJ, Colombier M, Lac G, Pequignot JM, et al. Food restriction, performance, biochemical, psychological, and endocrine changes in Judo athletes. Int J Sports Med. 2006;27:9–18. https://doi.org/10.1055/s-2005-837505.

127. Mårtensson S, Nordebo K, Malm C. High training volumes are associated with a low number of self-reported sick days in elite endurance athletes. J Sports Sci Med. 2014;13:929–33.

128. Logue DM, Madigan SM, Heinen M, McDonnell S-J, Delahunt E, Corish CA. Screening for risk of low energy availability in athletic and recreationally active females in Ireland. Eur J Sport Sci. 2019;19:112–22. https://doi.org/10.1080/17461391.2018.1526973.

129. Papageorgiou M, Dolan E, Elliott-Sale KJ, Sale C. Reduced energy availability: implications for bone health in physically active populations. Eur J Nutr. 2018;57:847–59. https://doi.org/10.1007/s00394-017-1498-8.

130. Barrack MT, Gibbs JC, De Souza MJ, Williams NI, Nichols JF, Rauh MJ, et al. Higher incidence of bone stress injuries with increasing female athlete triad–related risk factors: a prospective multisite study of exercising girls and women. Am J Sports Med. 2014;42:949–58. https://doi.org/10.1177/0363546513520295.

131. Bojsen-Møller J, Magnusson SP, Rasmussen LR, Kjaer M, Aagaard P. Muscle performance during maximal isometric and dynamic contractions is influenced by the stiffness of the tendinous structures. J Appl Physiol. 2005;99:986–94. https://doi.org/10.1152/japplphysiol.01305.2004.

132. Chidi-Ogbolu N, Baar K. Effect of estrogen on musculoskeletal performance and injury risk. Front Physiol. 2018;9:1834. https://doi.org/10.3389/fphys.2018.01834.

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3. General discussion

This dissertation aimed to investigate the health effects of a low dose, moderate intensity, aerobic and resistance training program alone versus the exercise program combined with a healthy diet or CO intake on body composition, glucose and lipid metabolism, and sirtuin activity in elderly untrained individuals. Additionally, the impact of immune cell senescence on insulin sensitivity was analyzed. Therefore, a randomized, controlled 12-week interventional trial was conducted with 134 elderly individuals (50-70 years), who were assigned to either a control group (CON), 2x/week aerobic and resistance training only (EX), exercise routine combined with a healthy diet (EXDC) or exercise routine combined with the intake of 2g/day CO (EXCO). At the beginning and the end of the study, dietary intake, physical activity outside of the intervention, body composition, and biochemical markers were analyzed. In addition to the interventional study, a literature search was performed to evaluate health from exercise and dietary habits in performance-oriented athletes. The results are presented in four original research papers and one review.

3.1 Exercise- versus exercise-plus diet-induced changes on body composition and metabolism in elderly untrained individuals

Results from **Paper I-III** demonstrate that health effects of a low dose, moderate intensity exercise program combined with dietary measures are more effective in impacting different health markers in untrained healthy older adults than exercise training alone.

3.1.1 Dietary intake and physical activity

Health markers are largely impacted by physical activity and the diet (see chapters **1.2-1.4**). Therefore, dietary intake was assessed not only to evaluate compliance of the EXDC group, but also to assess whether dietary intake changed in the other study groups during the intervention. Physical activity outside of the intervention was also monitored.

From the beginning to the end of the study, caloric intake decreased non-significantly about 200 kcal in the three intervention groups EX, EXDC, and EXCO. Within the EXDC group, the effectiveness of the dietary counseling was shown by an improvement in diet quality. More precisely, an increase in consumed portions of fruit (1.43 ± 0.98 to 2.14 ± 1.26 portions/day), vegetables (0.86 ± 0.34 portions to 1.49 ± 1.25 portions/day), fish (1.77 ± 1.53 to 2.69± 1.71 portions/week) and increase in fiber intake (19.0 ± 5.97 g/day to 22.0 ± 6.43 g/day) could be detected when compared to the other study groups. However, it should be noted that the FFQ used to assess food group intake did not differentiate between the consumption of fatty fish versus lean fish. This would have been a valuable information to compare the outcomes between the EXDC and EXCO group. Other changes observed within the other study groups

are negligible (e.g., slightly decreased carbohydrate and fiber intake in EX) and the physical activity outside of the intervention did not differ significantly. Therefore, changes in the health markers measured can be linked to the exercise routine and dietary changes made in accordance with the guidelines of the DGE or CO intake.

3.1.2 Body composition

As outlined in chapter **1.4.1**, body composition can be favorably influenced by exercise. The present work focused on combined aerobic and resistance training, which was shown to elicit favorable effects on LBM and FM [137].

Although not significantly different between the four study groups, it still has to be pointed out that a more pronounced reduction in body weight was observed among the study groups that performed the exercise program. In addition, those groups also showed an increase in the phase angle, which indicates improvent of cellular health [209]. Aside from that, no further significant effects could be observed in the EX group (*Paper I*). However, when exercise was combined with either of the dietary modifications, some significant changes in body composition were detected. The most profound changes were observed in the EXDC and the EXCO group, who both showed significantly decreased FM at the end of the intervention (EXDC: -1.41± 2.13 kg; EXCO: -1.70± 2.45 kg).

In the EXDC group, it can be assumed that the improved diet quality supported body fat loss. The underlying mechanisms are discussed to include increased satiety due to higher intakes of vegetables, fruit and fiber as well as overall lower consumption of energy dense foods [141,210]. Yet, it is surprising that no changes in LBM (more precisely BCM) were found. Particularly, as available comparable studies who used 2x/week of resistance training and healthy diet recommendations (defined as n-6/n-3 PUFA ratio <2 and a n-3 PUFA rich diet) showed an increase in leg lean mass and increased hypertrophy of type 2 muscle fibers after 24 weeks in elderly women [24,211]. However, only women were analyzed in those studies and caloric as well as macronutrient intake were adjusted, whereas the present work used general dietary guidelines. Yet, it also needs to be acknowledged that those studies used dual energy X-ray absorptiometry for body composition analysis whereas we used BIA.

In any case, the observed fat loss in the EXCO group may have been mediated by CO intake. This is supported by findings from a meta-analysis, which reported that n-3 PUFA supplementation can promote moderate weight loss, including a reduction in body fat [121]. Although not statistically significant results obtained from this analysis for the reduction of fat mass was showed a similar direction.

In that regard, several potential mechanisms including increased fat oxidation or improved adipocyte function (i.e., increased lipolysis and reduced lipogenesis) have been proposed [212–214]. Increased fat oxidation is further discussed as a consequence of improved β-oxidation and biogenesis of mitochondria [215], likely mediated by AMP-activated protein kinase (AMPK) resulting in activation of different transcription factors. However, as such mechanisms were mainly investigated in animal models or *in vitro* [213,216], the dose of n-3 PUFAs that may exert beneficial effects in humans remains unclear. Yet, when compared to previous human studies using supplements with >2 g of EPA+DHA, CO contains comparatively low amounts with only ~200 mg EPA+DHA per 2 g serving. This is lower than the 250 mg/day recommended by the EFSA [96] and less than half of the 500 mg/day recommended by the ISSFAL [97]. Therefore, other components present in CO may play a role in modulation of body composition too. In that regard, the carotene astaxanthin may potentially have beneficial effects on counteracting obesity [217]. However, as the doses used in other studies [218,219] highly exceed the amounts present in CO, it is more likely that the effect of CO is due to the combined effect of multiple components.

Nonetheless, when interpreting the results obtained from the study it should be noted that although BIA is an accepted tool for estimation of body composition in longitudinal studies [220–222], this method has some limitations. It is susceptible to confounders such as vigorous physical activity, dietary intake, or hydration status [220,223,224]. To minimize confounding effects, all measurements were performed after an overnight fast. Yet, hydration status was not controlled. In addition, other anthropometrical measurements such as e.g., measurement of skin folds could have been valuable.

3.1.3 Glucose and lipid metabolism

Although this study was conducted in metabolically healthy participants, with no diagnosed health conditions, assessing markers of glucose and lipid metabolism can still yield valuable information. In this study, it is also of interest, as specific body composition traits are linked to metabolic markers [225]. Moreover, both metabolic pathways are also known to be impacted by dietary intake and exercise [5,6,53].

Results from **Paper I** demonstrate no significant changes in markers of glucose- and lipid metabolism within any of the intervention groups. However, some trends could be observed. For example, the EXDC and EXCO groups both showed a slight decrease in fasting insulin levels (EXDC: -2.14 ± 4.59 µU/ml; EXCO: -1.42 ± 2.86 µU/ml). Regarding the EXDC group, those results would support that an overall healthy diet favourably impacts insulin levels and may prevent the development of type-2 diabetes [226]. Results from the EXCO group are supported by findings from an animal study, which reported improved glucose metabolism after

CO supplementation [17]. In addition, this group also showed the highest reduction of triglycerides (-10.5 ± 23.6 mg/dl), which falls in line with results showing a triglyceride-lowering effect of n-3 PUFAs supplementation [117]. Altogether, results from this study did not show any significant impact of exercise alone on metabolic markers, but showed some trends, particularly when exercise was combined with CO supplementation.

Altogether, results from **Paper I** showed that exercise combined with CO supplementation may have potential health benefits. However, when examining the physiological effects of marine oils it is important to ensure that the active ingredients (meaning the FAs) enter the body in sufficiently high amounts [104]. As CO contains the n-3 PUFAs SDA, EPA, and DHA and some beneficial effects may be led back to those FAs, investigation of ingestion of those PUFAs from CO on PUFA blood levels is important. Particularly, because unlike other commercial marine n-3 PUFA supplements, >80% of FAs in CO are bound as wax esters. In the past, this FA binding form was discussed as poorly digestible in mammals [227]. However, a single-dose study conducted by Cook et al. (2016) demonstrated comparable bioavailability of EPA and DHA from CO to ethyl ester bound EPA and DHA from fish oil when measured over a time course of 72 hours [228]. To date, no data on the long-term effects of CO intake on blood levels exist. Therefore, the present work investigated the long-term effects using the O3I.

Results presented in **Paper II** are the first data to show that a 12-week intake of 2 g CO (providing ~200 mg of EPA+DHA), significantly increased the O3I from 6.07 ± 1.29% to 7.37 ± 1.10%, which is an increase of 1.29 ± 0.66%. To account for the potential effects of the exercise program on the O3I, the EX group served as a control group in this analysis. However, as no difference in O3I was found in this group, the results indicate that a moderate exercise program does not influence the O3I. Anyhow, the increase in the O3I observed in the EXCO group is comparable with results from other studies that used a similar dose of EPA+DHA [168,229–231]. Interestingly, of those four comparable studies, one used EPA+DHA bound as ethyl esters [230], one used phospholipid-bound EPA+DHA [231] and the other two used triglyceride-bound EPA+DHA [168,229]. However, the other studies reported low baseline values of an O3I of ~3-4%, while the participants of the present work had a baseline value of ~6%. This is of interest, because the response of the O3I to n-3 PUFA supplementation is dependent on the baseline O3I, meaning a lower baseline O3I results in a greater O3I response [232,233]. Yet, the low dose of EPA+DHA from CO successfully improved the O3I to an extent comparable with the other studies.

Anyhow, it is unlikely that the increase in the O3I is solely due to the gastrointestinal uptake of preformed EPA and DHA. In fact, as the most abundant n-3 PUFA in CO is SDA, which is one of the precursors of EPA and DHA, it is likely that ingestion of this FA contributed to the

observed increase in the O3I. This is further supported by results from a single-dose study that reported increased EPA (47%) and DHA (21%) blood plasma levels 72 hours after ingestion of echium oil (providing ~3 g SDA) [234]. In addition, a long-term study found an increase of the O3I by 19.5% after a 16-week supplementation with SDA enriched soybean oil (providing ~3.7 g SDA) [235]. However, regarding the single FAs analyzed in the RBCs, the long-term study reported only significant increases of EPA and SDA. In comparison, the present work found increases of 41% in SDA, 44% in EPA and 17% in DHA. Another FA present in CO, which may further contribute to an increase in the O3I is the 22:1n11 (cetoleic acid). This FA was demonstrated to stimulate the conversion of ALA to EPA and DHA in human hepatocytes [236]. Taken together, the results from *Paper II* show that CO is a good source of n-3 PUFAs and can successfully contribute to a better supply.

One limitation in the analysis was the estimation of EPA and DHA intake from the background diet solely via 3-day food logs. As this data is self-reported, it is prone to over- or underestimation and is dependent on the food selection of the participant on the recording days. For example, consumption of a fish meal on one day during the three recording days may lead to fluctuations in the mean EPA+DHA levels. However, as only the EXCO group showed significant changes in RBC PUFA levels while no changes were detected in the EX group, dietary intake of EPA+DHA from the background diet can also be excluded as a confounder in this analysis.

3.1.4 Sirtuins

Sirtuins regulate numerous metabolic pathways such as energy metabolism and have been shown to be up-regulated during exercise [184,237]. A previous study found that diet may have an impact on the activation of sirtuins (namely SIRT1, SIRT3 and SIRT5) in response to acute exercise [238]. Therefore, the effect of chronic exercise combined with a healthy diet or CO supplementation was hypothesized to differently impact sirtuin activity. Results presented in *Paper III* are the first to show that chronic exercise over 12 weeks can induce changes in the activity of SIRT1 and SIRT3 in an elderly population and that upregulation of those sirtuins might be additionally impacted by dietary modifications.

With regards to SIRT1, exercise induced an increase in enzyme activity in all three intervention groups. However, when compared among each other, this increase differed only significantly between CON and EXDC and CON and EXCO. As chronic exercise elicits many favorable metabolic effects such as an increased capacity to use substrates for energy production [239], it seems evident that the activity of SIRT1 as "master regulator of metabolic pathways" [240] is increased as well. The improved substrate utilization is of particular interest, as it also

includes fat oxidation – a metabolic process which is discussed to be positively influenced by the n-3 PUFAs [214]. SIRT1 along with n-3 PUFAs are both discussed to elicit beneficial effects via their impact on PPARs [176,214]. Therefore, it may be hypothesized that exercise combined with CO intake impacts sirtuin activity because both modulate the same pathways.

Similar to results observed for SIRT1, exercise also significantly induced activity of SIRT3 in EX, EXDC and EXCO. When compared to CON, all of the three intervention groups differed significantly. Results from previous studies already showed positive effects of chronic exercise on SIRT3, particularly in skeletal muscle [184,241]. However, results from this study are the first to show increased SIRT3 activity measured after chronic exercise in human blood. Our results further indicate that CO intake and a healthy diet may contribute to the beneficial effects of exercise. Regarding CO, it can be hypothesized that the n-3 PUFAs and the antioxidant astaxanthin present in the oil contribute to improved sirtuin function [191]. The strongest previously described modulators of sirtuin activity are the polyphenol resveratrol and caloric restriction [187]. Therefore, the increased fruit and vegetable intake in the EXDC group may have additionally contributed to increased sirtuin activity, as both foods contain polyphenols.

Overall, the results presented give first insights into the effects of chronic exercise on sirtuin activity and potential dietary modulators in healthy elderly subjects. However, as sirtuin activity was measured in peripheral blood, this does not necessarily reflect activities in other relevant organs. Moreover, as the enzyme activity was measured *in vitro* under substrate saturation, this may be different from the activity *in vivo*. In addition, protein levels of sirtuins were not measured in this study. Taken together the results demonstrate that the health promoting effects of exercise may be mediated by the activation of sirtuins and are further enhanced by dietary factors.

3.2 Cellular senescence as predictor of insulin resistance

As presented in chapters **1.2-1.3**, glucose metabolism is largely impacted by dietary intake and physical activity. Within the older population, age-related changes, such as the progressive decline in muscle mass, can further contribute to impaired glucose tolerance. Evidence from animal studies also suggests a link between T-cell aging, glucose metabolism, and the development of type-2 diabetes [205,206].

Results from a cross-sectional analysis performed on baseline data from the interventional study show that T-cell aging may predict insulin resistance in healthy elderly individuals (***Paper IV***). A direct effect on insulin resistance was found for the BMI and CD4+ immune age ratio. Further, BMI seemed to impact the CD8+ immune age ratio, which in turn indirectly influences insulin resistance. Regarding the effect of BMI, those results are not surprising as an effect of overweight on CD4+ and CD8+ immune age ratios was already demonstrated. More precisely, obesity accelerates thymic involution leading to a reduced production of naïve T-cells [242]. In addition, T-cell senescence is also mediated by pro-inflammatory signals secreted by adipose tissue [243]. In that regard, Yi et al. (2019) found that an accumulation of senescent T-cells drives the inflammatory response and leads to abnormal glucose homeostasis [205].
In line with existing evidence, CMV status was found to be another modulator of T-cell aging. In fact, data from this analysis were consistent with results from Lachmann et al. who reported that 51-63% of German adults are CMV seropositive [244]. This is of further interest, as it was already shown that very old CMV seropositive individuals are more likely to have type-2 diabetes and higher levels of HbA1c and non-fasting glucose [245]

Altogether, results from this cross-sectional analysis suggest that in addition to BMI, the individual immune age ratio may be a predictor of metabolic disturbances. This is of particular interest, as metabolic dysfunction commonly develops silently throughout the course of life. As exercise and diet were already acknowledged to accelerate or attenuate T-cell aging, lifestyle modifications could potentially help to prevent or delay the onset of metabolic dysfunction. Yet, it has to be kept in mind, that the application of path analysis to cross sectional data cannot conclude any causality between increasing T cell aging and insulin resistance. However, it reveals the likelihood of this relationship. In a next step, the direct effect of the present exercise program and dietary modifications on T-cell senescence and metabolic markers should be clarified.

3.3 Implications of low energy availability in athletes

Beyond the described positive health effects of exercise and adequate dietary intake, there are also scenarios in which exercise and (inadequate) dietary intake may negatively impact health. This is particularly true for athletes when they either increase their training volume (and thereby exercise energy expenditure) and/or decrease their dietary intake. An dietary energy intake which is too low relatively to energy expended through exercise ultimately results in LEA – a state during which there is too little energy left to maintain all physiological functions (***Paper V***) [207].

The reasons for inadequate dietary intake are manifold and may range from harmless reasons such as loss of appetite after training [246] up to intentional avoidance of certain foods and/or severe eating disorders [247,248]. Moreover, it can also be a result from the belief that a certain appearance or overall lower body weight will help to increase performance.

In that regard, it was demonstrated that the direct social environment (particularly coaches and team mates [249–251]) of an athlete can largely influence the dietary behavior. In any case, LEA has body-wide effects which encompass metabolic adaptations in the total daily energy expenditure, also mediated by lowered leptin levels [252,253] and thyroid suppression [254,255]; as well as changes in blood substrates such as lower glucose levels, higher free FAs, glycerol, cholesterol, and β-hydroxybutyrate [256,257]. Further, LEA impacts sex hormones in female and male athletes, leading to lower estradiol and progesterone levels in both genders and lowered testosterone in men. In females, impairment of the reproductive function also becomes noticeable as amenorrhea, which is also a part of the female athlete triad [258]. In male athletes, disruptions in the endocrine reproductive system are a part of the exercise-hypogonadal male condition [259]. As estradiol plays a role in the maintenance of bone mineral density (BMD), lower levels of estradiol will consequentially negatively impact BMD [260]. Moreover, LEA also negatively influences the growth hormone axis and can impair immune function.

Altogether it becomes evident, that despite all the positive health effects of exercise, higher training volumes can have adverse health effects if the athlete is not fueled with adequate dietary energy.

4. General conclusion and perspectives

Taken together results from this thesis give new insights into the extent healthy but untrained elderly individuals can benefit from a combined resistance and aerobic training when performed alone or in combination with dietary modifications.

It was demonstrated, that despite a slight loss in body weight and an increase in phase angle, the moderate exercise program seemed to neither elicit beneficial effects in terms of body composition, nor impact markers of glucose and lipid metabolism. However, the results presented suggest that sirtuins may be favorably influenced even by low dose moderate exercise. Activity of both, SIRT1 but particularly SIRT3 seemed to increase through exercise. On the contrary, exercise combined with a healthy diet or CO led not only to a reduced body weight but also contributed to loss of FM. While markers of glucose and blood lipids did not change significantly, supplementation of CO effectively increased the O3I. In addition, it was found that the O3I was not affected by exercise without diet modification. Further, both groups that received dietary modifications had increased sirtuin activity of SIRT1 and SIRT3. In addition to data from the interventional study, a cross-sectional analysis of the baseline data further revealed that T-cell senescence may be a mediator of insulin sensitivity in an elderly population.

It was previously reported that beneficial health effects of exercise occur in a dose-dependent manner [49,261]. Therefore, it can be assumed that the low dose of moderate exercise used in this study was not enough to elicit beneficial effects in an already healthy study population if not combined with an additional dietary modification. Although generally classified as healthy (meaning the absence of diagnosed diseases), it should be noted that the study collective was overweight. Therefore, the fat loss observed when exercise was combined with dietary modifications may still be beneficial, particularly with regards to cellular senescence and inflammatory markers. Further, NCDs commonly manifest silently throughout the life span, which is why even slight beneficial effects can be valuable. Moreover, maintenance of muscle mass and lower body fat is of additional importance in older adults, as a decrease of muscle mass with a concurrent increase in FM are hallmarks of the aging process.

Although the results from this investigation support the importance of a combination of exercise and dietary modifications, they need further investigation as participants were untrained but not physically inactive outside of the intervention. Particularly regarding the impact on body composition and physical fitness. Future approaches should measure the basal fitness status and improvements in fitness via examination of maximal oxygen uptake (VO_{2max}) and/or hand grip strength. Ultimately, because health benefits observed in the group supplementing CO

may be mediated by n-3 PUFAs, it would be of interest to compare CO intake versus a healthy n-3 PUFA rich diet. Additionally, future studies should investigate health effects of CO compared to a placebo with and without additional exercise. Lastly, a higher dose program would also be of interest.

5. References

[1]   World Health Organization. Non communicable diseases n.d.
https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases (accessed
October 1, 2020).

[2]   Daar AS, Singer PA, Leah Persad D, Pramming SK, Matthews DR, Beaglehole R, et
al. Grand challenges in chronic non-communicable diseases. Nature 2007;450:494–6.
https://doi.org/10.1038/450494a.

[3]   Franklin NC, Tate CA. Lifestyle and Successful Aging: An Overview. Am J Lifestyle
Med 2009;3:6–11. https://doi.org/10.1177/1559827608326125.

[4]   Garatachea N, Pareja-Galeano H, Sanchis-Gomar F, Santos-Lozano A, Fiuza-Luces
C, Morán M, et al. Exercise Attenuates the Major Hallmarks of Aging. Rejuvenation Res
2015;18:57–89. https://doi.org/10.1089/rej.2014.1623.

[5]   Tambalis K, Panagiotakos DB, Kavouras SA, Sidossis LS. Responses of blood lipids
to aerobic, resistance, and combined aerobic with resistance exercise training: a systematic
review of current evidence. Angiology 2009;60:614–32.
https://doi.org/10.1177/0003319708324927.

[6]   Buresh R. Exercise and glucose control. J Sports Med Phys Fitness 2014;54:373–82.

[7]   Liberman K, Forti LN, Beyer I, Bautmans I. The effects of exercise on muscle
strength, body composition, physical functioning and the inflammatory profile of older adults:
a systematic review. Curr Opin Clin Nutr Metab Care 2017;20:30–53.
https://doi.org/10.1097/MCO.0000000000000335.

[8]   Myers J. Exercise and Cardiovascular Health. Circulation 2003;107.
https://doi.org/10.1161/01.CIR.0000048890.59383.8D.

[9]   Pedersen BK, Hoffman-Goetz L. Exercise and the immune system: regulation,
integration, and adaptation. Physiol Rev 2000;80:1055–81.

[10]   Kohut ML, Senchina DS. Reversing age-associated immunosenescence via exercise.
Exerc Immunol Rev 2004;10:41.

[11]   Gielen S, Schuler G, Adams V. Cardiovascular Effects of Exercise Training: Molecular
Mechanisms. Circulation 2010;122:1221–38.
https://doi.org/10.1161/CIRCULATIONAHA.110.939959.

[12]   Jacques M, Hiam D, Craig J, Barrès R, Eynon N, Voisin S. Epigenetic changes in
healthy human skeletal muscle following exercise– a systematic review. Epigenetics
2019;14:633–48. https://doi.org/10.1080/15592294.2019.1614416.

[13]   Albracht-Schulte K, Kalupahana NS, Ramalingam L, Wang S, Rahman SM, Robert-
McComb J, et al. Omega-3 fatty acids in obesity and metabolic syndrome: a mechanistic
update. J Nutr Biochem 2018;58:1–16. https://doi.org/10.1016/j.jnutbio.2018.02.012.

[14]   Flachs P, Rossmeisl M, Kopecky J. The effect of n-3 fatty acids on glucose

homeostasis and insulin sensitivity. Physiol Res 2014;63:S93-118.
https://doi.org/10.33549/physiolres.932715.

[15]   Ruxton CHS, Reed SC, Simpson MJA, Millington KJ. The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. J Hum Nutr Diet 2004;17:449–59. https://doi.org/10.1111/j.1365-277X.2004.00552.x.

[16]   Höper AC, Salma W, Khalid AM, Hafstad AD, Sollie SJ, Raa J, et al. Oil from the marine zooplankton *Calanus finmarchicus* improves the cardiometabolic phenotype of diet-induced obese mice. Br J Nutr 2013;110:2186–93. https://doi.org/10.1017/S0007114513001839.

[17]   Höper AC, Salma W, Sollie SJ, Hafstad AD, Lund J, Khalid AM, et al. Wax Esters from the Marine Copepod Calanus finmarchicus Reduce Diet-Induced Obesity and Obesity-Related Metabolic Disorders in Mice. J Nutr 2014;144:164–9. https://doi.org/10.3945/jn.113.182501.

[18]   Aguiar EJ, Morgan PJ, Collins CE, Plotnikoff RC, Callister R. Efficacy of interventions that include diet, aerobic and resistance training components for type 2 diabetes prevention: a systematic review with meta-analysis. Int J Behav Nutr Phys Act 2014;11:2. https://doi.org/10.1186/1479-5868-11-2.

[19]   Zheng L, Wu J, Wang G, Persuitte G, Ma Y, Zou L, et al. Comparison of control fasting plasma glucose of exercise-only versus exercise-diet among a pre-diabetic population: a meta-analysis. Eur J Clin Nutr 2016;70:424–30. https://doi.org/10.1038/ejcn.2015.128.

[20]   Verreijen AM, Engberink MF, Memelink RG, van der Plas SE, Visser M, Weijs PJM. Effect of a high protein diet and/or resistance exercise on the preservation of fat free mass during weight loss in overweight and obese older adults: a randomized controlled trial. Nutr J 2017;16:10. https://doi.org/10.1186/s12937-017-0229-6.

[21]   Ngandu T, Lehtisalo J, Solomon A, Levälahti E, Ahtiluoto S, Antikainen R, et al. A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial. The Lancet 2015;385:2255–63. https://doi.org/10.1016/S0140-6736(15)60461-5.

[22]   Molnár A, Jónásné Sztruhár I, Csontos ÁA, Ferencz C, Várbíró S, Székács B. Special nutrition intervention is required for muscle protective efficacy of physical exercise in elderly people at highest risk of sarcopenia. Physiol Int 2016;103:368–76. https://doi.org/10.1556/2060.103.2016.3.12.

[23]   Gorostegi-Anduaga I, Corres P, MartinezAguirre-Betolaza A, Pérez-Asenjo J, Aispuru GR, Fryer SM, et al. Effects of different aerobic exercise programmes with nutritional intervention in sedentary adults with overweight/obesity and hypertension: EXERDIET-HTA

study. Eur J Prev Cardiol 2018;25:343–53. https://doi.org/10.1177/2047487317749956.

[24]   Strandberg E, Edholm P, Ponsot E, Wåhlin-Larsson B, Hellmén E, Nilsson A, et al. Influence of combined resistance training and healthy diet on muscle mass in healthy elderly women: a randomized controlled trial. J Appl Physiol 2015;119:918–25. https://doi.org/10.1152/japplphysiol.00066.2015.

[25]   Liao C-D, Tsauo J-Y, Wu Y-T, Cheng C-P, Chen H-C, Huang Y-C, et al. Effects of protein supplementation combined with resistance exercise on body composition and physical function in older adults: a systematic review and meta-analysis. Am J Clin Nutr 2017;106:1078–91. https://doi.org/10.3945/ajcn.116.143594.

[26]   Cornish SM, Myrie SB, Bugera EM, Chase JE, Turczyn D, Pinder M. Omega-3 supplementation with resistance training does not improve body composition or lower biomarkers of inflammation more so than resistance training alone in older men. Nutr Res 2018;60:87–95. https://doi.org/10.1016/j.nutres.2018.09.005.

[27]   Cornish SM, Chilibeck PD. Alpha-linolenic acid supplementation and resistance training in older adults. Appl Physiol Nutr Metab 2009;34:49–59. https://doi.org/10.1139/H08-136.

[28]   Caspersen CJ, Powell KE, Christenson GM. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. Public Health Rep Wash DC 1974 1985;100:126–31.

[29]   U.S. Department of Health and Human Services. 2018 Physical Activity Guidelines Advisory Committee Scientific Report 2018:779.

[30]   Franklin BA, Brinks J, Berra K, Lavie CJ, Gordon NF, Sperling LS. Using Metabolic Equivalents in Clinical Practice. Am J Cardiol 2018;121:382–7. https://doi.org/10.1016/j.amjcard.2017.10.033.

[31]   Howley ET. Type of activity: resistance, aerobic and leisure versus occupational physical activity: Med Sci Sports Exerc 2001;33:S364–9. https://doi.org/10.1097/00005768-200106001-00005.

[32]   Egan B, Zierath JR. Exercise metabolism and the molecular regulation of skeletal muscle adaptation. Cell Metab 2013;17:162–84. https://doi.org/10.1016/j.cmet.2012.12.012.

[33]   Burton DA, Stokes K, Hall GM. Physiological effects of exercise. Contin Educ Anaesth Crit Care Pain 2004;4:185–8. https://doi.org/10.1093/bjaceaccp/mkh050.

[34]   Heinonen I, Kalliokoski KK, Hannukainen JC, Duncker DJ, Nuutila P, Knuuti J. Organ-Specific Physiological Responses to Acute Physical Exercise and Long-Term Training in Humans. Physiology 2014;29:421–36. https://doi.org/10.1152/physiol.00067.2013.

[35]   Voss MW, Nagamatsu LS, Liu-Ambrose T, Kramer AF. Exercise, brain, and cognition across the life span. J Appl Physiol 2011;111:1505–13. https://doi.org/10.1152/japplphysiol.00210.2011.

[36]   Thyfault JP, Bergouignan A. Exercise and metabolic health: beyond skeletal muscle. Diabetologia 2020;63:1464–74. https://doi.org/10.1007/s00125-020-05177-6.

[37]   Lee I-M, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. The Lancet 2012;380:219–29. https://doi.org/10.1016/S0140-6736(12)61031-9.

[38]   Bergouignan A, Rudwill F, Simon C, Blanc S. Physical inactivity as the culprit of metabolic inflexibility: evidence from bed-rest studies. J Appl Physiol 2011;111:1201–10. https://doi.org/10.1152/japplphysiol.00698.2011.

[39]   Booth FW, Roberts CK, Laye MJ. Lack of Exercise Is a Major Cause of Chronic Diseases. In: Terjung, editor. Compr. Physiol., Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2012, p. c110025. https://doi.org/10.1002/cphy.c110025.

[40]   McArdle WD, Katch FI, Katch VL. Exercise physiology: nutrition, energy, and human performance. Eighth edition. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2015.

[41]   World Health Organization. Global recommendations on physical activity for health. Genève: WHO; 2010.

[42]   Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee I-M, et al. Quantity and Quality of Exercise for Developing and Maintaining Cardiorespiratory, Musculoskeletal, and Neuromotor Fitness in Apparently Healthy Adults: Guidance for Prescribing Exercise. Med Sci Sports Exerc 2011;43:1334–59. https://doi.org/10.1249/MSS.0b013e318213fefb.

[43]   World Health Organization. Prevalence of insufficient physical activity among adults Data by WHO region 2018. https://apps.who.int/gho/data/view.main.2482?lang=en.

[44]   RKI. Gesundheitsfördernde körperliche Aktivität in der Freizeit bei Erwachsenen in Deutschland 2017. https://doi.org/10.17886/RKI-GBE-2017-027.

[45]   Arena R, Myers J, Williams MA, Gulati M, Kligfield P, Balady GJ, et al. Assessment of Functional Capacity in Clinical and Research Settings: A Scientific Statement From the American Heart Association Committee on Exercise, Rehabilitation, and Prevention of the Council on Clinical Cardiology and the Council on Cardiovascular Nursing. Circulation 2007;116:329–43. https://doi.org/10.1161/CIRCULATIONAHA.106.184461.

[46]   JafariNasabian P, Inglis JE, Reilly W, Kelly OJ, Ilich JZ. Aging human body: changes in bone, muscle and body fat with consequent changes in nutrient intake. J Endocrinol 2017;234:R37–51. https://doi.org/10.1530/JOE-16-0603.

[47]   Hall KS, Cohen HJ, Pieper CF, Fillenbaum GG, Kraus WE, Huffman KM, et al. Physical Performance Across the Adult Life Span: Correlates With Age and Physical Activity. J Gerontol A Biol Sci Med Sci 2017;72:572–8. https://doi.org/10.1093/gerona/glw120.

[48]   King AC. Interventions to Promote Physical Activity by Older Adults. J Gerontol A Biol Sci Med Sci 2001;56:36–46. https://doi.org/10.1093/gerona/56.suppl_2.36.

[49]   Moxley E, Habtzghi D. A Systematic Review Comparing Dose Response of Exercise on Cardiovascular and All-Cause Mortality. Home Health Care Manag Pract 2019;31:263–73. https://doi.org/10.1177/1084822319831929.

[50]   Fisher JP, Steele J, Gentil P, Giessing J, Westcott WL. A minimal dose approach to resistance training for the older adult; the prophylactic for aging. Exp Gerontol 2017;99:80–6. https://doi.org/10.1016/j.exger.2017.09.012.

[51]   Popkin BM. Global nutrition dynamics: the world is shifting rapidly toward a diet linked with noncommunicable diseases. Am J Clin Nutr 2006;84:289–98. https://doi.org/10.1093/ajcn/84.2.289.

[52]   Hauner H, Bechthold A, Boeing H, Brönstrup A, Buyken A, Leschik-Bonnet E, et al. Evidence-Based Guideline of the German Nutrition Society: Carbohydrate Intake and Prevention of Nutrition-Related Diseases. Ann Nutr Metab 2012;60:1–58. https://doi.org/10.1159/000335326.

[53]   Hahn A, Ströhle A, Wolters M. Ernährung - Physiologische Grundlagen, Prävention, Therapie. 3rd ed. WVG Stuttgart; 2016.

[54]   Yeum K-J, Russell RM. Biological Functions of Plant Pigment Phytochemicals in Humans. In: Laher I, editor. Syst. Biol. Free Radic. Antioxid., Berlin, Heidelberg: Springer Berlin Heidelberg; 2014, p. 4023–45. https://doi.org/10.1007/978-3-642-30018-9_161.

[55]   Haller D, Rimbach G, Grune T. Vitamine und Vitaminoide. In: Haller D, Rimbach G, Grune T, editors. Biofunktionalität Leb., Berlin, Heidelberg: Springer Berlin Heidelberg; 2013, p. 235–47. https://doi.org/10.1007/978-3-642-29374-0_16.

[56]   Institute of Medicine. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington, D.C.: National Academies Press; 2001. https://doi.org/10.17226/10026.

[57]   Institute of Medicine. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Washington, D.C.: National Academies Press; 1997. https://doi.org/10.17226/5776.

[58]   Institute of Medicine. Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. Washington, D.C.: National Academies Press; 2005. https://doi.org/10.17226/10925.

[59]   Institute of Medicine. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington, D.C.: National Academies Press; 1998. https://doi.org/10.17226/6015.

[60]   DGE - ÖGE - SGE, Deutsche Gesellschaft für Ernährung e.V. D-A-C-H-

Referenzwerte für die Nährstoffzufuhr. 2019.

[61]   U.S. Department of Health and Human Services and U.S. Department of Agriculture. Dietary guidelines for americans 2015-2020. 2016.

[62]   National Health and Medical Research Council, Department of Health and Ageing. Eat for health, Australian dietary guidelines. 2013.

[63]   Bechthold A, Breidenassel C, Egert S, Gahl A, Michel M, Oberritter H, et al. Vollwertig essen und trinken nach den 10 Regeln der DGE. Bonn: Deutschen Gesellschaft für Ernährung e. V.; 2018.

[64]   Wu H, Flint AJ, Qi Q, van Dam RM, Sampson LA, Rimm EB, et al. Association Between Dietary Whole Grain Intake and Risk of Mortality: Two Large Prospective Studies in US Men and Women. JAMA Intern Med 2015;175:373. https://doi.org/10.1001/jamainternmed.2014.6283.

[65]   Aune D, Giovannucci E, Boffetta P, Fadnes LT, Keum N, Norat T, et al. Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality—a systematic review and dose-response meta-analysis of prospective studies. Int J Epidemiol 2017;46:1029–56. https://doi.org/10.1093/ije/dyw319.

[66]   Miller V, Mente A, Dehghan M, Rangarajan S, Zhang X, Swaminathan S, et al. Fruit, vegetable, and legume intake, and cardiovascular disease and deaths in 18 countries (PURE): a prospective cohort study. The Lancet 2017;390:2037–49. https://doi.org/10.1016/S0140-6736(17)32253-5.

[67]   McRae MP. Health Benefits of Dietary Whole Grains: An Umbrella Review of Meta-analyses. J Chiropr Med 2017;16:10–8. https://doi.org/10.1016/j.jcm.2016.08.008.

[68]   Yip CSC, Chan W, Fielding R. The Associations of Fruit and Vegetable Intakes with Burden of Diseases: A Systematic Review of Meta-Analyses. J Acad Nutr Diet 2019;119:464–81. https://doi.org/10.1016/j.jand.2018.11.007.

[69]   He FJ, Nowson CA, Lucas M, MacGregor GA. Increased consumption of fruit and vegetables is related to a reduced risk of coronary heart disease: meta-analysis of cohort studies. J Hum Hypertens 2007;21:717–28. https://doi.org/10.1038/sj.jhh.1002212.

[70]   Key TJ, Appleby PN, Bradbury KE, Sweeting M, Wood A, Johansson I, et al. Consumption of Meat, Fish, Dairy Products, and Eggs and Risk of Ischemic Heart Disease: A Prospective Study of 7198 Incident Cases Among 409 885 Participants in the Pan-European EPIC Cohort. Circulation 2019;139:2835–45. https://doi.org/10.1161/CIRCULATIONAHA.118.038813.

[71]   Rohrmann S, Overvad K, Bueno-de-Mesquita HB, Jakobsen MU, Egeberg R, Tjønneland A, et al. Meat consumption and mortality - results from the European Prospective Investigation into Cancer and Nutrition. BMC Med 2013;11:63. https://doi.org/10.1186/1741-7015-11-63.

[72]   The InterAct Consortium. Association between dietary meat consumption and incident type 2 diabetes: the EPIC-InterAct study. Diabetologia 2013;56:47–59. https://doi.org/10.1007/s00125-012-2718-7.

[73]   Afshin A, Sur PJ, Fay KA, Cornaby L, Ferrara G, Salama JS, et al. Health effects of dietary risks in 195 countries, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet 2019;393:1958–72. https://doi.org/10.1016/S0140-6736(19)30041-8.

[74]   Max Rubner Institut. Nationale Verzehrsstudie II - Ergebnisbericht, Teil 2. Natl Verzehrsstudie II - Ergeb Teil 2 2008.

[75]   Calder PC, Yaqoob P. Understanding Omega-3 Polyunsaturated Fatty Acids. Postgrad Med 2009;121:148–57. https://doi.org/10.3810/pgm.2009.11.2083.

[76]   Azrad M, Turgeon C, Demark-Wahnefried W. Current Evidence Linking Polyunsaturated Fatty Acids with Cancer Risk and Progression. Front Oncol 2013;3. https://doi.org/10.3389/fonc.2013.00224.

[77]   Gabbs M, Leng S, Devassy JG, Monirujjaman M, Aukema HM. Advances in Our Understanding of Oxylipins Derived from Dietary PUFAs. Adv Nutr 2015;6:513–40. https://doi.org/10.3945/an.114.007732.

[78]   Vigor C, Bertrand-Michel J, Pinot E, Oger C, Vercauteren J, Le Faouder P, et al. Non-enzymatic lipid oxidation products in biological systems: Assessment of the metabolites from polyunsaturated fatty acids. J Chromatogr B 2014;964:65–78. https://doi.org/10.1016/j.jchromb.2014.04.042.

[79]   Serhan CN, Levy BD. Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. J Clin Invest 2018;128:2657–69. https://doi.org/10.1172/JCI97943.

[80]   Buckley CD, Gilroy DW, Serhan CN. Proresolving Lipid Mediators and Mechanisms in the Resolution of Acute Inflammation. Immunity 2014;40:315–27. https://doi.org/10.1016/j.immuni.2014.02.009.

[81]   Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature 2014;510:92–101. https://doi.org/10.1038/nature13479.

[82]   Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother 2002;56:365–79. https://doi.org/10.1016/S0753-3322(02)00253-6.

[83]   Simopoulos AP. Evolutionary Aspects of Diet: The Omega-6/Omega-3 Ratio and the Brain. Mol Neurobiol 2011;44:203–15. https://doi.org/10.1007/s12035-010-8162-0.

[84]   Phillips CM, Chen L-W, Heude B, Bernard JY, Harvey NC, Duijts L, et al. Dietary Inflammatory Index and Non-Communicable Disease Risk: A Narrative Review. Nutrients 2019;11:1873. https://doi.org/10.3390/nu11081873.

[85]   León-Pedroza JI, González-Tapia LA, del Olmo-Gil E, Castellanos-Rodríguez D,

Escobedo G, González-Chávez A. Low-grade systemic inflammation and the development of metabolic diseases: From the molecular evidence to the clinical practice. Cir Cir Engl Ed 2015;83:543–51. https://doi.org/10.1016/j.circen.2015.11.008.

[86]   Calder PC, Yaqoob P. Omega-3 polyunsaturated fatty acids and human health outcomes. BioFactors 2009;35:266–72. https://doi.org/10.1002/biof.42.

[87]   Kris-Etherton PM, Harris WS, Appel LJ. Fish Consumption, Fish Oil, Omega-3 Fatty Acids, and Cardiovascular Disease. Circulation 2002;106:2747–57. https://doi.org/10.1161/01.CIR.0000038493.65177.94.

[88]   von Schacky C. Omega-3 Index and Cardiovascular Health. Nutrients 2014;6:799–814. https://doi.org/10.3390/nu6020799.

[89]   Von Schacky C. Omega-3 Index and Sudden Cardiac Death. Nutrients 2010;2:375–88. https://doi.org/10.3390/nu2030375.

[90]   Endo J, Arita M. Cardioprotective mechanism of omega-3 polyunsaturated fatty acids. J Cardiol 2016;67:22–7. https://doi.org/10.1016/j.jjcc.2015.08.002.

[91]   Souci SW, Fachmann W, Kraut H, Andersen G, Deutsche Forschungsanstalt für Lebensmittelchemie, Deutschland, editors. Food composition and nutrition tables; Zusammensetzung der Lebensmittel, Nährwert-Tabellen. 8th revised and completed edition-8., revidierte und ergänzte Auflage. Stuttgart: MedPharm Scientific Publishers, an imprint of Wissenschaftliche Verlagsgesellschaft; 2016.

[92]   Deckelbaum RJ, Torrejon C. The Omega-3 Fatty Acid Nutritional Landscape: Health Benefits and Sources. J Nutr 2012;142:587S-591S. https://doi.org/10.3945/jn.111.148080.

[93]   Burdge GC, Jones AE, Wootton SA. Eicosapentaenoic and docosapentaenoic acids are the principal products of α-linolenic acid metabolism in young men. Br J Nutr 2002;88:355–63. https://doi.org/10.1079/BJN2002662.

[94]   Burdge GC, Wootton SA. Conversion of α-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. Br J Nutr 2002;88:411–20. https://doi.org/10.1079/BJN2002689.

[95]   Institute of Medicine (US). Panel on Macronutrients. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington, D.C: National Academies Press; 2005.

[96]   Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA J 2010;8. https://doi.org/10.2903/j.efsa.2010.1461.

[97]   International Society for the Study of Fatty Acids and Lipids. Report of the Sub-Committee on Recommendations for intake of polyunsaturated fatty acids in healthy adults 2004.

[98]   EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on

the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). EFSA J 2012;10. https://doi.org/10.2903/j.efsa.2012.2815.

[99] Micha R, Khatibzadeh S, Shi P, Fahimi S, Lim S, Andrews KG, et al. Global, regional, and national consumption levels of dietary fats and oils in 1990 and 2010: a systematic analysis including 266 country-specific nutrition surveys. BMJ 2014;348:g2272–g2272. https://doi.org/10.1136/bmj.g2272.

[100] Harris WS, von Schacky C. The Omega-3 Index: a new risk factor for death from coronary heart disease? Prev Med 2004;39:212–20. https://doi.org/10.1016/j.ypmed.2004.02.030.

[101] Harris WS. The Omega-3 Index: Clinical Utility for Therapeutic Intervention. Curr Cardiol Rep 2010;12:503–8. https://doi.org/10.1007/s11886-010-0141-6.

[102] Stark KD, Van Elswyk ME, Higgins MR, Weatherford CA, Salem N. Global survey of the omega-3 fatty acids, docosahexaenoic acid and eicosapentaenoic acid in the blood stream of healthy adults. Prog Lipid Res 2016;63:132–52. https://doi.org/10.1016/j.plipres.2016.05.001.

[103] Thuppal SV, von Schacky C, Harris WS, Sherif KD, Denby N, Steinbaum SR, et al. Discrepancy between Knowledge and Perceptions of Dietary Omega-3 Fatty Acid Intake Compared with the Omega-3 Index. Nutrients 2017;9. https://doi.org/10.3390/nu9090930.

[104] Schuchardt JP, Hahn A. Bioavailability of long-chain omega-3 fatty acids. Prostaglandins Leukot Essent Fatty Acids 2013;89:1–8. https://doi.org/10.1016/j.plefa.2013.03.010.

[105] Vaisali C, Charanyaa S, Belur PD, Regupathi I. Refining of edible oils: a critical appraisal of current and potential technologies. Int J Food Sci Technol 2015;50:13–23. https://doi.org/10.1111/ijfs.12657.

[106] Dyerberg J, Madsen P, Møller JM, Aardestrup I, Schmidt EB. Bioavailability of marine n-3 fatty acid formulations. Prostaglandins Leukot Essent Fatty Acids 2010;83:137–41. https://doi.org/10.1016/j.plefa.2010.06.007.

[107] Schuchardt J, Schneider I, Meyer H, Neubronner J, von Schacky C, Hahn A. Incorporation of EPA and DHA into plasma phospholipids in response to different omega-3 fatty acid formulations - a comparative bioavailability study of fish oil vs. krill oil. Lipids Health Dis 2011;10:145. https://doi.org/10.1186/1476-511X-10-145.

[108] Pedersen AM, Vang B, Olsen RL. Oil from *Calanus finmarchicus* —Composition and Possible Use: A Review. J Aquat Food Prod Technol 2014;23:633–46. https://doi.org/10.1080/10498850.2012.741662.

[109] Falk-Petersen S, Sargent JR, Tande KS. Lipid composition of zooplankton in relation to the sub-arctic food web. Polar Biol 1987;8:115–20. https://doi.org/10.1007/BF00297065.

[110]   Pedersen AM, Salma W, Höper AC, Larsen TS, Olsen RL. Lipid profile of mice fed a high-fat diet supplemented with a wax ester-rich marine oil: Digestion of marine wax esters by mice. Eur J Lipid Sci Technol 2014;116:1718–26. https://doi.org/10.1002/ejlt.201400052.

[111]   Falk-Petersen S, Mayzaud P, Kattner G, Sargent JR. Lipids and life strategy of Arctic *Calanus*. Mar Biol Res 2009;5:18–39. https://doi.org/10.1080/17451000802512267.

[112]   Schots PC, Pedersen AM, Eilertsen K-E, Olsen RL, Larsen TS. Possible Health Effects of a Wax Ester Rich Marine Oil. Front Pharmacol 2020;11:961. https://doi.org/10.3389/fphar.2020.00961.

[113]   Harris WS. Stearidonic Acid–Enhanced Soybean Oil: A Plant-Based Source of (n-3) Fatty Acids for Foods. J Nutr 2012;142:600S-604S. https://doi.org/10.3945/jn.111.146613.

[114]   Guil-Guerrero JL. Stearidonic acid (18:4n-3): Metabolism, nutritional importance, medical uses and natural sources. Eur J Lipid Sci Technol 2007;109:1226–36. https://doi.org/10.1002/ejlt.200700207.

[115]   Park J, Chyun J, Kim Y, Line LL, Chew BP. Astaxanthin decreased oxidative stress and inflammation and enhanced immune response in humans. Nutr Metab 2010;7:18. https://doi.org/10.1186/1743-7075-7-18.

[116]   Chew W, Mathison B, Kimble L, Mixter P, Chew B. Astaxanthin Decreases Inflammatory Biomarkers Associated with Cardiovascular Disease in Human Umbilical Vein Endothelial Cells. Am J Adv Food Sci Technol 2013. https://doi.org/10.7726/ajafst.2013.1001.

[117]   Weber P, Raederstorff D. Triglyceride-lowering effect of omega-3 LC-polyunsaturated fatty acids--a review. Nutr Metab Cardiovasc Dis NMCD 2000;10:28–37.

[118]   Davidson MH. Mechanisms for the Hypotriglyceridemic Effect of Marine Omega-3 Fatty Acids. Am J Cardiol 2006;98:27–33. https://doi.org/10.1016/j.amjcard.2005.12.024.

[119]   Smith GI, Atherton P, Reeds DN, Mohammed BS, Rankin D, Rennie MJ, et al. Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial. Am J Clin Nutr 2011;93:402–12. https://doi.org/10.3945/ajcn.110.005611.

[120]   Smith GI, Atherton P, Reeds DN, Mohammed BS, Rankin D, Rennie MJ, et al. Omega-3 polyunsaturated fatty acids augment the muscle protein anabolic response to hyperinsulinaemia-hyperaminoacidaemia in healthy young and middle-aged men and women. Clin Sci Lond Engl 1979 2011;121:267–78. https://doi.org/10.1042/CS20100597.

[121]   Bender N, Portmann M, Heg Z, Hofmann K, Zwahlen M, Egger M. Fish or n3-PUFA intake and body composition: a systematic review and meta-analysis: Fish and body composition. Obes Rev 2014;15:657–65. https://doi.org/10.1111/obr.12189.

[122]   Hill AM, Buckley JD, Murphy KJ, Howe PR. Combining fish-oil supplements with regular aerobic exercise improves body composition and cardiovascular disease risk factors.

Am J Clin Nutr 2007;85:1267–74. https://doi.org/10.1093/ajcn/85.5.1267.

[123]   Noreen EE, Sass MJ, Crowe ML, Pabon VA, Brandauer J, Averill LK. Effects of supplemental fish oil on resting metabolic rate, body composition, and salivary cortisol in healthy adults. J Int Soc Sports Nutr 2010;7:31. https://doi.org/10.1186/1550-2783-7-31.

[124]   Tiryaki-Sönmez G, Schoenfeld B, Vatansever-Ozen S. Omega-3 fatty acids and exercise: a review of their combined effects on body composition and physical performance. Biomed Hum Kinet 2011;3. https://doi.org/10.2478/v10101-011-0007-4.

[125]   Eilertsen K-E, Mæhre HK, Jensen IJ, Devold H, Olsen JO, Lie RK, et al. A Wax Ester and Astaxanthin-Rich Extract from the Marine Copepod Calanus finmarchicus Attenuates Atherogenesis in Female Apolipoprotein E–Deficient Mice. J Nutr 2012;142:508–12. https://doi.org/10.3945/jn.111.145698.

[126]   Jansen KM, Moreno S, Garcia-Roves PM, Larsen TS. Dietary Calanus oil recovers metabolic flexibility and rescues postischemic cardiac function in obese female mice. Am J Physiol-Heart Circ Physiol 2019;317:H290–9. https://doi.org/10.1152/ajpheart.00191.2019.

[127]   Seidell JC, Kahn HS, Williamson DF, Lissner L, Valdez R. Report from a Centers for Disease Control and Prevention Workshop on Use of Adult Anthropometry for Public Health and Primary Health Care. Am J Clin Nutr 2001;73:123–6. https://doi.org/10.1093/ajcn/73.1.123.

[128]   Data Input GmbH. Das BIA-Kompendium 3. Ausgabe 2005.

[129]   Sarin HV, Lee JH, Jauhiainen M, Joensuu A, Borodulin K, Männistö S, et al. Substantial fat mass loss reduces low-grade inflammation and induces positive alteration in cardiometabolic factors in normal-weight individuals. Sci Rep 2019;9:3450. https://doi.org/10.1038/s41598-019-40107-6.

[130]   Nishida C, Ko GT, Kumanyika S. Body fat distribution and noncommunicable diseases in populations: overview of the 2008 WHO Expert Consultation on Waist Circumference and Waist–Hip Ratio. Eur J Clin Nutr 2010;64:2–5. https://doi.org/10.1038/ejcn.2009.139.

[131]   Heo, Jin-Yeong, Park, Yoon Jeong, Ham, Mira, Kim, Jae Bum. Crosstalk between Adipocytes and Immune Cells in Adipose Tissue Inflammation and Metabolic Dysregulation in Obesity. Mol Cells 2014;37:365–71. https://doi.org/10.14348/MOLCELLS.2014.0074.

[132]   Oikonomou EK, Antoniades C. The role of adipose tissue in cardiovascular health and disease. Nat Rev Cardiol 2019;16:83–99. https://doi.org/10.1038/s41569-018-0097-6.

[133]   Choe SS, Huh JY, Hwang IJ, Kim JI, Kim JB. Adipose Tissue Remodeling: Its Role in Energy Metabolism and Metabolic Disorders. Front Endocrinol 2016;7. https://doi.org/10.3389/fendo.2016.00030.

[134]   Chia CW, Egan JM, Ferrucci L. Age-Related Changes in Glucose Metabolism, Hyperglycemia, and Cardiovascular Risk. Circ Res 2018;123:886–904.

https://doi.org/10.1161/CIRCRESAHA.118.312806.

[135]   Kyle U, Genton L, Hans D, Karsegard L, Slosman D, Pichard C. Age-related differences in fat-free mass, skeletal muscle, body cell mass and fat mass between 18 and 94 years. Eur J Clin Nutr 2001;55:663–72. https://doi.org/10.1038/sj.ejcn.1601198.

[136]   Kamel HK. Sarcopenia and Aging. Nutr Rev 2003;61:157–67. https://doi.org/10.1301/nr.2003.may.157-167.

[137]   Wilhelm EN, Pinto RS. Concurrent Aerobic and Strength Training for Body Composition and Health. In: Schumann M, Rønnestad BR, editors. Concurr. Aerob. Strength Train., Cham: Springer International Publishing; 2019, p. 293–307. https://doi.org/10.1007/978-3-319-75547-2_19.

[138]   Strasser B, Spreitzer A, Haber P. Fat Loss Depends on Energy Deficit Only, Independently of the Method for Weight Loss. Ann Nutr Metab 2007;51:428–32. https://doi.org/10.1159/000111162.

[139]   Guo X, Warden BA, Paeratakul S, Bray GA. Healthy Eating Index and obesity. Eur J Clin Nutr 2004;58:1580–6. https://doi.org/10.1038/sj.ejcn.1601989.

[140]   Drenowatz C, Shook RP, Hand GA, Hébert JR, Blair SN. The independent association between diet quality and body composition. Sci Rep 2015;4:4928. https://doi.org/10.1038/srep04928.

[141]   Xu F, Greene GW, Earp JE, Adami A, Delmonico MJ, Lofgren IE, et al. Relationships of Physical Activity and Diet Quality with Body Composition and Fat Distribution in US Adults. Obesity 2020;28:2431–40. https://doi.org/10.1002/oby.23018.

[142]   Wirth J, Hillesheim E, Brennan L. The Role of Protein Intake and its Timing on Body Composition and Muscle Function in Healthy Adults: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. J Nutr 2020;150:1443–60. https://doi.org/10.1093/jn/nxaa049.

[143]   Röder PV, Wu B, Liu Y, Han W. Pancreatic regulation of glucose homeostasis. Exp Mol Med 2016;48:e219–e219. https://doi.org/10.1038/emm.2016.6.

[144]   Nathan DM, Turgeon H, Regan S. Relationship between glycated haemoglobin levels and mean glucose levels over time. Diabetologia 2007;50:2239–44. https://doi.org/10.1007/s00125-007-0803-0.

[145]   American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2010;33:S62–9. https://doi.org/10.2337/dc10-S062.

[146]   Gayoso-Diz P, Otero-González A, Rodriguez-Alvarez MX, Gude F, García F, De Francisco A, et al. Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: effect of gender and age: EPIRCE cross-sectional study. BMC Endocr Disord 2013;13:47. https://doi.org/10.1186/1472-6823-13-47.

[147]   Andrade MIS de, Oliveira JS, Leal VS, Lima NMS da, Costa EC, Aquino NB de, et al.

Identification of cutoff points for Homeostatic Model Assessment for Insulin Resistance index in adolescents: systematic review. Rev Paul Pediatr Engl Ed 2016;34:234–42. https://doi.org/10.1016/j.rppede.2016.01.004.

[148]   Micha R, Peñalvo JL, Cudhea F, Imamura F, Rehm CD, Mozaffarian D. Association Between Dietary Factors and Mortality From Heart Disease, Stroke, and Type 2 Diabetes in the United States. JAMA 2017;317:912. https://doi.org/10.1001/jama.2017.0947.

[149]   Hawley JA, Lessard SJ. Exercise training-induced improvements in insulin action: Exercise and insulin action. Acta Physiol 2007;192:127–35. https://doi.org/10.1111/j.1748-1716.2007.01783.x.

[150]   Sato Y, Nagasaki M, Nakai N, Fushimi T. Physical Exercise Improves Glucose Metabolism in Lifestyle-Related Diseases. Exp Biol Med 2003;228:1208–12. https://doi.org/10.1177/153537020322801017.

[151]   Sato Y. Diabetes and life-styles: role of physical exercise for primary prevention. Br J Nutr 2000;84:187–90. https://doi.org/10.1079/096582197388662.

[152]   Rader DJ. Lipoprotein Metabolism. In: Offermanns S, Rosenthal W, editors. Encycl. Mol. Pharmacol., Berlin, Heidelberg: Springer Berlin Heidelberg; 2008, p. 696–700. https://doi.org/10.1007/978-3-540-38918-7_190.

[153]   Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J 2017;38:2459–72. https://doi.org/10.1093/eurheartj/ehx144.

[154]   Abdullah SM, Defina LF, Leonard D, Barlow CE, Radford NB, Willis BL, et al. Long-Term Association of Low-Density Lipoprotein Cholesterol With Cardiovascular Mortality in Individuals at Low 10-Year Risk of Atherosclerotic Cardiovascular Disease: Results From the Cooper Center Longitudinal Study. Circulation 2018;138:2315–25. https://doi.org/10.1161/CIRCULATIONAHA.118.034273.

[155]   Linsel-Nitschke P, Tall AR. HDL as a target in the treatment of atherosclerotic cardiovascular disease. Nat Rev Drug Discov 2005;4:193–205. https://doi.org/10.1038/nrd1658.

[156]   Fernandez ML, Webb D. The LDL to HDL cholesterol ratio as a valuable tool to evaluate coronary heart disease risk. J Am Coll Nutr 2008;27:1–5. https://doi.org/10.1080/07315724.2008.10719668.

[157]   Ingelsson E, Schaefer EJ, Contois JH, McNamara JR, Sullivan L, Keyes MJ, et al. Clinical utility of different lipid measures for prediction of coronary heart disease in men and women. JAMA 2007;298:776–85. https://doi.org/10.1001/jama.298.7.776.

[158]   Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, et al. Diet

and Lifestyle Recommendations Revision 2006: A Scientific Statement From the American Heart Association Nutrition Committee. Circulation 2006;114:82–96. https://doi.org/10.1161/CIRCULATIONAHA.106.176158.

[159] Authors/Task Force Members:, Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, et al. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012): The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts) * Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Eur Heart J 2012;33:1635–701. https://doi.org/10.1093/eurheartj/ehs092.

[160] Zhuang P, Zhang Y, He W, Chen X, Chen J, He L, et al. Dietary Fats in Relation to Total and Cause-Specific Mortality in a Prospective Cohort of 521 120 Individuals With 16 Years of Follow-Up. Circ Res 2019;124:757–68. https://doi.org/10.1161/CIRCRESAHA.118.314038.

[161] Kromhout D, Menotti A, Bloemberg B, Aravanis C, Blackburn H, Buzina R, et al. Dietary Saturated and transFatty Acids and Cholesterol and 25-Year Mortality from Coronary Heart Disease: The Seven Countries Study. Prev Med 1995;24:308–15. https://doi.org/10.1006/pmed.1995.1049.

[162] Siri-Tarino PW, Sun Q, Hu FB, Krauss RM. Saturated Fatty Acids and Risk of Coronary Heart Disease: Modulation by Replacement Nutrients. Curr Atheroscler Rep 2010;12:384–90. https://doi.org/10.1007/s11883-010-0131-6.

[163] Jakobsen MU, O'Reilly EJ, Heitmann BL, Pereira MA, Bälter K, Fraser GE, et al. Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. Am J Clin Nutr 2009;89:1425–32. https://doi.org/10.3945/ajcn.2008.27124.

[164] Skulas-Ray AC, Wilson PWF, Harris WS, Brinton EA, Kris-Etherton PM, Richter CK, et al. Omega-3 Fatty Acids for the Management of Hypertriglyceridemia: A Science Advisory From the American Heart Association. Circulation 2019;140. https://doi.org/10.1161/CIR.0000000000000709.

[165] Mann S, Beedie C, Jimenez A. Differential effects of aerobic exercise, resistance training and combined exercise modalities on cholesterol and the lipid profile: review, synthesis and recommendations. Sports Med Auckl NZ 2014;44:211–21. https://doi.org/10.1007/s40279-013-0110-5.

[166] Harris WS. The omega-3 index as a risk factor for coronary heart disease. Am J Clin Nutr 2008;87:1997S-2002S. https://doi.org/10.1093/ajcn/87.6.1997S.

[167] Block RC, Harris WS, Reid KJ, Sands SA, Spertus JA. EPA and DHA in blood cell membranes from acute coronary syndrome patients and controls. Atherosclerosis 2008;197:821–8. https://doi.org/10.1016/j.atherosclerosis.2007.07.042.

[168]   Flock MR, Skulas-Ray AC, Harris WS, Etherton TD, Fleming JA, Kris-Etherton PM. Determinants of Erythrocyte Omega-3 Fatty Acid Content in Response to Fish Oil Supplementation: A Dose–Response Randomized Controlled Trial. J Am Heart Assoc 2013;2. https://doi.org/10.1161/JAHA.113.000513.

[169]   Kaeberlein M, McVey M, Guarente L. The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. Genes Dev 1999;13:2570–80. https://doi.org/10.1101/gad.13.19.2570.

[170]   Buler M, Andersson U, Hakkola J. Who watches the watchmen? Regulation of the expression and activity of sirtuins. FASEB J 2016;30:3942–60. https://doi.org/10.1096/fj.201600410RR.

[171]   Carrico C, Meyer JG, He W, Gibson BW, Verdin E. The Mitochondrial Acylome Emerges: Proteomics, Regulation by Sirtuins, and Metabolic and Disease Implications. Cell Metab 2018;27:497–512. https://doi.org/10.1016/j.cmet.2018.01.016.

[172]   Flick F, Lüscher B. Regulation of Sirtuin Function by Posttranslational Modifications. Front Pharmacol 2012;3. https://doi.org/10.3389/fphar.2012.00029.

[173]   Mei Z, Zhang X, Yi J, Huang J, He J, Tao Y. Sirtuins in metabolism, DNA repair and cancer. J Exp Clin Cancer Res 2016;35. https://doi.org/10.1186/s13046-016-0461-5.

[174]   Rodriguez RM, Fernandez AF, Fraga MF. Role of Sirtuins in Stem Cell Differentiation. Genes Cancer 2013;4:105–11. https://doi.org/10.1177/1947601913479798.

[175]   Lombard DB, Tishkoff DX, Bao J. Mitochondrial sirtuins in the regulation of mitochondrial activity and metabolic adaptation. Handb Exp Pharmacol 2011;206:163–88. https://doi.org/10.1007/978-3-642-21631-2_8.

[176]   Ye X, Li M, Hou T, Gao T, Zhu W, Yang Y. Sirtuins in glucose and lipid metabolism. Oncotarget 2017;8:1845–59. https://doi.org/10.18632/oncotarget.12157.

[177]   Grabowska W, Sikora E, Bielak-Zmijewska A. Sirtuins, a promising target in slowing down the ageing process. Biogerontology 2017;18:447–76. https://doi.org/10.1007/s10522-017-9685-9.

[178]   Feldman JL, Dittenhafer-Reed KE, Denu JM. Sirtuin catalysis and regulation. J Biol Chem 2012;287:42419–27. https://doi.org/10.1074/jbc.R112.378877.

[179]   Turkmen K. Sirtuins as novel players in the pathogenesis of diabetes mellitus. World J Diabetes 2014;5:894. https://doi.org/10.4239/wjd.v5.i6.894.

[180]   Al-Khaldi A, Sultan S. The expression of sirtuins, superoxide dismutase, and lipid peroxidation status in peripheral blood from patients with diabetes and hypothyroidism. BMC Endocr Disord 2019;19:19. https://doi.org/10.1186/s12902-019-0350-y.

[181]   Tarantino G, Finelli C, Scopacasa F, Pasanisi F, Contaldo F, Capone D, et al. Circulating Levels of Sirtuin 4, a Potential Marker of Oxidative Metabolism, Related to Coronary Artery Disease in Obese Patients Suffering from NAFLD, with Normal or Slightly

Increased Liver Enzymes. Oxid Med Cell Longev 2014;2014:1–10.
https://doi.org/10.1155/2014/920676.

[182]   Cencioni C, Spallotta F, Mai A, Martelli F, Farsetti A, Zeiher AM, et al. Sirtuin function in aging heart and vessels. J Mol Cell Cardiol 2015;83:55–61.
https://doi.org/10.1016/j.yjmcc.2014.12.023.

[183]   Bayod S, del Valle J, Lalanza JF, Sanchez-Roige S, de Luxán-Delgado B, Coto-Montes A, et al. Long-term physical exercise induces changes in sirtuin 1 pathway and oxidative parameters in adult rat tissues. Exp Gerontol 2012;47:925–35.
https://doi.org/10.1016/j.exger.2012.08.004.

[184]   Vargas-Ortiz K, Pérez-Vázquez V, Macías-Cervantes MH. Exercise and Sirtuins: A Way to Mitochondrial Health in Skeletal Muscle. Int J Mol Sci 2019;20:2717.
https://doi.org/10.3390/ijms20112717.

[185]   Cohen HY, Miller C, Bitterman KJ, Wall NR, Hekking B, Kessler B, et al. Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. Science 2004;305:390–2. https://doi.org/10.1126/science.1099196.

[186]   Bordone L, Guarente L. Calorie restriction, SIRT1 and metabolism: understanding longevity. Nat Rev Mol Cell Biol 2005;6:298–305. https://doi.org/10.1038/nrm1616.

[187]   Villalba JM, Alcaín FJ. Sirtuin activators and inhibitors. BioFactors 2012;38:349–59.
https://doi.org/10.1002/biof.1032.

[188]   Ajami M, Pazoki-Toroudi H, Amani H, Nabavi SF, Braidy N, Vacca RA, et al. Therapeutic role of sirtuins in neurodegenerative disease and their modulation by polyphenols. Neurosci Biobehav Rev 2017;73:39–47.
https://doi.org/10.1016/j.neubiorev.2016.11.022.

[189]   Inoue T, Tanaka M, Masuda S, Ohue-Kitano R, Yamakage H, Muranaka K, et al. Omega-3 polyunsaturated fatty acids suppress the inflammatory responses of lipopolysaccharide-stimulated mouse microglia by activating SIRT1 pathways. Biochim Biophys Acta BBA - Mol Cell Biol Lipids 2017;1862:552–60.
https://doi.org/10.1016/j.bbalip.2017.02.010.

[190]   Xue B, Yang Z, Wang X, Shi H. Omega-3 Polyunsaturated Fatty Acids Antagonize Macrophage Inflammation via Activation of AMPK/SIRT1 Pathway. PLoS ONE 2012;7:e45990. https://doi.org/10.1371/journal.pone.0045990.

[191]   Leger T, Azarnoush K, Traoré A, Cassagnes L, Rigaudière J-P, Jouve C, et al. Antioxidant and Cardioprotective Effects of EPA on Early Low-Severity Sepsis through UCP3 and SIRT3 Upholding of the Mitochondrial Redox Potential. Oxid Med Cell Longev 2019;2019:1–21. https://doi.org/10.1155/2019/9710352.

[192]   Janeway CAJ, Travers P, Walport M, Shlomchik MJ. Principles of innate and adaptive immunity. Immunobiol Immune Syst Health Dis 5th Ed 2001.

[193]   Aiello A, Farzaneh F, Candore G, Caruso C, Davinelli S, Gambino CM, et al. Immunosenescence and Its Hallmarks: How to Oppose Aging Strategically? A Review of Potential Options for Therapeutic Intervention. Front Immunol 2019;10. https://doi.org/10.3389/fimmu.2019.02247.

[194]   Le Page A, Dupuis G, Larbi A, Witkowski JM, Fülöp T. Signal transduction changes in CD4+ and CD8+ T cell subpopulations with aging. Exp Gerontol 2018;105:128–39. https://doi.org/10.1016/j.exger.2018.01.005.

[195]   Golubovskaya V, Wu L. Different Subsets of T Cells, Memory, Effector Functions, and CAR-T Immunotherapy. Cancers 2016;8. https://doi.org/10.3390/cancers8030036.

[196]   Littman DR, Rudensky AY. Th17 and Regulatory T Cells in Mediating and Restraining Inflammation. Cell 2010;140:845–58. https://doi.org/10.1016/j.cell.2010.02.021.

[197]   Diller ML, Kudchadkar RR, Delman KA, Lawson DH, Ford ML. Balancing Inflammation: The Link between Th17 and Regulatory T Cells. Mediators Inflamm 2016;2016:1–8. https://doi.org/10.1155/2016/6309219.

[198]   Berard M, Tough DF. Qualitative differences between naive and memory T cells. Immunology 2002;106:127–38. https://doi.org/10.1046/j.1365-2567.2002.01447.x.

[199]   Xu W, Larbi A. Markers of T Cell Senescence in Humans. Int J Mol Sci 2017;18:1742. https://doi.org/10.3390/ijms18081742.

[200]   Rea IM, Gibson DS, McGilligan V, McNerlan SE, Alexander HD, Ross OA. Age and Age-Related Diseases: Role of Inflammation Triggers and Cytokines. Front Immunol 2018;9:586. https://doi.org/10.3389/fimmu.2018.00586.

[201]   Müller L, Hamprecht K, Pawelec G. The Role of CMV in Immunosenescence. In: Bueno V, Lord JM, Jackson TA, editors. Ageing Immune Syst. Health, Cham: Springer International Publishing; 2017, p. 53–68. https://doi.org/10.1007/978-3-319-43365-3_4.

[202]   Cytomegalovirus (CMV) and Congenital CMV Infection 2020. https://www.cdc.gov/cmv/overview.html (accessed September 30, 2020).

[203]   Franceschi C, Garagnani P, Parini P, Giuliani C, Santoro A. Inflammaging: a new immune–metabolic viewpoint for age-related diseases. Nat Rev Endocrinol 2018;14:576–90. https://doi.org/10.1038/s41574-018-0059-4.

[204]   Calder PC, Ahluwalia N, Albers R, Bosco N, Bourdet-Sicard R, Haller D, et al. A Consideration of Biomarkers to be Used for Evaluation of Inflammation in Human Nutritional Studies. Br J Nutr 2013;109:S1–34. https://doi.org/10.1017/S0007114512005119.

[205]   Yi H-S, Kim SY, Kim JT, Lee Y-S, Moon JS, Kim M, et al. T-cell senescence contributes to abnormal glucose homeostasis in humans and mice. Cell Death Dis 2019;10:249. https://doi.org/10.1038/s41419-019-1494-4.

[206]   Lau EYM, Carroll EC, Callender LA, Hood GA, Berryman V, Pattrick M, et al. Type 2 diabetes is associated with the accumulation of senescent T cells. Clin Exp Immunol

2019;197:205–13. https://doi.org/10.1111/cei.13344.

[207]   Mountjoy M, Sundgot-Borgen JK, Burke LM, Ackerman KE, Blauwet C, Constantini N, et al. IOC consensus statement on relative energy deficiency in sport (RED-S): 2018 update. Br J Sports Med 2018;52:687–97. https://doi.org/10.1136/bjsports-2018-099193.

[208]   Loucks AB, Kiens B, Wright HH. Energy availability in athletes. J Sports Sci 2011;29:S7–15. https://doi.org/10.1080/02640414.2011.588958.

[209]   Souza MF, Tomeleri CM, Ribeiro AS, Schoenfeld BJ, Silva AM, Sardinha LB, et al. Effect of resistance training on phase angle in older women: A randomized controlled trial. Scand J Med Sci Sports 2017;27:1308–16. https://doi.org/10.1111/sms.12745.

[210]   Fung TT, Pan A, Hou T, Chiuve SE, Tobias DK, Mozaffarian D, et al. Long-Term Change in Diet Quality Is Associated with Body Weight Change in Men and Women. J Nutr 2015;145:1850–6. https://doi.org/10.3945/jn.114.208785.

[211]   Strandberg E, Ponsot E, Piehl-Aulin K, Falk G, Kadi F. Resistance Training Alone or Combined With N-3 PUFA-Rich Diet in Older Women: Effects on Muscle Fiber Hypertrophy. J Gerontol A Biol Sci Med Sci 2019;74:489–94. https://doi.org/10.1093/gerona/gly130.

[212]   Albracht-Schulte K, Kalupahana NS, Ramalingam L, Wang S, Rahman SM, Robert-McComb J, et al. Omega-3 fatty acids in obesity and metabolic syndrome: a mechanistic update. J Nutr Biochem 2018;58:1–16. https://doi.org/10.1016/j.jnutbio.2018.02.012.

[213]   Kopecky J, Rossmeisl M, Flachs P, Kuda O, Brauner P, Jilkova Z, et al. *n*-3 PUFA: bioavailability and modulation of adipose tissue function: Symposium on 'Frontiers in adipose tissue biology.' Proc Nutr Soc 2009;68:361–9. https://doi.org/10.1017/S0029665109990231.

[214]   Flachs P, Rossmeisl M, Bryhn M, Kopecky J. Cellular and molecular effects of n−3 polyunsaturated fatty acids on adipose tissue biology and metabolism. Clin Sci 2009;116:1–16. https://doi.org/10.1042/CS20070456.

[215]   Flachs P, Horakova O, Brauner P, Rossmeisl M, Pecina P, Franssen-van Hal N, et al. Polyunsaturated fatty acids of marine origin upregulate mitochondrial biogenesis and induce β-oxidation in white fat. Diabetologia 2005;48:2365–75. https://doi.org/10.1007/s00125-005-1944-7.

[216]   Todorčević M, Hodson L. The Effect of Marine Derived n-3 Fatty Acids on Adipose Tissue Metabolism and Function. J Clin Med 2015;5. https://doi.org/10.3390/jcm5010003.

[217]   Mounien L, Tourniaire F, Landrier J-F. Anti-Obesity Effect of Carotenoids: Direct Impact on Adipose Tissue and Adipose Tissue-Driven Indirect Effects. Nutrients 2019;11:1562. https://doi.org/10.3390/nu11071562.

[218]   Canas JA, Lochrie A, McGowan AG, Hossain J, Schettino C, Balagopal PB. Effects of Mixed Carotenoids on Adipokines and Abdominal Adiposity in Children: A Pilot Study. J Clin Endocrinol Metab 2017;102:1983–90. https://doi.org/10.1210/jc.2017-00185.

[219]   Kakutani R, Hokari S, Nishino A, Ichihara T, Sugimoto K, Takaha T, et al. Effect of

Oral Paprika Xanthophyll Intake on Abdominal Fat in Healthy Overweight Humans: A Randomized, Double-blind, Placebo-controlled Study. J Oleo Sci 2018;67:1149–62. https://doi.org/10.5650/jos.ess18076.

[220]   Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Manuel Gómez J, et al. Bioelectrical impedance analysis—part II: utilization in clinical practice. Clin Nutr 2004;23:1430–53. https://doi.org/10.1016/j.clnu.2004.09.012.

[221]   Verdich C, Barbe P, Petersen M, Grau K, Ward L, Macdonald I, et al. Changes in body composition during weight loss in obese subjects in the NUGENOB study: comparison of bioelectrical impedance vs. dual-energy X-ray absorptiometry. Diabetes Metab 2011;37:222–9. https://doi.org/10.1016/j.diabet.2010.10.007.

[222]   Thomson R, Brinkworth GD, Buckley JD, Noakes M, Clifton PM. Good agreement between bioelectrical impedance and dual-energy X-ray absorptiometry for estimating changes in body composition during weight loss in overweight young women. Clin Nutr 2007;26:771–7. https://doi.org/10.1016/j.clnu.2007.08.003.

[223]   Tinsley GM, Morales E, Forsse JS, Grandjean PW. Impact of Acute Dietary Manipulations on DXA and BIA Body Composition Estimates: Med Sci Sports Exerc 2017;49:823–32. https://doi.org/10.1249/MSS.0000000000001148.

[224]   Thompson DL, Thompson WR, Prestridge TJ, Bailey JG, Bean MH, Brown SP, et al. Effects of hydration and dehydration on body composition analysis: a comparative study of bioelectric impedance analysis and hydrodensitometry. J Sports Med Phys Fitness 1991;31:565–70.

[225]   Lang P-O, Trivalle C, Vogel T, Proust J, Papazian J-P. Markers of metabolic and cardiovascular health in adults: Comparative analysis of DEXA-based body composition components and BMI categories. J Cardiol 2015;65:42–9. https://doi.org/10.1016/j.jjcc.2014.03.010.

[226]   McNaughton SA, Dunstan DW, Ball K, Shaw J, Crawford D. Dietary Quality Is Associated with Diabetes and Cardio-Metabolic Risk Factors. J Nutr 2009;139:734–42. https://doi.org/10.3945/jn.108.096784.

[227]   Place AR. Comparative aspects of lipid digestion and absorption: physiological correlates of wax ester digestion. Am J Physiol 1992;263:R464-471. https://doi.org/10.1152/ajpregu.1992.263.3.R464.

[228]   Cook CM, Larsen TS, Derrig LD, Kelly KM, Tande KS. Wax Ester Rich Oil From The Marine Crustacean, Calanus finmarchicus, is a Bioavailable Source of EPA and DHA for Human Consumption. Lipids 2016;51:1137–44. https://doi.org/10.1007/s11745-016-4189-y.

[229]   Sarter B, Kelsey KS, Schwartz TA, Harris WS. Blood docosahexaenoic acid and eicosapentaenoic acid in vegans: Associations with age and gender and effects of an algal-derived omega-3 fatty acid supplement. Clin Nutr 2015;34:212–8.

https://doi.org/10.1016/j.clnu.2014.03.003.

[230]   Köhler A, Heinrich J, von Schacky C. Bioavailability of Dietary Omega-3 Fatty Acids Added to a Variety of Sausages in Healthy Individuals. Nutrients 2017;9:629. https://doi.org/10.3390/nu9060629.

[231]   Berge K, Musa-Veloso K, Harwood M, Hoem N, Burri L. Krill oil supplementation lowers serum triglycerides without increasing low-density lipoprotein cholesterol in adults with borderline high or high triglyceride levels. Nutr Res 2014;34:126–33. https://doi.org/10.1016/j.nutres.2013.12.003.

[232]   Cao J, Schwichtenberg KA, Hanson NQ, Tsai MY. Incorporation and Clearance of Omega-3 Fatty Acids in Erythrocyte Membranes and Plasma Phospholipids. Clin Chem 2006;52:2265–72. https://doi.org/10.1373/clinchem.2006.072322.

[233]   Keenan AH, Pedersen TL, Fillaus K, Larson MK, Shearer GC, Newman JW. Basal omega-3 fatty acid status affects fatty acid and oxylipin responses to high-dose n3-HUFA in healthy volunteers. J Lipid Res 2012;53:1662–9. https://doi.org/10.1194/jlr.P025577.

[234]   Greupner T, Koch E, Kutzner L, Hahn A, Schebb NH, Schuchardt JP. Single-Dose SDA-Rich Echium Oil Increases Plasma EPA, DPAn3, and DHA Concentrations. Nutrients 2019;11:2346. https://doi.org/10.3390/nu11102346.

[235]   Harris WS, Lemke SL, Hansen SN, Goldstein DA, DiRienzo MA, Su H, et al. Stearidonic Acid-Enriched Soybean Oil Increased the Omega-3 Index, an Emerging Cardiovascular Risk Marker. Lipids 2008;43:805–11. https://doi.org/10.1007/s11745-008-3215-0.

[236]   Østbye T-KK, Berge GM, Nilsson A, Romarheim OH, Bou M, Ruyter B. The long-chain monounsaturated cetoleic acid improves the efficiency of the n-3 fatty acid metabolic pathway in Atlantic salmon and human HepG2 cells. Br J Nutr 2019;122:755–68. https://doi.org/10.1017/S0007114519001478.

[237]   Villanova L, Vernucci E, Pucci B, Pellegrini L, Nebbioso M, Mauri C, et al. Influence of age and physical exercise on sirtuin activity in humans. J Biol Regul Homeost Agents 2013;27:497–507.

[238]   Potthast AB, Nebl J, Wasserfurth P, Haufe S, Eigendorf J, Hahn A, et al. Impact of Nutrition on Short-Term Exercise-Induced Sirtuin Regulation: Vegans Differ from Omnivores and Lacto-Ovo Vegetarians. Nutrients 2020;12:1004. https://doi.org/10.3390/nu12041004.

[239]   Sylow L, Richter EA. Current advances in our understanding of exercise as medicine in metabolic disease. Curr Opin Physiol 2019;12:12–9. https://doi.org/10.1016/j.cophys.2019.04.008.

[240]   Li X. SIRT1 and energy metabolism. Acta Biochim Biophys Sin 2013;45:51–60. https://doi.org/10.1093/abbs/gms108.

[241]   Palacios OM, Carmona JJ, Michan S, Chen KY, Manabe Y, Iii JLW, et al. Diet and

exercise signals regulate SIRT3 and activate AMPK and PGC-1α in skeletal muscle. Aging 2009;1:771–83. https://doi.org/10.18632/aging.100075.

[242]   Yang H, Youm Y-H, Vandanmagsar B, Rood J, Kumar KG, Butler AA, et al. Obesity accelerates thymic aging. Blood 2009;114:3803–12. https://doi.org/10.1182/blood-2009-03-213595.

[243]   Shirakawa K, Yan X, Shinmura K, Endo J, Kataoka M, Katsumata Y, et al. Obesity accelerates T cell senescence in murine visceral adipose tissue. J Clin Invest 2016;126:4626–39. https://doi.org/10.1172/JCI88606.

[244]   Lachmann R, Loenenbach A, Waterboer T, Brenner N, Pawlita M, Michel A, et al. Cytomegalovirus (CMV) seroprevalence in the adult population of Germany. PLOS ONE 2018;13:e0200267. https://doi.org/10.1371/journal.pone.0200267.

[245]   Chen S, de Craen AJ, Raz Y, Derhovanessian E, Vossen AC, Rudi WG, et al. Cytomegalovirus seropositivity is associated with glucose regulation in the oldest old. Results from the Leiden 85-plus Study. Immun Ageing 2012;9:18. https://doi.org/10.1186/1742-4933-9-18.

[246]   Heaney S, O'Connor H, Naughton G, Gifford J. Towards an Understanding of the Barriers to Good Nutrition for Elite Athletes. Int J Sports Sci Coach 2008;3:391–401. https://doi.org/10.1260/174795408786238542.

[247]   Giel KE, Hermann-Werner A, Mayer J, Diehl K, Schneider S, Thiel A, et al. Eating disorder pathology in elite adolescent athletes: Eating Disorder Pathology in Young Elite Athletes. Int J Eat Disord 2016;49:553–62. https://doi.org/10.1002/eat.22511.

[248]   Condon EM, Dube KA, Herbold NH. The Influence of the Low-Carbohydrate Trend on Collegiate Athletes' Knowledge, Attitudes, and Dietary Intake of Carbohydrates: Top Clin Nutr 2007;22:175–84. https://doi.org/10.1097/01.TIN.0000270136.22969.d7.

[249]   Beckner BN, Record RA. Navigating the thin-ideal in an athletic world: influence of coach communication on female athletes' body image and health choices. Health Commun 2016;31:364–73. https://doi.org/10.1080/10410236.2014.957998.

[250]   Stirling AE, Kerr GA. The perceived effects of elite athletes' experiences of emotional abuse in the coach–athlete relationship. Int J Sport Exerc Psychol 2013;11:87–100. https://doi.org/10.1080/1612197X.2013.752173.

[251]   Arthur-Cameselle JN, Quatromoni PA. Factors Related to the Onset of Eating Disorders Reported by Female Collegiate Athletes. Sport Psychol 2011;25:1–17. https://doi.org/10.1123/tsp.25.1.1.

[252]   Fedewa MV, Hathaway ED, Ward-Ritacco CL, Williams TD, Dobbs WC. The Effect of Chronic Exercise Training on Leptin: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Sports Med 2018;48:1437–50. https://doi.org/10.1007/s40279-018-0897-1.

[253]   Ahima RS, Saper CB, Flier JS, Elmquist JK. Leptin regulation of neuroendocrine systems. Front Neuroendocrinol 2000;21:263–307. https://doi.org/10.1006/frne.2000.0197.

[254]   Müller MJ, Enderle J, Pourhassan M, Braun W, Eggeling B, Lagerpusch M, et al. Metabolic adaptation to caloric restriction and subsequent refeeding: the Minnesota Starvation Experiment revisited. Am J Clin Nutr 2015;102:807–19. https://doi.org/10.3945/ajcn.115.109173.

[255]   Loucks AB, Heath EM. Induction of low-T3 syndrome in exercising women occurs at a threshold of energy availability. Am J Physiol 1994;266:R817-823. https://doi.org/10.1152/ajpregu.1994.266.3.R817.

[256]   Loucks AB, Thuma JR. Luteinizing Hormone Pulsatility Is Disrupted at a Threshold of Energy Availability in Regularly Menstruating Women. J Clin Endocrinol Metab 2003;88:297–311. https://doi.org/10.1210/jc.2002-020369.

[257]   Koehler K, Hoerner NR, Gibbs JC, Zinner C, Braun H, De Souza MJ, et al. Low energy availability in exercising men is associated with reduced leptin and insulin but not with changes in other metabolic hormones. J Sports Sci 2016;34:1921–9. https://doi.org/10.1080/02640414.2016.1142109.

[258]   Nattiv A, Loucks AB, Manore MM, Sanborn CF, Sundgot-Borgen J, Warren MP, et al. American College of Sports Medicine position stand. The female athlete triad. Med Sci Sports Exerc 2007;39:1867–82. https://doi.org/10.1249/mss.0b013e318149f111.

[259]   Hackney AC. Hypogonadism in Exercising Males: Dysfunction or Adaptive-Regulatory Adjustment? Front Endocrinol 2020;11:11. https://doi.org/10.3389/fendo.2020.00011.

[260]   Sale C, Elliott-Sale KJ. Nutrition and Athlete Bone Health. Sports Med 2019. https://doi.org/10.1007/s40279-019-01161-2.

[261]   Geidl W, Schlesinger S, Mino E, Miranda L, Pfeifer K. Dose–response relationship between physical activity and mortality in adults with noncommunicable diseases: a systematic review and meta-analysis of prospective observational studies. Int J Behav Nutr Phys Act 2020;17:109. https://doi.org/10.1186/s12966-020-01007-5.

Appendix Paper I

Table S 1: Questionnaire based physical activity levels of the participants at baseline (0), after six weeks (6) and 12 weeks after the intervention (12).

	t	CON	EX	EXDC	EXCO	*p*
Basal activity	0	4.26 ± 3.81	4.17 ± 6.02	2.14 ± 1.86	3.12 ± 2.85	
	6	4.84 ± 4.70	4.50 ± 3.76	2.95 ± 2.51	5.18 ± 4.34	0.257
	12	6.73 ± 10.0	6.80 ± 8.97	4.99 ± 4.31	6.66 ± 5.13	
Leisure time activity	0	3.04 ± 3.95	2.14 ± 2.74	1.16 ± 1.10	1.90 ± 2.26	
	6	3.69 ± 4.66	2.39 ± 2.20	2.67 ± 2.00	1.71 ± 2.28	0.184
	12	4.36 ± 6.46	3.89 ± 5.59	2.65 ± 2.59	3.22 ± 4.48	
Sport activity	0	0.38 ± 1.04	0.33 ± 0.52	0.30 ± 0.69	0.31 ± 0.95	
	6	0.40 ± 0.90	0.54 ± 1.21	0.32 ± 0.71	0.53 ± 0.85	0.468
	12	0.20 ± 0.47	0.50 ± 0.95	0.32 ± 0.70	0.19 ± 0.46	
Total activity [hours/week]	0	7.69 ± 5.34	6.63 ± 7.60	3.59 ± 2.56	5.33 ± 3.88	
	6	8.93 ± 7.37	7.43 ± 5.66	5.94 ± 3.94	7.42 ± 4.80	0.151
	12	11.3 ± 14.5	11.2 ± 12.2	7.97 ± 6.02	10.1 ± 7.0	

Data shown as mean ± SD. Data were analyzed using two way repeated measures ANOVA. P values represent time*intervention interaction. t=time in weeks

Table S 2: Dietary Intake of food groups at baseline (0), after six weeks (6) and at the end of the intervention (12).

	t	CON	EX	EXDC	EXCO	*p*
Fruit intake [portion/day]	0	1.39 ± 1.06	1.85 ± 1.09	1.43 ± 0.98	2.02 ± 2.48	
	6	1.27 ± 0.92	1.78 ± 1.42	1.96 ± 0.96	1.78 ± 1.47	**0.006**
	12	1.32 ± 0.78	1.83 ± 1.89	2.14 ± 1.26‡	1.86 ± 1.52	
Vegetable intake [portion/day]	0	1.32 ± 1.87	1.10 ± 0.79	0.86 ± 0.34	0.95 ± 0.76	
	6	0.95 ± 0.63	0.97 ± 0.70	1.17 ± 0.74	0.97 ± 0.59	**0.001**
	12	0.77 ± 0.48*	1.10 ± 0.97	1.49 ± 1.25‡	0.96 ± 0.73	
Meat intake [portion/day]	0	1.63 ± 1.63	2.02 ± 2.04	1.33 ± 1.07	1.57 ± 1.18	
	6	1.77 ± 2.31	1.57 ± 1.68	1.08 ± 0.77	1.08 ± 0.79	0.480
	12	1.51 ± 1.63	1.41 ± 1.45	1.06 ± 0.85	1.08 ± 0.88	
Meat intake [g/week]	0	621.9 ± 333.2	667.0 ± 494.9	528.81± 346.6	523.2 ± 268.3	
	6	605.1 ± 384.2	485.3± 385.6	443.82± 256.3	383.4 ± 231.0	0.225
	12	536.4 ± 322.8	504.8 ± 327.1	418.1± 258.0	383.8 ± 207.9	
Cereal intake [portion/day]	0	3.38 ± 1.97	3.38 ± 2.37	3.03 ± 1.77	3.17 ± 1.44	
	6	2.80 ± 1.71	2.81 ± 1.56	2.67 ± 1.03	3.28 ± 1.85	0.521
	12	2.52 ± 1.06†	2.73 ± 1.55	2.75 ± 1.26	2.84 ± 1.30	
Milk intake [portion/day]	0	2.35± 1.80	3.02± 1.82	3.22± 2.15	3.01± 2.32	
	6	2.22± 1.62	2.51± 1.41	3.20± 1.07	2.83± 2.39	0.366
	12	2.08± 1.48	2.51± 1.56	3.71± 2.47	2.91± 2.03	
Fish intake [portion/week]	0	1.23± 1.10	1.49± 2.18	1.77± 1.53	1.26± 1.05	
	6	1.28± 0.99	1.16± 1.00	2.51± 1.94	1.70± 1.53	**0.010**
	12	1.08± 0.72	1.23± 1.06	2.69± 1.71‡	1.36± 1.04	

Data shown as mean ± SD. P values represent time*intervention interaction analyzed with two way repeated measure ANOVA. In case of significance, asterisks indicate statistical differences within groups detected with post hoc Bonferroni (* *p* < 0.05; † *p* < 0.01; ‡ *p* < 0.001).t=time in weeks.

Appendix Acknowledgements

Appendix Curriculum vitae

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Berufserfahrung

Seit 04/2017	Wissenschaftliche Mitarbeiterin, Institut für Lebensmittelwissenschaft und Humanernährung, Gottfried Wilhelm Leibniz Universität Hannover
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Seit 04/2017	Promotionsstudium am Institut für Lebensmittelwissenschaft und Humanernährung, Gottfried Wilhelm Leibniz Universität Hannover
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06/2013-08/2013	Praktikum an der Deakin University Australia am Institut für Ökophysiologie
12/2010-02/2011	Arbeit als wissenschaftliche Hilfskraft in der Abteilung für experimentelle Verhaltens- und Evolutionsforschung an der Tierärztlichen Hochschule Hannover

Schulabschluss

08/2002-08/2009	Allgemeine Hochschulreife, Bischöfliches Gymnasium Josephinum, Hildesheim

List of publications

Boßlau T.K., **Wasserfurth P.**, Krüger B., Reichel T., Palmowski J., Josefine Nebl J., Weyh C., Schenk A., Joisten N., Stahl F., Thoms S., Gebhardt K., Hahn A., Krüger K., 2021. Abdominal Obesity-Related Disturbance of Insulin Sensitivity Is Associated with CD8+ EMRA Cells in the Elderly. *Cells*, 10(5):998. https://doi.org/10.3390/cells10050998

Wasserfurth, P*., Palmowski, J*., Hahn, A., Krüger, K., 2020. Reasons for and Consequences of Low Energy Availability in Female and Male Athletes: Social Environment, Adaptations, and Prevention. Sports Med - Open 6, 44. https://doi.org/10.1186/s40798-020-00275-6

Wasserfurth, P., Nebl, J., Boßlau, T.K., Krüger, K., Hahn, A., Schuchardt, J.P., 2020. 12-weeks of *Calanus finmarchicus* oil intake improves omega-3-index in healthy older subjects engaging in an exercise program. Br J Nutr 1–17. https://doi.org/10.1017/S0007114520002809

Wasserfurth, P., Nebl, J., Schuchardt, J.P., Müller, M., Boßlau, T.K., Krüger, K., Hahn, A., 2020. Effects of Exercise Combined with a Healthy Diet or *Calanus finmarchicus* Oil Supplementation on Body Composition and Metabolic Markers—A Pilot Study. Nutrients 12, 2139. https://doi.org/10.3390/nu12072139

Potthast, A.B*., Nebl, J*., **Wasserfurth, P**., Haufe, S., Eigendorf, J., Hahn, A., Das, A., 2020. Impact of Nutrition on Short-Term Exercise-Induced Sirtuin Regulation: Vegans Differ from Omnivores and Lacto-Ovo Vegetarians. Nutrients 12, 1004. https://doi.org/10.3390/nu12041004

Nebl, J., Schuchardt, J.P., **Wasserfurth, P**., Haufe, S., Eigendorf, J., Tegtbur, U., Hahn, A., 2019. Characterization, dietary habits and nutritional intake of omnivorous, lacto-ovo vegetarian and vegan runners – a pilot study. BMC Nutrition 5, 51. https://doi.org/10.1186/s40795-019-0313-8

Nebl, J., Drabert, K., Haufe, S., **Wasserfurth, P**., Eigendorf, J., Tegtbur, U., Hahn, A., Tsikas, D., 2019. Exercise-Induced Oxidative Stress, Nitric Oxide and Plasma Amino Acid Profile in Recreational Runners with Vegetarian and Non-Vegetarian Dietary Patterns. Nutrients 11, 1875. https://doi.org/10.3390/nu11081875

Nebl, J., Haufe, S., Eigendorf, J., **Wasserfurth, P**., Tegtbur, U., Hahn, A., 2019. Exercise capacity of vegan, lacto-ovo-vegetarian and omnivorous recreational runners. J Int Soc Sports Nutr 16, 23. https://doi.org/10.1186/s12970-019-0289-4

Nebl, J., Schuchardt, J.P., Ströhle, A., **Wasserfurth, P**., Haufe, S., Eigendorf, J., Tegtbur, U., Hahn, A., 2019. Micronutrient Status of Recreational Runners with Vegetarian or Non-Vegetarian Dietary Patterns. Nutrients 11. https://doi.org/10.3390/nu11051146

Schuchardt, J.P., Hahn, A., Greupner, T., **Wasserfurth, P**., Rosales-López, M., Hornbacher, J., Papenbrock, J., 2019. Watercress – cultivation methods and health effects. https://doi.org/10.15488/9310

Wasserfurth, P., Schneider, I., Ströhle, A., Nebl, J., Bitterlich, N., Hahn, A., 2019. Effects of mineral waters on acid–base status in healthy adults: results of a randomized trial. Food Nutr Res 63. https://doi.org/10.29219/fnr.v63.3515

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