MASTERARBEIT | MASTER'S THESIS

Titel | Title

Analysis of fat-soluble vitamins in the NUMOQUA study using high performance liquid chromatography (HPLC): Focusing on vitamin A, vitamin E and beta-carotene

verfasst von | submitted by

Anna Bauer BSc

angestrebter akademischer Grad | in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien | Vienna, 2025

Studienkennzahl lt. Studienblatt | Degree programme code as it appears on the student record sheet:	UA 066 838
Studienrichtung lt. Studienblatt | Degree programme as it appears on the student record sheet:	Masterstudium Ernährungswissenschaften
Betreut von | Supervisor:	Univ.-Prof. Mag. Dr. Karl-Heinz Wagner

Acknowledgements

First and foremost, I would like to express my sincere gratitude to my supervisor, Univ.-Prof. Mag. Dr. Karl-Heinz Wagner, for his continuous support throughout the entire process. His expertise, and insightful feedback were invaluable to the development of this thesis.

I am also deeply grateful to Lina Maqboul, MSc, for her patient guidance in the laboratory and her constant support during every stage of this project. My warm thanks also go to Hannah Schnopfhagen, MSc, for her generous training in the laboratory.

Furthermore, I would like to thank Markus Spannbruckner, whose help with the analytical method, as well as his technical support and advice during my laboratory work, were of great importance. I am also very thankful for the entire team at the Department of Nutritional Sciences in Vienna for creating such a supportive and encouraging environment.

I would also like to thank my dear colleague and friend Katrina Stachowitz for the wonderful time we had in the laboratory, the great collaboration and support. Many funny moments made this time special.

Finally, a huge thank you goes out to my beloved family and friends, who have supported and motivated me throughout my studies and during the demanding process of completing this master´s thesis.

This accomplishment would not have been possible without you.

Thank you!

List of contents

List of abbreviations

BMI	*Body Mass Index*
BCO1/2	*β-Carotene oxygenase 1/2*
CG	*Control group*
CRBP	*Cellular retinol-binding protein*
DCM	*Dichloromethane*
ECW	*Extracellular water*
EtOH	*Ethanol*
FM	*Fat mass*
FMI	*Fat mass index*
FFM	*Fat-free mass*
HPLC	*High performance liquid chromatography*
IG	*Intervention group*
LDL	*Low-density lipoprotein*
MeOH	*Methanol*
OA	*Osteoarthritis*
PAL	*Physical activity level*
QC	*Quality control*
RAR	*Retinoic acid receptor*
RCT	*Randomised controlled trial*
ROS	*Reactive oxygen species*
RXR	*Retinoid X receptor*
SMM	*Skeletal muscle mass*
SMI	*Skeletal muscle index*
SSMM	*Segmental skeletal muscle mass*
TEE	*Total energy expenditure*
TBW	*Total body water*
α-TTP	*α-Tocopherol transfer protein*
VLDL	*Very low-density lipoprotein*

List of figures

List of tables

1 Introduction

Fat-soluble vitamins play a central role in numerous physiological processes in the human body. This group includes vitamins A, D, E and K, as well as the provitamin β-carotene, which acts as a precursor to vitamin A. They are essential for functions such as cell growth, antioxidant protection, immune defence or vision. Since the human organism cannot synthesise many of these vitamins itself, or can only do so insufficiently, an adequate supply through food is important [Andres et al., 2024].

Disorders in fat metabolism, an unbalanced diet or certain diseases can affect the absorption, transport and storage of fat-soluble vitamins. A deficiency can lead to health consequences, such as impaired vision, increased oxidative stress or reduced immune function. On the other hand, excessive intake, especially through supplements, can cause toxic effects. Therefore, the accurate determination of the concentration of these vitamins in biological samples is an important part of nutritional and clinical research [Youness et al., 2022; Traber & Atkinson, 2019].

High-performance liquid chromatography (HPLC) is a reliable and sensitive method for the quantitative determination of fat-soluble vitamins. It enables the precise separation and analysis of compounds with different chemical characteristics. Different detection systems, such as fluorescence or UV/VIS detection, can be used to determine vitamin A (retinol), vitamin E (tocopherols) and β-carotene in particular [Karazniewicz-Lada & Glowka, 2016; Moldoveanu & David, 2013].

As part of the randomized controlled intervention NUMOQUA study biological samples were collected to investigate the effect of a combined nutritional therapy and an evidence-based exercise program on the quality of life of patients with knee osteoarthritis (OA). The analysis of fat-soluble vitamins in these samples provides information about the nutritional status of the study participants and can help to provide preventive nutritional strategies [Höld et al., 2024].

The aim of this master thesis is to determine and evaluate the concentrations of vitamin A (retinol), vitamin E (α- & γ-tocopherol) and β-carotene in the samples from the NUMOQUA study. One main focus was also the re-establishment of the HPLC method, to guarantee reliable and reproducible results.

2 Literature review

2.1 Fat-soluble vitamins

Fat-soluble vitamins are a group of essential micronutrients characterized by their lipophilic nature, which causes them to be mainly found in fat-containing foods. In the body, they are absorbed, transported and stored primarily via lipid metabolism. Key storage sites are the liver and adipose tissue. Important fat-soluble vitamins are vitamin A and provitamin A carotenoids, vitamin E, vitamin K as well as vitamin D [Kono & Arai, 2014, O'Byrne & Blaner, 2013, Schmölzl et al., 2026]. These vitamins play important roles in many physiological processes, including the regulation of vision, antioxidant protection, cell growth and differentiation, bone health, blood coagulation and support of the immune system. A balanced intake of these vitamins is essential, as deficient or excessive intake can lead to health risks. [Andres et al., 2024]

2.1.1 Vitamin A

Vitamin A includes a group of fat-soluble compounds with similar biological functions that differ by modifications on the β-ionone ring and the polyunsaturated side chain. The main forms are all-trans-retinol, retinyl esters, retinal and retinoic acids. All-trans-retinol is the alcohol transport form in the body, whereas retinyl esters such as retinyl palmitate serve primarily as stable storage forms in the liver [O'Byrne & Blaner 2013]. Retinal, particularly 11-cis-retinal, is essential for visual function. It acts as the light sensitive chromophore bound to opsin in the retina [Friedman, 2007]. Retinoic acids, including all-trans- and 9-cis-retinoic acid, function as active ligands for nuclear receptors (RAR, RXR) and regulate gene expression involved in cell growth, differentiation and immune function [Rochette-Egly, 2015; Napoli, 2012]. Other derivates such as 13, 14-dihydroretinol and retro- or anhydroretinoids may have actions in regulating immune function, but their precise physiological roles are not clear right now [Kedishvili, 2016; O'Byrne & Blaner 2013].

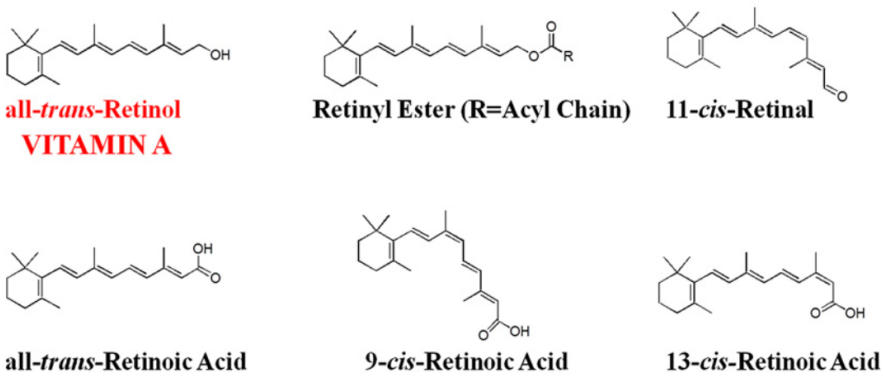

Figure 1: *Vitamin A structure [O'Byrne & Blaner 2013]*

2.1.1.1 Absorption and intestinal metabolism

The metabolism of vitamin A begins with the intake of preformed vitamin A (retinol or retinylesters) from animal sources and provitamin A carotenoids from plant-based foods (discussed in detail in section 2.1.3). The lipophilic retinylesters from animal foods are hydrolyzed during fat digestion in the small intestine, primarily by carboxylester lipase (CEL) from the pancreas [Weng et al., 1999; D'Ambrosio et al., 2011]. The resulting retinol is absorbed into the enterocytes of the small intestinal mucosa. There, with physiological intake, retinol binds to the cellular retinol-binding protein II (CRBPII) and is reesterified to retinylesters. Approximately 90% of this reesterification is catalized by lecithin retinol acyltransferase (LRAT), while the remaining 10% is mediated by acyl-CoA retinol acyltransferase (DGAT1). With very high intake, direct esterification can also take place without prior binding to CRBPII [Chelstowska et al., 2016; D'Ambrosio et al., 2011].

The newly formed retinylesters are incorporated into chylomicrons, together with dietary lipids and cholesterol, and transported into the systemic circulation via the lymphatic system. Hydrolysis of chylomicron triglycerides by lipoprotein lipase (LPL) releases some retinyl esters, which can be taken up directly by peripheral tissues, providing an RBP-independent vitamin A source. The majority of chylomicron remnants are, however, cleared by the liver through receptor-mediated uptake [O'Byrne & Blaner 2013].

2.1.1.2 Hepatic metabolism and systemic transport

In the liver, retinyl esters can be hydrolyzed to retinol, which is either bound to cellular retinol-binding protein (CRBP) for metabolic processing or transported to hepatic stellate cells for storage. Within these cells, retinol is re-esterified to retinylesters, forming the main storage pool of vitamin A in the body [Senoo et al., 2010; D'Ambrosio et al., 2011].

Mobilization of vitamin A from hepatic stores occurs via binding to retinol-binding protein (RBP). Due to its small molecular size, free RBP would be eliminated renally, however, complex formation with transthyretin (TTR) prevents this loss. The RBP-TTR-retinol complex formed in this way circulates in the plasma and delivers retinol to target cells by binding to the membrane receptor STRA6 [Steinhoff et al., 2022]. Cellular uptake of retinol is strictly regulated and occurs only in presence of intracellular binding proteins such as CRBP. Once inside the cell, retinol can bind to CRBP for intracellular transport and metabolism. It may also be oxidized to retinal, which, in specific tissues such as the retina, plays a role in the visual cycle or further oxidized to retinoic acid, which acts as a ligand for nuclear receptors such as RAR and RXR and regulated gene expression [O'Byrne & Blaner 2013].

Alternatively, retinol can be reesterified to retinylesters by the enzyme acyl-CoA retinol acyltransferase (ARAT) for intracellular storage, from which it can be remobilized by enzymatic hydrolysis when needed. Retinoic acid itself can also be taken up directly from the circulation, where it is bound to albumin and transported intracellularly by cellular retinoic acid-binding protein (CRABP) [Napoli, 2016; O'Byrne & Blaner 2013].

2.1.1.3 Biological functions

Physiologically, vitamin A plays an important role in numerous biological processes. Retinoic acid functions as a ligand for the nuclear receptors RAR (retinoic acid receptor) and RXR (retinoid X receptor), thereby regulating the expression of a wide range of genes. [Rochette-Egly & Germain, 2009] Vitamin A is also essential for vision, as 11-cis-retinal enables visual signal transduction. Additionally, it regulates cell proliferation and differentiation, particularly in skin and mucous membranes and supports immune defence by enhancing T- and B-cell function and maintaining barrier integrity [Al Tanoury

et al., 2013; Stephensen, 2001] Vitamin A metabolites also influence lipid metabolism and promote adipocyte differentiation [D'Ambrosio, 2011]. A deficiency in vitamin A can result in night blindness, xerophthalmia and increased sensitivity to infections, whereas excessive intake may lead to toxic effects [Andrés, 2024].

2.1.2 Vitamin E

Vitamin E is a group of fat-soluble compounds mostly known as tocopherols and tocotrienols. These compounds differ in the number and position of methyl groups on the chromanol ring, as well as in the saturation of the side chain. The most important biologically active form is RRR-α-tocopherol, which has three centres of chirality. Other isoforms such as β-, γ-, and δ-tocopherol occur in smaller quantities [Traber & Stevens, 2011]. Vitamin E primarily acts as a lipid antioxidant, protecting cell membranes from oxidative stress by scavenging reactive oxygen species and preventing lipid peroxidation [Traber & Atkinson, 2007].

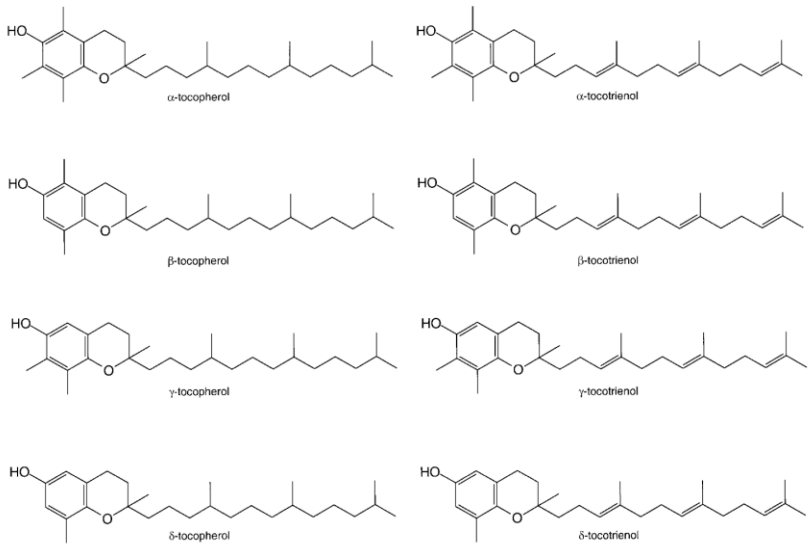

Figure 2: *Vitamin E structure [Brigelius-Flohé & Traber, 1999]*

2.1.2.1 Metabolism

Vitamin E is mainly found in plant oils, nuts and seeds. In the small intestine it is absorbed with dietary fats. This absorption works similarly to other fat-soluble vitamins, through micelle formation. Tocopheryl esters must first be hydrolyzed by lipases to release free tocopherols for absorption [Brigelius-Flohé & Traber, 1999; Schmölzl, 2016]. After

absorption vitamin E is incorporated into chylomicrons and transported via the lymphatic system into the bloodstream. The liver plays a central role in its distribution. The hepatic α-tocopherol transfer protein (α-TTP) prefers α-tocopherol and incorporates it into very-low-density lipoproteins (VLDL), which are then released into the circulation for distribution to peripheral tissues. A dynamic equilibrium is maintained by its exchange between various lipoprotein fractions. Uptake into target cells occurs either through lipoprotein lipase-mediated release, receptor-mediated endocytosis (e.g. LDL receptors) or selective α-tocopherol uptake from lipoproteins [Mardones & Rigotti, 2004]. Vitamin E is preferentially stored in adipose tissue and skeletal muscle. Other forms like γ- and δ-tocopherol are less well recognized by α-TTP and metabolized and excreted more rapidly [Traber & Stevens, 2011]. The breakdown process starts with ω-hydroxylation by the enzyme CYP4F2, followed by β-oxidation of the side chain. This leads to water-soluble compounds such as CEHCs (carboxyethyl-hydroxychromans), which are excreted in the urine. When intake is high, these metabolites can also be conjugated for a more efficient elimination [Jiang, 2014].

2.1.2.2 Biological functions

Physiologically vitamin E is especially important for its antioxidant function, as it protects cell membranes from oxidative damage. It achieves this by scavenging reactive oxygen species (ROS), thereby preventing lipid peroxidation. Polyunsaturated fatty acids in membrane lipids are especially susceptible to attack by free radicals such as hydroxyl and peroxyl radicals, leading to chain reactions that compromise membrane integrity [Brigelius-Flohé & Traber, 1999]. Vitamin E interrupts these chain reactions by donating an electron or hydrogen atom from its hydroxyl group to lipid radicals, forming a relatively stable tocopherol radical. This tocopherol radical can be regenerated to its active form by other antioxidants, especially vitamin C, which in turn is regenerated by glutathione. This interconnected antioxidant network ensures continuous protection against oxidative stress. [Traber & Stevens, 2011]

Due to its lipophilic nature, vitamin E integrates into biological membranes and is therefore also important for stabilization and maintenance of membrane structure. This protective function is particularly important in tissues with high oxygen turnover or high

levels of unsaturated fatty acids, such as in the brain, eyes, or lungs [Schmölzl et al., 2016; Lemaire-Ewing et al., 2010]

Beyond its antioxidant properties it also shows anti-inflammatory effects and modulates immune responses. It plays a key role in infection defense and the regulation of chronic inflammation [Meydani, 2005]. Vitamin E influences signal transduction and gene expression, for example by effecting protein kinases and transcription factors [Jiang, 1014; Traber & Atkinson, 2007].

A deficiency in vitamin E is rare but can occur in cases of fat malabsorption disorders or genetic defects. Such deficiencies can lead to neurological disorders characterized by nerve cell damage, including ataxia and peripheral neuropathy [Traber, 2024]. Excessive vitamin E is also uncommon but may cause coagulation disturbances. High levels can interfere with vitamin K dependent clotting factors, potentially leading to bleeding complications [Schmölzl et al., 2016].

2.1.3 β -Carotene

β -carotene is a fat-soluble provitamin A and belongs to the group of carotenoids, which are found in plants, algae and some microorganisms. Chemically, β-carotene consists of a long polyene hydrocarbon chain with conjugated double bonds, responsible for its characteristic orange colour. It is a C40 tetraterpenoid with two β-Ione rings at both ends of the molecule. As a provitamine A, β-carotene can be enzymatically converted in human cells to retinal, the biologically active aldehyde form of vitamin A [Geune et al., 2010]

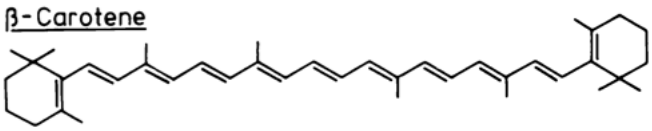

***Figure 3**: β-Carotene structure [Sies & Stahl, 1995]*

2.1.3.1 Metabolism

The absorption of β-carotene occurs in the small intestine, where it is incorporated into micelles along with other lipophilic substances and then absorbed by the scavenger receptor class B type 1 (SR-B1) in enterocytes. The receptors expression is regulated in response to the vitamin A status. The key enzymatic cleavage is performed by β-carotene-15,15'dioxygenase (BCO1), which splits the molecule into two molecules of retinal. This reaction is also regulated by vitamin A levels. When sufficient vitamin A is available, the expression of BCO1 is reduced. Alternatively, asymmetric cleavage via BCO2 can occur, producing apocarotenoids that can be further metabolized to retinoids. Some of the ß-carotene and the retinol formed is packed in chylomicrons and transported to the liver via the lymphatic system. There, ß-carotene can be converted to vitamin A or stored in peripheral tissues, especially in subcutaneous fat. Polar vitamin A metabolites such as retinoic acid, leave the enterocytes directly via the portal vein. [D'Ambrosio et al., 2011; Grune et al., 2010]

2.1.3.2 Biological functions

While ß-carotene serves as a vital source of vitamin A, it also provides independent health benefits. Due to its unique structure β-carotene acts as a potent antioxidant. It is particularly effective at quenching singlet oxygen and scavenging ROS, especially peroxyl radicals. [Grune et al., 2010] This antioxidant activity protects cellular membranes from oxidative stress, especially in light-exposed tissues such as the skin, where ß-carotene accumulates and supports photoprotection by neutralizing UV-induced oxidative damage [Sies & Stahl, 2003; Krinsky et al., 2003]. Unlike antioxidants such as tocopherols, ß carotene is broken down during the radical scavenging process and cannot be regenerated, which limits its long-term antioxidant capacity. For this reason, its principal function in human nutrition is that of a provitamin A, rather than a sustained radical scavenger [Grune et al., 2010].

In addition to its antioxidant properties, ß carotene supports immune function, modulates inflammation and plays a protective role for eyes and the skin. It plays a role in protecting against age-related degenerative diseases, including cardiovascular disease or certain cancers [Tanaka et al., 2012; Grune et al., 2010] In the immune system,

ß-carotene preserves the structural integrity of immune cells and helps modulate inflammatory signalling pathways, potentially reducing the risk of chronic inflammatory conditions [Chew & Park, 2004]. Furthermore, it influences intracellular communication and plays a role in the regulation of cell growth and differentiation. This is partly mediated through its conversion to retinoids, such as retinoic acid, which function as ligands for nuclear receptors and regulate gene expression [Grune et al., 2010].

Unlike preformed vitamin A, β-carotene is generally non-toxic because its conversation to vitamin A is regulated by the body, making overdose unlikely [Penniston & Tanumihardjo 2006]. In the diet, β-carotene is mainly found in orange and dark green vegetables such as carrots, sweet potatoes, spinach and kale. Its bioavailability can be increased by processing food (e.g. cooking) and the presence of fats in the meal. [Grune et al., 2010]

2.2 Relevance of fat-soluble vitamins in knee osteoarthritis

Osteoarthritis (OA), particularly knee OA, is a degenerative joint disease characterized by progressive cartilage degradation, inflammatory processes, and changes in the subchondral bone leading to functional decline and loss of quality in life. Worldwide OA is the most common arthritic disease and the 11th highest global reason of disability leading to significant morbidity and high costs for the healthcare system [Bruyere et al., 2019] [Tan et al., 2020]. Due to ongoing demographic changes, the prevalence of knee OA is expected to increase in the future [Davis et al., 2018]. Physical activity and exercise therapy represent important and effective components of early treatment, which are highly recommended as first-line treatment of OA [Wellsandt & Golightly, 2018]. Besides that, there is accumulating evidence that inflammation processes play an essential role in OA pathogenesis [Berenbaum, 2018]. The composition of the diet has an important influence on inflammation and plays a decisive role in inflammation related diseases [Minihane et al., 2015]. In the context of nutritional therapy, as a supplement to physical treatment, it makes sense to focus on foods with a high content of inflammation-modulating components. These include in particular polyphenols and vitamins from fruits and vegetables, polyunsaturated fatty acids from vegetable oils and fish and a diet

rich in fibre [Cena & Calder, 2020] [Bärebring et al., 2018] [Messina et al., 2019]. Of particular interest in the prevention and modulation of disease progression are antioxidants, such as vitamin E or carotenoids that reduce lipid peroxidation and free radical damage. These nutrients have been linked to longevity mainly due to their antioxidant and anti-inflammatory effects [Thomas 2006] [Ames 2018].

Several RCTs have been conducted on vitamin E, particularly α-tocopherol, but these have not been able to demonstrate any clear benefits. Brand et al. reported that six months of supplementation with 500 IU of α-tocopherol had no significant effect on pain, stiffness or function in patients with knee OA. Wluka et al. found similar results, observing no effect on cartilage volume or symptoms a two-year period [Brand et al., 2001] [Wulka et al., 2002]. Only short-term improvements in oxidative stress markers and symptoms were described, but the clinical relevance remains unclear [Tantavisut et al., 2017]. For vitamin A, both epidemiological and mechanistic data point to a rather adverse association. In an NHANES analysis, elevated serum vitamin A levels were associated with higher prevalence of OA. Vitamin A showed a protective effect within a certain concentration range, while higher levels can contribute to the development of OA [Wu et al., 2024]. The results to β-carotene are mixed. In McAlindon et al. higher β-carotene intake was associated with lower risk of progression, especially cartilage loss [McAlindon et al., 1996]. On the contrary, the Johnston County Osteoarthritis Project reported increased OA odds for high β-carotene levels, while other carotenoids such as β-cryptoxanthin were less likely to have OA [De Roos et al., 2001].

Due to varying results of scientific studies, targeted supplementation of fat-soluble vitamins for OA is currently not recommended. Since data on the status of fat-soluble vitamins are limited, particularly in subjects with OA, the aim of this thesis is to evaluate the status of retinol, γ-tocopherol, α-tocopherol and β-carotene after a nine-month lasting nutrition intervention. Studies of whole foods and diets, instead of supplements are needed to provide realistic recommendations for dietary patterns that may improve OA outcomes. Previous evidence suggests that dietary patterns based on the Mediterranean diets are related to a better treatment of OA [Buck et al., 2022].

3 Methods

For the purpose of this study, plasma samples were collected from the NUMOQUA study in Austria, specially centered around the David Institute in Krems and the St.Pölten University of Applied Sciences. The concentrations of fat-soluble vitamins were measured using high performance liquid chromatography (HPLC) from February to July 2025 at the University of Vienna. The data sets included nutritional, inflammatory and oxidative stress markers, as well as anthropometric, behavioural and clinical data [Höld et al., 2024].

3.1 List of materials and equipment

All the required equipment and materials for the measurements are given in the following tables.

Table 1: *Equipment used for this master thesis*

Equipment	Description
HPLC Setup	Shimadzu Nexera (as shown in Table 4)
Vortex	Heidolph REAX 2000
Rotator Mixer	Premiere RM-4D
Centrifuge	Thermo Fisher Scientific Heraeus Megafuge 40R
Pipette	Biohit Proline 20-200 µl
Pipette	Biohit Proline 1-5 ml
Waterbath	Gesellschaft für Labortechnik GFL 1002
Ultrasonic bath	Allpax Palssonic

Table 2: *Materials used for this master thesis*

Material	Description
Methanol	Supelco, LiChrosolv Reag. Ph Eur Methanol gradient grade for liquid chromatography, product-code: 1.06007.2500
Ethanol	Merck, LiChrosolv Ethanol gradient grade for liquid chromatography, product-code: 1.11727.2500
Dichloromethane	Supelco, LiChrosolv Dichloromethane for liquid chromatography, product-code: 1.06044.2500
Hexane	Merck, LiChrosolv n-Hexane for liquid chromatography, product-code: 1.04391.2500
Mobile Phase	78% MeOH / 22% DCM
Calibration Standards	(+)-γ-tocopherol, analytical standard, Supelco (Merck), Product no. 47785; (±)-α-tocopherol, 1 ml ampule, Supelco (Merck), Product no. V-020-1ML, Retinol solution 1 ml ampule, Supelco (Merck), product no. V-011-1ML; β-carotene EP Reference Standard, Supelco (Merck), product no. Y0002050
Quality Controls	Chromesystems, Serum Control Bi-Level (I+II) ß-carotene in serum/plasma REF 0025, Serum Control Bi-Level (I+II) Vitamins A and E in serum/plasma REF 0032
Glastubes	Tube pyrex 10 ml, 16x100 mm, with stopper SVL 15
Glas Pasteur Pipettes	VWR, disposable glass pasteur pipettes, 230 mm, product-code: 612-1702
Vials	Bruckner Analysetechnik, 1,5 ml Kurzgewindeflasche, 32 x 11,6 mm, Braunglas, 1. hydrolytische Klasse, weite Öffnung, Schriftfeld und Füllmarkierung
Inserts	Shimadzu 0,2 ml micro insert (31x6 mm), product-code: 961-10020-12
Helium	Messer, Helium 5.0, >99,999%

3.2 NUMOQUA study samples

The NUMOQUA study is a randomized controlled intervention study investigating the effect of a combined nutritional therapy ("Austrian Osteoarthritis Cuisine") and an evidence-based exercise program (GLAD:D) on the quality of life of patients with mild to moderate knee OA. The aim is to investigate whether this holistic, inflammation-modulating therapy may improve quality of life, pain perception, joint function, and nutritional and inflammatory status. The recruitment took place from autumn 2022 to January 2024, followed by the nine-month intervention until October 2024. The study was implemented in Lower Austria, which has a high prevalence of OA (14,8% of residents aged ≥15 years) [Höld et al., 2024] [Klimont, 2019]. Trainings and medical examinations were conducted at the David Institute in Krems and the St. Pölten University of Applied Sciences, while biochemical analyses were performed at the University of Vienna and the University for Continuing Education Krems.

OA has long been considered a purely degenerative disease, but new findings suggest the role of chronic low-grade inflammation in its pathogenesis. Knee OA is the most common form of this disease worldwide, especially in older people. In addition to exercise therapy, nutrition is also considered a key factor in modulating inflammatory processes [Höld et al., 2024].

3.2.1 Intervention and study design

The study followed a two-arm, parallel, randomized design with 62 participants (31 per group), aged 50-75 years and diagnosed with gonarthrosis (Kellgren-Lawrence grade 1-3). The intervention group received the GLAD:D training program (Good Life with OsteoArthritis in Denmark) over a period of nine months, consisting of exercise and education sessions. In addition, they received nutritional therapy based on the "Austrian OA Cuisine", an adaption of the New Nordic Diet that takes into account Austrian food culture, sustainability and inflammatory principles. This included group training, individual nutritional counselling, a recipe booklet and practical exercises. The control group also received the GLA:D program, but only general information on a healthy lifestyle [Höld et al., 2024].

Inclusion and exclusion criteria

Participants were eligible if they were between 50 and 75 years of age and had a diagnosis of tibiofemoral OA with gonarthrosis (Kellgren-Lawrence grade 1-3). Adequate German language skills and the ability to understand the intervention were required. Exclusion criteria included diabetes mellitus, uncontrolled hypertension, acute or unstable neurological or psychiatric conditions, systematic inflammatory diseases and alcohol or drug abuse. Individuals with obesity (BMI ≥ 35 kg/m^2) or self-reported vegetarian or vegan dietary habits were excluded. Additional criteria were a history of cortisol therapy or autologous conditioned plasma therapy within the past 6 weeks, hyaluronic acid injections in the past 12 weeks, chronic intake of anti-inflammatory medication or use of high-dose nutritional supplements within 6 weeks before study start [Höld et al., 2024].

Data collection

Data were collected at five time points over 12 months: baseline (T0), after 6 weeks (T1), after 3 months (T2), after 9 months (T3) and after 12 months (T4, follow-up). Biochemical, anthropometric and behavioural parameters were assessed at T0, T2, T3 and T4.

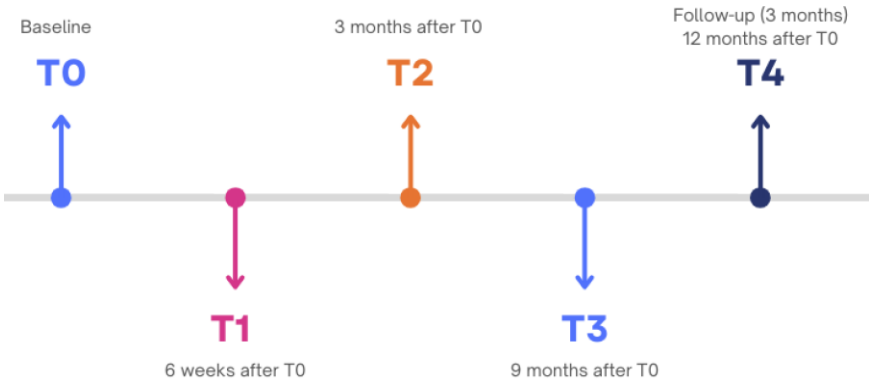

Figure 4: *NUMOQUA data collection timepoints*

Knee OA symptoms

The primary target criteria is the knee-related quality of life, measured using the KOOS (Knee Injury and Osteoarthritis Outcome Score), especially the subscale for quality of life, which is evaluated through four items rated on a five-point Likert scale. The subscale score is calculated according to the standardised score calculation by transforming the raw item scores into a 0-100 scale, where 100 indicates no symptoms and 0 represents extreme symptoms [Höld et al., 2024].

Dietary assessment

Dietary behaviour was measured using 24-hour recalls and an adapted version of the Vienna Food record (VFR), which is a paper-based prospective food record and can be completed without an interview or oral instruction. All 24-hour dietary recalls were analysed using the programme nut.s nutritional software [Höld et al., 2024].

Anthropometry

Body height, body weight and body fat were measured using Seca mBCA 555, which measures body weight with a scale, body height with ultrasound length measurement and body composition by the voltage drop of the alternating current in one step. Waist circumference was measured to calculate the waist-to-height ratio [Höld et al., 2024].

Biochemical parameters

Fasting blood samples were collected and analysed for biochemical markers, including lipid profile, fasting glucose, vitamins, carotenoids, inflammatory parameters (e.g., CRP, interleukins, TNF-α, MMP-3) and oxidative stress markers (e.g., MDA, glutathione status, DNA damage) [Höld et al., 2024].

Physical function

Functional status of the participants knee was evaluated using the 40-m fast-paced walk test (40 MFPWT) and the 30-s chair stand test (30 SCST) [Höld et al., 2024]. The 40 MFPWT is a tool to measure gait speed and changes in knee OA patients' physical function over time. The 30 SCST is a valid tool to assess lower limb strength in patients with knee OA. [Motyl et al., 2023] [Kroman et al., 2014]

Additional assessments

In addition to the main outcome parameters, demographic and anamnestic data such as sex, gender, employment status, household characteristics, income, lifestyle factors and clinical information were collected. Participants also reported their perceived health, pain levels and physical activity during work and leisure time, as well as general physical activity [Höld et al., 2024]

3.3 Measurement of fat-soluble vitamins

For the purpose to determine the concentration of fat-soluble vitamins in each serum sample, high performance liquid chromatography (HPLC) was used.

3.3.1 HPLC methodology

The method is based on the principle of reverse-phase chromatography, which enables the separation of sample components between two phases. An isocratic mobile phase consisting of 78% methanol (MeOH) and 22% dichloromethane (DCM) is used in combination with a stationary phase represented by a LiChrospher C18 HPLC column (4,0 x 250mm, 5 µm particle size). After dissolution in the polar mobile phase, the serum sample is introduced into the HPLC system and passed through the non-polar stationary phase under high pressure (85 bar). The individual components of the sample are separated based on difference in their molecular structure, polarity, and affinity of the stationary phase. Detection occurs via a PDA detector, with each compound eluting at a specific retention time. Table 3 shows the typical retention times of the analytes. These can vary slightly depending on the composition of the mobile phase (e.g. MeOH/DCM ratio), column material (e.g. C18) and flow rate. The HPLC system is interfaced with a computer running LabSolution software, which is used to control instrument parameters, evaluate chromatographic peaks, and quantify the concentration of the compounds in each sample.

Table 3: *Retention times of the analytes under the applied HPLC conditions*

Compound	**Retention time (min.)**
Retinol	3,6
γ-Tocopherol	5,3
α- Tocopherol	5,7
β-Carotene	14,5

Due to their molecular structure, the fat-soluble vitamins are highly sensitive to degradation caused by external factors such as light, heat, alkine substances and/or metal ions. Therefore, it is essential to store standard solutions and samples in a cool,

dark place and to carry out the analysis under controlled conditions. HPLC represents an ideal analytical method for this purpose, as it allows for a non-destructive, rapid, and reproducible determination at low temperatures. The following figure visualizes the used HPLC setup and analysis.

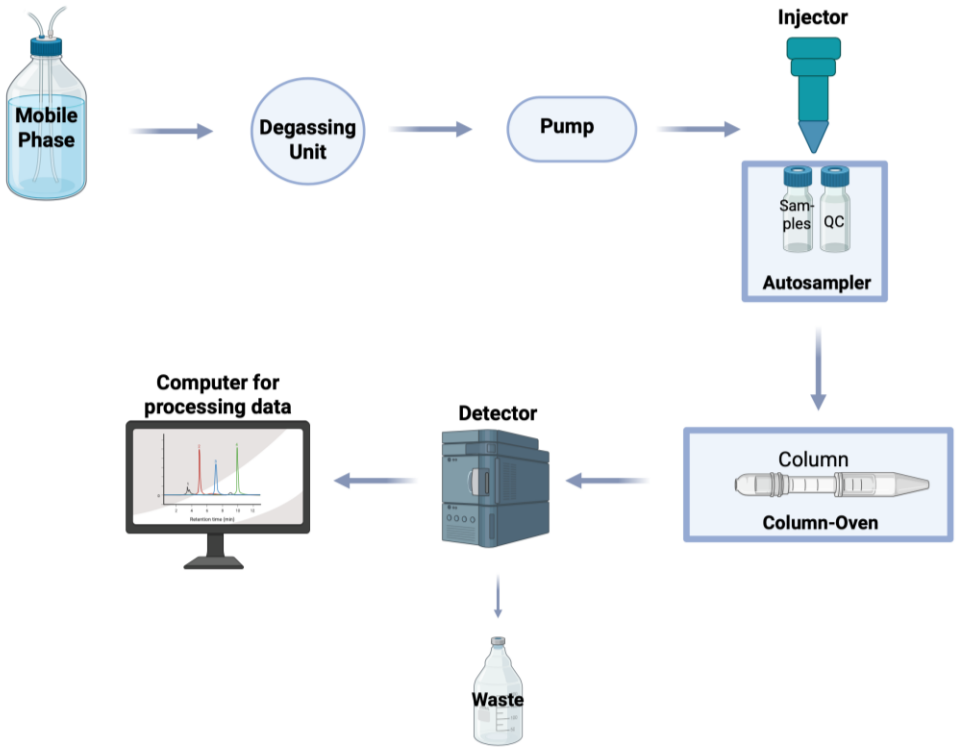

Figure 5: *Schematic illustration of the HPLC analysis* (self-created with biorender.com)

3.3.2 HPLC setup and preparations

A detailed overview of all components and specifications of the HPLC system used in this study is provided in table 4. To ensure reliable and reproducible results, strict control of the HPLC setup was required. The operational conditions for each component listed in table 5 had to be verified daily, in order to start with sample preparation. Once these parameters were confirmed the system was powered on and a standard mixture containing all target vitamins at known concentrations was measured. This step served to verify system performances, peak resolution, and retention times. Only after ensuring consistent and accurate detection of the standard compounds, sample preparation started.

Table 4: *HPLC setup used in this master thesis*

Component	Description
System Controller	Shimadzu Nexera HPLC/UHPLC, SCL-40 System Controller
Degassing Unit	Shimadzu Nexera HPLC/UHPLC, DGU-405 Degassing Unit
Pump	Shimadzu Nexera HPLC/UHPLC, LC-40D Solvent Delivery Module
Autosampler	Shimadzu Nexera HPLC/UHPLC, SIL-40C Autosampler, 5°C, Injection Volume: 50 µl
Column Oven	Shimadzu Nexera HPLC/UHPLC Column Oven CTO-40C
Column	LiChrospher® 100 RP-18 (5 µm) HPLC Column Lot. L 55120617 No. 615381
Detector	Shimadzu Nexera HPLC/UHPLC, SPD-M40 Photo Diode Array Detector
Software	LabSolutions

Table 5: *HPLC conditions used in this master thesis*

Component	Description
Autosampler	Temperature: 15°C
Column Oven	Temperature: 25°C
Column/Pre-Column	No leakage
Flow	0,8 ml/minute
Pump	Pressure: 85 bar

3.3.2.1 Preparation of mobile phase

The mobile phase used in this master thesis consisted of a mixture of 78% MeOH and 22% DCM. The solvents were measured separately using a graduated cylinder and subsequently combined in a clean glass container. To ensure optimal chromatographic performance and to prevent bubble formation in the HPLC system, the mobile phase was degassed prior to use. Degassing was performed in two steps: first, the solvent mixture was placed in an ultrasonic bath for 5 minutes to remove dissolved gases. This was followed by an additional degassing step using helium sparging for 20 minutes.

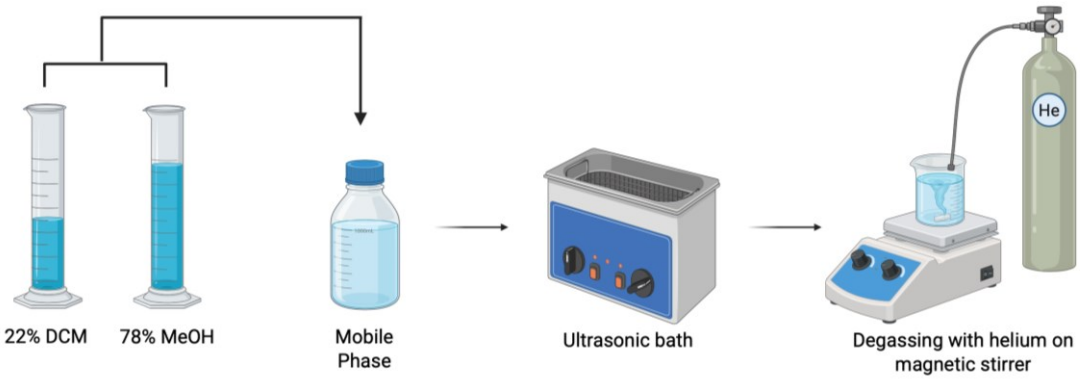

Figure 6: *Preparation of mobile phase* (self-created with biorender.com)

3.3.2.2 Preparation of calibration standards and calibration curves

For quantitative evaluation of the HPLC measurements, specific calibration standards were prepared for each target compound. Defined stock solutions were used for this purpose, which were prepared either from commercially available standards or from diluted concentrations of these. The ß-carotene stock solution was prepared in DCM and further diluted in ethanol (EtOH), whereas all other analytes were dissolved in EtOH only.

The concentrations of the stock solutions were checked photometrically with a specific wavelength for each compound, which are shown in table 6. From these stock solutions, five calibration standards with increasing concentrations were prepared by pipetting defined volumes into volumetric flasks and diluting them to the final volume with the

mobile phase. This covered the expected measurement range of the analytes. Table 7 summarizes the concentrations of the stock solutions and the calibration ranges used for each analyte.

Table 6: *Detection wavelength per compound*

Compound	**Wavelength (nm)**
Retinol	325
γ-Tocopherol	298
α- Tocopherol	290
β-Carotene	472

Table 7: *Stock solutions and calibration ranges for each analyte*

Compound	***Stock Solution (µg/ml)***	***Calibration Range (µg/ml)***
Retinol	100	0,2 - 2
γ-Tocopherol	1000	0,4 - 4
α- Tocopherol	500	2 - 20
β-Carotene	100	0,2 - 2

The resulting calibration standards were then transferred to HPLC vials and analysed under identical chromatographic conditions. Calibration curves were then created from the peak area and used for the quantitative interpretation of the sample data. Figure 7 shows the calibration curve using retinol as an example.

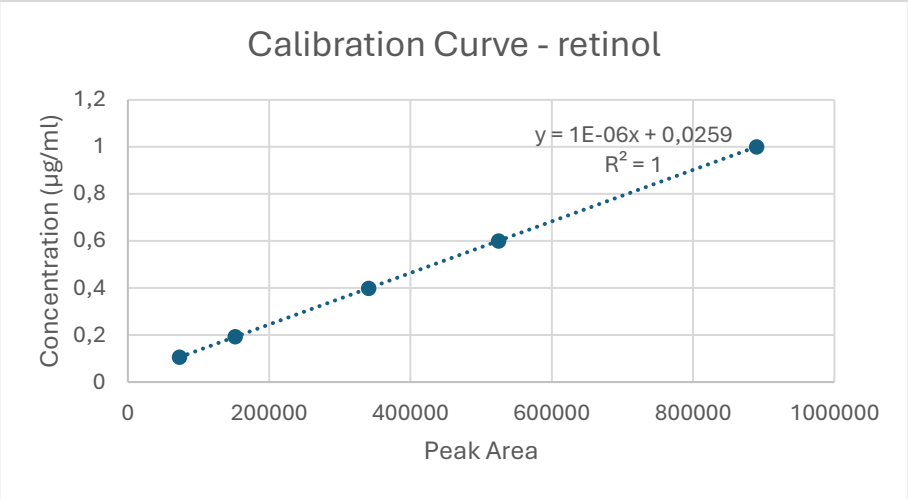

Figure 7: *Calibration curve of retinol*

3.3.2.3 Preparation of quality controls

To ensure the accuracy and precision of the HPLC analyses, we analysed quality control serum samples from Chromesystems with each HPLC run. These were prepared and analyed in the same way as the samples (see chapter 4.2.4). The QC´s were checked for concentration and retention time throughout the entire analysis to ensure the reproducibility of the method. The following table shows the concentration ranges for retinol, α-tocopherol and β-carotene in Chromesystems serum quality control materials (Level I and II).

Table 8: *Concentration ranges of the analytes in QC serum samples*

	Serum Control Level I (µg/ml)	**Serum Control Level II (µg/ml)**
Retinol	0,350 – 0,524	1,02 – 1,54
α- Tocopherol	6,87 – 10,3	13,9 – 20,36
β-Carotene	0,282 – 0,424	0,620 – 0,930

3.3.2.4 Preparation of samples

The serum samples were taken from the freezer and stored on ice until further processing. Two glass tubes were labelled for each sample. Then, 100 µl of the sample was pipetted into the first tube and mixed with 100 µl EtOH. After vortexing for 5 seconds, 3 ml of hexane was added and the mixture was vortexed again for 20 seconds. The samples were then mixed in a rotator mixer (shaker) for 15 minutes and subsequently

centrifuged at 3000 rpm and 4 °C for 3 minutes. The organic supernatant (approx. 2,5 ml) was carefully transferred to the second, previously labelled glass tube using a pasteur-pipette. For complete extraction, 3 ml hexane was added to the first glass tube and the extraction process (vortexing, shaking, centrifuging) was repeated. This supernatant was also transferred to the second glass tube so that both extracts were combined. The combined extracts were then placed in a 40 °C water bath for about 10 minutes and evaporated under nitrogen (N2) until the solvent was completely removed. The residue was resuspended with 200 µl mobile phase (MeOH:DCM in a ratio of 85:15) and vortexed briefly. Finally, 120 µl of the solution was transferred to appropriate HPLC vials and subjected to chromatographic analysis.

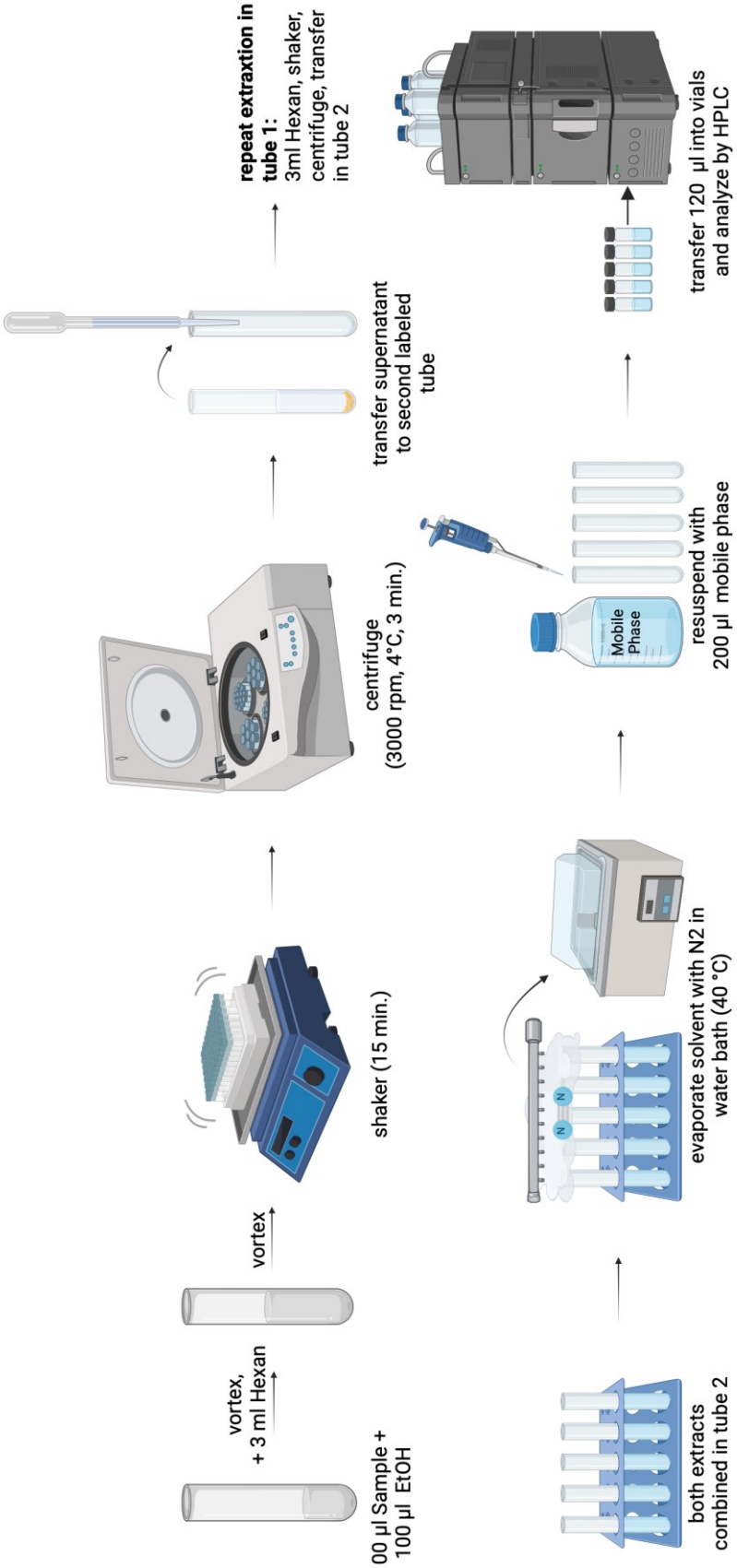

Figure 8: *Schematic preparation of the samples* (self-created with biorender.com)

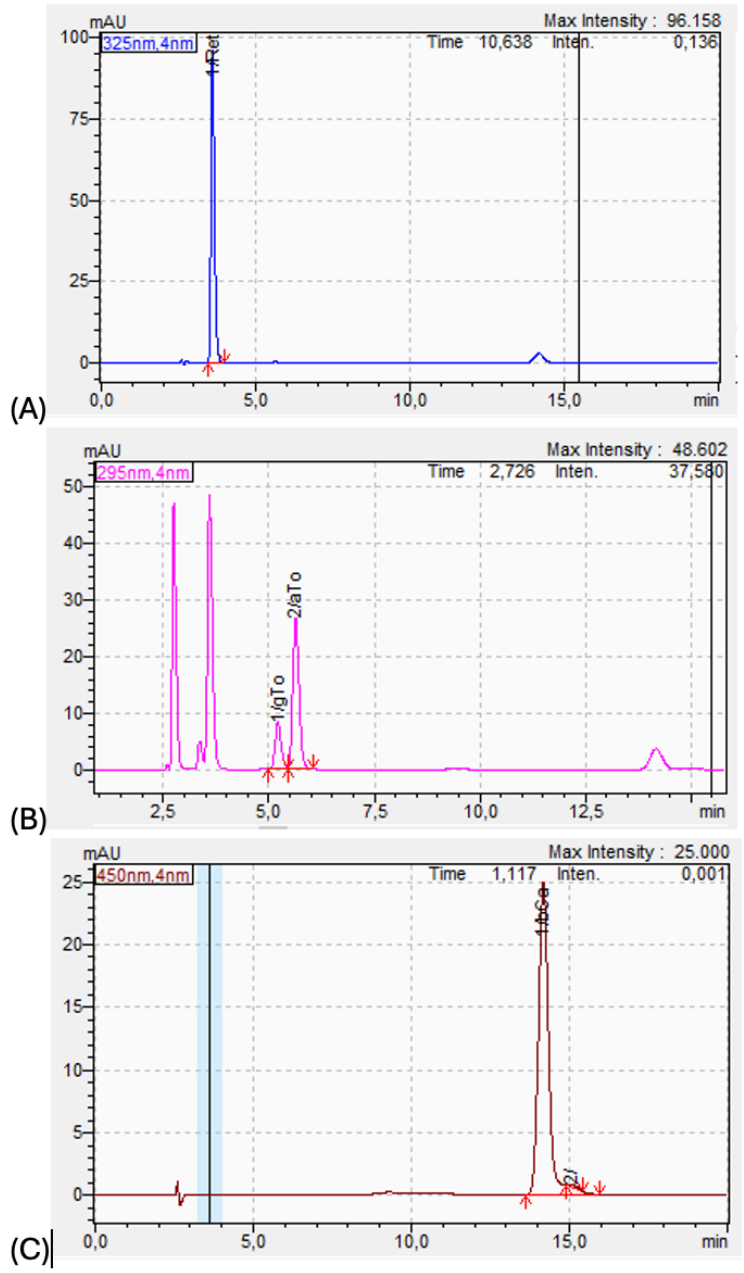

***Figure 9**: Representative chromatograms of fat-soluble vitamins: (A) retinol, (B) α- & γ-tocopherol, (C) β-carotene*

3.3.3 Data interpretation

The HPLC measurements were analysed using the LabSolution software, which was used to record, integrate and quantitatively analyse chromatographic data. After injecting the samples, the resulting chromatograms were evaluated in terms of retention time and peak area. The target compounds were identified by comparing the measured retention times with those of the calibration standards. Quantification was performed by automatic integration of the peak areas, which were converted into concentrations using the previously created calibration curves.

The LabSolution software also offers the option of marking all peaks individually, checking them and correcting them manually if the automatic integration produces inaccurate results (e.g. in the case of baseline disturbances, picking a wrong peak or overlapping peaks). All calculated concentrations were given in µg/ml and finally documented in µmol/L.

4 Statistical analysis

All statistical analyses were carried out using IBM SPSS 29 software. Baseline characteristics were compared by sex (women vs. men) and by study group (IG vs. CG) using one-way ANOVA. Changes in fat-soluble vitamins were analysed with repeated-measures general linear model (GLM) with time (T0, T2, T3, T4) as the within-subject factor and group as the between-subject factor. Main effects for time and group and their interaction (timexgroup) were reported. The same models were run stratified by sex. A Greenhouse-Geisser correction was performed if sphericity was not present. Post-hoc tests with Bonferroni corrections were then conducted to compare the individual time points. The correlations between delta-BMI and changes in fat-soluble vitamins was determined using both Pearson and Spearman (two-sided) supplemented by 95% bootstrapping CIs (1000 resamples). The level of statistical significance was defined as $p < 0.05$.

5 Results

A total of 62 participants were randomized in the NUMOQUA study (intervention group n = 31; control group n = 31). At baseline (T0), after accounting for dropouts, laboratory samples were available for n = 56. Further participation was n = 51 at T2, n = 49 at T3 and n = 48 at T4. The complete-case dataset (data available for T0-T4) comprised n = 39 and was used for statistical analysis using IBM SPSS Statistics 29. The significance level was set at $p < 0.05$.

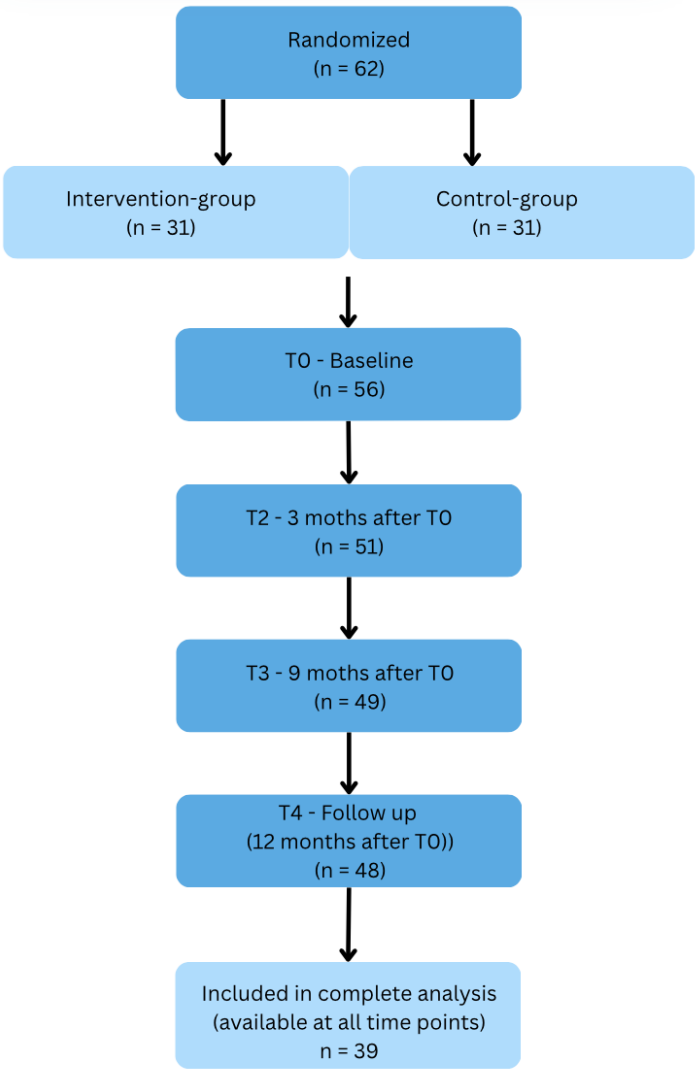

Figure 10: *Participant flow diagram*

5.1 Descriptive analysis of the samples

Descriptive statistics were used to characterize the study population at baseline (T0). The samples used for analysis consisted of 62 participants with a mean age of 62.4 years (SD 6.3) and the majority were female (n = 40; 64.5%). The average BMI was 28.0 kg/m^2 (SD 3.5), with the following distribution across categories: 22 participants (35.5%) where of normal weight, 21 (33.9%) were classified as pre-obese and 19 (30.6%) as obese. Further anthropometric and body composition measures, including fat mass, lean mass and waist circumference are summarized in table 9. Although 62 participants were enrolled at baseline, not all subjects attended every follow-up visit. Consequently, the sample size varies slightly between time points.

Table 9: *Baseline characteristics of the study population (n = 62)*

Parameters	**n (%)**	**Mean ± SD / n**
Age (years)	-	62.4 ± 6.3
Gender (female/male)	40 (64.5) / 22 (35.5)	-
Fat mass (%)	-	28.7 ± 8.9
Lean mass (%)	-	51.6 ± 9.5
Waist circumference (cm)	-	99.9 ± 10.0
BMI categories (n/%)		
normal weight	22 (35.5)	
pre-obesity	21 (33.9)	
obesity	19 (30.6)	

Data are presented as mean ± standard deviation. Categorical variables as n (%). BMI categories follow WHO definitions: normal weight (18.5-24.9 kg/m^2), pre-obesity (25.0-29.9 kg/m^2), obesity (> 30.0 kg/m^2)

As shown in the following table, at the start of the study, there were no differences between women and men in terms of age, physical activity level (PAL) and body mass index (BMI). However, men were heavier (86.7 ± 12.3 kg vs. 76.8 ± 12.6 kg; p = 0.004) and in terms of gender-typical body composition, had significantly lower fat mass indices and percentages as well as higher fat-free and muscular parameters. Especially, the percentage fat mass (FMP) and fat mass index (FMI) in men were significantly lower than in women (FMP: 28.2 ± 6.2 % vs. 39.4 ± 5.9 %; FMI: 8.0 ± 2.3 vs. 11.2 ± 3.1 kg/m^2); both p < 0.001). In contrast, the percentage of fat-free mass (FFMP), the fat-free mass index (FFMI) and the skeletal muscle index (SMI) were higher in men (all p < 0.001). The percentage of total body water (TBWP) was also higher in men (53.0 ± 4.5 % vs. 45.6 ± 4.4 %; p < 0.001), while the ECW/TBW ratio was higher in women (47.1 ± 1.7 % vs. 43.2 ± 1.4 %; p < 0.001). Segmental skeletal muscle mass (SSMM) in the arms and legs was consistently greater in men (all p < 0.001). As expected, estimated total energy expenditure (TEE) was higher in men (2877 ± 285 kcal/d vs. 2274 ± 255 kcal/d; p < 0.001). For standardised skeletal muscle index (Z-SMI), there was only a borderline difference (p = 0.059). Overall, the baseline data indicate comparable age and activity levels, but a distinctly more muscle/water-rich and less fat-rich body composition in men.

Table 10: *Baseline characteristics divided by sex*

women (n=40) men (n=22) total (n=62)

Parameter	Women Mean ± SD	Men Mean ± SD	Total Mean ± SD	p-value
Age [years]	63.07 ± 5.94	61.14 ± 6.83	62.39 ± 6.28	0.248
Weight [kg]	76.77 ± 12.57	86.71 ± 12.33	80.30 ± 13.28	0.004
Waist circumference [cm]	98.83 ± 10.63	101.83 ± 8.79	99.89 ± 10.05	0.264
PAL [-]	1.65 ± 0.08	1.65 ± 0.10	1.65 ± 0.09	0.836
BMI [kg/m^2]	28.03 ± 3.87	27.82 ± 2.87	27.95 ± 3.52	0.827
FM percent (FMP) [%]	39.37 ± 5.90	28.17 ± 6.15	35.39 ± 8.03	<.001
Fat mass index (FMI) [kg/m^2]	11.23 ± 3.05	7.96 ± 2.33	10.07 ± 3.21	<.001
FFM percent (FFMP) [%]	60.63 ± 5.90	71.83 ± 6.15	64.61 ± 8.03	<.001
FFM index (FFMI) [kg/m^2]	16.80 ± 1.20	19.86 ± 1.40	17.89 ± 1.94	<.001
TBW percent (TBWP) [%]	45.59 ± 4.43	52.96 ± 4.54	48.20 ± 5.69	<.001
ECW percent (ECWP) [%]	21.46 ± 2.07	22.90 ± 2.16	21.97 ± 2.20	0.012
ECW/TBW [%]	47.12 ± 1.70	43.24 ± 1.43	45.74 ± 2.46	<.001
SMM percent (SMMP) [%]	26.96 ± 2.54	34.61 ± 2.81	29.67 ± 4.52	<.001
Skeletal muscle index (SMI) [kg/m^2]	7.48 ± 0.69	9.58 ± 0.81	8.23 ± 1.25	<.001
Z-SMI [z]	0.65 ± 0.86	0.20 ± 0.90	0.49 ± 0.89	0.059
SSMM Right Arm [kg]	1.22 ± 0.21	1.94 ± 0.31	1.47 ± 0.42	<.001
SSMM Left Arm [kg]	1.17 ± 0.22	1.86 ± 0.30	1.42 ± 0.42	<.001
SSMM Right Leg [kg]	4.46 ± 0.60	5.85 ± 0.83	4.95 ± 0.96	<.001
SSMM Left Leg [kg]	4.52 ± 0.62	5.94 ± 0.81	5.02 ± 0.97	<.001
TEE [kcal/d]	2274.35 ± 255.43	2877.37 ± 284.67	2488.32 ± 392.71	<.001

Data are presented as mean standard deviation. P-values refer to between-group comparison (IG vs. CG) at baseline from one-way ANOVA. BMI = body mass index, PAL = physical activity level; FM = fat mass, FMP = fat mass percent, FMI = fat mass index, FFM = fat-free mass, FFMP = fat-free mass percent, FFMI = fat-free mass index, TBW = total body water, TBWP = total body water percent, ECW = extracellular water, ECWP = extracellular water percent; ECW/TBW = ratio of extracellular to total body water, SSM = skeletal muscle mass, SMMP = skeletal muscle mass percent, SMI = skeletal muscle index, Z-SMI = z-standardized SMI (vs. reference population), SSMM = segmental skeletal muscle mass, TEE = total energy expenditure.

As described in table 11, the two randomised groups were largely well balanced at baseline. No significant differences were found for age, body weight, waist circumference, PAL, BMI, fat and fat-free indices or proportions, water compartments, segmental muscle mass or TEE (all $p < 0.131$). Only the Z-SMI differed slightly (IG 0.26 ± 0.99 vs. CG 0.72 ± 0.72; $p = 0.045$), while all other parameters showed very similar mean values in both groups (e.g. BMI 27.3 ± 3.7 vs. 28.6 ± 3.2 kg/m^2); FFMI 17.6 ± 1.9 vs. 18.2 ± 1.9 kg/ m^2). The baseline comparisons therefore suggest that the groups started on a comparable level in terms of relevant anthropometric and compartment-specific parameters.

Table 11: *Baseline characteristics divided by group (IG, CG)*

IG (n=31) CG (n=31) total (n=62)

Parameter	IG Mean ± SD	CG Mean ± SD	Total Mean ± SD	p-value
Age [years]	62.84 ± 6.20	61.94 ± 6.43	62.39 ± 6.28	0.576
Weight [kg]	79.51 ± 14.16	81.08 ± 12.52	80.30 ± 13.28	0.646
Waist circumference [cm]	98.41 ± 9.35	101.37 ± 10.65	99.89 ± 10.05	0.248
PAL [-]	1.65 ± 0.08	1.65 ± 0.10	1.65 ± 0.09	0.889
BMI [kg/m^2]	27.28 ± 3.74	28.63 ± 3.21	27.95 ± 3.52	0.131
FM percent (FMP) [%]	34.91 ± 7.59	35.88 ± 8.53	35.39 ± 8.03	0.638
Fat mass index (FMI) [kg/m^2]	9.69 ± 3.09	10.44 ± 3.33	10.07 ± 3.21	0.365
FFM percent (FFMP) [%]	65.09 ± 7.59	64.12 ± 8.53	64.61 ± 8.03	0.638
FFM index (FFMI) [kg/m^2]	17.58 ± 1.93	18.19 ± 1.94	17.89 ± 1.94	0.221
TBW percent (TBWP) [%]	48.50 ± 5.40	47.90 ± 6.03	48.20 ± 5.69	0.681
ECW percent (ECWP) [%]	17.59 ± 2.66	17.63 ± 2.47	17.61 ± 2.54	0.950
ECW/TBW [%]	45.88 ± 2.58	45.60 ± 2.37	45.74 ± 2.46	0.663
SMM percent (SMMP) [%]	29.66 ± 4.32	29.69 ± 4.79	29.67 ± 4.52	0.975
Skeletal muscle index (SMI) [kg/m^2]	8.03 ± 1.29	8.43 ± 1.21	8.23 ± 1.25	0.216
Z-SMI [z]	0.26 ± 0.99	0.72 ± 0.72	0.49 ± 0.89	0.045
SSMM Right Arm [kg]	1.47 ± 0.42	1.48 ± 0.43	1.47 ± 0.42	0.886
SSMM Left Arm [kg]	1.41 ± 0.41	1.43 ± 0.42	1.42 ± 0.42	0.826
SSMM Right Leg [kg]	4.95 ± 1.02	4.96 ± 0.91	4.95 ± 0.96	0.964
SSMM Left Leg [kg]	5.03 ± 1.03	5.01 ± 0.92	5.02 ± 0.97	0.952
TEE [kcal/d]	2469.66 ± 395.07	2506.99 ± 395.96	2488.32 ± 392.71	0.711

Data are presented as mean ± standard deviation. P-values refer to between-group comparison (IG vs. CG) at baseline from one-way ANOVA. BMI = body mass index, PAL = physical activity level; FM = fat mass, FMP = fat mass percent, FMI = fat mass index, FFM = fat-free mass, FFMP = fat-free mass percent, FFMI = fat-free mass index, TBW = total body water, TBWP = total body water percent, ECW = extracellular water, ECWP = extracellular water percent; ECW/TBW = ratio of extracellular to total body water, SSM = skeletal muscle mass, SMMP = skeletal muscle mass percent, SMI = skeletal muscle index, Z-SMI = z-standardized SMI (vs. reference population), SSMM = segmental skeletal muscle mass, TEE = total energy expenditure.

5.2 Serum concentrations of fat-soluble vitamins in relation to reference studies

The mean serum concentrations of retinol, γ-tocopherol, α-tocopherol and β-carotene were relatively stable across all time points. Retinol values ranged from 1.66 ± 0.49 to 1.76 ± 0.50 µmol/L, while γ-tocopherol concentrations remained around 2.2 µmol/L with standard deviations of approximately 0.8-0.9. For α-tocopherol as usual, mean concentrations were higher with 26.82 ± 8.23 to 27.42 ± 7.28 µmol/L, reflecting moderate variability. β-carotene values were consistently close to 0.9 µmol/L with SDs of about 0.5.

Table 12: *Mean serum concentrations of fat-soluble vitamins during all timepoints*

Vitamin [µmol/L]	**T0 (n=56) Mean ± SD**	**T2 (n=51) Mean ± SD**	**T3 (n=49) Mean ± SD**	**T4 (n=48) Mean ± SD**
Retinol	1.76 ± 0.50	1.71 ± 0.46	1.74 ± 0.51	1.66 ± 0.49
γ-Tocopherol	2.22 ± 0.94	2.21 ± 0.79	2.23 ± 0.90	2.18 ± 0.86
α- Tocopherol	27.42 ± 7.28	26.99 ± 7.28	26.62 ± 8.53	26.82 ± 8.23
β-Carotene	0.90 ± 0.48	0.93 ± 0.47	0.90 ± 0.50	0.90 ± 0,48

Data are presented as mean ± standard deviation. Due to participant dropouts and missing data, the number of available observations (n) varies between time points.

In the present cohort, the mean serum concentrations of retinol, α- and γ-tocopherol, and β-carotene remained stable across all four measurement points (retinol: 1.66-1.76 µmol/L; α-tocopherol: 26.6-27-4 µmol/L; α- tocopherol: 2.18-2.23 µmol/L; β-carotene: 0.90-0,93 µmol/L). These values were compared with published reference data from population-based studies and intervention studies in elderly people in order to classify vitamin status.

Stuetz et al. (2016) reported plasma concentrations of retinol, tocopherols and carotenoids in the large European MARK-AGE study (35-74 years, n=2118) and reports geometric mean values of 1.71 µmol/L for retinol, 27.8 µmol/L for α-tocopherol, 1.29 µmol/L for γ-tocopherol and 0.54 µmol/L for β-carotene. The concentrations in the NUMOQUA study correspond almost exactly to these European reference values for retinol and α- tocopherol. The values for γ-tocopherol were moderately higher, but within the percentile range reported in MARK-AGE (0.40-3.55 µmol/L). At around 0.9 µmol/L on

average, β-carotene was higher in the present cohort than in the European comparison (0.54 µmol/L), which indicates an overall better carotenoid status. These differences could be explained by dietary habits, a lower proportion of smokers or seasonal fluctuations, as carotenoids are known to exhibit high intra-individual variability [Stuetz et al., 2016].

There is a clear contrast when compared to the Vienna Active Aging Study (VAAS), which was conducted on institutionalised elderly people (average age 83). There, the baseline medium values were 2.20 µmol/L for retinol, 16.5 µmol/L for α- tocopherol, 2.63 µmol/L for γ-tocopherol and 0.42 µmol/L for β-carotene. In comparison, the subjects in the NUMOQUA study had lower retinol levels but significantly higher α- tocopherol and β-carotene levels. The γ-tocopherol levels were slightly below the VAAS values. These differences are plausible, as the populations differ, the VAAS study examined very old, institutionalised individuals with potentially restricted diets and a higher burden of disease, while the NUMOQUA cohort was significantly younger (average age 62) and likely had a better overall diet [Franzke et al., 2019].

Table 13: *Fat-soluble vitamin concentrations in NUMOQUA and reference cohorts*

Parameter [µmol/L]	**NUMOQUA baseline (n=56; Mean ± SD; Min-Max**	**Stuetz et al. (n=2118; GM; 2.5-97.5th P)**	**VAAS baseline (n=117; Median; Min-Max)**
Retinol	1.76 ± 0.50 (0.71-2.94)	1.71 (0.98-2.73)	2.20 (1.05-4.16)
γ-Tocopherol	2.22 ± 0.94 (0.42-4.71)	1.29 (0.40-3.55)	2.63 (1.80-9.14)
α- Tocopherol	27.42 ± 7.28 (4.36-44.61)	27.8 (17.5-48.5)	16.51 (n.d.-26.8)
β-Carotene	0.90 ± 0.48 (0.15-2.26)	0.54 (0.13-1.86)	0.42 (n.d.-2.40)

NUMOQUA data are presented as mean ± standard deviation and with observed minimum and maximum values. European population data from Stuetz et al. (2016) are reported as geometric means with 2.5-97th percentiles; VAAS data from Franzke et al. (2019) as medians with minimum-maximum values

Overall, the results indicate the NUMOQUA vitamin levels are consistent with or slightly above those of the general European population. Compared to institutionalised elderly people, they have higher α- tocopherol and β-carotene levels, a slightly lower retinol status and slightly lower γ-tocopherol levels. These differences reflect both age structure and living situation as well as methodological differences in data collection and evaluation. The baseline data show that the NUMOQUA population had an overall adequate to favourable status of fat-soluble vitamins at the start of the study.

5.3 Comparison of the subgroups

Table 14: *Fat-soluble vitamins at different timepoints for both sexes (IG, CG)*

Parameter [µmol/L]	Group	T0 Mean ± SD	T2 Mean ± SD	T3 Mean ± SD	T4 Mean ± SD	p-value Time	p-value Time×Group	p-value Group
Retinol	IG	1.67 ± 0.53	1.66 ± 0.45	1.77 ± 0.39	1.68 ± 0.45	0.098	0.776	0.583
	CG	1.81 ± 0.51	1.74 ± 0.56	1.84 ± 0.56	1.72 ± 0.50			
	Total	1.74 ± 0.52	1.70 ± 0.50	1.80 ± 0.47	1.70 ± 0.47			
γ-Tocopherol	IG	2.28 ± 0.83	2.49 ± 0.96	2.34 ± 0.77	2.42 ± 0.84	0.796	0.056 (trend)	0.393
	CG	2.40 ± 1.00	2.00 ± 0.68	2.29 ± 1.01	2.04 ± 0.83			
	Total	2.34 ± 0.91	2.25 ± 0.86	2.32 ± 0.88	2.24 ± 0.85			
α-Tocopherol	IG	26.92 ± 8.65	27.12 ± 8.54	27.09 ± 8.33	27.26 ± 8.66	0.885	0.845	0.885
	CG	28.09 ± 7.30	26.90 ± 7.63	27.01 ± 8.45	27.82 ± 8.75			
	Total	27.49 ± 7.94	27.02 ± 8.00	27.05 ± 8.28	27.53 ± 8.59			
β-Carotene	IG	0.97 ± 0.50	0.96 ± 0.49	1.02 ± 0.59	1.01 ± 0.57	0.900	0.559	0.310
	CG	0.85 ± 0.44	0.85 ± 0.40	0.80 ± 0.39	0.86 ± 0.43			
	Total	0.91 ± 0.47	0.91 ± 0.45	0.91 ± 0.51	0.94 ± 0.51			

Data are presented as mean ± standard deviation, the p-values were determined using a repeated measures ANOVA. A p-value of 0.05 is considered significant.

As can be seen in the table above, retinol showed no significant time trend across all time points (p-time = 0.098), no interaction between time and group (p-timexgroup = 0.776) and no group difference (p-group = 0.583). In both groups, retinol levels were close to each other and remained stable over the measurement timepoints. In the IG, they ranged between 1.66 and 1.77, and in the CG between 1.72 and 1.84 µmol/L, each with a slight peak at T3 and a return to near T0 levels at T4. Overall, the pattern indicates stable retinol levels without group-specific changes. As shown in Figure 11, IG and CG run almost parallel across all time points. The CIs overlap significantly in each case and support the missing group/intervention effect.

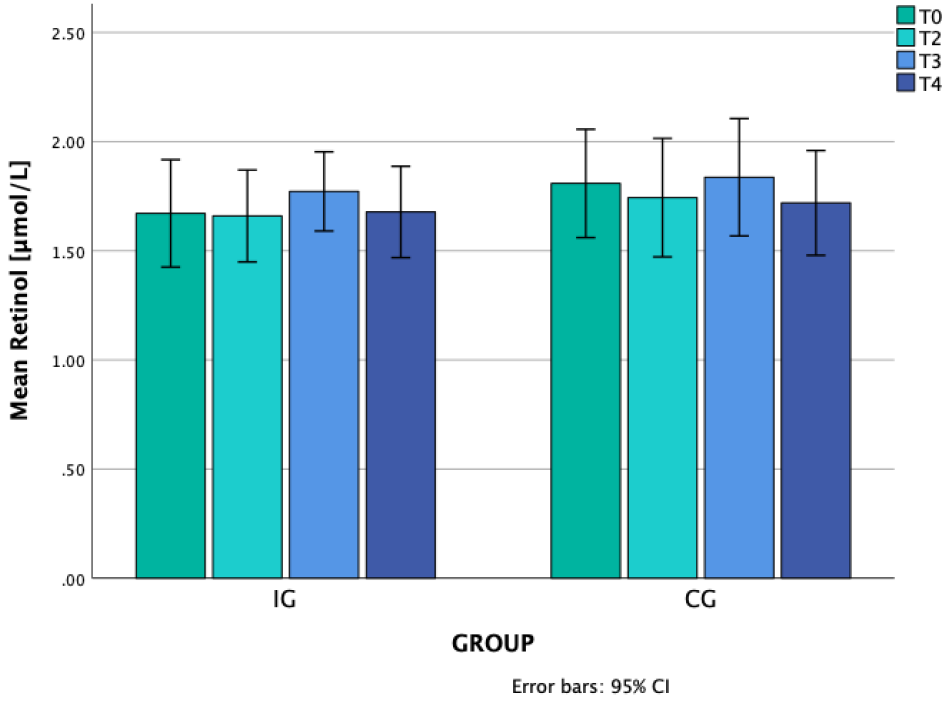

Figure 11: *Serum retinol concentrations over time by group*

(data show group means with 95% CIs)

For γ-tocopherol, the time effect was not significant (p-time = 0.796), as well as the group effect (p-group = 0.393). However, there was a borderline trend for a time-group interaction (p-timexgroup = 0.056). In the IG, γ-tocopherol increased from T0 (2.28) to T2 (2.49), while the CG decreased in the same interval (2.40 to 2.00). In later measurements, the difference becomes smaller again. This pattern indicates different temporal courses between the IG and CG, without the overall test (time or group) becoming significant. Figure 12 visualises the opposite movement from T0 to T2 (IG rises, CG falls). The CIs converge again from T3 onwards, which corresponds to the borderline interaction trend, with no clear separation of the CIs between the groups.

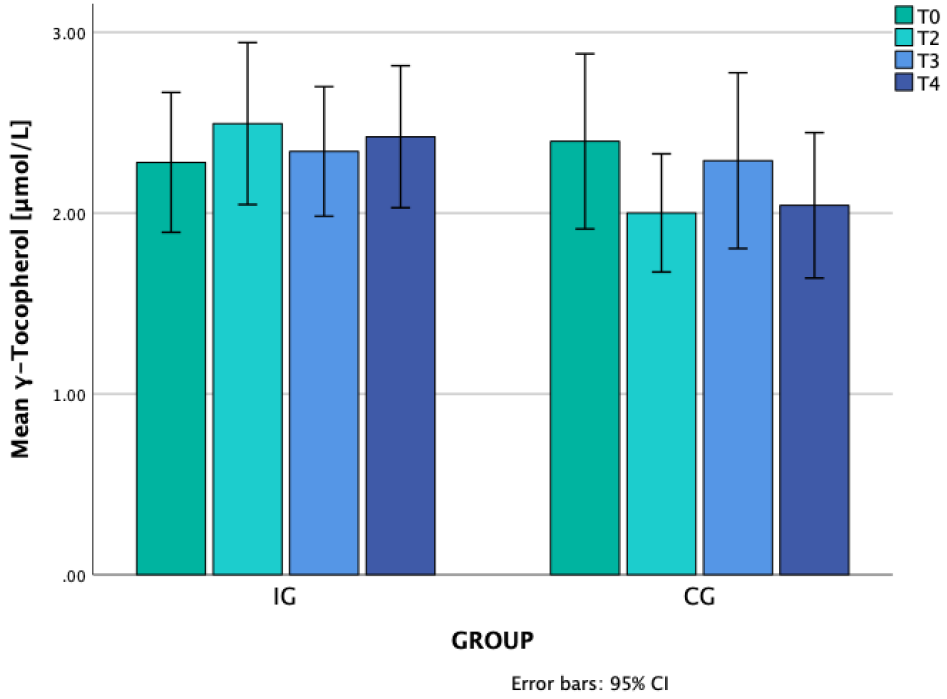

Figure 12: *Serum γ-tocopherol concentrations over time by group*

(data show group means with 95% CIs)

The values for α- tocopherol were stable across all time points and comparable between groups, with no time effect (p-time = 0.885), no time-group interaction (p-timexgroup = 0.845) and no group effect (p-group = 0.885). Mean values consistently ranged between 27 and 30 µmol/L in both groups, with no apparent trend. In figure 13, the group means remain close together. Broad, overlapping 95% CIs underscore the absence of time, group and interaction effects.

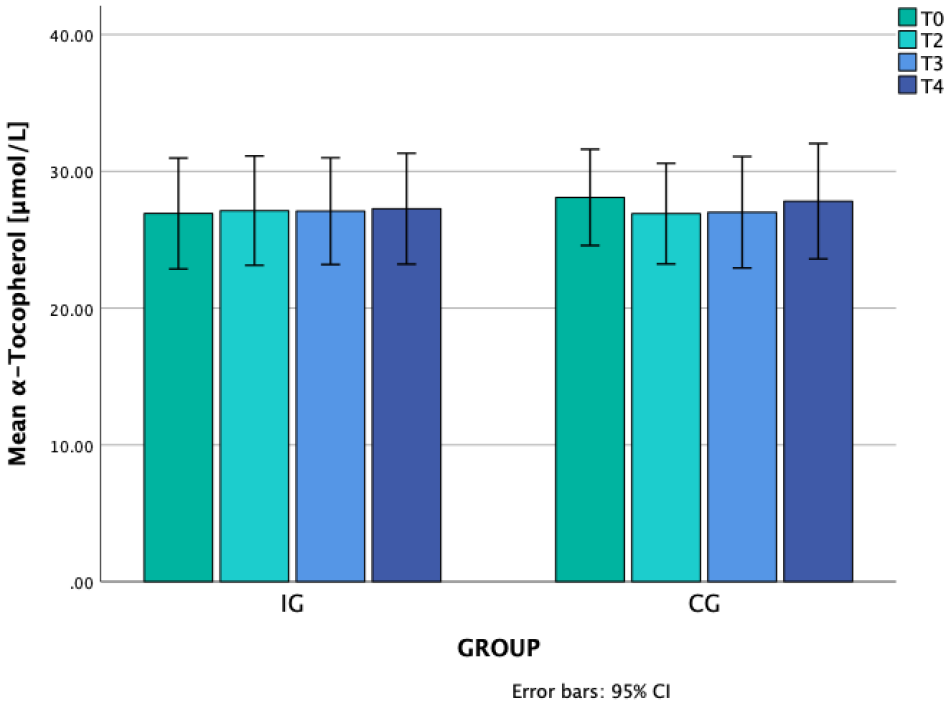

Figure 13: *Serum α- tocopherol concentrations over time by group*

(data show group means with 95% CIs)

For β-carotene, there was no time effect (p-time = 0.900), no interaction (p-timexgroup = 0.559) and no group effect (p-group = 0.310). The IG was slightly higher than the CG at all timepoints, but these differences were not statistically significant. Figure 14 shows consistently slightly higher mean values for the IG. The clear overlap of the 95% CIs at all timepoints corresponds to the lack of significance.

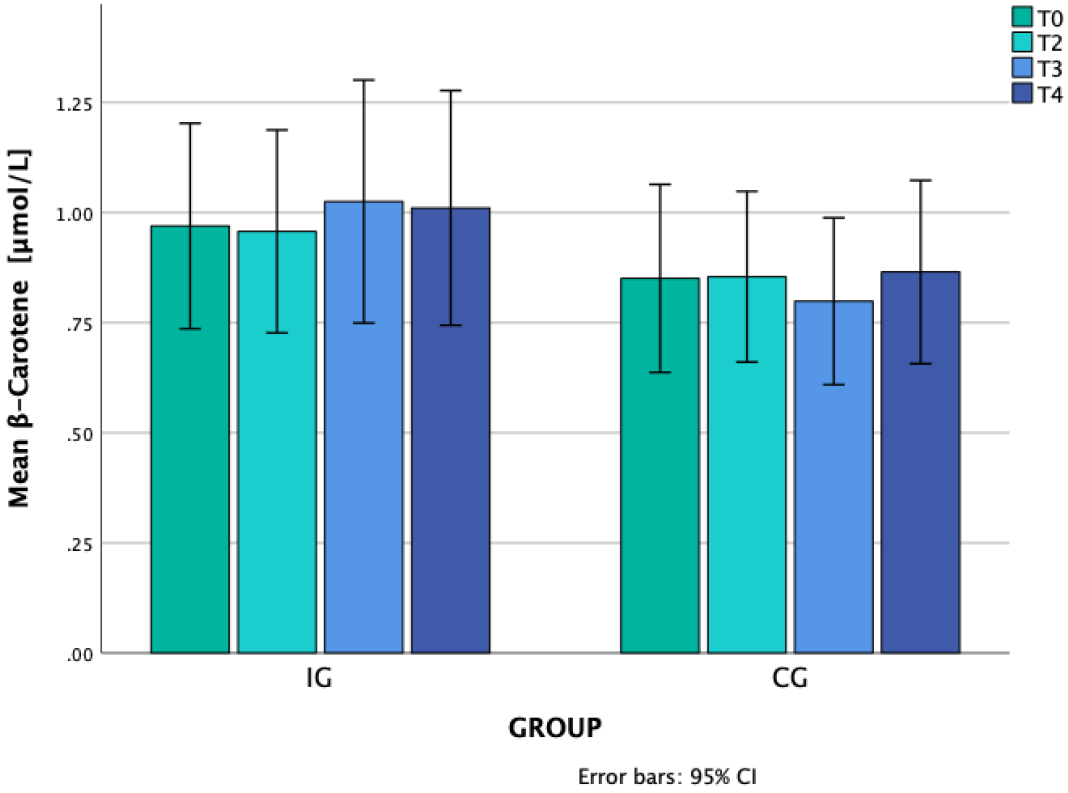

Figure 14: *Serum β-carotene concentrations over time by group*

(data show group means with 95% CIs)

Overall, the data for the fat-soluble vitamins retinol, α- tocopherol and β-carotene show stable concentrations without significant group differences or changes over time. For γ-tocopherol, there is evidence of a borderline time-group interaction (increase in IG vs. decrease in CG from T0 to T2), which flattens out again at later time points. The findings suggest comparable baseline and progression profiles for fat-soluble vitamins in the IG and CG over T0-T4.

5.4 Differences regarding gender

No group-specific intervention effects were observed for fat-soluble vitamins overall. Most markers remained stable over time and comparable between the IG and CG.

For retinol, the course differed depending on gender. In women, there was no time effect ($p = 0.392$), no time-group interaction ($p = 0.763$) and no group effect ($p = 0.498$). The mean values varied slightly in both groups. In men, however, there was a significant time effect ($p = 0.030$) without interaction ($p = 0.899$) and without group effect ($p = 0.933$). Both groups increased until T3 (IG 1.69-1.9; CG 1.77-1.92 µmol/L) and were close to the baseline level again at T4. This pattern suggests a general temporal course rather than an intervention-related difference.

For γ-tocopherol, a significant time-group interaction ($p = 0.036$) was observed in women, with no main effects (p-time = 0.353; p-group = 0.998). From T0 to T2, the IG rose from 2.19 to 2.35 µmol/L, while the CG decreased from 2.67 to 2.03 µmol/L. At T3/T4, the mean values converged again (IG 2.27/2.32; CG 2.29/2.15 µmol/L). In men, time ($p = 0.814$) and interaction ($p = 0.551$) were not significant. The group effect was just below the threshold for significamce ($p = 0.077$), with a tendency towards higher values in the IG over the course of the study.

α-Tocopherol remained stable in both sexes over T0-T4 and comparable between groups. Women showed no significant effects (p-time = 0.096, p-timexgroup = 0.855, p-group = 0.613) with mean values around 27-30 µmol/L. Men also showed no effects (p-time = 0.416, p-timexgroup = 0.486, p-group = 0.580), with only a temporary peak at T2 in the IG.

β-Carotene also showed no significant time, interaction or group effects in either women or men (woman: p-time = 0.533, p-timexgroup = 0.713, p-group = 0.456; men: p-time = 0.353, p-timexgroup = 0.442, p-group = 0.369). The values showed no clear trend. There was a tendency towards slightly higher values in the IG, but this was statistically insignificant.

In summary, retinol, α- tocopherol and β-carotene remain largely constant in both sexes and show no reliable IG-CG-differences. Two findings stand out: a pure time-effect for retinol in men (increase until T3, decrease T4) and a time-group interaction for γ-tocopherol in women (divergent changes from T0 to T2 followed by convergence). Both patterns suggest gender-specific time dynamics rather than a robust intervention effect.

Table 15: *Fat-soluble vitamins at different timepoints for females (IG, CG)*

Parameter [µmol/L]	Group	T0 Mean± SD	T2 Mean± SD	T3 Mean± SD	T4 Mean± SD	p-value Time	p-value Time×Group	p-value Group
Retinol	IG	1.66± 0.46	1.58± 0.43	1.66± 0.36	1.65± 0.52	0.392	0.763	0.498
	CG	1.83± 0.48	1.72± 0.54	1.79± 0.52	1.69± 0.49			
	Total	1.75± 0.47	1.65± 0.48	1.73± 0.44	1.67± 0.49			
γ-Tocopherol	IG	2.19± 0.88	2.35± 1.02	2.27± 0.86	2.32± 1.02	0.353	0.036	0.998
	CG	2.67± 1.05	2.03± 0.71	2.29± 1.03	2.15± 0.87			
	Total	2.43± 0.98	2.19± 0.87	2.28± 0.93	2.23± 0.93			
α-Tocopherol	IG	28.56± 9.74	26.43± 8.62	27.61± 7.26	29.21± 9.40	0.096	0.855	0.613
	CG	30.70± 6.84	28.27± 7.54	29.61± 8.75	29.79± 8.64			
	Total	29.63± 8.30	27.35± 7.97	28.61± 7.93	29.50± 8.83			
β-Carotene	IG	1.15± 0.50	1.03± 0.46	1.14± 0.61	1.16± 0.60	0.533	0.713	0.456
	CG	1.00± 0.39	0.98± 0.35	0.94± 0.37	1.04± 0.40			
	Total	1.07± 0.44	1.00± 0.40	1.04± 0.50	1.10± 0.50			

Data are presented as mean ± standard deviation, the p-values were determined using a repeated measures ANOVA. A p-value of 0.05 is considered significant.

Table 16: *Fat-soluble vitamins at different timepoints for males (IG, CG)*

Parameter [µmol/L]	Group	T0 Mean± SD	T2 Mean± SD	T3 Mean± SD	T4 Mean± SD	p-value Time	p-value Time×Group	p-value Group
Retinol	IG	1.69± 0.64	1.78± 0.48	1.94± 0.39	1.73± 0.34	0.030	0.899	0.933
	CG	1.77± 0.60	1.78± 0.64	1.92± 0.66	1.77± 0.55			
	Total	1.73± 0.60	1.78± 0.54	1.93± 0.51	1.74± 0.43			
γ-Tocopherol	IG	2.41± 0.77	2.71± 0.87	2.44± 0.64	2.57± 0.49	0.814	0.551	0.077
	CG	1.93± 0.78	1.95± 0.66	2.30± 1.04	1.86± 0.79			
	Total	2.19± 0.79	2.36± 0.85	2.37± 0.82	2.24± 0.72			
α-Tocopherol	IG	24.46 ±6.53	28.17± 8.90	26.31± 10.22	24.34± 6.96	0.416	0.486	0.580
	CG	23.62 ±6.07	24.56± 7.78	22.54± 6.11	24.43± 8.47			
	Total	24.06 ±6.11	26.48± 8.30	24.55± 8.49	24.38± 7.41			
β-Carotene	IG	0.70± 0.39	0.85± 0.54	0.84± 0.54	0.79± 0.48	0.353	0.442	0.369
	CG	0.60± 0.44	0.65± 0.42	0.56± 0.33	0.57± 0.31			
	Total	0.65± 0.40	0.75± 0.49	0.71± 0.47	0.69± 0.41			

Data are presented as mean ± standard deviation, the p-values were determined using a repeated measures ANOVA. A p-value of 0.05 is considered significant.

5.5 Fat-soluble vitamins and BMI

Previous studies have described associations between fat-soluble vitamin concentrations and the BMI [Chai et al., 2010]. Therefore, this link was also tested with our data set.

Table 17: *Association between delta-BMI (T3-T0) and changes in fat-soluble vitamins*

Parameter [µmol/L] (T3–T0)	Spearman ρ (95% CI)	p-value	Pearson r (95% CI)	p-value	N
Δ-Retinol	0.049 (−0.293, 0.398)	0.767	0.141 (−0.222, 0.459)	0.391	39
Δ-γ-Tocopherol	−0.111 (−0.455, 0.226)	0.500	0.009 (−0.406, 0.292)	0.956	39
Δ-α-Tocopherol	0.111 (−0.264, 0.457)	0.500	0.234 (−0.191, 0.541)	0.151	39
Δ-β-Carotene	−0.282 (−0.574, 0.026)	0.082	−0.255 (−0.479, −0.018)	0.117	39

Values are correlation coefficients with 95% bootstrap confidence intervals (1,000 samples) and two-sided p-values. N = 39.

For retinol, Spearman (ρ = -0.049, p = 0.767) and Pearson (r = 0.141, p = 0.391) are close to zero, indicating no evidence of a correlation. γ-Tocopherol also shows no correlation (ρ = -0.111, p = 0.500; r = 0.009, p = 0.956). For α-tocopherol, the estimators indicate at most a weak positive relationship (ρ = 0.111, r =0.234) but this is not significant (p ≥ 0.151). The largest, but still small negative correlations are found in β-carotene (ρ = -0.282, p = 0.082; r = -0.255; p = 0.117). This shows a not reliable inverse relationship. A decrease in BMI could be linked to slight decreases in β-carotene, but this is not statistically proven. The 95% bootstrap confidence intervals (1000 resamples) enclose zero for most estimators. If the confidence intervals are close to zero, this is consistent with p-values above 0.05 and indicates only weak evidence.

6 Discussion

In this master´s thesis, serum concentrations of fat-soluble vitamins (retinol, α-/γ-tocopherol and β-carotene) were determined using HPLC at four measurement points as part of the NUMOQUA study to characterise the temporal course and potential differences between the intervention and control group. Measurements from 56 individuals were available for laboratory analysis. The complete case analysis across all time points included 39 data sets. The baseline characteristics were well balanced between the IG and CG (no significant differences in age, anthropometry, body composition and water compartments), which indicates successful randomisation. As expected, gender-specific differences in body composition were observed (men with higher FFM/SSM/TBW, women with higher FM/FMI). These findings support the internal validity of the measurements.

The primary finding is that the fat-soluble vitamins remained largely stable over the observation period and that there were no significant group differences between IG and CG. For retinol, there were no significant time, interaction or group effects in the overall collective. However, a significant time effect was observed in the male subgroup (pxtime = 0.030). The values increased in both groups until T3 and approached the baseline level again at T4 with no time-group interaction or group differences. This suggests a general temporal course rather than an intervention-specific effect. Similar stability patterns have also been observed in previous intervention studies, in which neither retinol nor α-tocopherol levels increased significantly after moderate supplementation. These results indicate strong homeostatic regulation of fat-soluble vitamins, which can mask short-term changes in serum status [Joris et al., 2015; Youness et al., 2022]

No time, group or interaction effects were observed for α-tocopherol. Concentrations remained largely unchanged between T0 and T4 and were comparable between the IG and CG in both the overall collective and in the gender-specific analysis. β-Carotene behaved similarly. Small, clinically insignificant deviations without significant time, group or interaction effects. IG was slightly higher in some cases, but this was not statistically significant. This corresponds with observations by White et al., who showed

that differences in vitamin E serum status are more likely to be caused by factors such as BMI or supplementation and dietary factors playing a smaller role [White et al., 2001].

γ-Tocopherol showed an exceptional pattern. In the overall collective, there was a hint of a borderline time-group interaction ($p \approx 0.056$), which became significant in the women´s subgroup (p-timexgroup = 0.036). Especially γ-tocopherol increased in the IG from T0 to T2, while it decreased in the CG. At later time points, the groups converged again. This pattern suggests different temporal patterns between the IG and CG in women, but without main effects of time or group and without confirmation in men (p-group = 0.077, trend towards higher IG values, but not significant). Taken together, the data suggests short-term, group-specific dynamics in women rather than a consistent intervention effect over the course. Reports on gender-specific differences in γ-tocopherol metabolism and on the decrease in γ-tocopherol during α-tocopherol supplementation support our observation of dynamics modulated by biological factors in women, without a consistent intervention effect [Frank et al., 2008; Huang & Appel, 2003].

The fact that retinol, α-tocopherol and β-carotene changed only minimally over time in both groups suggests that the intervention in the present implementation and setting did not significantly change vitamin levels. The parallel increase in retinol in men up to T3 could reflect temporal or other influences such as measurement or sampling variability. Since the trends in the IG and CG are almost identical, an intervention-related effect is unlikely. The gender-specific γ-tocopherol pattern in women could be related to differences in diet, lipid transport, tissue storage or compliance. According to Joris et al., intervention effects on fat-soluble vitamins are usually small and depend on various covariates, which is why larger replication studies are needed. Since there is no evidence of an effect at group level and the effect is not consistent over time, the clinical relevance is limited and should be investigated in larger studies [Joris et al., 2015].

No reliable correlation between delta-BMI and changes in fat-soluble vitamins was found for T0-T3. Overall, the correlations between delta-BMI and delta-vitamins show small, insignificant effects (CI mostly at or close to zero), with at most a trend-like inverse pattern for β-carotene. Possible small effects should be verified in larger analyses.

The strengths of the study are its randomisation, the good baseline balance between the IG and CG and the repeated measurement design over several time points. Limitations include the moderate sample size (especially in the gender-specific subgroups) and dropouts with reduction to complete case analysis (n = 39), which may limit power and precision. In addition, depending on the study setting, residual confounding factors due to dietary intake, seasonality, supplement use, or measurement variability cannot be ruled out.

Overall, the data show stable levels of retinol, α-tocopherol and β-carotene with no significant differences between IG and CG. Retinol increases over time in men (without group effect) and γ-tocopherol shows temporary IG-CG difference in women. Altogether there is no consistent evidence of robust intervention effects on the status of fat-soluble vitamins in the NUMOQUA study samples.

7 Summary

Background: Fat-soluble vitamins play an important role in antioxidant metabolism and can potentially be modulated through dietary or lifestyle interventions. Inflammatory processes are also a central aspect of osteoarthritis pathogenesis. The aim of this study was to investigate the progression of serum levels of fat-soluble vitamins in the NUMOQUA study.

Method: The concentration of fat-soluble vitamins was measured using HPLC. In the randomised NUMOQUA study, 62 subjects were included in an intervention and control group (IG = 31, CG = 31). Laboratory measurements were available for 56 individuals. The complete case analysis (T0-T4) included 39 data sets. Retinol, α-, γ-tocopherol and β-carotene were determined. Group, time and interaction effects were analysed using repeated measurements. In addition, a subgroup analysis was performed separately for each gender. Correlations between delta-BMI (T3-T0) and delta-vitamins (T3-T0) were calculated using Pearson and Spearman correlations with 95% bootstrap confidence intervals (1000 resamples).

Results: The concentrations of fat-soluble vitamins remained largely stable over the timepoints. No significant time, group or interaction effects were observed for retinol, α-tocopherol and β-carotene ($p > 0.05$). In men, a moderate increase in retinol was observed up to T3 (p-time = 0.030), which then returned to the baseline value (without group differences). For γ-tocopherol there was a borderline time-group interaction in the overall group ($p \approx 0.056$), which became significant in the women´s subgroup (p-timexgroup = 0.036). In the IG, γ-tocopherol increased until T2, while it decreased in the CG before both curves converged again. In men, there was only a non-significant trend towards higher IG values (p-group = 0.077). The correlations between delta-BMI and changes in vitamin levels were weak and statistically insignificant. The bootstrap confidence intervals included zero in all cases.

Conclusion: Overall, the observed results show that the fat-soluble vitamin levels remained largely stable and were only minimally affected by the intervention.

8 Zusammenfassung

Hintergrund: Fettlösliche Vitamine spielen eine wichtige Rolle im antioxidativen Stoffwechsel und sind potenziell durch Ernährungs- oder Lebensstilinterventionen modulierbar. Auch in der Osteoarthritis Pathogenese sind Entzündungsprozosse ein zentraler Aspekt. Ziel dieser Arbeit war es, den Verlauf der Serum-Spiegel fettlöslicher Vitamine in der NUMOQUA-Studie zu untersuchen.

Methode: Die Konzentration fettlöslicher Vitamine wurden mittels HPLC gemessen. In der randomisierten NUMOQUA Studie wurden 62 Probanden in eine Interventions- und Kontrollgruppe eingeschlossen (IG = 31, CG = 31). Für 56 Personen lagen Labormessungen vor. Die Complete-Case-Analyse (T0-T4) umfassten 39 Datensätze. Bestimmt wurden Retinol, α-, γ-Tocopherol und β-Carotin. Gruppen-, Zeit- und Interaktionseffekte wurden über wiederholte Messungen analysiert. Zusätzlich wurde eine Subgruppenanalyse getrennt nach Geschlecht durchgeführt. Zusammenhänge zwischen delta-BMI (T3-T0) und delta-Vitamin (T3-T0) wurden mittels Pearson- und Spearman-Korrelationen mit 95%-Bootstrap-Konfidenzintervallen (1000 Resamples) berechnet.

Ergebnisse: Die Konzentrationen der fettlöslichen Vitamine blieben über den Beobachtungszeitraum weitgehend stabil. Für Retinol, α-Tocopherol und β-Carotin zeigten sich keine signifikanten Zeit-, Gruppen- oder Interaktionseffekte ($p > 0.05$). Bei Männern ließ sich für Retinol ein moderater Anstieg bis T3 (p-time = 0.030) zeigen, Welcher sich anschließend wieder dem Ausgangswert annäherte (ohne Gruppenunterschied. Für γ-Tocopherol deutete sich in der Gesamtgruppe eine grenzwertige Zeit-Gruppen-Interaktion an ($p \approx 0.056$), die in der Frauen-Subgruppe signifikant wurde (p-timexgroup = 0.036). In der IG stieg γ-Tocopherol bis T2 an, während es in der CG abfiel, bevor sich beide Verläufe wieder anglichen. Bei Männern zeigte sich lediglich ein nicht signifikanter Trend zu höheren IG-werten (p-group = 0.077.) Die Korrelationen zwischen delta-BMI und Änderungen der Vitaminspiegel waren schwach und statistisch nicht signifikant. Die Bootstrap-Konfidenzintervalle umschlossen in allen Fällen die Null.

Zusammenfassung: Die beobachteten Ergebnisse zeigen, dass die fettlöslichen Vitamine insgesamt weitgehend stabil blieben und durch die Intervention nur gering beeinflusst wurden.

9 References

Al Tanoury Z, Piskunoc A, Rochette-Egly C. (2013). Vitamin A and retinoid signaling: genomic and nongenomic effects. Journal of Lipid Research. 54(7), 1761-1775. doi: 10.1194/jlr.R030833

Ames BN. (2018). Prolonging healthy aging: Longevity vitamins and proteins. PNAS, 114(43), 10839-10844. doi: 10.1073/pnas.1809045115

Andres E, Lorenzo-Villalba N, Terrade JE, Mendez-Bailon M. Fat-Soluble Vitamin A, D, E, and K: Review of the Literature and Points of Interest for the Clinician. Journal of Clinical Medicine, 2024, 13, 3641; https://doi.org/10.3390/jcm13133641

Bärebring L, Winkvist A, Gjertsson I, Lindqvist H. (2018). Poor dietary quality is associated with increased inflammation in Swedish patients with rheumatoid arthritis. Nutrients, 10(10), 1535. https://doi.org/10.3390/nu10101535

Berenbaum F. (2013). Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). Osteoarthritis Cartilage, 21(1), 16-21. https://doi.org/10.1016/j.joca.2012.11.012

Blaner WS, Obunike JC, Kurlandsky SB, Al-Haideri M, Piantedosi R, Deckelbaum RJ, Goldberg IJ. Lipoprotein Lipase Hydrolysis of Retinyl Ester. The Journal of Biological Chemistry, 1994, 269(24), 6559-16565. https://doi.org/10.1016/S0021-9258(19)89425-5

Brand C, Snaddon J, Cicuttini F. (2001). Vitamin E is ineffective for symptomatic relief of knee osteoarthritis: a six month double blind, randomised, placebo controlled study. Annals of rheumatic disease, 60(10), 946-949. Doi: 10.1136/ard.60.10.946

Brigelius-Flohe R, Traber MG. Vitamin E: function and metabolism. The FASEB Journal, 1999, Vol. 13, Issue 10, p.1145-1155; doi: 10.1096/fasebj.13.10.1145

Bruyere O, Honvo G, Veronese N, Arden NK, Branco J, Curtis EM, et al. (2019). An updated algorithm recommendation for the management of knee osteoarthritis from the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO). Semin Arthritis Rheum, 49(3). 337-50. https://doi.org/10.1016/j.semarthrit.2019.04.008

Buck AN, Shultz SP, Huffman KF, Vincent HK, Batsis JA, Newman CB, Beresic N, Abbate LM, Callahan LF. (2022). Mind the Gap: Exploring Nutritional Health Compared with

weight Management Interests of Individuals with Osteoarthritis. Currents Developments in Nutrition, 6, nzac084. https://doi.org/10.1093/cdn/nzac084

Cena H, Calder PC. (2020). Defining a healthy diet: evidence for the role of contemporary dietary patterns in health and disease. Nutrients, 12(2), 334. https://doi.org/10.3390/nu12020334

Chelstowska S, Widjaja-Adhi MAK, Silvaroli JA, Golczak M. (2016). Molecular Basis for Vitamin A Uptake and Storage in Vertebrates. Nutrients, 8, 676. doi: 10.3390/nu8110676

Chew BP & Park JS. Carotenoid Action on the Immune Response. The Journal of nutrition, 2004, Vol.134 (1), p.257S-261; doi: 10.1093/jn/134.1.257S

D'Ambrosio DN, Clugston RD, Blaner WS. Vitamin A Metabolism: An Update. Nutrients, 2011, 3, p.63-103; doi: 10.3390/nu3010063

Davis AM, Kennedy D, Wong R, Robarts S, Skou ST, McGlasson R, et al. (2018). Cross-cultural adaptation and implementation of Good Life with osteoarthritis in Denmark (GLA:DTM): group education and exercise for hip and knee osteoarthritis is feasible in Canada. Osteoarthritis and Cartilage, 26(2), 211-9. https://doi.org/10.1016/j.joca.2017.11.005

De Roos AJ, Arab L, Renner JB, Craft N, Luta G, Helmick CG, Hochberg MC, Jordan JM. (2001). Serum carotenoids and radiographic knee osteoarthritis: the Johnston County Osteoarthritis Project. Public Health Nutrition, 4(5), 935-942. doi: 10.1079/PHN2001132

Frank J, Lee S, Leonard SW, Atkinson JK, Kamal-Eldin A, Traber MG. (2008). Sex differences in the inhibition of -tocopherol metabolism by a single dose of dietary sesame oil in healthy subjects. Journal of Clinical Nutrition, 87(6), 1723-9. doi: 10.1093/ajcn/87.6.1723

Grune T, Lietz G, Palou A, Ross AC, Stahl W, Tang G, Thurnham D, Yin S, Biesalski HK. b-Carotene Is an Important Vitamin A Source for Humans. The Journal of Nutrition, 2010, 140 (12), 2268-2285S; doi: 10.3945/jn.109.119024.

Huang HY, Appel LJ. (2003). Supplementation of Diets with alpha-Tocopherol reduces Serum Concentrations of gamma- and delta-Tocopherol in Humans. The Journal of Nutrition, 133(10), 3137-3140. https://doi.org/10.1093/jn/133.10.3137

Jiang Q. Natural forms of vitamin E: metabolism, antioxidant and anti-inflammatory activities and the role in disease prevention and therapy. Free Radical Biology and Medicine, 2014, 72: 76-90; doi: 10.1016/j.freeradbiomed.2014.03.035.

Joris PJ, Mensink RP. (2015). Effects of supplementation with the fat-soluble vitamins E and D on fasting flow-mediated vasodilation in adults: a meta-analysis of randomized controlled trials. DOI: 10.3390/nu7031728

Karazniewicz-Lada M, Glowka A. (2016). A review of chromatographic methods for the determination of water- and fat-soluble vitamins in biological fluids. J Sep Sci, 39, 132-148. doi: 10.1002/jssc.201501038

Klimont J. (2020). Österreichische Gesundheitsbefragung 2019. Hauptergebnisse des Austrian Health Interview Surve (ATHIS) und methodische Dokumentation. Bundesministerium für Soziales, Gesundheit, Pflege und Konsumentenschutz, Statistik Austria. https://www.statistik.at/fileadmin/publications/Oesterreichische-Gesundheitsbefragung2019_Hauptergebnisse.pdf

Krinsky NI & Yeum KJ. Carotenoid-radical interactions. Biochemical and Biophysical Research Communications, 2003, Vol. 305, p.754–760; doi: 10.1016/S0006-291X(03)00816-7

Kroman SL, Roos EM, Bennell KL, Hinman RS, Dobson F. (2014). Measurement properties of performance-based outcome measures to assess physical function in young and middle-aged people known to be at high risk of hip and/or knee osteoarthritis: a systematic review. Osteoarthritis Cartilage, 22(1), 26–39. doi: 10.1016/j.joca.2013.10.021

Lemaire-Ewing S, Desrumaux C, Neel D, Lagrost L. (2010). Vitamin E transport, membrane incorporation and cell metabolism: Is α-tocopherol in lipid rafts an oar in the lifeboat. Molecular nutrition & food research, 54(5), 631-640. doi: 10.1002/mnfr.200900445

Maoka T. (2020). Carotenoids as natural functional pigments. Journal of Natural Medicines, 74, 1–16. https://doi.org/10.1007/s11418-019-01364-x

Mardones P, Rigotti A. (2004). Cellular mechanisms of vitamin E uptake: relevance in -tocopherol metabolism and potential implications for disease. The Journal of Nutritional Biochemistry, 15, 252-260. doi: 10.1016/j.jnutbio.2004.02.006

McAlindon TE, Jaques P, Zhang Y, Hannan MT, Aliabadi P, Weissman B, Rush D, Levy D, Felson DF. (1996). Do antioxidant micronutrients protect against the development and progression of knee osteoarthritis? Arthritis & Rheumatism, 39(4), 648-656 doi: 10.1002/art.1780390417

Messina OD, Vidal Wilman M, Vidal Neira NF. (2019). Nutrition, osteoarthritis and cartilage metabolism. Aging Clinical and Experimental Research, 31(6), 807-813 https://doi.org/10.1007/s40520-019-01191-w

Meydani SN, Han SN, Wu D. (2005). Vitamin E and immune response in the aged: molecular mechanisms and clinical implications. Immunological Reviews, 205 (1), 269-284. doi: 10.1111/j.0105-2896.2005.00274.x

Minihane AM, Vinoy S, Russell WR, Baka A, Roche HM, Tuohy KM et al. (2015). Low-grade inflammation, diet composition and health: current research evidence and its translation. British Journal of Nutrition, 114(7), 999-1012. doi: 10.1017/S0007114515002093

Moldoveanu SC, David V. (2013). Essentials in Modern HPLC Separations. Elsevier, https://doi.org/10.1016/B978-0-12-385013-3.00001-X

Motyl JM, Driban JB, McAdams E, Price LL, McAlindon TE. (2013). Test-retest reliability and sensitivity of the 20-meter walk test among patients with knee osteoarthritis. BMC Musculoskelet Disorders, 14, 166. doi: 10.1186/1471-2474-14-166

Napoli JL. (2016). Functions of Intracellular Retinoid Binding-Proteins. Subcellular Biochemistry, 81, 21-76. doi: 10.1007/978-94-024-0945-1_2

O'Byrne SM & Blaner WS. (2013). Retinol and retinyl esters: biochemistry and physiology. Journal of Lipid Research, 54(7), 1731-1743; doi: 10.1194/jlr.R037648

Penniston KL & Tanumihardjo SA. (2006). The acute and chronic toxic effects of vitamin A. The American Journal of Clinical Nutrition, 83 (2), 191-201. https://doi.org/10.1093/ajcn/83.2.191

Rochette-Egly C. (2015). Retinoid acid signaling and mouse embryonic stem cell differentiation: Cross talk between genomic and non-genomic effects of RA. Biochemica et Biophysica Acta 1851, 66-75. http://dx.doi.org/10.1016/j.bbalip.2014.04.003

Rochette-Egly C & Germain P. (2009). Dynamic and combinatorial control of gene expression by nuclear retinoic acid receptors (RARs). Nuclear Receptor Signaling, 7. doi: 10.1621/nrs.07005

Schmölzl L, Birringer M, Lorkowski S, Wallert M. (2016). Complexity of vitamin E metabolism. World Journal of Biological Chemistry, 7(1), 14-43. doi: http://dx.doi.org/10.4331/wjbc.v7.i1.14

Senoo H, Yoshikawa K, Morii M, Miura M, Imai K, Mezaki Y. (2010). Hepatic stellate cell (vitamin A-storing cell) and its relative – past, present and future. Cell Biology International, 34, 1247-1272. doi:10.1042/CBI20100321

Stahl W & Sies H. (2003). Antioxidant activity of carotenoids. Molecular Aspects of Medicine, 24, 345–351. doi:10.1016/S0098-2997(03)00030-X

Sies H & Stahl W. (1995). Vitamins E and C, ß-carotene, and other carotenoids as antioxidants. The American Journal of Clinical Nutrition, 62(6), 1315S-1321S.

Steinhoff JS, Lass A, Schupp M. (2022). Retinoid Homeostasis and Beyond: How Retinol Binding Protein 4 Contributes to Health and Disease. Nutrients, 14, 1236. https://doi.org/10.3390/nu14061236

Stephensen CB. (2001). Vitamin A, Infection, and Immune Function. Annual Reviews of Nutrition, 21, 167–92. doi: 10.1146/annurev.nutr.21.1.167

Tan BY, Ding BTK, Pereira MJ, Skou ST, Thumboo J, Car J. (2020). Collaborative model of care between Orthopaedics and allied healthcare professionals trial (CONNACT) – a feasibility study in patients with knee osteoarthritis using a mixed method approach. BMC Musculoskelet Disorders, 21(1), 592. https://doi.org/10.1186/s12891-020-03611-9

Tanaka T, Shnimizi M, Moriwaki H. (2012). Cancer Chemoprevention by Carotenoids. Molecules, 17(3), 3202-3242. doi: 10.3390/molecules17033202

Tantavisut S, Tanavalee A, Honsawek S, Suantawee T, Ngarmukos S, Adisakwatana S, Callaghan JJ. (2017). Effect of vitamin E on oxidative stress level in blood, synovial fluid, and synovial tissue in severe knee osteoarthritis: a randomized controlled study. BMC Musculoskeletal Disorders, 18, 281. doi: 10.1186/s12891-017-1637-7

Thomas DR. (2006). Vitamins in aging, health, and longevity. Clinical Intervention in Aging, 1(1), 81-91. doi: 10.2147/ciia.2006.1.1.81

Traber MG. (2007). Vitamin E Regulatory Mechanisms. Annual Reviews of Nutrition, 27 347-62. doi: 10.1146/annurev.nutr.27.061406.093819

Traber MG. (2024). Human Vitamin E Deficiency, and what is and is NOT vitamin E? Free Radical Biology & Medicine, 213, 285-292. doi: 10.1016/j.freeradbiomed.2024.01.024

Traber MG, Atkinson J. (2007). Vitamin E, Antioxidant and Nothing More. Free Radical Biology & Medicine, 43(1), 4-15. doi: 10.1016/j.freeradbiomed.2007.03.024

Traber MG, Stevens JF. (2011). Vitamin C and E: Beneficial effects from a mechanistic perspective. Free Radical Biology & Medicine, 51, 1000-1013. doi: 10.1016/j.freeradbiomed.2011.05.017

Wellsandt E, Golightly Y. (2018). Exercise in the management of knee and hip osteoarthritis. Current opinion in rheumatology, 30, 151-159 doi: 10.1097/BOR.0000000000000478

Weng W, Li L, van Bennekum AM, Potter SH, Harrison EH, Blaner WS, Breslow JL, Fisher EA. (1999). Intestinal Absorption of Dietary Cholosteryl Ester Is Decreased but Retinyl Ester Absorption Is Normal in Carboxyl Ester Lipase Knockout Mice. Biochemistry, 38, 4143-4149. doi: 10.1021/bi981679a

White E, Kristal AR, Shikany JM, Wilson AC, Chen C, Mares-Perlman JA, Masaki KH, Caan BJ. (2001). Correlates of serum alpha- and gamma-tocopherol in the Women´s Health Initiative. Ann. Epidemiol, 11(2), 136-144. doi: 10.1016/s1047-2797(00)00189-7

Wluka AE, Stuckey S, Brand C, Cicuttini FM. (2002). Supplementary Vitamin E Does Not Affect the Loss of Cartilage Volume in Knee Osteoarthritis: A 2 Year Double Blind Randomized Placebo Controlled Study. The Journal of Rheumatology, 29(12): 2585-2591

Wu A, Wu N, Xu P, Jin Y, Yang Z, Teng J. (2024). Association of blood vitamin A with osteoarthritis: a nationally representative cross-sectional study. Frontiers in Nutrition, 11, 1459332. doi: 10.3389/fnut.2024.1459332

Youness RA, Dawoud A, ElTahtawy O, Farag MA. (2022). Fat-soluble vitamins: updated review of their role and orchestration in human nutrition throughout life cycle with sex differences. Nutrition & Metabolism, 19(1), 60. doi: 10.1186/s12986-022-00696-y