

Aus dem Institut für Epidemiologie
der Christian-Albrechts-Universität zu Kiel

**Plasma ferritin, zinc and copper in the general population –
metabolic correlates and associations with body composition**

Dissertation
zur Erlangung des Doktorgrades
der Agrar- und Ernährungswissenschaftlichen Fakultät
der Christian-Albrechts-Universität zu Kiel

vorgelegt von
M.Sc. Cara Sophie Övermöhle
aus Weener
Kiel, 2024

Dekan: Prof. Dr. Georg Thaller

1. Berichterstatter: Prof. Dr. Wolfgang Lieb

2. Berichterstatter: Prof. Dr. Gerald Rimbach

Tag der mündlichen Prüfung: 27.05.2024

Acknowledgments

I would like to thank Prof. Dr. Wolfgang Lieb for the opportunity to write my dissertation at the Institute of Epidemiology at Kiel University. I am grateful for the insightful discussions, the constructive criticism and the support during the preparation of this thesis.

Special thanks are due to Dr. Katharina S. Weber for her guidance and tireless commitment in supervising my work. Her always open ear and constructive suggestions were invaluable.

I would also like to thank Prof. Dr. Gerald Rimbach from the Institute of Human Nutrition and Food Science at Kiel University, who co-supervised my work and provided me with his invaluable expertise and feedback on my manuscript.

I would like to thank all my colleagues at the Institute of Epidemiology for the friendly working atmosphere and their willingness to help. My special thanks go to my doctoral colleagues Tatjana Liedtke and Paula Stürmer for their friendly support throughout my doctoral studies. I would also like to thank Dr. Eike Strathmann, who joined our institute as a postdoc last year and has given me a lot of support, especially with my scientific writing.

I would also like to thank all the other co-authors for their constructive contributions to the manuscripts of this thesis. I would also like to thank all the study participants in the *pogen-controls* cohort, whose participation made this work possible.

Finally, I would like to express my deepest gratitude to my family, especially my parents and my partner. Their unwavering support and encouragement have been a constant source of strength in my life. I am very grateful to my partner for his understanding and support, especially during the most difficult stages of this dissertation.

Table of Contents

List of publications and presentations	3
Index of abbreviations.....	5
1 General introduction	1
1.1 Iron in human health	1
1.1.1 Plasma ferritin as parameter for iron status	2
1.1.2 Sources of dietary iron and recommendations for dietary iron intake	2
1.1.3 Circulating ferritin concentrations in epidemiological studies.....	3
1.2 Zinc in human health	4
1.2.1 Sources of dietary zinc and recommendations for dietary zinc intake	6
1.2.2 Zinc status and possible associations with body composition and blood lipids.....	6
1.3 Copper in human health	8
1.3.1 Sources of dietary copper and recommendations for dietary copper intake .	8
1.3.2 Copper status and possible associations with body composition and blood lipids.....	8
1.4 Aims	9
1.5 Methods.....	10
1.5.1 Study sample.....	10
1.5.2 Covariate assessment	11
1.5.3 Measurements of ferritin, zinc and copper	12
1.5.4 Assessment of dietary intake to estimate the intake of micronutrients, macronutrients and food groups	13

Table of Contents

1.5.5	Assessment of body composition by magnetic resonance imaging for the investigation of associations of zinc and copper with adipose tissue.....	13
1.5.6	Statistics	15
1.6	References	17
2	Plasma ferritin concentrations in the general population: A cross-sectional analysis of anthropometric, metabolic and dietary correlates.....	27
3	Association of plasma zinc and copper with body composition, lipids and inflammation in a cross-sectional general population sample from Germany.	40
4	General discussion	59
4.1	Plasma ferritin, zinc and copper status	60
4.2	Dietary correlates of plasma ferritin, zinc and copper.....	62
4.3	Plasma ferritin, zinc and copper and body composition	64
4.3.1	Ferritin as marker of iron status and inflammation	67
4.3.2	Heterogeneity between studies as an important contributor to conflicting results for correlates of zinc and copper	69
4.3.3	Homeostasis of zinc and copper	71
4.4	Methodological considerations and potential biases	73
4.4.1	Validity and limitations of food frequency questionnaires	73
4.4.2	Potential biases	75
4.5	Future research	77
4.6	Conclusion.....	77
4.7	References	79
5	Summary	87
6	Zusammenfassung	89

List of publications and presentations

Articles incorporated into this thesis

Övermöhle C, Waniek S, Rimbach G, Weber KS, Lieb W. *Plasma Ferritin Concentrations in the General Population: A Cross-Sectional Analysis of Anthropometric, Metabolic, and Dietary Correlates*. The Journal of Nutrition 153 (5), S. 1524–1533. DOI: 10.1016/j.tjnut.2023.03.008.

Övermöhle C, Rimbach G, Waniek S, Strathmann EA, Liedtke T, Stürmer P, Both M, Weber KS, Lieb W. *Association of plasma zinc and copper with body composition, lipids and inflammation in a cross-sectional general population sample from Germany*. Nutrients 15 (20), S. 4460. DOI: 10.3390/nu15204460.

Articles not incorporated into this thesis

Stürmer P, Ratjen I, Weber KS, **Övermöhle C**, Liedtke T, Waniek S, Strathmann EA, Lieb W. *Greater adherence to the Healthy Nordic Food Index is associated with lower all-cause mortality in a population-based sample from northern Germany*. European Journal of Nutrition, S. 1–11. DOI: 10.1007/s00394-023-03271-0.

Presentations at scientific conferences

Oral

Plasma zinc and copper/zinc ratio in the general population: A cross-sectional analysis of anthropometric and metabolic correlates. 18. Jahrestagung der Deutschen Gesellschaft für Epidemiologie; 26.-28. September 2023, Würzburg, Germany

Poster

Associations of plasma ferritin with patterns of food intake and anthropometric measures in a general population sample. 17. Jahrestagung der Deutschen Gesellschaft für Epidemiologie; 26.-29. September 2022, Greifswald, Germany

Associations of dietary patterns with plasma ferritin concentrations in a general population sample. 60. Wissenschaftlicher Kongress der Deutschen Gesellschaft für Ernährung e. V.; 15.-17. March 2023, Bonn, Germany

Index of abbreviations

BMI	Body mass index
DGE	Deutsche Gesellschaft für Ernährung
EPIC	European Prospective Investigation into Cancer and Nutrition
FFQ	Food frequency questionnaire
MRI	Magnetic resonance imaging
NHANES	National Health and Nutrition Examination Survey
WHO	World Health Organization
WHR	Waist-to-hip ratio

1 General introduction

Iron, zinc and copper are essential trace elements for humans. Iron deficiency and zinc deficiency are among the most relevant nutritional deficiencies globally [1–5]. In Europe, however, malnutrition is rather rare and usually only affects vulnerable groups such as children, pregnant women and the elderly [1, 4]. However, in Germany, the population status of these trace elements is not well documented yet. Furthermore, it is largely unclear, how these trace elements relate to classic cardiometabolic risk factors and particularly to parameters of body composition, such as subcutaneous or visceral fat or to measures of general adiposity.

It is well established that adiposity is a major global health problem [6]. If current trends persist, the economic impact of overweight and obesity is projected to exceed four trillion US\$ in 2035, representing almost 3% of the current global gross domestic product [6]. By 2035 approximately four billion people will be living with overweight and obesity [6].

The aim of this thesis, therefore, was to determine the iron, zinc and copper status of an elderly observational cohort from Northern Germany (*popgen controls*, Kiel, Schleswig-Holstein) and to assess, in particular, whether circulating concentrations of iron, zinc and copper relate to dietary intake, metabolic risk factors (e. g. circulating blood lipids) and body composition.

1.1 Iron in human health

Iron is a trace element that is crucial for human health and plays a role in several important physiological processes [1, 7]. As the main component of hemoglobin in red blood cells, it is essential for oxygen transport [1]. In addition, iron is necessary for the immune system, as it functions as an acute phase protein [8–10]. Iron also plays a role in cognitive function and brain development [11]. Furthermore, iron is essential for normal growth and development, especially during periods of rapid growth, such as infancy, childhood and adolescence [11]. Finally, iron is essential for DNA synthesis, which is

necessary for cell division and tissue repair [1]. Inadequate iron concentrations can hinder these processes.

However, also elevated circulating iron concentrations can be harmful [1, 11]. Iron deposits in organs such as the liver, heart, pancreas and joints, may cause progressive damage of these organs [1, 11] and foster the development of chronic diseases like diabetes [12–14] and cardiovascular diseases [15–17]. Therefore, a balanced iron intake is critical for overall health.

1.1.1 Plasma ferritin as parameter for iron status

Since iron is highly reactive, it is bound to proteins in the human body [1, 11]. In the blood stream, iron is bound to transferrin, while within cells it is bound to ferritin, a protein that stores and releases iron as needed. Therefore, plasma ferritin concentrations serve as an indicator of iron store in the body [1, 11]. Adequate iron stores are usually reflected in normal ferritin concentrations. Conversely, low ferritin concentrations indicate a possible iron deficiency and excessively high ferritin concentrations may indicate iron overload. According to the World Health Organization (WHO), circulating ferritin concentrations should range from <15 µg/L to >150 µg/L in females and <15 µg/L to >200 µg/L in males [1]. Moderately increased plasma ferritin may be induced by lifestyle factors such as alcohol intake, meat consumption or use of iron supplements [18]. The health status also influences plasma ferritin with chronic inflammation, cancer, liver disease and metabolic syndrome being associated with moderately increased concentrations of ferritin [18]. However, plasma ferritin concentrations above 600 µg/L are more likely to be caused by hereditary hemochromatosis, a genetically determined disorder leading to an increased iron storage [1, 11, 18].

1.1.2 Sources of dietary iron and recommendations for dietary iron intake

Dietary iron is found in two distinct forms, heme and non-heme iron. Heme iron is obtained from animal sources, such as meat and meat products, while non-heme iron is predominantly present in plant-based foods, primarily vegetables and bread [7, 19]. Iron

absorption is inhibited by certain factors, including dairy products, black tea, coffee and inhibiting components commonly found in plant-based foods like tannins, oxalic acids, phytates and phosphates [7, 19]. On the other hand, iron absorption is improved in the presence of ascorbic acid. Non-heme iron tends to have a lower bioavailability of around 5%, while heme iron may have a higher bioavailability, possibly exceeding 20% [19].

Dietary iron intake recommendations vary based on factors such as age, sex and life stage. The German Nutrition Society (*Deutsche Gesellschaft für Ernährung*; DGE) recommends an iron intake of 10 mg/day for male adults aged 19 and older while women between the ages of 19 and 51 should consume 15 mg of iron per day [19]. This is due to increased blood loss caused by menstrual bleeding. After menopause, the recommended daily amount for women is reduced to 10 mg/day [19].

1.1.3 Circulating ferritin concentrations in epidemiological studies

Iron deficiency, characterized by insufficient ferritin concentrations ($<15 \mu\text{g/L}$), represents the most prevalent nutritional deficiency worldwide and poses a risk of anemia [1]. 29% of non-pregnant women and 43% of children worldwide exhibit anemia [1]. The impact of nutritional iron deficiency extends to approximately two billion individuals worldwide [2].

Even though iron deficiency is globally common, the prevalence of elevated ferritin in western societies is considerably high. Within the *European Prospective Investigation into Cancer and Nutrition* (EPIC) cohort ($n = 828$), 35% of males and 20% of females had high concentrations of ferritin when compared to the reference values of the WHO [9].

Interestingly, the prevalence of elevated ferritin was higher in Northern countries compared to Southern countries [9]. However, within the *Copenhagen City Heart Study* ($n = 8,988$), an observational cohort study, geographically close to the Kiel *popgen controls* cohort, only 16% had elevated plasma ferritin concentrations ($\geq 200 \mu\text{g/L}$) [18]. Within the original cohort of the North American *Framingham Heart Study* ($n = 1,016$), higher threshold values were used ($>300 \mu\text{g/L}$ in males and $>200 \mu\text{g/L}$ in females) and

elevated ferritin concentrations were observed in 12.9% of the participants at the 20th examination cycle (individuals aged 67 to 96 years) [8].

Typically, health status deteriorates with age, an association that is often driven by chronic inflammation. In accordance, age was found to be associated with higher concentrations of ferritin in several studies, possibly triggered by inflammation [8–10, 20]. Besides that, in middle-aged females, the onset of menopause may lead to an increase in plasma ferritin concentrations, attributed to the cessation of blood loss during menstruation [10], while age-related deficiency in ferroportin expression (the cellular iron exporter in mammals), can lead to iron accumulation in both sexes [8–10, 20].

Elevated ferritin appears to be a predictor variable for unhealthy aging. Within the *Copenhagen City Heart Study* moderately elevated plasma ferritin was associated with increased risk of mortality, including the overall mortality or disease-specific risk of mortality [18]. The *English Longitudinal Study of Ageing* (n = 5,471) also observed an association of elevated circulating ferritin with all-cause mortality in males and females and with cardiovascular mortality in males [21]. Therefore, monitoring circulating ferritin concentrations may be important not only with respect to iron status *per se*, but also as a predictor for mortality.

In accordance with established associations of ferritin and aging, one of the multiple functions of ferritin is the role as an acute phase protein [22, 23]. The association of inflammation and circulating ferritin concentrations appears to be caused by a variety of processes that are as diverse as the other functions of ferritin [24]. One potential underlying mechanism is the impact of inflammatory cytokines like interferon gamma, interleukin-6 and tumor necrosis factor-alpha prompting on ferritin synthesis and release into the blood stream [24–26].

1.2 Zinc in human health

Like iron, zinc is an essential trace element that plays an important role in various physiological processes and ensures the proper functioning of the organism [27, 28]. Zinc

is contained in approximately 300 enzymes, illustrating its multifunctionality in human metabolism [7, 27, 28].

Zinc is especially important for the immune system, as it is involved in the production and activation of immune cells [29, 30]. In addition, zinc serves as an antioxidant that protects cells from oxidative damage caused by free radicals [29, 30]. Moreover, zinc plays a critical role in growth, especially during childhood, adolescence and pregnancy, by enabling proper cell division and DNA synthesis to promote normal growth and development [27, 28]. While the impact of zinc deficiency on health is well-established, discussions about zinc excess are infrequent and typically revolve around zinc toxicity. Cases of poisoning have been associated with the consumption of an estimated dose of 325 – 650 mg of zinc [3]. Such toxicity manifests in individuals with symptoms including nausea, abdominal cramping, vomiting, tenesmus and diarrhea, with or without bleeding [3]. It is important to note that zinc toxicity is relatively rare and results from excessive intake of zinc supplements or environmental exposure rather than from habitual dietary intake [31]. However, oral zinc intake may also have adverse health effects especially when zinc intake is significantly higher than copper intake [3]. Copper is an important antagonist of zinc [3, 5, 32]. Zinc and copper have a similar transport mechanism in the small intestine [5, 32]. An excess of one mineral in the diet or in the form of supplements can interfere with the absorption of the other [5, 32]. This means that high zinc intake can decrease copper intake and *vice versa*. Therefore, negative health associations of elevated zinc concentrations could also be negative consequences of copper deficiency, triggered by imbalance between both trace elements due to increased absorption of zinc [31]. In addition, zinc and copper are cofactors for various enzymes in the human body and in some cases, they compete for binding sites, hence an imbalance can impact metabolic processes [5, 32]. Therefore, zinc should be assessed along with copper, as the two trace elements interact in a way that is often referred to as antagonistic. An imbalance between zinc and copper concentrations can be detrimental to health [3, 5, 32]. The mode of action of copper is explained in more detail in a later chapter (**page 8 f.**).

1.2.1 Sources of dietary zinc and recommendations for dietary zinc intake

Zinc is mainly contained in fatty animal-based foods like meat (especially beef), liver, fish, eggs and dairy products [19, 33]. However, also plant-based foods, such as seeds, legumes and oats contain zinc [33]. Like for iron, bioavailability of zinc is higher for zinc from animal-based foods [33]. Several components – e. g. fibers, iron and copper – decrease the bioavailability of zinc [33]. Especially phytate is associated with decreased absorption of zinc and thus included in intake recommendations for zinc [19, 33]. For adult males, the DGE recommends a daily intake of 11 – 16 mg/day (depending on the intake of phytate), while recommendations for adult females are ranging from 7 – 10 mg/day (depending on the intake of phytate) [19].

1.2.2 Zinc status and possible associations with body composition and blood lipids

Besides iron deficiency, zinc deficiency is a common nutritional deficiency on a global scale. It is estimated that approximately 25% of the world population is at risk of zinc deficiency [3]. In Europe, especially hospitalized or institutionalized persons are at risk for zinc deficiency [4]. In free-living elderly adults from Europe, prevalence of zinc deficiency is approximately 5% [4]. As for iron and ferritin, in western societies the elevated intake of zinc or high circulating concentrations of zinc may be more of concern nowadays. Zinc toxicity can be caused by an excessive use of zinc supplements or exposure due to inhalation at the workplace, while high dietary intake of zinc may cause copper deficiency but not zinc toxicity [3]. Thresholds for adverse shifts in the ratio of zinc to copper are unknown [3]. Besides zinc toxicity and copper deficiency, several studies suggest that zinc *per se* may be associated with various adverse health effects, such as unfavorable shifts in body composition [34–38]. However, a challenge within these studies is the heterogeneity in the study designs and the limited evidence from epidemiological studies [34–38].

The potential influence of zinc on adipose tissues has been mainly investigated in experimental *in-vivo* and *in-vitro* studies [34–37]. For example, zinc supplementation (200 mg/kg) for six weeks increased body fat in mice [37]. It is suggested that zinc may exhibit an insulin-like effect and therefore suppresses lipolysis in adipocytes or engages in interactions with thyroid hormone activities [37, 39]. In general, lipolysis in adipocytes is suppressed by insulin, leading to accumulation of fat depots [39]. Moreover, zinc may be associated with the upregulation of lipogenic enzymes and therefore promotes fat storage [35, 36, 40].

Some analyses on the association of zinc with parameters of body composition from population-based studies have been reported. For instance, within the Korean *National Health and Nutrition Examination Survey* (NHANES) (n = 1,896) a direct association of zinc with the quantity and distribution of body fat, measured by dual-energy X-ray absorptiometry has been reported [38]. A similar positive association of zinc with body fat was also observed in 87 hemodialysis patients [41]. Yet, other observational studies rather suggest an inverse association between zinc concentrations and obesity parameters. Namely, in 19,952 participants from the US NHANES (2007 – 2014), dietary zinc intake was inversely associated with anthropometric measures of body composition [42]. Comparing details of the two population-based studies from the US and Korea illustrate important differences in study designs, as the NHANES study from Korea uses more precise measures like serum zinc concentrations and body fat, compared to estimated dietary zinc intake and simple anthropometric measures like body mass index (BMI) and waist circumference in the US NHANES.

The published literature on the relation between zinc and blood lipids is rather conflicting and quite heterogeneous. The use of different measures of zinc exposure might be an important contributing factor. Some studies assessed dietary zinc intake (assessed e. g. by 24 h recall protocols), whereas others measured circulating zinc concentrations in plasma or serum. If zinc had an adipogenic effect, it could be expected, that blood lipids

will also be affected in an unfavorable manner by higher zinc concentrations. In an observational study from China (n = 12,028) higher dietary zinc intake was associated with higher triglyceride concentrations [43]. Whereas a meta-analysis of 24 studies from 2015 reported a lipid-lowering effect of zinc [44]. Therefore, the parallel investigation of adipose tissue and blood lipids in relation to plasma zinc concentrations may offer new perspectives.

1.3 Copper in human health

The third essential trace element on which this work focuses on is copper. It is also required for various physiological processes [7]. Copper is important for the formation of collagen and elastin, proteins that provide structure and elasticity to the skin, blood vessels and connective tissue [45–47]. Therefore, adequate copper concentrations are necessary to maintain skin and tissue health. Like zinc, copper is important for antioxidant protection [45–47]. Copper is a component of superoxide dismutase, an antioxidant enzyme that protects cells from oxidative damage caused by free radicals [5, 7]. In addition, copper is involved in the regulation of iron metabolism [45–47].

1.3.1 Sources of dietary copper and recommendations for dietary copper intake

Like zinc, copper is mainly contained in fatty animal-based foods like offal, meat (pork and beef) and fish [19, 33]. However, also nuts, legumes, whole grain products and cocoa contain high concentrations of copper. Bioavailability of copper is mainly decreased by high intake of iron, zinc, phytate and vitamin C [33]. The DGE recommends a daily intake of 1.0 – 1.5 mg copper per day for adolescents and adults [19].

1.3.2 Copper status and possible associations with body composition and blood lipids

While iron and zinc deficiencies are globally common, copper deficiency is rare and predominately found in infants or in persons with the inherited Menkes syndrome, a genetically determined X-chromosomal linked recessive disorder [48, 49]. Intake of dietary copper is estimated well above recommendations in European countries [50]. Therefore,

elevated circulating copper concentrations and high copper intake may be more of an issue. Like zinc, copper may also be associated with body weight and body fat in an unfavorable way [47, 51–53]. In a meta-analysis of eight cross-sectional studies and 27 case-control studies including children and adults, higher serum copper concentrations were associated with overweight [51]. Similarly, in the US NHANES (2011 – 2016, n = 7,285) linear associations of serum copper with BMI, waist circumference and body fat, assessed by dual-energy X-ray absorptiometry, were reported [52]. In addition, another NHANES survey (2011 – 2014, n = 3,211) also reported higher copper serum concentrations to be associated with an increased prevalence of non-alcoholic fatty liver disease [47]. A cohort study from China (n = 4,812) reported a direct associations of copper concentrations with triglycerides [54]. However, a meta-analysis of five clinical trials, including 176 participants in total, reported no association of copper supplementation with blood lipids [55]. Interestingly, higher concentrations of circulating serum copper were associated with all-cause mortality and with cardiovascular mortality in a direct linear way within the US NHANES 2011 – 2016 survey (n = 5,412) [56]. This underlines once again that it is also of scientific interest to examine the potential health effects of higher micronutrient concentrations and not only micronutrient deficiencies.

1.4 Aims

The aims of the present thesis were, therefore, to investigate the iron (assessed by plasma ferritin concentrations), zinc and copper status of an elderly general-population sample from Northern Germany and to assess the association of ferritin, zinc and copper with anthropometric and cardiometabolic traits. These analyses might contribute to a better understanding of how high concentrations of these trace elements might contribute to the development of chronic disease conditions, like adiposity, metabolic syndrome and cardiovascular diseases.

The following scientific questions should be answered within the first publication on plasma ferritin concentrations:

1. How is the distribution plasma ferritin concentrations in the elderly general population?
2. Which foods explain the maximum variance in plasma ferritin concentrations?
3. Which anthropometric and inflammatory traits are associated with elevated plasma ferritin concentrations?

The second publication on plasma zinc and copper concentrations addressed the following questions:

1. How is the distribution of plasma zinc and copper concentrations in the elderly general population?
2. How are plasma concentrations of zinc and copper associated with a broad spectrum of anthropometric traits?
3. How are plasma concentrations of zinc and copper associated with subcutaneous and visceral adipose tissue and liver fat?

1.5 Methods

All data from the present thesis were obtained from the *popgen controls* cohort. Plasma ferritin concentrations were measured at the second follow-up examination of *popgen controls* while concentrations of zinc and copper were analyzed in plasma samples from the first follow-up examination, as detailed below.

1.5.1 *Study sample*

In 2003, the biobank *popgen* for the target region Northern Schleswig-Holstein was founded in Kiel [57]. One of the central goals of the biobank was the collection of DNA samples (for subsequent genetic analyses) for a broad spectrum of disease conditions in a clearly defined region. Between 2005 and 2007, a reference sample (the *popgen controls*) for these genetic analyses was established (**Figure 1**) [58]. A total of 1,317 participants (45% females) were drawn from population registries from the city of Kiel and neighboring communities (n = 747) as well as from blood donor registries from the University Hospital in Kiel (n = 570) [58]. Between 2010 and 2012, a first follow-up

examination was conducted among 929 participants (42% females). Six years later, a second follow-up examination was implemented (2016 – 2018; n = 666; 43% females). The response rates for the first and the second follow-up examination were 70.5% and 71.7%, respectively. The study was conducted in accordance with the Declaration of Helsinki. The Ethics Committee of the Medical Faculty of the Christian-Albrechts-University Kiel has approved the study. Written informed consent was obtained from all study participants.

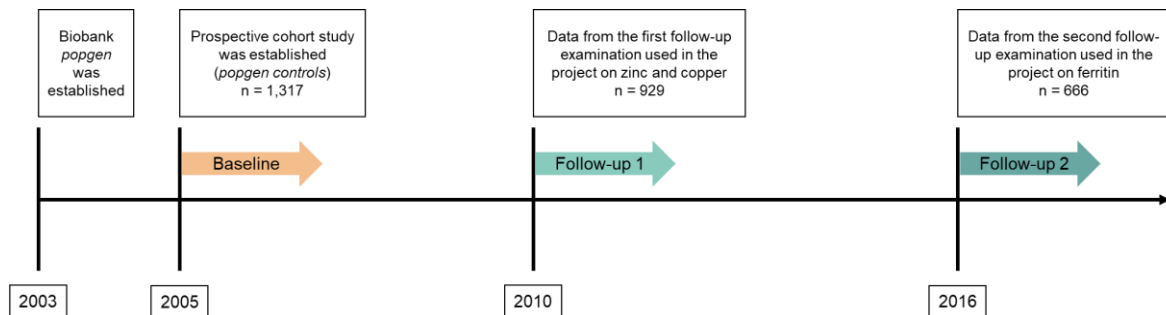


Figure 1: Timeline for the prospective cohort study *popgen controls* from Kiel, Schleswig-Holstein, Germany.

1.5.2 Covariate assessment

Trained personnel conducted all measurements in accordance with established standard operating procedures, consistent across all examination cycles [58]. Anthropometric assessments, encompassing body weight, height, waist and hip circumference, were carried out while participants were clad in lightweight clothing and without footwear. BMI was computed as the ratio of body weight (kg) to the square height (m²) and waist-to-hip ratio (WHR) was calculated as the division of waist and hip circumference [59–61].

Data on demographic information, including age, sex, education, smoking habits and medical history was collected through self-administered questionnaires [59, 62].

Additionally, participants provided details about their habitual physical activity over the past 12 months, specifying the time (in hours) spent on activities such as walking, cycling, sports, gardening, housework, home repair and stair climbing [59–62]. The physical

activity components were then multiplied by the corresponding metabolic equivalent of task (MET) values and summed across all activities [59–63].

Blood samples were drawn from participants while in a seated position [60, 61].

Individuals were instructed to indicate the time elapsed since their last meal to determine whether the blood draw occurred in a fasting or postprandial state [59].

Detailed information on categorization of variables and certain use in the specific research project can be found in the included publications (**page 27 ff. and page 40 ff.**).

1.5.3 Measurements of ferritin, zinc and copper

The measurement of plasma ferritin concentrations was conducted at the Institute for Clinical Chemistry, University Hospital Schleswig-Holstein Campus Kiel, on the same day of sample collection using unfrozen blood samples [60, 61]. By immunoturbidimetry, plasma ferritin was measured in heparin plasma ($\mu\text{g/L}$) [64]. In this method, antibodies were introduced to the sample, forming light-absorbing immune complexes with ferritin. The photometric measurement of absorbed light allowed the derivation of the ferritin concentration in the sample [65].

The concentrations of plasma zinc and copper concentrations ($\mu\text{g/L}$) were determined in frozen plasma samples in 2022 by SGS Analytics Germany GmbH, utilizing the inductively coupled plasma-mass spectrometry ICAP Q instrument (Thermo Fisher Scientific, Waltham, MA, USA) in accordance with DIN EN ISO 17294-2: 2017-01. Prior to measurement, plasma sample aliquots underwent decomposition (nitric acid and hydrogen peroxide in a 4:1 ratio) through microwave digestion [66]. Calibration utilized a multi-element standard and rhodium ($2 \mu\text{g/L}$) was added as an internal standard. The use of a collision/reaction cell for detection helped minimize spectroscopic interferences [66].

1.5.4 Assessment of dietary intake to estimate the intake of micronutrients, macronutrients and food groups

At the first and second follow-up examination, each individuals' habitual dietary intake over the past 12 months was evaluated using a self-administered, semi-quantitative food frequency questionnaire (FFQ), which has been validated for the German population as part of the EPIC-Potsdam study by the German institute for nutritional research (Potsdam-Rehbrücke) [67]. At the second follow-up examination, an updated version from January 2013 was used to account for contemporary changes in the nutritional landscape, including the addition of vegetarian options. From the data gathered through the FFQ, information on food group consumption, nutrient content and total energy intake was computed following the guidelines of the German Food Code and Nutrient Database (version II.3) [67, 68].

To filter out participants who may have inaccurately reported their food intake in the FFQ, cutoffs for implausible energy intake, as defined by Walter Willett were employed [69]. For females, the acceptable range for energy intake was set at 500 – 3500 kcal, while for males, it was defined as 800 – 4000 kcal [69].

Within the publication on ferritin (**page 27 ff.**), food groups were used as proxy of iron intake, since derived data on iron intake did not distinguished between heme and non-heme iron. For the project on zinc and copper (**page 40 ff.**), intake of both micronutrients was estimated, but also intake of fat and saturated fatty acids was derived, since copper and especially zinc are contained in fatty animal-based foods.

1.5.5 Assessment of body composition by magnetic resonance imaging for the investigation of associations of zinc and copper with adipose tissue

At the first follow-up examination, magnetic resonance imaging (MRI) was performed on >60% of participants, using a 1.5-T scanner (Magnetom Avanto; Siemens Medical solution, Erlangen, Germany). The exact measurement procedure has been described in detail elsewhere [70–72]. For the present analyses, data on subcutaneous and visceral

adipose tissue and on liver fat (measured as liver signal intensity) were derived [73], as detailed below.

From MRI images of the trunk, adipose tissue volumes (dm^3) were calculated by number of voxels multiplied by the voxel size [71, 73]. MRI images were analyzed using the software ATLAS (Automatic Tissue Labelling Analysis Software) [71]. The images were analyzed in cross-section and finally merged into a three-dimensional representation (**Figure 2**) [71]. For the assessment of subcutaneous adipose tissue, the voxels situated beneath the skin, encompassing the abdominal region from the upper extent of the liver to the femoral heads, were identified. Visceral adipose tissue was defined as the voxels counted within the abdominal muscular wall from the upper part of the liver to the femoral heads. Liver signal intensity (LSI) served as the metric for determining liver fat, calculated by computing the relative difference in LSI between out-phase and in-phase images, expressed in arbitrary units [70, 73]. Images for both phases were captured during a breath-holding maneuver using T1-weighted gradient echo sequences. LSI was quantified by measuring the average signal intensity within three circular regions of the liver parenchyma.

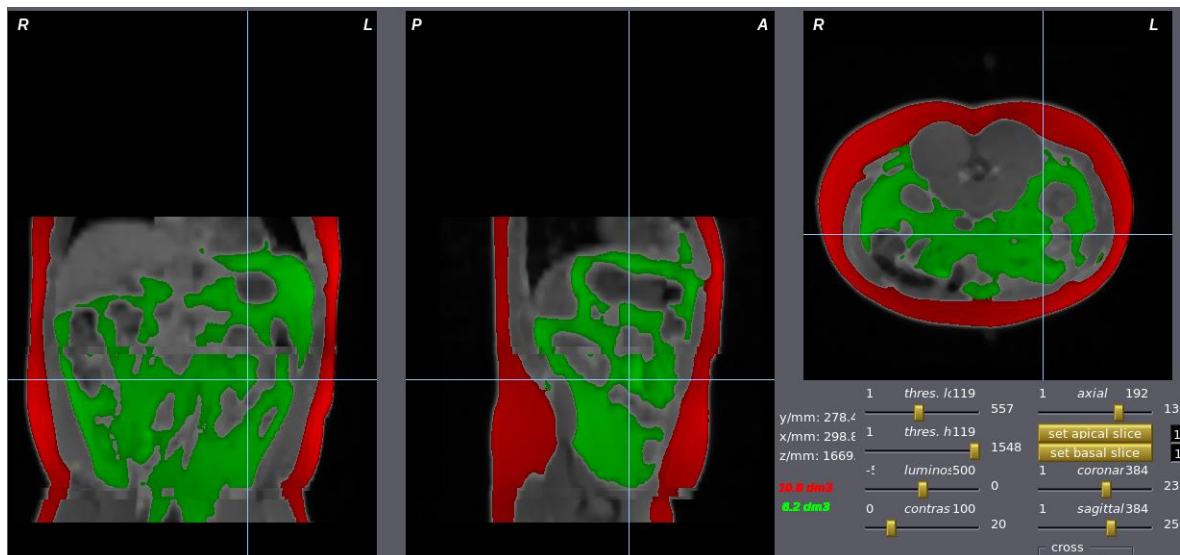


Figure 2: Exemplary *magnetic resonance image of the trunk*. Subcutaneous adipose tissue colored red and visceral adipose tissue colored green using ATLAS software (Automatic Tissue Labelling Analysis Software).

1.5.6 Statistics

The general principles of the statistical methods used in both publications are described below. Detailed descriptions of the models used with regard to exposure variables, outcome variables and confounders are described in detail in the corresponding publications (**page 27 ff. and page 40 ff.**). Within both projects, linear regression models were used to assess associations of exposures and outcomes of interest. Confounders for multivariable-adjusted linear regression models were chosen based on literature. Further more complex statistical methods, *i.e.* reduced rank regression and restricted cubic splines regression, are described below and within the respective papers.

1.5.6.1 Reduced rank regression

In observational studies, it can be challenging to isolate the impact of individual foods on disease development since people typically consume various foods in combination [74]. One approach to assess the collective influence of dietary choices is to create dietary patterns [74, 75]. Examples for hypothesis-based dietary patterns are the *Dietary Approaches to Stop Hypertension* or the *Mediterranean diet* pattern [76, 77]. However, it must be taken into account that these dietary patterns can have disadvantages. For instance, the *Mediterranean diet* was described in the 1960s based on the dietary habits in the southern regions of Greece, Italy, Spain and France [78]. Beneficial health effects associated with this diet are more evident in these regions than in more distant areas [78, 79].

By contrast, reduced rank regression is a statistical method, which offers more flexibility than predefined dietary patterns by computing data-driven dietary patterns. Reduced rank regression can be applied effectively in the field of nutritional epidemiology by selecting disease-related response variables and identifying combinations of food consumption that account for maximum variance of the response variable [74].

In the project on ferritin concentrations (**page 27 ff.**), reduced rank regression was utilized to identify a dietary pattern that explains a maximum variance in circulating ferritin

concentrations, using 42 food groups as predictor variables. These 42 predefined food groups were yielded out of the 112 food items of the FFQ based on their nutrient composition or culinary usage [59].

In this analysis, natural logarithm-transformed plasma ferritin concentrations were employed as the response variable. When establishing the dietary pattern, only those food groups with absolute factor loadings (meaning the strength of the correlation between the extracted factor and the food) equal to or greater than 0.2 were considered as components contributing to the dietary pattern score. This score was calculated following the simplified pattern approach [74].

1.5.6.2 Restricted cubic splines regression

Within the project on ferritin (**page 27 ff.**), restricted cubic splines regressions were used to test for non-linear associations of anthropometric and metabolic traits with circulating ferritin concentrations. The restricted cubic splines regression is a tool that enables the creation of seamless and credible dose-response curves, accommodating a diverse array of potential shapes that describe the relationship between a continuous exposure and a specific outcome [80]. Associations were modelled using restricted cubic splines regressions with four knots at 5%, 35%, 65% and 95%.

1.6 References

1. World Health Organization. WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations. Geneva, Switzerland: World Health Organization; 2020.
2. Zimmermann MB, Hurrell RF. Nutritional iron deficiency. *Lancet*. 2007;370:511–20. doi:10.1016/S0140-6736(07)61235-5.
3. Maret W, Sandstead HH. Zinc requirements and the risks and benefits of zinc supplementation. *Journal of Trace Elements in Medicine and Biology*. 2006;20:3–18. doi:10.1016/j.jtemb.2006.01.006.
4. Andriollo-Sanchez M, Hininger-Favier I, Meunier N, Toti E, Zaccaria M, Brandolini-Bunlon M, et al. Zinc intake and status in middle-aged and older European subjects: the ZENITH study. *Eur J Clin Nutr*. 2005;59 Suppl 2:S37-41. doi:10.1038/sj.ejcn.1602296.
5. Nishito Y, Kambe T. Absorption Mechanisms of Iron, Copper, and Zinc: An Overview. *J Nutr Sci Vitaminol (Tokyo)*. 2018;64:1–7. doi:10.3177/jnsv.64.1.
6. World Obesity Federation. World Obesity Atlas 2023. 2023. <https://data.worldobesity.org/publications/?cat=19>. Accessed 18 Dec 2023.
7. Jomova K, Makova M, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, et al. Essential metals in health and disease. *Chem Biol Interact*. 2022;367:110173. doi:10.1016/j.cbi.2022.110173.
8. Fleming DJ, Jacques PF, Tucker KL, Massaro JM, D'Agostino RB, Wilson PW, Wood RJ. Iron status of the free-living, elderly Framingham Heart Study cohort: an iron-replete population with a high prevalence of elevated iron stores. *The American Journal of Clinical Nutrition*. 2001;73:638–46. doi:10.1093/ajcn/73.3.638.
9. Iglesias-Vázquez L, Arija V, Aranda N, Aglago EK, Cross AJ, Schulze MB, et al. Factors associated with serum ferritin levels and iron excess: results from the EPIC-EurGast study. *Eur J Nutr* 2021. doi:10.1007/s00394-021-02625-w.

10. Liu J-M, Hankinson SE, Stampfer MJ, Rifai N, Willett WC, Ma J. Body iron stores and their determinants in healthy postmenopausal US women. *The American Journal of Clinical Nutrition*. 2003;78:1160–7. doi:10.1093/ajcn/78.6.1160.
11. Andrews NC. Disorders of iron metabolism. *N Engl J Med*. 1999;341:1986–95. doi:10.1056/NEJM199912233412607.
12. Gao H, Yang J, Pan W, Yang M. Iron Overload and the Risk of Diabetes in the General Population: Results of the Chinese Health and Nutrition Survey Cohort Study. *Diabetes Metab J*. 2022;46:307–18. doi:10.4093/dmj.2020.0287.
13. Yeap BB, Divitini ML, Gunton JE, Olynyk JK, Beilby JP, McQuillan B, et al. Higher ferritin levels, but not serum iron or transferrin saturation, are associated with Type 2 diabetes mellitus in adult men and women free of genetic haemochromatosis. *Clin Endocrinol (Oxf)*. 2015;82:525–32. doi:10.1111/cen.12529.
14. Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB. Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. *JAMA*. 2004;291:711–7. doi:10.1001/jama.291.6.711.
15. Klipstein-Grobusch K, Koster JF, Grobbee DE, Lindemans J, Boeing H, Hofman A, Witteman JC. Serum ferritin and risk of myocardial infarction in the elderly: the Rotterdam Study. *The American Journal of Clinical Nutrition*. 1999;69:1231–6. doi:10.1093/ajcn/69.6.1231.
16. van der A DL, Grobbee DE, Roest M, Marx JJM, Voorbij HA, van der Schouw YT. Serum ferritin is a risk factor for stroke in postmenopausal women. *Stroke*. 2005;36:1637–41. doi:10.1161/01.STR.0000173172.82880.72.
17. Kim MK, Baek KH, Song K-H, Kang MI, Choi JH, Bae JC, et al. Increased serum ferritin predicts the development of hypertension among middle-aged men. *Am J Hypertens*. 2012;25:492–7. doi:10.1038/ajh.2011.241.
18. Ellervik C, Marott JL, Tybjærg-Hansen A, Schnohr P, Nordestgaard BG. Total and cause-specific mortality by moderately and markedly increased ferritin concentrations:

- general population study and metaanalysis. Clin Chem. 2014;60:1419–28.
doi:10.1373/clinchem.2014.229013.
19. Deutsche Gesellschaft für Ernährung. Referenzwerte für die Nährstoffzufuhr. 2nd ed. Bonn: Deutsche Gesellschaft für Ernährung; 2016.
 20. Baudry J, Kopp JF, Boeing H, Kipp AP, Schwerdtle T, Schulze MB. Changes of trace element status during aging: results of the EPIC-Potsdam cohort study. Eur J Nutr. 2020;59:3045–58. doi:10.1007/s00394-019-02143-w.
 21. Kadoglou NPE, Biddulph JP, Rafnsson SB, Trivella M, Nihoyannopoulos P, Demakakos P. The association of ferritin with cardiovascular and all-cause mortality in community-dwellers: The English longitudinal study of ageing. PLoS ONE. 2017;12:e0178994. doi:10.1371/journal.pone.0178994.
 22. Zafon C, Lecube A, Simó R. Iron in obesity. An ancient micronutrient for a modern disease. Obes Rev. 2010;11:322–8. doi:10.1111/j.1467-789X.2009.00638.x.
 23. Khan A, Khan WM, Ayub M, Humayun M, Haroon M. Ferritin Is a Marker of Inflammation rather than Iron Deficiency in Overweight and Obese People. J Obes. 2016;2016:1937320. doi:10.1155/2016/1937320.
 24. Kernan KF, Carcillo JA. Hyperferritinemia and inflammation. Int Immunol. 2017;29:401–9. doi:10.1093/intimm/dxx031.
 25. Fahmy M, Young SP. Modulation of iron metabolism in monocyte cell line U937 by inflammatory cytokines: changes in transferrin uptake, iron handling and ferritin mRNA. Biochem J. 1993;296 (Pt 1):175–81. doi:10.1042/bj2960175.
 26. Mahroum N, Alghory A, Kiyak Z, Alwani A, Seida R, Alrais M, Shoenfeld Y. Ferritin - from iron, through inflammation and autoimmunity, to COVID-19. J Autoimmun. 2022;126:102778. doi:10.1016/j.jaut.2021.102778.
 27. Stefanidou M, Maravelias C, Dona A, Spiliopoulou C. Zinc: a multipurpose trace element. Arch Toxicol. 2006;80:1–9. doi:10.1007/s00204-005-0009-5.
 28. Livingstone C. Zinc: physiology, deficiency, and parenteral nutrition. Nutr Clin Pract. 2015;30:371–82. doi:10.1177/0884533615570376.

29. Malavolta M, Piacenza F, Basso A, Giacconi R, Costarelli L, Mocchegiani E. Serum copper to zinc ratio: Relationship with aging and health status. *Mech Ageing Dev.* 2015;151:93–100. doi:10.1016/j.mad.2015.01.004.
30. Chasapis CT, Ntouna P-SA, Spiliopoulou CA, Stefanidou ME. Recent aspects of the effects of zinc on human health. *Arch Toxicol.* 2020;94:1443–60. doi:10.1007/s00204-020-02702-9.
31. Fosmire GJ. Zinc toxicity. *The American Journal of Clinical Nutrition.* 1990;51:225–7. doi:10.1093/ajcn/51.2.225.
32. Cousins RJ. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. *Physiological Reviews.* 1985;65:238–309. doi:10.1152/physrev.1985.65.2.238.
33. Schek A. Ernährungslehre kompakt: Kompendium der Ernährungslehre für Studierende der Ernährungswissenschaft, Medizin und Naturwissenschaften und zur Ausbildung von Ernährungsfachkräften. 5th ed. Sulzbach im Taunus: Umschau Zeitschriftenverlag; 2013.
34. Ghosh C, Yang SH, Kim JG, Jeon T-I, Yoon BH, Lee JY, et al. Zinc-chelated Vitamin C Stimulates Adipogenesis of 3T3-L1 Cells. *Asian-Australas J Anim Sci.* 2013;26:1189–96. doi:10.5713/ajas.2013.13179.
35. Huang X, Jiang D, Zhu Y, Fang Z, Che L, Lin Y, et al. Chronic High Dose Zinc Supplementation Induces Visceral Adipose Tissue Hypertrophy without Altering Body Weight in Mice. *Nutrients* 2017. doi:10.3390/nu9101138.
36. Zhang HB, Wang MS, Wang ZS, Zhou AM, Zhang XM, Dong XW, Peng QH. Supplementation dietary zinc levels on growth performance, carcass traits, and intramuscular fat deposition in weaned piglets. *Biol Trace Elem Res.* 2014;161:69–77. doi:10.1007/s12011-014-0078-5.
37. Chen MD, Lin PY, Cheng V, Lin WH. Zinc supplementation aggravates body fat accumulation in genetically obese mice and dietary-obese mice. *Biol Trace Elem Res.* 1996;52:125–32. doi:10.1007/BF02789454.

38. Kim H-N, Song S-W, Choi W-S. Association between serum zinc level and body composition: The Korean National Health and Nutrition Examination Survey. *Nutrition*. 2016;32:332–7. doi:10.1016/j.nut.2015.09.006.
39. Santoro A, McGraw TE, Kahn BB. Insulin action in adipocytes, adipose remodeling, and systemic effects. *Cell Metab*. 2021;33:748–57. doi:10.1016/j.cmet.2021.03.019.
40. Li X, Guan Y, Shi X, Ding H, Song Y, Li C, et al. Effects of high zinc levels on the lipid synthesis in rat hepatocytes. *Biol Trace Elem Res*. 2013;154:97–102. doi:10.1007/s12011-013-9702-z.
41. Fukasawa H, Niwa H, Ishibuchi K, Kaneko M, Iwakura T, Yasuda H, Furuya R. The Impact of Serum Zinc Levels on Abdominal Fat Mass in Hemodialysis Patients. *Nutrients* 2020. doi:10.3390/nu12030656.
42. Jiang S, Ma X, Li M, Yan S, Zhao H, Pan Y, et al. Association between dietary mineral nutrient intake, body mass index, and waist circumference in U.S. adults using quantile regression analysis NHANES 2007-2014. *PeerJ*. 2020;8:e9127. doi:10.7717/peerj.9127.
43. Wang Y, Jia X-F, Zhang B, Wang Z-H, Zhang J-G, Huang F-F, et al. Dietary Zinc Intake and Its Association with Metabolic Syndrome Indicators among Chinese Adults: An Analysis of the China Nutritional Transition Cohort Survey 2015. *Nutrients*. 2018;10:572. doi:10.3390/nu10050572.
44. Ranasinghe P, Wathurapatha WS, Galappaththy P, Katulanda P, Jayawardena R, Constantine GR. Zinc supplementation in prediabetes: A randomized double-blind placebo-controlled clinical trial. *J Diabetes*. 2018;10:386–97. doi:10.1111/1753-0407.12621.
45. Danks DM. Copper deficiency in humans. *Annu Rev Nutr*. 1988;8:235–57. doi:10.1146/annurev.nu.08.070188.001315.
46. Myint ZW, Oo TH, Thein KZ, Tun AM, Saeed H. Copper deficiency anemia: review article. *Ann Hematol*. 2018;97:1527–34. doi:10.1007/s00277-018-3407-5.

47. Chen C, Zhou Q, Yang R, Wu Z, Yuan H, Zhang N, et al. Copper exposure association with prevalence of non-alcoholic fatty liver disease and insulin resistance among US adults (NHANES 2011-2014). *Ecotoxicol Environ Saf.* 2021;218:112295. doi:10.1016/j.ecoenv.2021.112295.
48. Abiaka C, Olusi S, Al-Awadhi A. Reference ranges of copper and zinc and the prevalence of their deficiencies in an Arab population aged 15-80 years. *Biol Trace Elem Res.* 2003;91:33–43. doi:10.1385/BTER:91:1:33.
49. Olivares M, Uauy R. Copper as an essential nutrient. *The American Journal of Clinical Nutrition.* 1996;63:791S-6S. doi:10.1093/ajcn/63.5.791.
50. Filippini T, Cilloni S, Malavolti M, Violi F, Malagoli C, Tesauro M, et al. Dietary intake of cadmium, chromium, copper, manganese, selenium and zinc in a Northern Italy community. *J Trace Elem Med Biol.* 2018;50:508–17. doi:10.1016/j.jtemb.2018.03.001.
51. Gu K, Li X, Xiang W, Jiang X. The Relationship Between Serum Copper and Overweight/Obesity: a Meta-analysis. *Biol Trace Elem Res.* 2020;194:336–47. doi:10.1007/s12011-019-01803-6.
52. Wu H, Li Q, Zhang K, Zhao J. The association between serum copper and obesity and all-cause mortality: the NHANES 2011-2016. *Environ Sci Pollut Res.* 2023;30:31395–407. doi:10.1007/s11356-022-24432-4.
53. Liang J, Chen F, Fang G, Zhang X, Li Y, Ma B, et al. Relationship Between Plasma Copper Concentration and Body Fat Distribution in Children in China: a Cross-Sectional Study. *Biol Trace Elem Res.* 2020;198:430–9. doi:10.1007/s12011-020-02105-y.
54. Ma J, Xie Y, Zhou Y, Wang D, Cao L, Zhou M, et al. Urinary copper, systemic inflammation, and blood lipid profiles: Wuhan-Zhuhai cohort study. *Environ Pollut.* 2020;267:115647. doi:10.1016/j.envpol.2020.115647.

55. Wang S, Wang N, Da Pan, Zhang H, Sun G. Effects of Copper Supplementation on Blood Lipid Level: a Systematic Review and a Meta-Analysis on Randomized Clinical Trials. *Biol Trace Elem Res.* 2021;199:2851–7. doi:10.1007/s12011-020-02423-1.
56. Li X, Ling J, Hu Q, Fang C, Mei K, Wu Y, et al. Association of serum copper (Cu) with cardiovascular mortality and all-cause mortality in a general population: a prospective cohort study. *BMC Public Health.* 2023;23:2138. doi:10.1186/s12889-023-17018-3.
57. Krawczak M, Nikolaus S, Eberstein H von, Croucher PJP, El Mokhtari NE, Schreiber S. PopGen: population-based recruitment of patients and controls for the analysis of complex genotype-phenotype relationships. *Community Genet.* 2006;9:55–61. doi:10.1159/000090694.
58. Nöthlings U, Krawczak M. PopGen. Eine populationsbasierte Biobank mit Langzeitverfolgung der Kontrollkohorte. [PopGen. A population-based biobank with prospective follow-up of a control group]. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz.* 2012;55:831–5. doi:10.1007/s00103-012-1487-2.
59. Barbaresko J, Siegert S, Koch M, Aits I, Lieb W, Nikolaus S, et al. Comparison of two exploratory dietary patterns in association with the metabolic syndrome in a Northern German population. *Br J Nutr.* 2014;112:1364–72. doi:10.1017/S0007114514002098.
60. Waniek S, Di Giuseppe R, Esatbeyoglu T, Plachta-Danielzik S, Ratjen I, Jacobs G, et al. Vitamin E (α - and γ -Tocopherol) Levels in the Community: Distribution, Clinical and Biochemical Correlates, and Association with Dietary Patterns. *Nutrients* 2017. doi:10.3390/nu10010003.
61. Enderle J, Klink U, Di Giuseppe R, Koch M, Seidel U, Weber K, et al. Plasma Lithium Levels in a General Population: A Cross-Sectional Analysis of Metabolic and Dietary Correlates. *Nutrients* 2020. doi:10.3390/nu12082489.
62. Weber KS, Ratjen I, Enderle J, Seidel U, Rimbach G, Lieb W. Plasma boron concentrations in the general population: a cross-sectional analysis of cardio-

- metabolic and dietary correlates. *Eur J Nutr.* 2022;61:1363–75. doi:10.1007/s00394-021-02730-w.
63. Ainsworth BE, Haskell WL, Herrmann SD, Meckes N, Bassett DR, Tudor-Locke C, et al. 2011 Compendium of Physical Activities: a second update of codes and MET values. *Med Sci Sports Exerc.* 2011;43:1575–81. doi:10.1249/MSS.0b013e31821ece12.
64. Institut für Klinische Chemie Universitätsklinikum Schleswig-Holstein. Leistungsverzeichnis Institut für Klinische Chemie. 04.07.2022. <http://svlabindex02.uksh.de:8080/labindex/>. Accessed 9 Aug 2022.
65. Töpfer G. Immunturbidimetrie. In: Gressner AM, Arndt T, editors. *Lexikon der medizinischen laboratoriumsdiagnostik*. [Place of publication not identified]: Springer; 2018. p. 1234–1235. doi:10.1007/978-3-662-48986-4_1556.
66. Weber KS, Ratjen I, Enderle J, Seidel U, Rimbach G, Lieb W. Plasma boron concentrations in the general population: a cross-sectional analysis of cardio-metabolic and dietary correlates. *Eur J Nutr.* 2022;61:1363–75. doi:10.1007/s00394-021-02730-w.
67. Nöthlings U, Hoffmann K, Bergmann MM, Boeing H. Fitting portion sizes in a self-administered food frequency questionnaire. *The Journal of Nutrition.* 2007;137:2781–6. doi:10.1093/jn/137.12.2781.
68. Dehne LI, Klemm C, Henseler G, Hermann-Kunz E. The German Food Code and Nutrient Data Base (BLS II.2). *Eur J Epidemiol.* 1999;15:355–9. doi:10.1023/a:1007534427681.
69. Willett W. *Nutritional epidemiology*. 3rd ed. New York: Oxford University Press; 2013.
70. Koch M, Borggrefe J, Barbaresko J, Groth G, Jacobs G, Siegert S, et al. Dietary patterns associated with magnetic resonance imaging-determined liver fat content in a general population study. *The American Journal of Clinical Nutrition.* 2014;99:369–77. doi:10.3945/ajcn.113.070219.

71. Fischer K, Moewes D, Koch M, Müller H-P, Jacobs G, Kassubek J, et al. MRI-determined total volumes of visceral and subcutaneous abdominal and trunk adipose tissue are differentially and sex-dependently associated with patterns of estimated usual nutrient intake in a northern German population. *The American Journal of Clinical Nutrition*. 2015;101:794–807. doi:10.3945/ajcn.114.101626.
72. Müller H-P, Raudies F, Unrath A, Neumann H, Ludolph AC, Kassubek J. Quantification of human body fat tissue percentage by MRI. *NMR Biomed*. 2011;24:17–24. doi:10.1002/nbm.1549.
73. Waniek S, Di Giuseppe R, Plachta-Danielzik S, Ratjen I, Jacobs G, Koch M, et al. Association of Vitamin E Levels with Metabolic Syndrome, and MRI-Derived Body Fat Volumes and Liver Fat Content. *Nutrients*. 2017;9:1143. doi:10.3390/nu9101143.
74. Hoffmann K, Schulze MB, Schienkiewitz A, Nöthlings U, Boeing H. Application of a new statistical method to derive dietary patterns in nutritional epidemiology. *Am J Epidemiol*. 2004;159:935–44. doi:10.1093/aje/kwh134.
75. Schulze MB, Hoffmann K, Kroke A, Boeing H. An approach to construct simplified measures of dietary patterns from exploratory factor analysis. *Br J Nutr*. 2003;89:409–19. doi:10.1079/BJN2002778.
76. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. *N Engl J Med*. 2003;348:2599–608. doi:10.1056/NEJMoa025039.
77. Fung TT, Chiuve SE, McCullough ML, Rexrode KM, Logroscino G, Hu FB. Adherence to a DASH-style diet and risk of coronary heart disease and stroke in women. *Arch Intern Med*. 2008;168:713–20. doi:10.1001/archinte.168.7.713.
78. Mattavelli E, Olmastroni E, Bonofiglio D, Catapano AL, Baragetti A, Magni P. Adherence to the Mediterranean Diet: Impact of Geographical Location of the Observations. *Nutrients* 2022. doi:10.3390/nu14102040.
79. Kouris-Blazos A, Gnardellis C, Wahlqvist ML, Trichopoulos D, Lukito W, Trichopoulou A. Are the advantages of the Mediterranean diet transferable to other populations? A

cohort study in Melbourne, Australia. *Br J Nutr.* 1999;82:57–61.

doi:10.1017/s0007114599001129.

80. Desquilbet L, Mariotti F. Dose-response analyses using restricted cubic spline

functions in public health research. *Stat Med.* 2010;29:1037–57.

doi:10.1002/sim.3841.

2 Plasma ferritin concentrations in the general population: A cross-sectional analysis of anthropometric, metabolic and dietary correlates

Cara Övermöhle¹, Sabina Waniek¹, Gerald Rimbach², Katharina S. Weber^{1*}, Wolfgang Lieb^{1*}

¹ Institute of Epidemiology, Kiel University, 24105 Kiel, Germany

² Institute of Human Nutrition and Food Science, Kiel University, 24118 Kiel, Germany

* These authors contributed equally to this work.

Published in: The Journal of Nutrition 153 (5), S. 1524–1533. DOI:
10.1016/j.tjnut.2023.03.008.



Nutritional Epidemiology

Plasma Ferritin Concentrations in the General Population: A Cross-Sectional Analysis of Anthropometric, Metabolic, and Dietary Correlates

Cara Övermöhle¹, Sabina Waniek¹, Gerald Rimbach², Katharina Susanne Weber^{1,*}, Wolfgang Lieb^{1,†}

¹ Institute of Epidemiology, Kiel University, Kiel, Germany; ² Institute of Human Nutrition and Food Science, Kiel University, Kiel, Germany

ABSTRACT

Background: Elevated concentrations of ferritin seem to be detrimental to human health while being quite common in the elderly. Data on dietary, anthropometric, and metabolic correlates of circulating ferritin levels in the elderly are scant.

Objectives: We aimed to identify a dietary pattern, anthropometric, and metabolic traits associated with plasma ferritin status in an elderly cohort ($n = 460$, 57% male, age: 66 ± 12 y) from Northern Germany.

Methods: Plasma ferritin levels were measured by immunoturbidimetry. Reduced rank regression (RRR) yielded a dietary pattern explaining 13% of the variation in circulating ferritin concentrations. Cross-sectional associations of anthropometric and metabolic traits with plasma ferritin concentrations were assessed using multivariable-adjusted linear regression analysis. Restricted cubic spline regression was used to identify nonlinear associations.

Results: The RRR pattern was characterized by a high intake of potatoes, certain vegetables, beef, pork, processed meat, fats (frying and animal fat), and beer and a low intake of snacks, representing elements of the traditional German diet. BMI, waist circumference, and CRP were directly, HDL cholesterol inversely, and age nonlinearly associated with plasma ferritin concentrations (all $P < 0.05$). After additional adjustment for CRP, only the association of ferritin with age remained statistically significant.

Conclusion: Higher plasma ferritin concentrations were associated with a traditional German dietary pattern. The associations of ferritin with unfavorable anthropometric traits and low HDL cholesterol were rendered statistically nonsignificant upon additional adjustment for chronic systemic inflammation (measured as elevated biomarker of the measurement of inflammation (CRP)), suggesting that these associations were largely driven by the proinflammatory role of ferritin (an acute-phase reactant).

Keywords: elderly population sample, ferritin overload, PopGen, reduced rank regression, trace mineral

Introduction

In the human body, iron functions as an important component of cytochromes, oxygen-binding molecules, and other enzymes [1]. Therefore, a sufficient iron status is necessary for oxygen transport, DNA synthesis, and muscle metabolism [2,3]. On the other hand, iron can promote the formation of free-radical ions, which then damage tissues, proteins, or even DNA [1]. Therefore, both iron deficiency and iron overload can be harmful [1, 2]. Because of its high reactivity, iron molecules are bound to

proteins in the human body. In serum, iron is bound to transferrin [1,2] and within cells, iron ions are bound to ferritin. Hence, the intracellular molecule ferritin serves as a marker of the total amount of iron stored in the human body [1,2].

Iron deficiency, defined as low levels of ferritin ($<15 \mu\text{g/L}$), is the most common nutritional deficiency in the world and might lead to anemia [2]. A total of 29% of nonpregnant women and 43% of children worldwide are considered anemic [2]. Approximately 2 billion people are affected by nutritional iron deficiency worldwide [4].

Abbreviations: RRR, reduced rank regression.

* Corresponding author. E-mail address: katharina.weber@epi.uni-kiel.de (K.S. Weber).

† KSW and WL contributed equally to this work.

<https://doi.org/10.1016/j.tjnut.2023.03.008>

Received 8 December 2022; Received in revised form 28 February 2023; Accepted 7 March 2023; Available online 10 March 2023
0022-3166/© 2023 American Society for Nutrition. Published by Elsevier Inc. All rights reserved.

On a parallel note, also increased storage of iron and elevated ferritin concentrations can be harmful and have been associated with the development of major chronic diseases, such as diabetes [5–7] and CVD [8–10]. Inflammatory processes [11] and hereditary predispositions for increased iron storage (hemochromatosis) [2] can contribute to elevated ferritin concentrations. Based on cut points from the WHO (circulating ferritin >200 µg/L in males and >150 µg/L in females), prevalence of 35% in males and 20% in females, respectively, have been reported, e.g., in the European Prospective Investigation into Cancer and Nutrition cohort, which collects data in multiple European countries. Interestingly, the prevalence of high-ferritin levels were higher in northern European than in southern European countries [12]. Using slightly higher cut points (i.e., >300 µg/L in males >200 µg/L in females), a prevalence of 12.9% has been reported in an elderly sample aged 67–96 y from the Framingham Original cohort [13]. Thus, abnormal (too low and too high) values of ferritin are common in the population and are associated with relevant morbidity. Therefore, understanding factors explaining interindividual variation in ferritin levels is of public health importance.

Besides known sex differences, age, body weight, and meat intake are established correlates of circulating ferritin concentrations [12].

There are two different forms of dietary iron, called heme and nonheme iron. While heme iron comes from animal-based foods, plant-based foods contain nonheme iron [14]. The main sources of heme iron are meat and meat products, whereas the main nonheme iron sources are vegetables and bread. Iron absorption is impaired by dairy products, black tea, coffee, and inhibiting components in plant foods (e.g., tannins, oxalic acids, phytates, and phosphates) and enhanced by ascorbic acid. Therefore, especially nonheme iron has a low bioavailability of approximately 5%, whereas the bioavailability of heme iron may be >20% [14].

Taking together, evidence exists that both low and elevated circulating ferritin concentrations 1) might have adverse effects on health, 2) appear to be more common in the population with relevant regional variation and 3) particularly affect the elderly people.

Therefore, in the present analysis, we aim to identify 1) an explorative dietary pattern explaining maximum variance in plasma ferritin concentrations and 2) anthropometric, inflammatory, and metabolic traits associated with elevated plasma ferritin concentrations within an elderly cohort from Northern Germany.

Methods

Study sample

We analyzed data from the second follow-up examination of the “PopGen controls,” a prospective population-based cohort recruited by the PopGen Biobank in the Kiel area in Northern Germany (state of Schleswig-Holstein). Between 2005 and 2007, a total of 1317 participants were recruited in the city of Kiel and in neighboring communities, using population registries ($n = 747$) as well as blood donor registries from the University Hospital in Kiel ($n = 570$). At the recruitment, participants were aged 19–77 y, 55% being male [15,16]. Between 2016 and 2018, the second follow-up examination was performed, which included a

standardized interview, questionnaires, and a physical examination by trained personnel. We used standardized questionnaires on medical history, demographics, and lifestyle factors, such as diet, physical activity, smoking habits, and education [16–18].

Our cross-sectional analysis was based on data from the second follow-up examination (2016–2018). In total, 666 participants attended the second follow-up examination. Of these, 460 subjects (57% male) were eligible for the cross-sectional analysis.

This study was conducted in accordance with the Declaration of Helsinki. The Ethics Committee of the Medical Faculty of the Christian-Albrechts University Kiel has approved the study. Written informed consent was obtained from all the study participants.

Clinical evaluation and laboratory analyses

All measurements were performed by specifically trained personnel according to standard operating procedures already used during the first follow-up examination.

Anthropometric measurements, including assessment of body weight, height, waist and hip circumference, were performed with participants wearing light clothing and no shoes. BMI was calculated as body weight (kg) divided by height (m^2), and WHR was determined as the quotient of waist and hip circumference [17–19].

Information on age, sex, education, smoking habits, and medical history was captured by self-administered questionnaires [17,20]. In addition, participants reported their habitual physical activity during the last 12 mo as time (hours) spent walking, cycling, sporting, gardening, doing housework, home repair, and stair climbing [17–20]. The physical activity components were multiplied by the corresponding metabolic equivalent of task value and summed up for all activities [17–21].

Blood samples were taken from participants in a sitting position [18,19]. Subjects were asked to report the time since their last meal to determine whether the blood was drawn in a fasting or fed state [17]. Measured as elevated biomarker of the measurement of inflammation (CRP), HbA1c, plasma glucose, TGs, total HDL and LDL cholesterol, and plasma ferritin were measured at the Institute for Clinical Chemistry, University Hospital Schleswig-Holstein Campus Kiel, the same day in unfrozen blood samples [18,19]. Plasma concentration of ferritin was measured in heparin plasma (µg/L) using immunoturbidimetry [22]. Here, antibodies were added to the sample, which together with the ferritin formed light-absorbing immune complexes. The amount of absorbed light was measured photometrically, and the ferritin concentration of the sample was derived [23].

Assessment of dietary intake

Dietary intake during the past 12 mo was assessed using a self-administered semiquantitative FFQ covering 112 food and beverage items. The FFQ has been validated for the German population within the European Prospective Investigation into Cancer and Nutrition-Potsdam study [24] and has been updated recently (version from January 2013, Deutsches Institut für Ernährungsforschung Potsdam-Rehbrücke), to cover current changes in the nutritional environment (i.e., vegetarian dishes). The assessed 112 food items were fitted into 42 predefined food

groups according to the similarity of nutrients or culinary usage [17]. Based on the FFQ data, food group intake, nutrient content, and energy intake were calculated according to the German Food Code and Nutrient Database (version II.3) [24,25].

To exclude participants who misreported data of food intake in their FFQ, cutoffs for implausible energy intake defined by Walter Willett [26] were used. For females, the range of acceptable energy intake is 500–3500 kcal, whereas the range for males is defined as 800–4000 kcal [26].

Dietary pattern derivation by reduced rank regression

In addition, reduced rank regression (RRR) was used to derive a dietary pattern, which explains maximal variation in plasma ferritin concentrations. In nutritional epidemiology, RRR is applied as a statistical method investigating potential mechanisms in diet-disease associations by integrating an intermediate response variable [19,20,27,28]. RRR is extracting linear functions of predictors (i.e., food items), which explain the maximal variation of predefined response variables (i.e., disease-related intermediate variables). The RRR analysis was performed with the SAS procedure PLS [27]. In the present project, RRR was used to obtain a dietary pattern of the 42 above-mentioned food groups as predictor variables, whereas ln-transformed plasma ferritin concentrations were taken as a response variable. For the dietary pattern, only food groups with absolute factor loadings (i.e., the correlation between the extracted factor and the food) ≥ 0.2 were considered as contributing to the dietary pattern score, which was calculated according to the simplified pattern approach [28].

Statistical analyses

Analyses were conducted with SAS Enterprise Guide 7.1 (SAS Institute) and *P* values of <0.05 were considered statistically significant.

Normally distributed continuous variables were reported as mean SD, continuous skewed variables as median and IQR, and categorical variables as absolute numbers and percentages. Characteristics of the participants were compared across sex-specific tertiles of plasma ferritin concentrations using chi-square test for categorical variables, general linear models for continuous variables, and Kruskal–Wallis test for continuous skewed variables.

Seasonal variation in levels of circulating ferritin was assessed using Kruskal–Wallis test. Seasons were generated based on the month of examination (winter, January–March; spring, April–June; summer, July–September; and fall, October–December).

To illustrate food intake, the 42 predefined food groups were presented as least square means and 95% CI (adjusted for age and sex), according to tertiles of plasma ferritin concentrations.

Regression models, analysis of covariance, and restricted cubic spline regression with anthropometric, metabolic, and inflammatory traits

Multivariable-adjusted regression analyses were used to evaluate the association of anthropometric and metabolic traits (as exposure variable; each trait separately) with ln-transformed ferritin (continuous dependent variable). To test for differences in anthropometric and metabolic traits according to tertiles of plasma ferritin concentrations, ANCOVA was performed. Anthropometric and metabolic traits included as independent

variables were age, BMI, waist circumference, WHR, CRP, plasma glucose, HbA1c, TGs, HDL, and LDL cholesterol.

Before analysis, all variables were standardized (mean = 0, SD = 1) so that all effects estimates are given per 1-SD increment in the exposure variable. In addition, CRP, plasma glucose, and TGs were ln-transformed and retransformed for data presentation. Model 1 was adjusted for age (except for age as an independent variable), sex, and menopausal status of the female participants. Model 2 was additionally adjusted for BMI (not for regression models with BMI, waist circumference, and WHR as an independent variable), smoking status, (never, former, and current), educational level (≤ 9 , 10, and ≥ 11 y), season (winter, spring, summer, and fall), lipid-lowering medication (for TGs, HDL, and LDL cholesterol as independent variables), and fasting status (for plasma glucose, TGs, HDL, and LDL cholesterol as independent variables). Model 3 was additionally adjusted for physical activity (metabolic equivalent of task-h/wk) and alcohol intake (g/d). Model 4 was additionally adjusted for CRP, a marker of systemic inflammation.

To assess nonlinear associations of anthropometric and metabolic traits with circulating ferritin concentrations, restricted cubic spline were used, adjusted for potential confounders of models 3 and 4 as described above.

Results

Participant characteristics

In total, 460 participants were eligible for analyses (Figure 1). There were no differences with respect to the main characteristics between the 460 participants included in the analysis compared with those who were excluded because of missing data (Supplemental Table 1).

The mean age of the total sample was 66 y, with 43% being female and with 82% of these being postmenopausal. In the overall sample, the mean BMI was within the overweight range (Table 1).

According to WHO guidelines for ferritin concentrations [2], 4.4% of the study population were ferritin deficient (<15 $\mu\text{g/L}$), whereas 20.4% were at risk for ferritin overload (>150 $\mu\text{g/L}$ in females and >200 $\mu\text{g/L}$ in males). A total of 94.4% and 83.5% of the population had sufficient plasma transferrin concentrations and transferrin saturation, respectively, whereas transferrin was high in 1.9% and transferrin saturation was low in 13%, both possibly indicating iron deficiency [22].

Participants in the highest (as compared with the lowest) sex-specific plasma ferritin tertile were significantly older, had higher values of BMI, waist circumference (in females only), WHR (in males only), CRP, HbA1c, and plasma glucose concentrations as well as higher plasma TG concentrations and were more likely to be current smokers and postmenopausal (females) (Table 1). Furthermore, circulating ferritin concentrations varied according to the season with the highest levels in autumn and the lowest levels during spring (Supplemental Table 2).

Food group correlates of circulating ferritin

When analyzing food groups individually, participants in the highest compared with the lowest tertile of plasma ferritin concentrations consumed less fruits, less cereals (others than bread, pasta, and rice), and less snacks (all adjusted for age and sex). In addition, they had higher age- and sex-adjusted intake of pork

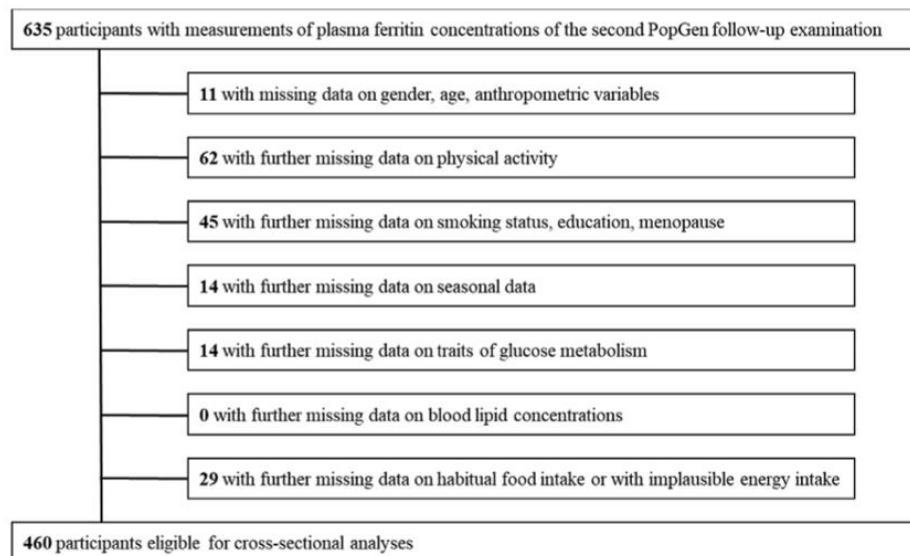


FIGURE 1. Flowchart showing the participants being eligible for analysis from those with measurements of plasma ferritin concentrations.

meat, poultry, other meats, fats (others than butter, margarine, and vegetable oils), and beer. Participants in the highest compared with the middle tertile of plasma ferritin concentrations showed a higher intake of processed meat (Supplemental Table 3).

Furthermore, a dietary pattern explaining 13% of the variation in circulating ferritin concentrations was generated by RRR analysis. The pattern was characterized by a high intake of potatoes, vegetables (others than leafy, fruiting or root vegetables, and cabbages), beef, pork, processed meat, fats (others than butter, margarine, and vegetable oils), and beer and low intake of snacks (Table 2). Especially, a high intake of meat and potatoes reflects traditional dietary habits in Germany.

Association of plasma ferritin concentrations with anthropometric traits

Age showed a nonlinear association with plasma ferritin concentrations, with circulating ferritin concentration reaching its maximum at the age of 75 y, followed by a decrease (Figure 2). Furthermore, BMI ($P < 0.05$) and waist circumference ($P < 0.01$) showed a direct linear association with circulating ferritin concentrations. Individuals in the highest compared with the middle tertile of plasma ferritin concentrations had larger WHR ($P < 0.01$), whereas regression analysis revealed no significant associations (model 3) (Table 3). Linear associations of circulating ferritin with BMI and waist circumference were attenuated and no longer statistically significant after additional adjustment for CRP (model 4) (Table 3), whereas the nonlinear association between ferritin and age remained significant (Figure 2).

Association of plasma ferritin concentrations with inflammatory and metabolic traits

CRP concentrations and HDL-cholesterol concentrations showed a direct and linear association with plasma ferritin concentrations ($P < 0.01$ and $P < 0.05$), whereas further

metabolic traits neither showed nonlinear/linear associations with circulating ferritin concentrations nor group differences for tertiles of ferritin concentrations (model 3) (Table 4). Additional adjustment for CRP attenuated the association of circulating ferritin with HDL cholesterol and rendered it statistically nonsignificant (model 4) (Table 4).

Discussion

In a moderate-sized elderly community-based sample from Northern Germany, we assessed the distributions of plasma ferritin concentrations as well as its nutritional, anthropometric, inflammatory, and metabolic correlates. Our principal observations were as follows: first, we observed that 20.4% of the study population was at risk of ferritin overload. Second, we identified a dietary pattern explaining 13% of the interindividual variation in circulating ferritin. This RRR pattern was characterized by high intakes of food groups that matches the traditional German cuisine (i.e., potatoes, meat, and beer). Third, plasma ferritin concentrations were directly and linearly associated with BMI, waist circumference, and CRP, whereas age showed a nonlinear relation with circulating ferritin, with an increase in plasma ferritin concentrations until approximately 75 y followed by a decrease. Of these, only the nonlinear association with age remained significant after additional adjustment for CRP. Fourth, no associations were observed for circulating ferritin concentrations with lipid traits and traits of glucose metabolism, except for an inverse linear association with HDL cholesterol. However, additional adjustment for CRP also attenuated this association, rendering it statistically nonsignificant.

In the context of the published literature

Food group correlates of plasma ferritin concentrations

Food groups characterizing the RRR pattern within the present cohort were largely consistent with prior findings. In

TABLE 1
General characteristics of the overall study sample and according to sex-specific tertiles of circulating plasma ferritin concentrations ($n = 460$)

	Total sample	T1 Females, 2.5–53.0 µg/L Males, 10.0–68.0 µg/L	T2 Females, 53.1–116.0 µg/L Males, 68.1–163.0 µg/L	T3 Females, 116.1–590.0 µg/L Males, 163.1–739.0 µg/L	P^7
n^1 (% male)	460 (57)	150 (58)	158 (57)	152 (56)	0.936
Plasma concentration of ferritin ² (µg/L)	96.0 (49.5–167.5)	32.0 (20.0–49.0)	96.0 (74.0–123.0)	191.0 (169.0–271.0)	<0.001
Plasma concentration of transferrin ² (g/L)	2.5 (2.3; 2.9)	2.9 (2.6–3.2)	2.5 (2.3–2.7)	2.4 (2.2–2.6)	<0.001
Plasma transferrin saturation ² (%)	25.9 (20.0–32.5)	20.8 (15.1–27.8)	26.0 (21.0–32.4)	29.0 (23.3–35.6)	<0.001
Plasma hemoglobin ^{2,4} (g/dL)	14.2 (13.4–15.1)	14.0 (13.2–14.8)	14.3 (13.5–15.1)	14.7 (13.5–15.4)	0.001
Age ³ (y)	66 ± 12	61 ± 13	68 ± 11	69 ± 9	<0.001
BMI ³ (kg/m ²)	27.5 ± 4.4	27 ± 4.2	27.2 ± 4.3	28.2 ± 4.6	0.035
Waist circumference ³ (cm)	88.1 ± 11.9	85.3 ± 11.9	86.5 ± 11.3	92.4 ± 11.5	0.001
WHR ³	0.86 ± 0.07	0.85 ± 0.08	0.85 ± 0.07	0.88 ± 0.06	0.173
	0.98 ± 0.08	0.97 ± 0.08	0.98 ± 0.07	1.00 ± 0.07	0.052
CRP ³ (mg/L)	1.12 (0.53–2.44)	0.95 (0.42–2.04)	1.05 (0.49–2.25)	1.52 (0.73–2.82)	0.021
HbA1c ³ (%)	5.71 ± 0.55	5.63 ± 0.54	5.70 ± 0.53	5.80 ± 0.57	0.002
Plasma glucose concentration ^{2,5} (mg/dL)	94.3 (85.1–104.7)	91.3 (82.7–102.9)	94.1 (84.5–106.0)	97.2 (88.5–105.1)	0.032
Plasma TG concentration ^{2,4} (mg/dL)	122.5 (87.5–175.0)	105.0 (78.8–166.3)	122.5 (87.5–175.0)	131.3 (96.3–175.0)	0.023
Plasma HDL-cholesterol concentration ³ (mg/dL)	62.4 ± 18.3	63.7 ± 17.7	62.7 ± 18.3	60.6 ± 19.0	0.020
Plasma LDL-cholesterol concentration ³ (mg/dL)	131.5 ± 37.1	129.5 ± 38.8	128.4 ± 36.1	136.5 ± 36.1	0.319
Lipid-lowering medication ¹ (yes/no)	97 (21)/372 (79)	28 (19)/122 (81)	37 (23)/121 (77)	32 (21)/120 (79)	0.115
Current smokers ¹	38 (8)	10 (7)	11 (7)	17 (11)	0.593
Alcohol intake ³ (g/d)	8.3 (2.3–19.7)	8.7 (2.3–18.3)	7.0 (2.2–17.8)	9.6 (2.8–26.8)	0.037
Physical activity ² (MET-h/wk)	82.3 (55.6–120.0)	76.5 (54.8–112.8)	84.6 (63.1–120.0)	84.3 (53.8–122.7)	0.198
Education level (low [<10 y], medium [10 y], high [≥ 11 y]) ¹	142 (31)/147 (32)/171 (37)	38 (25)/45 (30)/67 (45)	52 (33)/49 (31)/57 (36)	52 (34)/53 (35)/47 (31)	0.245
Menopause ^{1,6} (yes/no)	163/35	39/24	63/5	61/6	0.147
MET, metabolic equivalent of task; T, tertile.					<0.001

¹ n (%)
² Median (IQR)
³ Mean ± SD
⁴ Data only available for $n = 459$
⁵ Data only available for $n = 454$
⁶ Data only available for $n = 198$ females
⁷ P values for the comparison of T1, T2 and T3 based on chi-square test (categorical variables), Kruskal–Wallis test (continuous skewed variables), or general linear models (continuous normally distributed variables).

TABLE 2
Factor loadings in 42 food groups and explained variation in ln-transformed ferritin plasma concentration by using reduced rank regression ($n = 460$)

	Reduced rank regression factor loading
	Overall
Explained variance in food intake (%)	6.38
Explained variance in response variable (plasma ferritin concentration) (%)	12.64
Food group (g/d)	
Potatoes	0.350
Leafy vegetables	NR
Fruiting vegetables	NR
Root vegetables	NR
Cabbage	NR
Other vegetables	0.205
Legumes	NR
Fruits	NR
Nuts and seeds	NR
Milk	NR
Dairy products	NR
Cheese	NR
Bread	NR
Pasta and rice	NR
Other cereals	NR
Beef	0.254
Pork	0.308
Poultry	NR
Processed meat	0.346
Other meat	NR
Fish and fish products	NR
Eggs	NR
Butter	NR
Margarine	NR
Vegetable oils	NR
Other fats	0.288
Sugar products (e.g., syrups, candy, ice cream, desserts)	NR
Chocolate, sweets	NR
Cakes and cookies	NR
Nonalcoholic beverages	NR
Soft drinks	NR
Coffee	NR
Tea	NR
Beer	0.201
Wine	NR
Other alcoholic beverages	NR
Sauces	NR
Soups	NR
Bouillon	NR
Vegetarian dishes and soy products	NR
Snacks	−0.246
Condiments	NR

Food groups with absolute factor loadings ≥ 0.20 were considered as contributing to the dietary pattern. A total of 42 food groups were used as predictor variables and ln-transformed plasma ferritin concentrations as a response variable for reduced rank regression. NR: not relevant, food group with absolute factor loading < 0.20

particular, red meat and alcohol are known to correlate with higher ferritin concentrations [12,29,30]. A high intake of meat accompanied by potatoes and vegetables and a low intake of snacks may reflect traditional German dietary habits. Although the high intake of certain vegetables and the low intake of snacks contributing to the RRR pattern can be rated as healthful, the high intake of (processed) meat, fats, and alcohol needs to be considered as unhealthy dietary components.

Age and plasma ferritin concentrations

We identified a nonlinear association between age and plasma ferritin concentration characterized by increasing plasma ferritin concentrations until the age of approximately 75 y followed by a decrease in plasma ferritin concentrations. Within the third National Health and Nutrition Examination Survey conducted in the United States, a similar relationship between age and ferritin concentration was observed [31]. Other publications are rather contradictory. Some studies describe increasing ferritin concentrations being associated with older age [12,13,30,32], whereas other publications describe a decrease in ferritin concentrations with increasing age [33,34]. The observed nonlinear relationship between age and plasma ferritin concentrations in our cohort might be explained as follows: until a certain age, factors increasing plasma ferritin concentrations may be predominant. In females, the middle decade of life goes along with menopause. Consequently, plasma ferritin concentrations increase after menopause because of cessation of blood loss during menstruation [30,31]. In both sexes, an iron dyshomeostasis, which is caused by the lack of ferroportin expression, the cellular iron exporter in mammals, may lead to the accumulation of iron and subsequently plasma ferritin [35]. From the age of approximately 75 y on, plasma ferritin concentrations might decrease because of increasing frequency of multimorbidity with higher age. Poor diet quality, loss of appetite, and lower physical activity have been additionally shown to affect iron metabolism in the elderly [33]. Also, acute inflammation, occult bleeding, and the regular intake of medication, such as aspirin, can cause a decrease in plasma ferritin concentrations [33,34]. Because high-ferritin concentrations are also directly associated with mortality, the higher survival of subjects with lower plasma ferritin concentrations might in part explain the decrease in ferritin concentrations from the age of 75 y on [36].

Anthropometric and inflammatory correlates of plasma ferritin concentrations

Our findings of a direct association between plasma ferritin concentrations and BMI and waist circumference reinforce the results of existing publications [37–40]. In addition to BMI, plasma ferritin concentrations are directly associated with CRP in the present analysis. The direct association between ferritin concentrations and CRP is also well established [12,13,30]. Because ferritin also functions as an acute-phase protein, coexisting elevated values of ferritin and CRP could indicate an inflammatory state in affected subjects rather than the presence of overfilled body iron stores [11,41]. With the classification of obesity as a chronic inflammatory state [42,43], elevated ferritin concentrations are linked to the inflammatory state in overweight, thus focusing on ferritin as an acute-phase protein [11,39]. Therefore, the elevated ferritin levels in our cohort might also be evaluated as an inflammatory event rather than an actual increase in iron stores. Theoretically, an indication of the primary function of the circulating ferritin molecule could be provided by analyses of its subunits. Ferritin consists of 24 subunits, which are made up of H- and L- subunits. The L subunits seem to be primarily responsible for the storage of iron [44].

The relevant attenuation of the initially statistically significant associations between anthropometric variables and ferritin

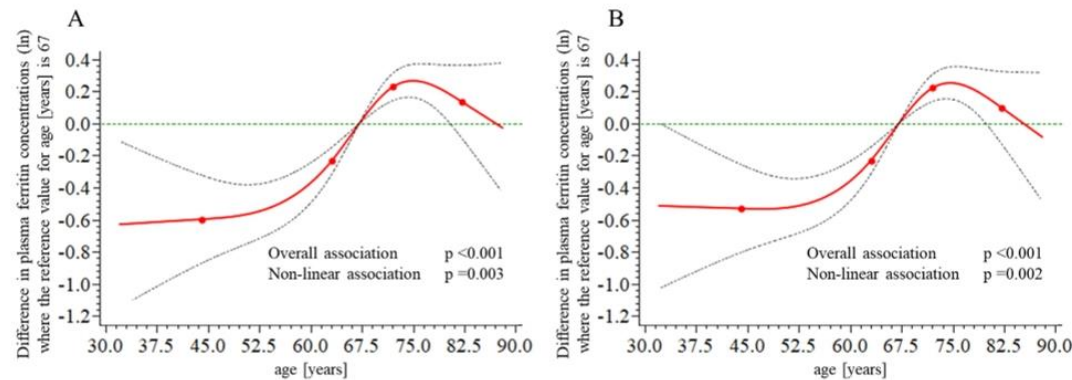


FIGURE 2. Nonlinear associations of plasma ferritin concentrations with age using restricted cubic splines adjusted for possible confounders (A) and additionally adjusted for CRP as confounder (B) ($n = 460$). — Estimate, Lower confidence limit, Upper confidence limit, ● Knots. (A) Adjusted for sex, menopause, BMI, smoking status, education level, season, physical activity, and alcohol intake. (B) Additionally adjusted for CRP concentration.

TABLE 3
Associations of anthropometric traits with plasma ferritin concentrations ($n = 460$)

	Least square means and 95% CI of anthropometric traits by tertiles of ferritin plasma concentrations			Percentage change (estimate and 95% CI) in plasma ferritin concentrations per 1-SD increment in trait	
	T1	T2	T3	β (95% CI) ¹	P
Age (y)					
Model 1	58.9 (57.2–60.7) ⁴	63.1 (61.1–65.0)	64.8 (62.8–66.7)	Nonlinear association presented in Figure 2	
Model 2	59.3 (57.3–61.4) ⁴	63.2 (61.1–65.2)	64.7 (62.7–66.7)		
Model 3	59.2 (57.2–61.2) ⁴	63.0 (61.0–65.1)	64.8 (62.8–66.8)		
Model 4	59.3 (57.3–61.3) ⁴	62.9 (60.8–64.9)	64.3 (62.3–66.2)		
BMI (kg/m ²)					
Model 1	26.7 (25.9–27.6) ³	27.2 (26.4–28.0) ²	28.2 (27.4–29.1)	10.80 (2.44–19.85)	0.010
Model 2	26.8 (25.8–27.7) ³	27.2 (26.3–28.2)	28.2 (27.3–29.0)	9.97 (1.59–19.04)	0.019
Model 3	26.7 (25.8–27.6) ³	27.2 (26.3–28.1)	28.2 (27.3–29.0)	10.26 (1.96–19.23)	0.015
Model 4	26.7 (25.8–27.6)	27.0 (26.2–27.9)	27.6 (26.8–28.5)	5.63 (–2.70–14.68)	0.191
Waist circumference (cm)					
Model 1	90.4 (88.2–92.5) ³	91.1 (88.8–93.3) ³	94.8 (92.6–97.0)	14.50 (5.08–24.76)	0.002
Model 2	90.4 (87.9–92.8) ³	91.3 (88.8–93.7) ³	94.7 (92.4–97.1)	13.79 (4.41–24.03)	0.003
Model 3	90.3 (87.8–92.8) ³	91.4 (88.9–93.8) ²	94.8 (92.4–97.1)	14.03 (4.70–24.18)	0.003
Model 4	90.2 (87.9–92.6) ²	90.9 (88.5–93.2)	93.4 (91.1–95.6)	9.22 (–0.13–19.45)	0.054
WHR					
Model 1	0.89 (0.88–0.91) ²	0.89 (0.88–0.90) ³	0.91 (0.90–0.93)	11.69 (0.75–23.82)	0.036
Model 2	0.91 (0.89–0.92)	0.90 (0.89–0.92) ²	0.92 (0.91–0.94)	8.18 (–2.48–20.00)	0.137
Model 3	0.91 (0.89–0.92)	0.90 (0.89–0.92) ²	0.92 (0.91–0.94)	8.21 (–2.32–19.88)	0.131
Model 4	0.91 (0.89–0.92)	0.90 (0.88–0.91) ²	0.92 (0.90–0.93)	5.01 (–5.22–16.34)	0.349

Model 1 adjusted for age (except for age as an independent variable), sex, and menopause. Model 2 additionally adjusted for BMI (just for age as an independent variable), smoking status, education level, and season. Model 3 additionally adjusted for physical activity and alcohol intake. Model 4 additionally adjusted for CRP.

Ferritin concentrations entered into the models as ln-transformed variables.

Data are least square means with 95% CIs of anthropometric traits by tertiles of plasma ferritin concentrations and regression coefficients (β) with 95% CIs.

T, tertile.

¹ Regression coefficients indicate % change in plasma ferritin per 1-SD increment in BMI, waist circumference, and WHR.

² $P < 0.05$ compared with T3 (ANCOVA).

³ $P < 0.01$ compared with T3 (ANCOVA).

⁴ $P < 0.001$ compared with T3 (ANCOVA).

after additional adjustment for CRP is consistent with the concept that these associations were largely driven by the proinflammatory role of ferritin rather than by its role as an indicator of body iron stores.

Metabolic correlates of plasma ferritin concentrations

Previous studies reported direct associations of ferritin concentrations with parameters of glucose metabolism (i.e., blood glucose concentrations and HbA1c) and with risk of type 2

TABLE 4
Associations of inflammatory and metabolic traits with plasma ferritin concentrations ($n = 460$)

	Least square means and 95% CI of metabolic traits by tertiles of ferritin plasma concentrations			Percentage change (estimate and 95% CI) in plasma ferritin concentrations per increment, as defined in the footnote	
	T1	T2	T3	β (95% CI) ³	P^4
CRP (mg/L)					
Model 1	1.12 (0.91–1.38) ⁶	1.25 (1.01–1.55) ⁵	1.65 (1.33–2.05) ⁵	10.21 (4.34–16.41)	<0.001
Model 2	1.24 (0.98–1.56) ⁵	1.33 (1.06–1.67)	1.63 (1.31–2.02)	8.63 (2.53–15.10)	0.005
Model 3	1.21 (0.96–1.53) ⁵	1.33 (1.06–1.67)	1.64 (1.32–2.03)	9.36 (3.28–15.80)	0.002
Model 4	No additional adjustment for CRP as confounder				
HbA1c (%)					
Model 1	5.7 (5.6–5.8)	5.7 (5.6–5.8)	5.8 (5.7–5.9)	5.55 (–2.85–14.67)	0.201
Model 2	5.7 (5.6–5.8)	5.7 (5.6–5.8)	5.8 (5.6–5.9)	3.80 (–4.60–12.94)	0.385
Model 3	5.7 (5.6–5.9)	5.7 (5.6–5.8)	5.8 (5.6–5.9)	3.23 (–5.04–12.21)	0.455
Model 4	5.7 (5.6–5.8)	5.7 (5.6–5.8)	5.7 (5.6–5.9)	2.85 (–5.31–11.72)	0.504
Plasma glucose concentration ¹ (mg/dL)					
Model 1	96.2 (92.5–99.9)	94.9 (91.2–98.8)	97.6 (93.7–101.7)	1.72 (–1.69–5.26)	0.326
Model 2	96.8 (91.7–102.2)	94.4 (89.4–99.6)	95.7 (90.8–100.8)	0.04 (–3.40–3.61)	0.981
Model 3	96.7 (91.5–102.1)	94.0 (89.0–99.3)	95.4 (90.6–100.6)	–0.10 (–3.50–3.43)	0.957
Model 4	96.5 (91.3–101.9)	93.7 (88.7–99.1)	95.0 (90.1–100.2)	–0.33 (–3.71–3.17)	0.851
Plasma TG concentration ² (mg/dL)					
Model 1	114.3 (104.3–125.3)	122.2 (111.1–134.3)	131.5 (119.6–144.5)	3.57 (0.31–6.93)	0.032
Model 2	111.2 (97.7–126.5)	116.6 (102.5–132.6)	119.5 (105.5–135.4)	1.92 (–1.54–5.50)	0.279
Model 3	110.4 (96.9–125.7)	116.8 (102.6–132.9)	120.0 (106.0–135.9)	2.39 (–1.06–5.95)	0.177
Model 4	110.0 (96.6–125.4)	116.3 (102.1–132.4)	119.3 (105.1–135.3)	2.19 (–1.22–5.72)	0.210
Plasma HDL-cholesterol concentration (mg/dL)					
Model 1	66.3 (63.2–69.4)	64.8 (61.6–67.9)	62.6 (59.5–65.8)	–10.23 (–17.71––2.07)	0.015
Model 2	62.7 (58.4–67.0)	62.0 (57.7–66.3)	61.4 (57.2–65.5)	–6.69 (–15.02–2.44)	0.146
Model 3	63.9 (59.7–68.2)	62.9 (58.7–67.2)	61.6 (57.5–65.7)	–9.70 (–17.75––0.86)	0.032
Model 4	64.3 (60.0–68.6)	63.4 (59.1–67.7)	62.3 (58.2–66.4)	–8.37 (–16.53–0.59)	0.066
Plasma LDL-cholesterol concentration (mg/dL)					
Model 1	128.5 (121.6–135.4)	126.5 (119.4–133.6) ⁵	134.8 (127.8–141.9)	4.68 (–3.24–13.25)	0.254
Model 2	125.3 (115.7–134.8)	122.9 (113.4–132.4)	128.9 (119.8–138.1)	2.59 (–5.82–11.76)	0.556
Model 3	126.2 (116.6–135.8)	123.5 (114.0–133.1)	129.0 (119.9–138.2)	1.47 (–6.77–10.43)	0.735
Model 4	125.6 (116.0–135.3)	122.8 (113.2–132.4)	127.8 (118.5–137.0)	1.47 (–6.77–10.43)	0.735

Model 1 adjusted for age, sex, and menopause. Model 2 additionally adjusted for BMI, smoking status, education level, season, and fasting status (for plasma glucose, TG, HDL, and LDL concentrations). Model 3 additionally adjusted for physical activity and alcohol intake. Model 4 additionally adjusted for CRP.

Ferritin, CRP, glucose, and TG concentrations entered into the models as ln-transformed variables.

Data are least square means with 95% CIs of anthropometric and metabolic traits by tertiles of ferritin plasma concentrations and regression coefficients (β) with 95% CIs.

T, tertile.

¹ Data only available for $n = 454$

² Data only available for $n = 459$

³ Regression coefficients indicate % change in plasma ferritin per 1-SD increment in HbA1c, HDL, and LDL. A doubling in CRP and a 50% increase in glucose and TG concentrations were associated with % change in plasma ferritin concentrations by β .

⁴ P values from linear regression analyses with continuous metabolic traits as the independent variable.

⁵ $P < 0.05$ compared with T3 (ANCOVA).

⁶ $P < 0.01$ compared with T3 (ANCOVA).

diabetes [5,7]. In contrast, we could not detect this association in the present analysis, which is in line with the population-based study by Krisai et al. [45] ($n = 2169$). Instead, Krisai et al. [45] reported a direct correlation of circulating ferritin with fasting insulin concentrations and insulin resistance. However, because these data are not available in our cohort, in-depth analyses between ferritin concentrations and glucose metabolism are not possible.

TGs and LDL-cholesterol concentrations were not associated with plasma ferritin concentrations in the present analysis, which is consistent with a previously published study [46]. However, the majority of previous studies on the association of blood lipids and circulating ferritin concentrations reported direct linear associations for ferritin concentrations with TGs and

LDL cholesterol, but inverse associations with HDL cholesterol [47–50]. In the present study, an inverse association of HDL cholesterol and ferritin was observed in multivariable-adjusted models. However, additional adjustment for CRP attenuated the association. In contrast, TG and LDL-cholesterol concentrations were not statistically significantly associated with ferritin in our sample upon multivariable adjustment, although we observed slightly higher TG and LDL-cholesterol levels with higher ferritin levels.

Strengths and limitations

The strengths of our analyses include the moderate-sized community-based elderly sample and the availability of detailed nutritional data and of multiple relevant confounders.

Furthermore, we assessed dietary intake using a validated FFQ and computed an explorative dietary pattern explaining 13% of the variation in plasma ferritin concentrations. Moreover, we applied restricted cubic spline regression to model nonlinear relationships.

However, the following limitations warrant consideration. First, we used BMI as a proxy for defining overweight due to the lack of data from, e.g., BIA. BMI has some shortcomings as a measurement for categorization into overweight and adiposity. In contrast to BIA, BMI cannot distinguish between body fat and muscle mass and might, therefore, lead to some misclassification of overweight and adiposity [51]. However, we believe this misclassification to be nondifferential (not related to ferritin levels), slightly reducing the statistical power of the analyses, including BMI. Second, elevated concentrations of circulating ferritin are common in patients with nonalcoholic fatty liver disease [52]. In our study, liver function tests were not evaluated. Therefore, we cannot rule out that ferritin concentrations could be elevated due to declining liver function in our elderly sample. Third, our sample comprised elderly individuals from Northern Germany. The generalizability of our observations to other samples from Germany, Europe, and the world is unknown. Therefore, similar analyses on the nutritional, metabolic and anthropometric correlates of ferritin, and on the role of subgrade inflammation, in other samples are warranted.

Conclusions

In our elderly population-based sample, a traditional German dietary pattern consisting of potatoes, meats, and vegetables was associated with higher plasma ferritin concentrations. In addition, higher plasma ferritin concentrations were related to higher BMI, waist circumference, and circulating concentrations of CRP, whereas there was a nonlinear relation with age, characterized by an increase in plasma ferritin concentrations until the age of approximately 75 y and a decrease afterward. Most associations of ferritin with anthropometric traits and HDL cholesterol were attenuated upon adjustment for CRP, suggesting that these associations were mediated through inflammation. Further studies are needed to distinguish between the correlates of clinically relevant iron overload and correlates of elevated ferritin concentrations because of inflammation.

Acknowledgments

We appreciate the voluntary contribution of all the study participants. We also thank the staff of the Institute of Epidemiology for excellent technical assistance and taking care of the patients.

Author contributions

The authors' responsibilities were as follows – WL, CÖ, KSW, SW, GR: designed the research; WL, SW, GR: conducted the research; KSW, SW: provided essential materials; CÖ: analyzed the data or performed the statistical analysis; CÖ: wrote the paper; CÖ, KSW, WL: had primary responsibility for the final content; all authors: read the manuscript, made significant

intellectual contributions, and read and approved the final manuscript.

Data Availability

Data described in the article, code book, and analytic code will be made available upon reasonable request through our P2N data application platform (<https://portal.poggen.de/>).

Funding

The authors reported no funding received for this study.

Author disclosures

CÖ, SW, GR, KSW, and WL, no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tjn.2023.03.008>.

References

- [1] N.C. Andrews, Disorders of iron metabolism, *N. Engl. J. Med.* 341 (1999) 1986–1995.
- [2] World Health Organization, WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations, World Health Organization, Geneva, Switzerland, 2020.
- [3] J.L. Beard, Iron biology in immune function, muscle metabolism and neuronal functioning, *J. Nutr.* 131 (2001) 568S–579S, discussion 580S.
- [4] M.B. Zimmermann, R.F. Hurrell, Nutritional iron deficiency, *Lancet* 370 (2007) 511–520.
- [5] H. Gao, J. Yang, W. Pan, M. Yang, Iron overload and the risk of diabetes in the general population: results of the Chinese Health and Nutrition Survey cohort study, *Diabetes Metab. J.* 46 (2022) 307–318.
- [6] B.B. Yeap, M.L. Divitini, J.E. Gunton, J.K. Olynyk, J.P. Beilby, B. McQuillan, et al., Higher ferritin levels, but not serum iron or transferrin saturation, are associated with type 2 diabetes mellitus in adult men and women free of genetic haemochromatosis, *Clin. Endocrinol. (Oxf)* 82 (2015) 525–532.
- [7] R. Jiang, J.E. Manson, J.B. Meigs, J. Ma, N. Rifai, F.B. Hu, Body iron stores in relation to risk of type 2 diabetes in apparently healthy women, *JAMA* 291 (2004) 711–717.
- [8] K. Klipstein-Grobusch, J.F. Koster, D.E. Grobbee, J. Lindemans, H. Boeing, A. Hofman, et al., Serum ferritin and risk of myocardial infarction in the elderly: the Rotterdam Study, *Am. J. Clin. Nutr.* 69 (1999) 1231–1236.
- [9] D.L. van der A, D.E. Grobbee, M. Roest, J.J.M. Marx, H.A. Voorbij, Y.T. van der Schouw, Serum ferritin is a risk factor for stroke in postmenopausal women, *Stroke* 36 (2005) 1637–1641.
- [10] M.K. Kim, K.H. Baek, K.-H. Song, M.I. Kang, J.H. Choi, J.C. Bae, et al., Increased serum ferritin predicts the development of hypertension among middle-aged men, *Am. J. Hypertens* 25 (2012) 492–497.
- [11] C. Zafon, A. Lecube, R. Simó, Iron in obesity. An ancient micronutrient for a modern disease, *Obes. Rev.* 11 (2010) 322–328.
- [12] L. Iglesias-Vázquez, V. Arijia, N. Aranda, E.K. Aglago, A.J. Cross, M.B. Schulze, et al., Factors associated with serum ferritin levels and iron excess: results from the EPIC-EurGast study, *Eur. J. Nutr.* 61 (2022) 101–114.
- [13] D.J. Fleming, P.F. Jacques, K.L. Tucker, J.M. Massaro, R.B. D'Agostino, P.W. Wilson, et al., Iron status of the free-living, elderly Framingham Heart Study cohort: an iron-replete population with a high prevalence of elevated iron stores, *Am. J. Clin. Nutr.* 73 (2001) 638–646.
- [14] Deutsche Gesellschaft für Ernährung, Referenzwerte für die Nährstoffzufuhr. 2. Auflage, 2. aktualisierte Ausgabe. Deutsche Gesellschaft für Ernährung, Bonn, 2016.

- [15] M. Krawczak, S. Nikolaus, H. von Eberstein, P.J.P. Croucher, N.E. El Mokhtari, S. Schreiber, PopGen: population-based recruitment of patients and controls for the analysis of complex genotype-phenotype relationships, *Community Genet* 9 (2006) 55–61.
- [16] U. Nöthlings, M. Krawczak, PopGen. A population-based biobank with prospective follow-up of a control group, *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* 55 (2012) 831–835.
- [17] J. Barbaresko, S. Siegert, M. Koch, I. Aits, W. Lieb, S. Nikolaus, et al., Comparison of two exploratory dietary patterns in association with the metabolic syndrome in a Northern German population, *Br. J. Nutr.* 112 (2014) 1364–1372.
- [18] S. Waniek, R. Di Giuseppe, T. Esatbeyoglu, S. Plachta-Danielzik, I. Ratjen, G. Jacobs, et al., Vitamin E (α - and γ -Tocopherol) levels in the community: distribution, clinical and biochemical correlates, and association with dietary patterns, *Nutrients* 10 (2017) 3.
- [19] J. Enderle, U. Klink, R. Di Giuseppe, M. Koch, U. Seidel, K. Weber, et al., Plasma lithium levels in a general population: a cross-sectional analysis of metabolic and dietary correlates, *Nutrients* 12 (2020) 2489.
- [20] K.S. Weber, I. Ratjen, J. Enderle, U. Seidel, G. Rimbach, W. Lieb, Plasma boron concentrations in the general population: a cross-sectional analysis of cardio-metabolic and dietary correlates, *Eur. J. Nutr.* 61 (2022) 1363–1375.
- [21] B.E. Ainsworth, W.L. Haskell, S.D. Herrmann, N. Meckes, D.R. Bassett, C. Tudor-Locke, et al., Compendium of Physical Activities: a second update of codes and MET values, *Med. Sci. Sports Exerc.* 43 (2011) 1575–1581, 2011.
- [22] Institut für Klinische Chemie Universitätsklinikum Schleswig-Holstein, Leistungsverzeichnis Institut für Klinische Chemie [Internet]. Available from: <http://svlabindex02.uksh.de:8080/labindex/> [cited August 9, 2022].
- [23] G. Töpfer, Immunturbidimetrie, in: A.M. Gressner, T. Arndt (Eds.), *Lexikon der medizinischen Laboratoriumsdiagnostik*, Springer, 2019, pp. 1234–1235 [Place of publication not identified].
- [24] U. Nöthlings, K. Hoffmann, M.M. Bergmann, H. Boeing, Fitting portion sizes in a self-administered food frequency questionnaire, *J. Nutr.* 137 (2007) 2781–2786.
- [25] L.I. Dehne, C. Klemm, G. Henseler, E. Hermann-Kunz, The German food code and nutrient data base (BLS II.2), *Eur. J. Epidemiol.* 15 (1999) 355–359.
- [26] W. Willett, *Nutritional epidemiology*, 3rd ed., Oxford University Press, New York, 2013.
- [27] K. Hoffmann, M.B. Schulze, A. Schienkiewitz, U. Nöthlings, H. Boeing, Application of a new statistical method to derive dietary patterns in nutritional epidemiology, *Am. J. Epidemiol.* 159 (2004) 935–944.
- [28] M.B. Schulze, K. Hoffmann, A. Kroke, H. Boeing, An approach to construct simplified measures of dietary patterns from exploratory factor analysis, *Br. J. Nutr.* 89 (2003) 409–419.
- [29] G.N. Ioannou, J.A. Dominitz, N.S. Weiss, P.J. Heagerty, K.V. Kowdley, The effect of alcohol consumption on the prevalence of iron overload, iron deficiency, and iron deficiency anemia, *Gastroenterology* 126 (2004) 1293–1301.
- [30] J.M. Liu, S.E. Hankinson, M.J. Stampfer, N. Rifai, W.C. Willett, J. Ma, Body iron stores and their determinants in healthy postmenopausal US women, *Am. J. Clin. Nutr.* 78 (2003) 1160–1167.
- [31] L.R. Zacharski, D.L. Ornstein, S. Woloshin, L.M. Schwartz, Association of age, sex, and race with body iron stores in adults: analysis of NHANES III data, *Am. Heart J.* 140 (2000) 98–104.
- [32] J. Baudry, J.F. Kopp, H. Boeing, A.P. Kipp, T. Schwerdtle, M.B. Schulze, Changes of trace element status during aging: results of the EPIC-Potsdam cohort study, *Eur. J. Nutr.* 59 (2020) 3045–3058.
- [33] A.A. Wawer, A. Jennings, S.J. Fairweather-Tait, Iron status in the elderly: a review of recent evidence, *Mech. Ageing Dev.* 175 (2018) 55–73.
- [34] S.J. Fairweather-Tait, A.A. Wawer, R. Gillings, A. Jennings, P.K. Myint, Iron status in the elderly, *Mech. Ageing Dev.* 136–137 (2014) 22–28.
- [35] J. Xu, Z. Jia, M.D. Knutson, C. Leeuwenburgh, Impaired iron status in aging research, *Int. J. Mol. Sci.* 13 (2012) 2368–2386.
- [36] N.P.E. Kadooglou, J.P. Biddulph, S.B. Rafnsson, M. Trivella, P. Nihoyannopoulos, P. Demakakos, The association of ferritin with cardiovascular and all-cause mortality in community-dwellers: the English longitudinal study of ageing, *PLoS One* 12 (2017), e0178994.
- [37] A.M. Heslin, A. O'Donnell, M. Buffini, A.P. Nugent, J. Walton, A. Flynn, et al., Risk of iron overload in obesity and implications in metabolic health, *Nutrients* 13 (2021) 1539.
- [38] O.R. Aderibigbe, P.T. Pisa, R.L. Mamabolo, H.S. Kruger, H.H. Vorster, The relationship between indices of iron status and selected anthropometric cardiovascular disease risk markers in an African population: the THUSA study, *Cardiovasc. J. Afr.* 22 (2011) 249–256.
- [39] F. Alam, A.S. Memon, S.S. Fatima, Increased body mass index may lead to hyperferritinemia irrespective of body iron stores, *Pak. J. Med. Sci.* 31 (2015) 1521–1526.
- [40] T. Iwasaki, A. Nakajima, M. Yoneda, Y. Yamada, K. Mukasa, K. Fujita, et al., Serum ferritin is associated with visceral fat area and subcutaneous fat area, *Diabetes Care* 28 (2005) 2486–2491.
- [41] A. Khan, W.M. Khan, M. Ayub, M. Humayun, M. Haroon, Ferritin is a marker of inflammation rather than iron deficiency in overweight and obese people, *J. Obes.* 2016 (2016) 1937320.
- [42] A. Kohlgruber, L. Lynch, Adipose tissue inflammation in the pathogenesis of type 2 diabetes, *Curr. Diab. Rep.* 15 (2015) 92.
- [43] T. Kawai, M.V. Autieri, R. Scalia, Adipose tissue inflammation and metabolic dysfunction in obesity, *Am. J. Physiol. Cell Physiol.* 320 (2021) C375–C391.
- [44] M. Plays, S. Müller, R. Rodriguez, Chemistry and biology of ferritin, *Metallomics* 13 (2021), mfab021.
- [45] P. Krisai, S. Leib, S. Aeschbacher, T. Kofler, M. Assadian, A. Maseli, et al., Relationships of iron metabolism with insulin resistance and glucose levels in young and healthy adults, *Eur. J. Intern. Med.* 32 (2016) 31–37.
- [46] K. Mörwald, E. Aigner, P. Bergsten, S.M. Brunner, A. Forslund, J. Kullberg, et al., Serum ferritin correlates with liver fat in male adolescents with obesity, *Front. Endocrinol. (Lausanne)* 11 (2020) 340.
- [47] M.F. Suárez-Ortegón, E. Enseldo-Carrasco, T. Shi, S. McLachlan, J.M. Fernández-Real, S.H. Wild, Ferritin, metabolic syndrome and its components: a systematic review and meta-analysis, *Atherosclerosis* 275 (2018) 97–106.
- [48] Y.E. Kim, D.H. Kim, Y.K. Roh, S.Y. Ju, Y.J. Yoon, G.E. Nam, et al., Relationship between serum ferritin levels and dyslipidemia in Korean adolescents, *PLoS One* 11 (2016), e0153167.
- [49] M. Jehn, J.M. Clark, E. Guallar, Serum ferritin and risk of the metabolic syndrome in U.S. adults, *Diabetes. Care.* 27 (2004) 2422–2428.
- [50] N.S. Al Akl, O. Khalifa, K. Errafii, A. Arredouani, Association of dyslipidemia, diabetes and metabolic syndrome with serum ferritin levels: a middle eastern population-based cross-sectional study, *Sci. Rep.* 11 (2021) 24080.
- [51] K.J. Rothman, BMI-related errors in the measurement of obesity, *Int. J. Obes. (Lond.)* 32 (Suppl 3) (2008) S56–S59.
- [52] S.Y. Ong, A.J. Nicoll, M.B. Delatycki, How should hyperferritinaemia be investigated and managed? *Eur. J. Intern. Med.* 33 (2016) 21–27.

SUPPLEMENTAL MATERIALS

Title: Plasma ferritin concentrations in the general population: A cross-sectional analysis of anthropometric, metabolic and dietary correlates

First author: Cara Övermöhle¹

¹Institute of Epidemiology, Kiel University, 24105 Kiel, Germany

Supplemental Table 1: Main characteristics of all participants who attended the second follow-up examination (n = 666), of all individuals included in the present analyses (n=406), and in those with missing data (n=206).

	Total sample n = 666	Individuals included in present analyses n = 460	Individuals excluded from present analysis due to missing data n = 206	P ⁴
n [% male] ^{1,5}	657 (43)	460 (43)	197 (42)	0.869
Plasma concentration of ferritin [µg/L] ^{2,6}	100.0 (50.0; 169.0)	96.0 (49.5; 167.5)	107.0 (52.0; 167.5)	0.250
Age [years] ^{3,7}	66±12	66±12	66±12	0.600
Body mass index [kg/m ²] ^{3,8}	27.4±4.5	27.5±4.4	27.3±4.8	0.583
Current smokers ^{1,9}	54 (8)	38 (8)	16 (9)	0.915
Education level [low (<10 years), medium (10 years), high (≥11 years)] ^{1,10}	209 (33) / 196 (31) /231 (36)	141 (31) / 147 (32) /172 (37)	68 (39) / 49 (28) /59 (34)	0.158

Values are ¹n (%), ²median (IQR), ³mean±SD, ⁴P-values for included individuals vs. excluded individuals based on chi-square test (categorical variables), Kruskal Wallis test (continuous skewed variables) or general linear models (continuous normally distributed variables).

Data available for ⁵n = 657, ⁶n = 635, ⁷n = 666, ⁸n = 665, ⁹n = 637, ¹⁰n = 636

Abbreviations: CI, confidence interval; IQR, interquartile range; MET, metabolic equivalent of task; SD, standard deviation; nmiss, missings.

Supplemental Table 2: Seasonal differences in plasma ferritin concentrations (n=460).

	Spring (n=166)	Summer (n=101)	Autumn (n=61)	Winter (n=131)	P ¹
Plasma concentration of ferritin [µg/L]	75.0 (34.0; 142.0)	120.0 (59.0; 181.0)	127.0 (64.0; 183.0)	94.0 (47.0; 164.5)	0.001

Values are median (IQR), ¹P-values based on Kruskal Wallis test (continuous skewed variable).

Boldface indicates significant associations (P<0.05).

Abbreviations: IQR, interquartile range.

Supplemental Table 3: Age- and sex-adjusted mean intake of 42 food groups [g/day] according to tertiles of plasma ferritin concentrations (n = 460).

Food groups [g/day]	Plasma concentration of ferritin [µg/L]		
	T1 females 2.5-53.0 µg/L males 10.0-68.0 µg/L n = 150	T2 females 53.1-116.0 µg/L males 68.1-163.0 µg/L n = 158	T3 females 116.1-590.0 µg/L males 163.1-739.0 µg/L n = 152
Potatoes	72.9 (65.8; 80.0)	78 (71.3; 84.7)	81.1 (74.2; 88.0)
Leafy vegetables	9.5 (7.9; 11)	9.2 (7.8; 10.7)	9.2 (7.8; 10.7)
Fruiting vegetables	55.5 (49.8; 61.3)	55.5 (50.0; 60.9)	56.5 (50.9; 62.1)
Root vegetables	13.8 (12.5; 15.0)	14.2 (12.9; 15.4)	13.4 (12.1; 14.6)
Cabbage	18.4 (16.1; 20.8)	18.8 (16.5; 21.0)	18.1 (15.8; 20.4)
Other vegetables	25.0 (23.0; 27.0)	24.5 (22.7; 26.4)	25.2 (23.2; 27.1)
Legumes	4.8 (4.2; 5.4)	4.0 (3.5; 4.6)	4.4 (3.8; 5.0)
Fruits	177.4 (160.2; 194.6) *	169.9 (153.6; 186.2)	148.9 (132.1; 165.7)
Nuts and seeds	3.8 (3.1; 4.6)	3.9 (3.2; 4.7)	3.0 (2.2; 3.7)
Milk	132.3 (112.8; 151.8)	129.7 (111.2; 148.1)	111.8 (92.8; 130.9)
Dairy products	95.6 (84.4; 106.8)	88.4 (77.8; 99.0)	93.3 (82.4; 104.2)
Cheese	29.5 (26.6; 32.3)	28.9 (26.2; 31.5)	29.7 (27.0; 32.5)
Bread	118.5 (105.7; 131.4)	118.8 (106.6; 131.0)	123.5 (110.9; 136.0)
Pasta and rice	41.0 (35.7; 46.4)	41.7 (36.6; 46.7)	45.8 (40.6; 51.1)
Other cereals	17.2 (14.8; 19.5) *	16.1 (13.9; 18.3)	13.4 (11.2; 15.7)
Beef	15.4 (13.0; 17.8)	14.4 (12.2; 16.7)	16.1 (13.8; 18.4)
Pork	23.5 (18.2; 28.9) **	24.9 (19.9; 30.0) *	33.7 (28.5; 38.9)
Poultry	0.3 (0.3; 0.4) *	0.3 (0.3; 0.4)	0.4 (0.4; 0.5)
Processed meat	57.1 (51.2; 63.0)	50.7 (45.1; 56.3) ***	64.7 (59.0; 70.5)
Other meat	18.8 (16.0; 21.6)	18.5 (15.8; 21.1) *	22.6 (19.8; 25.3)
Fish, fish products	21.3 (18.7; 24.0)	21.8 (19.3; 24.4)	20.8 (18.1; 23.4)
Eggs	13.4 (12.0; 14.8)	11.9 (10.6; 13.2) *	14.0 (12.7; 15.4)
Butter	10.9 (9.1; 12.7)	10.1 (8.5; 11.8)	11.6 (9.9; 13.4)
Margarine	9.8 (7.4; 12.2)	8.9 (6.7; 11.2)	9.5 (7.2; 11.8)
Vegetable oils	8.9 (7.2; 10.5)	10.4 (8.8; 11.9)	9.7 (8.1; 11.3)
Other fats	0.6 (0.5; 0.7) *	0.7 (0.5; 0.8)	0.8 (0.7; 0.9)
Sugar products (e.g. syrups, candy, ice cream, desserts)	39.6 (35.6; 43.7)	38.4 (34.5; 42.2)	35.4 (31.4; 39.3)
Chocolate, sweets	10.7 (9.0; 12.3)	9.7 (8.1; 11.3)	9.3 (7.7; 10.9)
Cake, cookies	56.3 (50.1; 62.6)	61.0 (55.1; 66.9)	56.8 (50.7; 62.9)
Non-alcoholic beverages	1082.5 (981.9; 1183.1)	1018.2 (922.9; 1113.5)	1047.3 (949.3; 1145.4)
Soft drinks	53.7 (22.8; 84.5)	47.8 (18.6; 77.1)	64.8 (34.7; 94.8)
Coffee	311.3 (270.1; 352.4)	298.9 (259.9; 337.8)	306.1 (266.0; 346.2)
Tea	195.7 (144.3; 247.0)	221.7 (173.1; 270.4)	209.4 (159.3; 259.4)
Beer	112.4 (57.5; 167.3) **	112.6 (60.6; 164.6) **	218.5 (165.0; 272.0)
Wine	75.8 (53.0; 98.6)	85.2 (63.6; 106.7)	97.9 (75.7; 120.1)
Other alcoholic beverages	10.3 (5.5; 15)	11.8 (7.3; 16.3)	13.9 (9.2; 18.5)
Sauces	88.6 (81.6; 95.5)	83.8 (77.2; 90.4)	91.9 (85.1; 98.6)
Soups	29.2 (23.9; 34.6)	28.7 (23.6; 33.7)	24.6 (19.4; 29.8)
Bouillon	11.5 (9.8; 13.2)	9.9 (8.3; 11.5)	11.1 (9.5; 12.8)
Vegetarian dishes, soy products	5.3 (-0.1; 10.8)	9.6 (4.4; 14.7)	4.9 (-0.4; 10.2)
Snacks	1.2 (1.1; 1.3) **	1.1 (1.1; 1.2) *	1.0 (0.9; 1.1)
Condiments	2.6 (2.0; 3.2)	3.1 (2.6; 3.7)	3.1 (2.5; 3.6)

Data are expressed as re-transformed least square means (95% CI) calculated based on ln-transformed food group intakes adjusted for age and sex; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to least square mean of T3.

Boldface indicates significant associations ($P < 0.05$).

Abbreviations: CI, confidence interval; T, tertile.

3 Association of plasma zinc and copper with body composition, lipids and inflammation in a cross-sectional general population sample from Germany.

Cara Övermöhle¹, Gerald Rimbach², Sabina Waniek¹, Eike A. Strathmann¹, Tatjana Liedtke¹, Paula Stürmer¹, Marcus Both³, Katharina S. Weber^{1*}, Wolfgang Lieb^{1*}

¹ Institute of Epidemiology, Kiel University, 24105 Kiel, Germany

² Institute of Human Nutrition and Food Science, Kiel University, 24118 Kiel, Germany

³ Department of Diagnostic Radiology, University Hospital Schleswig-Holstein, 24105 Kiel, Germany

* These authors contributed equally to this work.

Published in: Nutrients 15 (20), S. 4460. DOI: 10.3390/nu15204460.

Article

Association of Plasma Zinc and Copper with Body Composition, Lipids and Inflammation in a Cross-Sectional General Population Sample from Germany

Cara Övermöhle ^{1,*}, Gerald Rimbach ², Sabina Waniek ¹, Eike A. Strathmann ¹, Tatjana Liedtke ¹, Paula Stürmer ¹, Marcus Both ³, Katharina S. Weber ^{1,†} and Wolfgang Lieb ^{1,†}

¹ Institute of Epidemiology, Kiel University, 24105 Kiel, Germany; katharina.weber@epi.uni-kiel.de (K.S.W.); wolfgang.lieb@epi.uni-kiel.de (W.L.)

² Institute of Human Nutrition and Food Science, Kiel University, 24118 Kiel, Germany

³ Department of Diagnostic Radiology, University Hospital Schleswig-Holstein, 24105 Kiel, Germany

* Correspondence: cara.oevermoehle@epi.uni-kiel.de; Tel.: +49-(0)431-500-30223

† These authors contributed equally to this work.



Citation: Övermöhle, C.; Rimbach, G.; Waniek, S.; Strathmann, E.A.; Liedtke, T.; Stürmer, P.; Both, M.; Weber, K.S.; Lieb, W. Association of Plasma Zinc and Copper with Body Composition, Lipids and Inflammation in a Cross-Sectional General Population Sample from Germany. *Nutrients* **2023**, *15*, 4460. <https://doi.org/10.3390/nu15204460>

Academic Editor: Juan P. Liuzzi

Received: 22 September 2023

Revised: 15 October 2023

Accepted: 18 October 2023

Published: 20 October 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: We aimed to relate circulating plasma zinc and copper to a broad spectrum of adiposity-related traits in a cross-sectional Northern German study (n = 841, 42% female, age: 61 ± 12 years). Zinc and copper were measured by inductively coupled plasma–mass spectrometry. Subcutaneous (SAT) and visceral (VAT) adipose tissue and liver fat were derived from 534 and 538 participants, respectively, via magnet resonance imaging. Associations were assessed using multivariable-adjusted linear regression analysis. An increase per one standard deviation (SD) in zinc was associated with direct linear increases in body mass index (BMI) (1.17%; 95% confidence interval (95%CI) 0.15–2.20%), waist circumference (0.85%; 95%CI 0.04–1.67%) and waist-to-hip ratio (0.64%; 95%CI 0.18–1.09%). A 1-SD increment in copper was directly associated with BMI (1.64%; 0.41–2.88%) and waist circumference (1.22%; 95%CI 0.25–2.20%) but not waist-to-hip ratio. Independent of fat intake, zinc displayed associations with VAT (5.73%; 95%CI 2.04–9.56%) and with liver fat (3.84%; 95%CI 1.49–6.25%), the latter association being also independent of BMI. Copper was directly associated with SAT (4.64%; 95%CI 0.31–9.15%) before accounting for BMI, but showed no association with VAT or liver fat. Observed associations suggest a possible relevance of zinc and copper to adiposity. Particularly zinc displayed associations with traits of abdominal adiposity and liver fat.

Keywords: abdominal adiposity; magnet resonance imaging; popgen; trace elements

1. Introduction

Zinc (Zn) is an essential trace element for human health. Approximately 300 enzymes contain Zn. Thus, Zn is involved in a variety of biological processes, including growth, adequate immune response, protection against oxidative stress and aging [1,2]. Furthermore, Zn is involved in lipid storage and metabolism [3–5]. Pronounced Zn deficiency is rare in Europe (<5%) [6] and is clinically characterized by skin rash, impaired wound healing and susceptibility for infections [1,2].

A second essential trace element for human health is copper (Cu). Cu supports enzyme activity, iron metabolism, connective tissue formation and antioxidant defense [7–9]. Both a Cu deficiency and excess can have adverse health effects. While Cu deficiency is characterized by anemia, neurological impairments and impaired immune function [7,8], excess Cu may lead to liver damage, cognitive impairments and hemolysis [8,9].

In many biological processes, Zn and Cu act antagonistically, since they compete for absorption and metallothionein binding [10]. Evaluating both trace minerals together is therefore essential to fully understand their function in the human body.

Mounting evidence links Zn and Cu to adiposity. A variety of studies have reported associations of these trace minerals with different measures of body fat and body composition. In a Korean study ($n = 1896$), higher serum Zn was directly associated with increased waist circumference (WC) and body fat (measured by dual-energy X-ray absorptiometry (DEXA)) in men but not in women [11]. Similar associations were found for serum Cu in studies in US adults ($n = 7285$) [12] and in Chinese children ($n = 454$) [13].

However, results from previous studies have been conflicting. A study in Mexican adults ($n = 172$) reported inverse associations between serum Zn and body mass index (BMI) [14], while no differences in serum Zn and Cu were observed between children from Montenegro with normal weight and obesity ($n = 202$) [15].

Experimental studies corroborate the association of Zn with lipid storage and metabolism [5]. In mice models, chronic Zn supplementation led to more visceral adipose tissue and an increased adipocyte size [4]. In rat hepatocytes, treatment with high levels of Zn, led to an upregulation of different enzymes involved in lipid metabolism, including fatty acid synthase, stearyl-CoA desaturase-1, and acetyl-CoA carboxylase 1 [3].

As mentioned above, previous studies used DEXA to determine associations of Zn and Cu with body composition. However, it is known that magnet resonance imaging (MRI) is considered even more precise to determine body composition [16].

Therefore, we aimed to investigate in a community-based sample from Northern Germany whether (i) plasma Zn and Cu concentrations were related to a broad spectrum of anthropometric, lipid and inflammatory markers; (ii) both trace elements were associated with MRI traits of body composition; and (iii) the associations of both trace elements with these traits were in opposite directionality.

2. Subjects and Methods

2.1. Study Sample

The data for this cross-sectional study were obtained from the first follow-up examination (2010–2012) of the “popgen controls”, a prospective population-based cohort in Northern Germany. Initially, 1317 participants were enrolled through population registries ($n = 747$) and blood donor registries from the University Hospital in Kiel ($n = 570$). Out of these, 929 participants attended the first follow-up examination [17–19]. Data on Zn and Cu biomarkers, outcomes, and relevant confounders were analyzed in 841 participants (Figure 1). MRI data for subcutaneous and visceral adipose tissue, along with liver fat content, were analyzed in a subset of 534 (adipose tissue) and 538 (liver fat content) individuals, respectively.

The study adhered to the Declaration of Helsinki, and the Ethics Committee of the Medical Faculty of Kiel University approved the study (Project identification code A 156/03; P2N reference numbers 2021-036, 2022-048). All participants provided written informed consent.

2.2. Clinical Evaluation and Laboratory Analyses

Trained personnel conducted standardized physical examinations [18–20]. Self-administered questionnaires collected data on age, sex, education, medical history, and lifestyle factors, including smoking habits and habitual physical activity over the past 12 months. Total physical activity was calculated by summing up the metabolic equivalent of the task for each activity [18–21].

Weight and height were measured with participants wearing light clothing [18]. An amount of 2.0 kg was subtracted from the measured weight to correct for clothing. Based on these measurements, BMI (kg/m^2) was calculated. Additionally, WC and hip circumference were measured to calculate the waist-to-hip ratio (WHR).

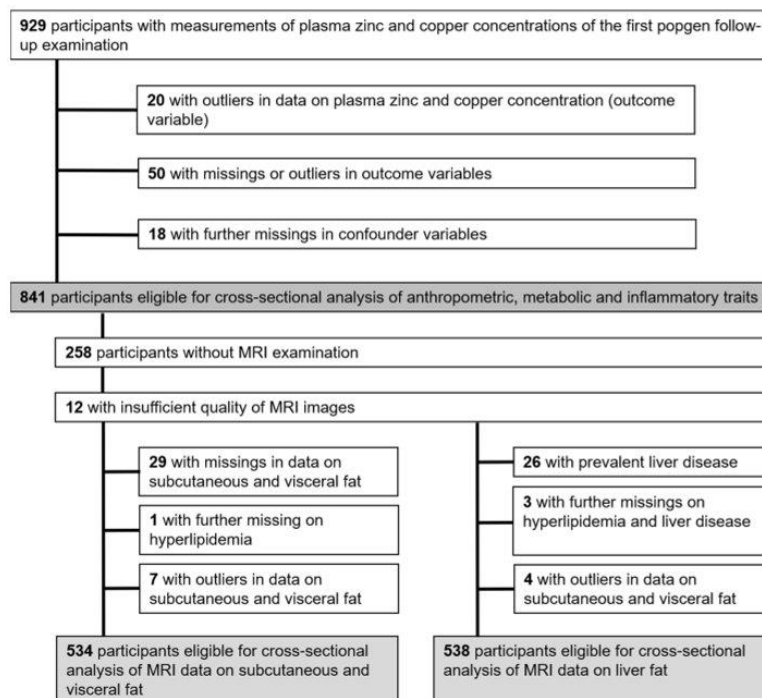


Figure 1. Flowchart showing the participants from the first follow-up examination (2010–2012) in the popgen cohort from Northern Germany being eligible for analysis from those with measurements of plasma zinc concentrations. Abbreviations: MRI, magnet resonance imaging.

Laboratory analyses, including assessment of triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol and plasma C-reactive protein (CRP), were performed using the unfrozen samples the same day in the Institute of Clinical Chemistry at the University Hospital Schleswig-Holstein, Campus Kiel [18–20].

2.2.1. Assessment of Plasma Zinc and Copper

Concentrations of Zn and Cu were measured from frozen plasma samples in 2022 by SGS Analytics Germany GmbH, via inductively coupled plasma–mass spectrometry ICAP Q instrument (Thermo Fisher Scientific, Waltham, MA, USA) in accordance with DIN EN ISO 17294-2: 2017-01 [22]. Prior to measurement, plasma sample aliquots were decomposed (nitric acid and hydrogen peroxide (4:1)) by microwave digestion [23]. Multi-element standard was used for calibration, while rhodium (2 µg/L) was added as internal standard. Spectroscopic interferences were minimized by use of a collision/reaction cell for detection.

2.2.2. Assessment of Dietary Intake of Zinc, Total Fat and Saturated Fatty Acids

Dietary intake of Zn, Cu, total fat and saturated fatty acids (SFAs) was approximated by a food frequency questionnaire (FFQ). The FFQ has been validated for the German population within the EPIC-Potsdam study [24]. Based on the FFQ data, nutrient content was calculated according to the German Food Code and Nutrient Database (version II.3) [24,25].

2.2.3. Assessment of Subcutaneous and Visceral Adipose Tissue and Liver Fat

MRI measurements of subcutaneous (SAT) and visceral (VAT) adipose tissue and liver fat were conducted using a 1.5 T scanner (Magnetom Avanto; Siemens Medical solution, Erlangen, Germany) [26]. Details of the procedure have been described elsewhere [20,27,28].

Adipose tissue volumes (dm^3) were calculated by the number of voxels multiplied by the voxel size [26,27]. For SAT, the voxels beneath the skin, surrounding the abdomen, from the top of the liver to the femoral heads, was determined. VAT was defined as voxels counted from the top of the liver to the femoral heads inside the abdominal muscular wall. Liver signal intensity (LSI) was used to determine liver fat by computing the relative difference of LSI on out-of-phase and in-phase images in arbitrary units [20,26]. Images of both phases were captured during breath hold using T1-weighted gradient echo sequences. LSI was determined by measurement of the average of three circular regions of the liver parenchyma.

2.3. Definitions

Fatty liver disease was defined as \ln -transformed LSI ≥ 3.0 [20,26]. The education of participants was given in three categories: low (≤ 9 years), middle (10 years), or high (≥ 11 years) [18,23]. Smoking behavior was presented as three groups: never-smokers, former smokers and current smokers [18,23,26]. Fasting status at time of blood sampling was defined as not fasting (ate or drank within the last 8 h) and fasting (did not eat or drink in the last 8 h). The season in which the examination took place was presented in the following categories: winter (January–March), spring (April–June), summer (July–September) and fall (October–December) [23].

2.4. Statistical Analyses

Data analyses were performed using SAS[®] (version 9.4; SAS Institute, Cary, NC, USA) and p values of <0.05 were considered statistically significant.

Only participants with complete and plausible data for independent, dependent and confounding variables were included. Participants with outliers in continuous independent or dependent variables were removed from the sample. Fences for outliers were defined as $\text{mean} \pm 3 \times \text{standard deviation (SD)}$. Potential confounders were defined a priori based on the literature.

In descriptive analysis, categorical variables were reported as absolute numbers and percentages, continuous normally distributed variables as mean and SD, and continuous skewed variables as median and interquartile range. Sample characteristics were compared across tertiles of plasma Zn concentrations ($\mu\text{g/L}$) using a chi-square test for categorical variables, unadjusted general linear models for continuous normally distributed variables and the Kruskal–Wallis test for continuous skewed variables [23].

2.4.1. Association of Zinc and Copper with Anthropometric, Metabolic and Inflammatory Parameters

Linear regression analyses (PROC GLM, SAS[®]) were used to assess the associations of Zn and Cu (each trace element considered as a separate exposure variable) with anthropometric parameters (BMI, WC, and WHR), lipid traits (TG, HDL, and LDL) and inflammation (CRP) (separate dependent outcome variables, all \ln -transformed). We performed the following adjustments: Model A1 was unadjusted. Model A2 was adjusted for age and sex. Model A3 additionally included BMI (not for WC and WHR), education, smoking habits, season, physical activity, alcohol consumption, Zn supplementation (for Zn as an independent variable), lipid-lowering medication (only for lipid traits) and fasting status (only for lipid traits). Model A4 was additionally adjusted for total fat intake and intake of SAFs.

The effect estimates from the linear regression models were re-transformed and given as % increase in the dependent variable per 1-SD increase in the independent variable (Zn or Cu).

2.4.2. Association of Zinc and Copper with MRI Traits of Body Composition

To model the associations of Zn and Cu (as separate exposure variables) with \ln -transformed MRI traits of body composition (SAT, VAT and LSI, each trait considered

as a separate outcome) we used multivariable-adjusted linear regression models (PROC GLM, SAS®) with the following adjustments: Model B1 was unadjusted. Model B2 was adjusted for age and sex. Model B3 was additionally adjusted for education, smoking habits, season, physical activity, alcohol consumption, Zn supplementation (for Zn as independent variable), lipid-lowering medication and prevalent fatty liver disease (for LSI as the outcome variable). Model B4 additionally included total fat intake and intake of SAFs, while Model B5 also included BMI.

The effect estimates from the linear regression models were re-transformed and given as % increase in the dependent variable per 1-SD increase in the independent variable (Zn or Cu).

2.4.3. Sensitivity Analysis

In order to assess if the observed results might have been affected by use of Zn supplementation, all analyses as described above were repeated in a subsample of participants not taking Zn supplements ($n = 784$) and in the corresponding MRI subsamples for adipose tissues ($n = 493$) and liver fat ($n = 497$).

3. Results

3.1. Participants' Characteristics

Baseline characteristics of our study sample are provided in Table 1. About 7% of the participants reported intake of Zn supplements. According to a previous classification [29], only 3.8% ($n = 32$) met the criterion for Zn deficiency ($<460 \mu\text{g/L}$), while 1.9% ($n = 16$) had low ($<760 \mu\text{g/L}$ in females, $<700 \mu\text{g/L}$ in males) and 2.1% ($n = 18$) high ($>1522 \mu\text{g/L}$ in females, $>1400 \mu\text{g/L}$ in males) plasma concentrations for Cu.

Table 1. General characteristics of participants from the first follow-up examination (2010–2012) in the popgen cohort from Northern Germany according to sex-specific tertiles of circulating plasma zinc concentrations ($n = 841$).

	Total	T1	T2	T3	<i>p</i> Values ^d
	434.9–958.7 $\mu\text{g/L}$	434.9–658.2 $\mu\text{g/L}$	658.3–724.9 $\mu\text{g/L}$	725.0–958.7 $\mu\text{g/L}$	
<i>n</i> (% female) ^a	841 (42)	280 (41)	281 (44)	280 (40)	0.501
Age (years) ^b	61 \pm 12	60 \pm 13	62 \pm 12	61 \pm 12	0.103
Plasma zinc ($\mu\text{g/L}$) ^b	695.0 \pm 83.1	607.7 \pm 40.7	690.9 \pm 18.9	786.3 \pm 52.4	<0.001
Plasma copper ($\mu\text{g/L}$) ^b	1037.9 \pm 192.5	1035.9 \pm 197.1	1043.9 \pm 183.5	1034.0 \pm 197.2	0.813
Zinc intake (mg/day) ^c	11.1 (9.4; 13.2)	11.2 (9.3; 13.4)	11.1 (9.7; 13.0)	11.0 (9.3; 13.2)	0.865
Intake of zinc supplements (yes, (%)) ^a	57 (7)	15 (5)	20 (7)	22 (8)	0.481
Copper intake (mg/day) ^c	2.5 (2.1; 2.9)	2.5 (2.1; 3.0)	2.4 (2.1; 2.9)	2.4 (2.1; 2.8)	0.370
Intake of total fat (g/day) ^c	94.1 (78.5; 115.9)	95.6 (77.7; 119.7)	94.1 (78.2; 112.8)	93.2 (79.1; 112.5)	0.783
Intake of saturated fatty acids (g/day) ^c	38.0 (30.8; 46.9)	38.4 (29.8; 48.7)	38.1 (31.6; 45.5)	37.6 (30.6; 46.4)	0.838
Subcutaneous adipose tissue (dm^3) ($n = 534$) ^c	6.0 (4.7; 8.2)	5.9 (4.8; 8.1)	6.1 (4.5; 8.3)	6.1 (4.8; 8.2)	0.732
Visceral adipose tissue (dm^3) ($n = 538$) ^c	3.8 (2.4; 5.2)	3.5 (2.2; 5.1)	3.8 (2.5; 5.0)	4.1 (2.7; 5.2)	0.179
Liver signal intensity ^c	18.5 (14.9; 23.5)	17.5 (14.5; 22.1)	19.0 (14.9; 23.8)	18.8 (15.3; 24.4)	0.067
Body mass index (kg/m^2) ^b	27.2 \pm 4.2	27.0 \pm 4.3	27.3 \pm 4.2	27.4 \pm 4.2	0.484
Waist circumference (cm) ^b					
Females	90.3 \pm 12.8	88.5 \pm 12.4	91.5 \pm 12.8	90.8 \pm 13.2	0.172
Males	100.4 \pm 10.8	100.6 \pm 11.0	100.3 \pm 10.9	100.4 \pm 10.7	0.998
Waist-to-hip ratio ^b					
Females	0.87 \pm 0.07	0.86 \pm 0.07	0.88 \pm 0.06	0.88 \pm 0.07	0.151
Males	0.98 \pm 0.06	0.98 \pm 0.06	0.99 \pm 0.06	0.98 \pm 0.07	0.661

Table 1. Cont.

	Total	T1	T2	T3	<i>p</i> Values ^d
	434.9–958.7 µg/L	434.9–658.2 µg/L	658.3–724.9 µg/L	725.0–958.7 µg/L	
Plasma triglyceride concentration (mg/dL) ^c	105.0 (76.0; 138.0)	101.5 (77.5; 134.5)	103.0 (76.0; 131.0)	110.0 (73.0; 149.5)	0.301
Plasma high-density lipoprotein cholesterol (mg/dL) ^b	65.1 ± 17.2	66.2 ± 17.7	64.2 ± 17.2	64.8 ± 16.8	0.397
Plasma low-density lipoprotein cholesterol (mg/dL) ^b	131.6 ± 33.0	125.6 ± 34.2	133.8 ± 30.7	135.6 ± 33.4	<0.001
Lipid-lowering medication (yes, (%)) ^a	114 (14)	30 (11)	36 (13)	48 (17)	0.077
C-reactive protein (mg/L) ^c	1.2 (0.5; 2.4)	1.3 (0.5; 2.8)	1.2 (0.5; 2.4)	1.1 (0.5; 2.1)	0.203
Current smokers (yes, (%)) ^a	108 (13)	44 (16)	32 (11)	32 (11)	0.263
Alcohol intake (g/day) ^c	9.0 (3.2; 18.5)	10.7 (3.9; 21.9)	8.5 (3.0; 17.6)	8.4 (2.8; 16.4)	0.022
Physical activity (MET-hours/week) ^c	90.5 (59.0; 131.5)	90.5 (59.7; 132.3)	88.3 (56.9; 126.3)	93.8 (59.1; 135.6)	0.694
Education level (low [<10 years], medium [10 years], high [≥ 11 years]) ^a	292 (35); 271 (32); 278 (33)	87 (31); 93 (33); 100 (36)	106 (38); 90 (32); 85 (30)	99 (35); 88 (31); 93 (33)	0.517

Values are as follows: ^a n (%) (categorical variables), ^b mean ± SD (continuous normally distributed variables), ^c median (IQR) (continuous skewed variables), ^d *p* values based on chi-square test (categorical variables), Kruskal–Wallis test (continuous skewed variables) or general linear models (continuous normally distributed variables). Abbreviations: IQR, interquartile range; MET, metabolic equivalent of task; SD, standard deviation; T, tertile.

Across Zn tertiles, comparable plasma Cu and FFQ extracted intakes of dietary Zn, Cu, fat and SAFs were established (Table 1). Participants in the top Zn tertile had higher concentrations of plasma LDL as compared to the lower tertiles. Additionally, alcohol intake was highest in the lowest tertile of Zn concentrations.

Due to missing and implausible data, not all participants were included in our main analyses. Compared to the excluded participants ($n = 88$), participants within our initial study sample ($n = 841$) had comparable circulating Zn concentrations, lower circulating Cu concentrations, were older, had lower BMI, were less likely smokers and had higher physical activity (Table S1).

3.2. Association of Zinc and Copper with Anthropometric, Metabolic and Inflammatory Traits

In multivariable-adjusted linear regression models, Zn was directly associated with different anthropometric measures (i.e., BMI, WC and WHR) and LDL, and inversely associated with CRP (Table 2, Model A3, $p = 0.023$; 0.041 ; 0.007 ; <0.001 and 0.007 , respectively). Cu displayed direct associations with BMI, WC, HDL, LDL, and CRP (Table 2, Model A3, $p = 0.008$; 0.015 ; 0.002 ; 0.010 and <0.001 , respectively). Including intake of total fat and SAFs into the model did not change results considerably (Table 2, Model A4).

Table 2. Associations of plasma zinc and copper concentrations with anthropometric, metabolic and inflammatory traits (n = 841).

Anthropometric, Metabolic and Inflammatory Outcome Variables	Zinc		Copper	
	Estimates (95% CI) ^a	p Values	Estimates (95% CI) ^a	p Values
Body mass index (kg/m ²)				
Model A1	1.35 (0.30; 2.40)	0.011	0.38 (−0.66; 1.42)	0.475
Model A2	1.19 (0.16; 2.23)	0.024	1.60 (0.38; 2.84)	0.010
Model A3	1.18 (0.17; 2.21)	0.023	1.65 (0.43; 2.89)	0.008
Model A4	1.17 (0.15; 2.20)	0.024	1.64 (0.41; 2.88)	0.009
Waist circumference (cm)				
Model A1	1.15 (0.23; 2.07)	0.014	−1.79 (−2.67; −0.89)	<0.001
Model A2	0.83 (0.02; 1.65)	0.046	1.26 (0.30; 2.24)	0.011
Model A3	0.84 (0.03; 1.66)	0.041	1.20 (0.23; 2.18)	0.015
Model A4	0.85 (0.04; 1.67)	0.040	1.22 (0.25; 2.20)	0.014
Waist-to-hip ratio				
Model A1	0.88 (0.25; 1.51)	0.006	−2.45 (−3.03; −1.85)	<0.001
Model A2	0.58 (0.12; 1.04)	0.014	0.67 (0.13; 1.22)	0.016
Model A3	0.63 (0.18; 1.09)	0.007	0.47 (−0.07; 1.02)	0.090
Model A4	0.64 (0.18; 1.09)	0.006	0.48 (−0.06; 1.03)	0.084
Plasma triglyceride concentration (mg/dL)				
Model A1	2.79 (−0.33; 6.00)	0.080	−0.89 (−3.90; 2.21)	0.569
Model A2	2.16 (−0.87; 5.29)	0.164	2.24 (−1.34; 5.97)	0.223
Model A3	1.58 (−1.33; 4.57)	0.290	−0.08 (−3.41; 3.36)	0.963
Model A4	1.52 (−1.39; 4.51)	0.310	−0.18 (−3.51; 3.26)	0.917
Plasma HDL concentration (mg/dL)				
Model A1	−1.58 (−3.33; 0.20)	0.082	7.49 (5.64; 9.37)	<0.001
Model A2	−1.26 (−2.85; 0.35)	0.124	1.40 (−0.53; 3.37)	0.156
Model A3	−0.50 (−1.99; 1.02)	0.517	2.85 (1.06; 4.67)	0.002
Model A4	−0.50 (−1.99; 1.03)	0.520	2.87 (1.07; 4.69)	0.002
Plasma LDL concentration (mg/dL)				
Model A1	3.32 (1.48; 5.18)	<0.001	3.17 (1.33; 5.03)	<0.001
Model A2	3.16 (1.34; 5.02)	<0.001	3.62 (1.46; 5.84)	0.001
Model A3	3.77 (1.99; 5.59)	<0.001	2.73 (0.66; 4.84)	0.010
Model A4	3.75 (1.97; 5.57)	<0.001	2.70 (0.63; 4.81)	0.010
C-reactive protein (mg/L)				
Model A1	−6.12 (−12.05; 0.20)	0.058	38.98 (30.70; 47.78)	<0.001
Model A2	−6.59 (−12.42; −0.36)	0.039	47.09 (36.86; 58.08)	<0.001
Model A3	−7.75 (−13.02; −2.17)	0.007	37.34 (28.41; 46.90)	<0.001
Model A4	−7.81 (−13.08; −2.22)	0.007	37.33 (28.38; 46.91)	<0.001

Model A1 was unadjusted. Model A2 was adjusted for age and sex. Model A3 was additionally adjusted for BMI (not for BMI, waist circumference and waist-to-hip ratio as exposure variable), education, smoking habits, season, zinc supplementation (for plasma zinc as dependent variable), lipid-lowering medication (for triglycerides, HDL and LDL as independent variables), fasting status (for triglycerides, HDL and LDL as independent variables) and physical activity and alcohol consumption. Model A4 was additionally adjusted for total fat intake and intake of saturated fatty acids. ^a Regression coefficients indicate the percentage change in outcome variables per 1-SD increment in plasma zinc and copper. Abbreviations: BMI, body mass index; CI, confidence interval; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

Restricting the sample to participants without intake of Zn supplements led to essentially unchanged results (Table S2).

3.3. Associations of Zinc and Copper with Adipose Tissue and Liver Fat

Zn displayed direct associations with SAT, VAT and LSI in multivariable-adjusted model B3 (Table 3, Model B3, $p = 0.049$; 0.002 and 0.001, respectively). Further adjustment for total fat intake and intake of SAFs attenuated the association with SAT (Table 3, Model B4, $p = 0.065$). Upon further adjustment for BMI, the association with LSI remained statistically significant (Table 3, Model B5, $p = 0.006$), but the associations with VAT were rendered statistically non-significant (Table 3, Model B5, $p = 0.057$).

Table 3. Associations of plasma Zn and Cu with subcutaneous and visceral fat (n = 534), and with liver signal intensity (n = 538).

MRI Traits as Outcome Variables	Zinc		Copper	
	Estimates (95% CI) ^a	p Values	Estimates (95% CI) ^a	p Values
Subcutaneous adipose tissue (dm ³)				
Model B1	3.17 (−0.54; 7.03)	0.095	9.89 (6.02; 13.91)	<0.001
Model B2	3.77 (0.18; 7.48)	0.039	3.65 (−0.63; 8.11)	0.096
Model B3	3.60 (0.01; 7.31)	0.049	4.77 (0.44; 9.29)	0.031
Model B4	3.38 (−0.21; 7.10)	0.065	4.64 (0.31; 9.15)	0.036
Model B5	−0.37 (−2.22; 1.51)	0.697	0.46 (−1.77; 2.74)	0.688
Visceral adipose tissue (dm ³)				
Model B1	7.60 (3.01; 12.40)	0.001	−12.07 (−15.74; −8.23)	<0.001
Model B2	5.78 (2.02; 9.68)	0.002	2.31 (−2.06; 6.88)	0.305
Model B3	5.69 (2.02; 9.50)	0.002	3.56 (−0.78; 8.08)	0.109
Model B4	5.73 (2.04; 9.56)	0.002	3.57 (−0.78; 8.10)	0.109
Model B5	2.40 (−0.07; 4.93)	0.057	−0.06 (−2.96; 2.91)	0.966
Liver signal intensity				
Model B1	6.32 (2.73; 10.04)	<0.001	−2.17 (−5.50; 1.29)	0.216
Model B2	5.83 (2.32; 9.45)	0.001	−3.70 (−7.53; 0.29)	0.069
Model B3	3.92 (1.57; 6.32)	0.001	−1.48 (−4.16; 1.29)	0.291
Model B4	3.84 (1.49; 6.25)	0.001	−1.49 (−4.18; 1.27)	0.287
Model B5	3.20 (0.90; 5.54)	0.006	−2.11 (−4.72; 0.56)	0.121

Model B1 was unadjusted. Model B2 was adjusted for age and sex. Model B3 was additionally adjusted for education, smoking habits, season, zinc supplementation (plasma zinc as exposure variable), lipid-lowering medication, prevalent fatty liver disease (for liver signal intensity as outcome variable), physical activity and alcohol consumption. Model B4 was additionally adjusted for total fat intake and intake of saturated fatty acids. Model B5 was additionally adjusted for BMI. Subcutaneous and visceral adipose tissue and liver signal intensity entered into the models as ln-transformed variables. ^a Regression coefficients indicate the % change in subcutaneous and visceral adipose tissue and liver signal intensity per 1-SD increment in plasma zinc and copper concentrations. Abbreviations: BMI, body mass index; CI, confidence interval; MRI, magnet resonance imaging; SD, standard deviation.

Cu was directly associated with SAT upon adjustment for fat and SAFs intake (Table 3, Models B3 and B4, $p = 0.031$ and 0.036). However, additional adjustment for BMI rendered this association statistically non-significant (Table 3, Model B5, $p = 0.688$). VAT and LSI were not related to plasma Cu levels (Table 3).

In sensitivity analysis restricted to participants not taking Zn supplements, associations of Zn and Cu with SAT, VAT and LSI remained essentially unchanged. However, exclusion of participants taking Zn supplements led to a more pronounced association of Zn with VAT, which now remained statistically significant upon further adjustment for BMI (Table S3). Cu was no longer associated with SAT in this subsample (Table S3).

MRI examinations were conducted in a subsample. Compared to the excluded participants, participants in the MRI subsample had lower Cu blood concentrations, were slightly older and less likely smokers, had lower BMI, drank more alcohol and had higher education (Table S1).

Restricting the sample to participants without intake of Zn supplements led to essentially unchanged results (Table S2).

4. Discussion

In a moderate-sized sample from Northern Germany, we evaluated the associations of the trace elements Zn and Cu with a broad spectrum of anthropometric, metabolic and inflammatory markers, and with MRI traits of body composition (SAT, VAT, and liver fat).

Our principal observations were as follows: first, Zn and Cu both displayed direct associations with anthropometric and lipid traits. Second, Zn displayed direct associations with MRI traits of body composition, including SAT, VAT and liver fat in multivariable-adjusted models excluding BMI. However, upon additional adjustment for BMI, only the association with liver fat remained statistically significant in the overall sample. In a sample

excluding participants on Zn supplements, both, liver fat and VAT remained statistically significantly associated with Zn concentrations in multivariable-adjusted models including BMI. Third, Cu levels were not related to SAT, VAT and liver fat in multivariable-adjusted models including BMI. Fourth, intake of total fat or SAFs could not explain the associations between Zn and Cu, and features of lipid metabolism and fat storage. Fifth, Zn and Cu showed an opposing effect on CRP, while all other associations were consistently in the same direction.

4.1. Associations of Zinc and Copper with Anthropometric Traits and MRI Measures of Body Composition

Prior analyses on the association of Zn and Cu with anthropometric traits were not entirely consistent and used different measures of Zn and Cu exposure, including dietary intake and circulating Zn and Cu concentrations.

In 19952 participants from the US National Health and Nutrition Examination Survey (NHANES) (2007–2014), dietary Zn intake was inversely associated with BMI and WC [30]. Additionally, in a recent meta-analysis, serum Zn concentrations were inversely related to BMI [31]. In our sample, however, plasma levels of both Zn and Cu were directly associated with BMI and WHR in multivariable-adjusted models, which is in part in line with results from a large observational survey from Korea (NHANES, 2010) ($n = 1896$), where higher serum Zn was associated with larger WC in male participants, but not in females [11].

Also, the associations of Cu with body composition and body weight are in part conflicting [12,30,32,33]. A meta-analysis from 2019 reported an association of higher Cu concentrations and overweight [34], which is consistent with our observations.

We expand these prior analyses by relating plasma Zn and Cu levels to MRI traits of body composition. In these analyses, we observed direct linear associations of circulating Zn levels with SAT, VAT and liver fat in multivariable-adjusted models. Upon additional adjustment for BMI, only the association with liver fat remained statistically significant in the overall sample. However, in a sample excluding 57 individuals on Zn supplements, liver fat and VAT remained statistically significantly associated with Zn in multivariable-adjusted models including BMI. Cu concentrations were not consistently associated with VAT, SAT or liver fat. Some smaller studies on the association of these trait elements with MRI measures of obesity or with DEXA traits of body composition have been published. In 87 hemodialysis patients, a direct association of circulating Zn with VAT has been reported [35]. This agrees well with our observation in a much larger population-based sample. Furthermore, within the above mentioned Korean NHANES ($n = 1896$), a direct association of Zn with the quantity and distribution of body fat (assessed by DEXA) and abdominal obesity was found in men (but not in women) [11]. Other studies, however, reported inverse associations between Zn levels and body fat % (assessed by DEXA) [36] or found no association of Zn concentrations with excess fat as measured by DEXA [15].

The association of Zn with adiposity is also supported by experimental studies and in animal models [3,5,37,38]. For example, Zn supplementation (200 mg/kg) for six weeks increased body fat by 49.4% and 18.9% in genetically obese mice and high-fat diet-induced obese mice, suggesting that body fat deposition can be aggravated by Zn supplementation [38]. In this context it has been hypothesized that Zn exerts an insulin-like effect or that it interacts with thyroid hormone activities [38]. In general, insulin suppresses lipolysis in adipocytes, leading to the accumulation of fat depots [39]. Therefore, insulin-like effects of Zn may also suppress lipolysis. Another explanation might be the regulation of Zn transporters within adipocytes, which differs between lean and obese subjects, and also between SAT and VAT, suggesting that metabolic activity in adipocytes may be dependent upon Zn and Zn transporters or vice versa [40]. Furthermore, the upregulation of lipogenic enzymes may be responsible for the observed associations in our sample [3–5]. For example, supplementation of Zn for 40 days increased the expression of fatty acid synthase, stearoyl-CoA desaturase-1, acetyl-CoA carboxylase 1, peroxisome proliferator-activated receptor

gamma, and sterol-regulatory element binding protein in a study in piglets compared to a control diet without Zn supplementation [5].

We also observed a direct association of Zn with MRI-determined liver fat, further supporting an adipogenic role of Zn. This contrasts, however, with a recent meta-analysis reporting lower Zn concentrations in patients with non-alcoholic fatty liver disease (NAFLD) [41]. Nevertheless, the expression of lipogenic enzymes was shown to be upregulated in an in vitro study on rat hepatocytes [4], supporting our observed association of circulating Zn with increased liver fat.

Initial associations between Cu and SAT were weakened after further adjustment with BMI, while no associations emerged with VAT or liver fat (as measured by LSI). Also, the evidence regarding Cu's influence on adipokines is partially conflicting [12]. NHANES data (2011–2016) from the USA ($n = 7285$) reported linear associations of Cu with anthropometric measures (BMI, and WC) and with adipose tissue (assessed by DEXA) [12]. Similarly, in children ($n = 454$), Cu was associated with increased body fat (measured by DEXA), partly mediated through inflammation [13]. In the US, NHANES 2011–2014 ($n = 3211$) reported higher Cu to be associated with an increased likelihood for NAFLD [9]. By contrast, a 2017 review suggests that reduced Cu availability might contribute to the development of NAFLD [42]. In our analyses, we observed no statistically significant relation of Cu with liver fat.

4.2. Association of Zinc and Copper with Blood Lipids

Both, Zn and Cu have been studied in relation to blood lipids in experimental, interventional and observational studies, in part with controversial results.

In a large Chinese observational study ($n = 12028$), higher Zn intake was associated with higher TG levels [43]. Another study by Kim and Choi (2013) reported no association of Zn intake with blood lipids in healthy adults with self-selected diet ($n = 258$) [44]. In 600 African Americans, Zn and also Cu were not associated with LDL, HDL or TG [45]. With respect to Cu, another cohort study from China reported that urinary Cu was directly associated with TG, but not with other blood lipids ($n = 4812$) [46].

It is noteworthy to mention that using dietary Zn intake as a measure of Zn status may be problematic because circulating Zn concentrations are tightly homeostatically regulated and, therefore, dietary intake of Zn may be a poor predictor of blood Zn levels [47]. In line with this concept, we observed no differences in dietary Cu and Zn intake across tertiles of plasma Zn in our sample.

Also, the results from the interventional studies and meta-analysis were partly conflicting. An intervention ($n = 387$) using 15 mg/day or 30 mg/day Zn supplement over 6 months raised LDL in middle-aged individuals taking 30 mg, but not in elderly or in the 15 mg group [48]. In contrast, an interventional study on Zn supplementation (50 mg Zn gluconate) in type 2 diabetes patients found reduced TG levels after 8 weeks [49].

It has to be acknowledged, however, that the daily intake of 30 mg or 50 mg per day is much higher than the average daily intake in our sample.

A 2015 meta-analysis of 24 studies with a total of $n = 14515$ participants supports Zn's lipid-lowering effects [50].

On a parallel note, a meta-analysis of five randomized clinical trials (a total of $n = 176$ participants) reported no effect of Cu supplementation on blood lipids (TG, HDL and LDL) [51].

4.3. Diet

Dietary exposures are unlikely to play a significant role in observed associations of plasma Zn with blood lipids and body composition. Zn is mainly contained in meat, eggs and milk products, which are rich in fat and SAFs [52]. While Cu is also prevalent in fatty foods like liver, fish, nuts and chocolate, it is also contained in grain products [52]. In our analyses, the associations of Zn and Cu with anthropometric traits and MRI traits of body

composition were not attenuated after adjustments for fat and SAFs, even though both nutrients have a strong influence on lipid metabolism and fat deposition themselves [53].

However, total fat and SAF intake were estimated by FFQ data. The concept behind the FFQ approach is to capture an individual's dietary habits over an extended period, such as weeks, months, or even years, by assessing the habitual intake of a certain number of food items. Therefore, the FFQ-derived intake of specific single nutrients is rather a rough proxy for the actual intake. For macronutrients, the FFQ is an acceptable approximation of actual intake but, for subgroups such as SAFs and micronutrients, the agreement is rather weak [54,55]. It is possible that the use of FFQ data is not sufficient to completely rule out the possibility that the real driver behind the associations of obesity and body fat is fat intake.

4.4. Association of Zinc and Copper with Inflammation

While we observed directionality-consistent associations of Zn and Cu with anthropometric traits and lipids (see above), we observed opposing associations of the two trace elements—a direct association of Cu and an inverse association of Zn—with CRP, a marker of chronic systemic inflammation. The association of CRP with Cu is in line with previous reports and may be promoted by the upregulation of ceruloplasmin, the major carrier for Cu [56,57]. It has been furthermore suggested that inflammation may mediate the association of Cu with body composition.

A potential explanation for the inverse association of Zn with CRP could be that Zn levels may be lower in an inflammatory state, since Zn is needed to induce intracellular antioxidant pathways [57,58] in response to inflammation or that Zn levels are lower due to the downregulation of albumin, the carrier protein for Zn, in states of inflammation [56,57].

This opposing behavior of Zn and Cu may be due to an opposing reaction to certain inflammatory stimuli. For example, the cytokine interleukin 1 is produced under inflammatory conditions. On the one hand, interleukin 1 alters Zn distribution and thereby decreases circulating Zn levels and, on the other hand, the same cytokine increases ceruloplasmin, the major carrier for circulating Cu [59]. This mechanism illustrates why Zn and Cu behave differently under inflammatory conditions.

4.5. Strengths and Limitations

The strengths of our present study include the moderate-sized general-population sample and the availability of detailed information on multiple relevant confounders. Furthermore, MRI data were available for over 60% of participants, which enabled us to investigate traits of adipose tissue and liver fat. According to current knowledge, MRI is the most reliable method to analyze the body composition of humans [16]. The relatively large MRI subsample is therefore particularly valuable. Furthermore, plasma Zn is considered a reliable biomarker for Zn status in humans [60]. Evidence for plasma Cu as a reliable biomarker is not as clear [61]; however, it is used in large studies like NHANES from the US [9].

The following limitations warrant consideration. First, our cross-sectional design and the strongly pronounced multifunctionality of both trace elements does not allow causal inferences. Second, measuring dietary Zn, Cu, fat and SAF intake by FFQ is only a rough proxy for actual intake and the values should therefore only be viewed and evaluated with caution. Third, there were differences between included and excluded subjects, both in the large study sample and in the subsample for the MRI analysis. Overall, the included subjects were leaner than the excluded subjects and individuals with pronounced adiposity were not included in the present sample. It is known that severe adiposity is accompanied by chronic inflammation, which in consequence would lead to a decrease in Zn [62]. Thus, in a sample comprised by a large number of participants with severe adiposity, the observations we made could be masked. Fourth, our data were obtained in an elderly sample from Northern Germany. The generalizability of our observations to other age groups, ethnicities or geographic areas is unknown.

5. Conclusions

In our population-based sample from Northern Germany, plasma concentrations of Zn displayed a direct association with anthropometric traits (BMI, WC, and WHR) and MRI traits of body composition, i.e., SAT, VAT and liver fat. Cu displayed a direct association only with SAT, suggesting links of Cu and Zn to adiposity and body composition. Furthermore, we observed an association of Zn and Cu with LDL suggesting that both trace elements might be involved in lipid homeostasis. Finally, both trace elements were related with CRP, although with opposite directionality, indicating opposing roles of Zn and Cu in chronic systemic inflammation. The underlying physiological mechanisms need to be identified.

Supplementary Materials: The following supporting information on selection bias and sensitivity analyses can be downloaded at: <https://www.mdpi.com/article/10.3390/nu15204460/s1>, Table S1: Main characteristics of participants, separate for those individuals included and excluded in analyses in participants from the first follow-up examination (2010–2012) in the popgen-cohort from Northern Germany.; Table S2: Associations of plasma zinc and copper concentrations with anthropometric, metabolic and inflammatory traits in participants not taking zinc supplements (n = 784); Figure S3: Associations of plasma Zn and Cu with subcutaneous and visceral fat (n = 534) and with liver signal intensity in participants not taking zinc supplements.

Author Contributions: C.Ö., G.R., K.S.W. and W.L.: designed the research; S.W., E.A.S., T.L., P.S., M.B. and K.S.W.: provided essential materials; C.Ö.: performed the statistical analysis; C.Ö.: wrote the paper; C.Ö., K.S.W. and W.L.: had primary responsibility for the final content. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study adhered to the Declaration of Helsinki, and the Ethics Committee of the Medical Faculty of Kiel University approved the study (Project identification code A 156/03; P2N reference numbers 2021-036, 2022-048).

Informed Consent Statement: All participants provided written informed consent.

Data Availability Statement: Data described in the article, code book, and analytic code will be made available upon reasonable request.

Acknowledgments: We appreciate the voluntary contribution of all study participants. We also thank the staff of the Institute of Epidemiology for excellent technical assistance and taking care of the patients.

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

Abbreviations

BMI	Body mass index
CI	Confidence interval
CRP	C-reactive protein
Cu	Copper
DEXA	Dual-energy X-ray absorptiometry
FFQ	Food frequency questionnaire
HDL	High-density lipoprotein cholesterol
LDL	Low-density lipoprotein cholesterol
LSI	Liver signal intensity
MRI	Magnet resonance imaging
NAFLD	Non-alcoholic fatty liver disease
NHANES	National Health and Nutrition Examination Survey
SAFs	Saturated fatty acids
SAT	Subcutaneous adipose tissue
TG	Triglycerides
SD	Standard deviation
VAT	Visceral adipose tissue

WC	Waist circumference
WHR	Waist-to-hip ratio
Zn	Zinc

References

- Stefanidou, M.; Maravelias, C.; Dona, A.; Spiliopoulou, C. Zinc: A multipurpose trace element. *Arch. Toxicol.* **2006**, *80*, 1–9. [\[CrossRef\]](#) [\[PubMed\]](#)
- Livingstone, C. Zinc: Physiology, deficiency, and parenteral nutrition. *Nutr. Clin. Pract.* **2015**, *30*, 371–382. [\[CrossRef\]](#)
- Huang, X.; Jiang, D.; Zhu, Y.; Fang, Z.; Che, L.; Lin, Y.; Xu, S.; Li, J.; Huang, C.; Zou, Y.; et al. Chronic High Dose Zinc Supplementation Induces Visceral Adipose Tissue Hypertrophy without Altering Body Weight in Mice. *Nutrients* **2017**, *9*, 1138. [\[CrossRef\]](#) [\[PubMed\]](#)
- Li, X.; Guan, Y.; Shi, X.; Ding, H.; Song, Y.; Li, C.; Liu, R.; Liu, G. Effects of high zinc levels on the lipid synthesis in rat hepatocytes. *Biol. Trace Elem. Res.* **2013**, *154*, 97–102. [\[CrossRef\]](#)
- Zhang, H.B.; Wang, M.S.; Wang, Z.S.; Zhou, A.M.; Zhang, X.M.; Dong, X.W.; Peng, Q.H. Supplementation dietary zinc levels on growth performance, carcass traits, and intramuscular fat deposition in weaned piglets. *Biol. Trace Elem. Res.* **2014**, *161*, 69–77. [\[CrossRef\]](#) [\[PubMed\]](#)
- Andriollo-Sanchez, M.; Hininger-Favre, I.; Meunier, N.; Toti, E.; Zaccaria, M.; Brandolini-Bunlon, M.; Polito, A.; O'Connor, J.M.; Ferry, M.; Coudray, C.; et al. Zinc intake and status in middle-aged and older European subjects: The ZENITH study. *Eur. J. Clin. Nutr.* **2005**, *59* (Suppl. S2), S37–S41. [\[CrossRef\]](#) [\[PubMed\]](#)
- Danks, D.M. Copper deficiency in humans. *Annu. Rev. Nutr.* **1988**, *8*, 235–257. [\[CrossRef\]](#)
- Myint, Z.W.; Oo, T.H.; Thein, K.Z.; Tun, A.M.; Saeed, H. Copper deficiency anemia: Review article. *Ann. Hematol.* **2018**, *97*, 1527–1534. [\[CrossRef\]](#)
- Chen, C.; Zhou, Q.; Yang, R.; Wu, Z.; Yuan, H.; Zhang, N.; Zhi, M.; Zhang, Y.; Ni, X.; Wang, Z.; et al. Copper exposure association with prevalence of non-alcoholic fatty liver disease and insulin resistance among US adults (NHANES 2011–2014). *Ecotoxicol. Environ. Saf.* **2021**, *218*, 112295. [\[CrossRef\]](#)
- Cousins, R.J.; Ayaz, M.; Turan, B.; Grosell, M.; McGeer, J.C.; Wood, C.M.; Miller, L.V.; Krebs, N.F.; Hambidge, K.M.; Cappelli-Bigazzi, M.; et al. Absorption, transport, and hepatic metabolism of copper and zinc: Special reference to metallothionein and ceruloplasmin. *Physiol. Rev.* **1985**, *65*, 238–309. [\[CrossRef\]](#)
- Kim, H.-N.; Song, S.-W.; Choi, W.-S. Association between serum zinc level and body composition: The Korean National Health and Nutrition Examination Survey. *Nutrition* **2016**, *32*, 332–337. [\[CrossRef\]](#) [\[PubMed\]](#)
- Wu, H.; Li, Q.; Zhang, K.; Zhao, J. The association between serum copper and obesity and all-cause mortality: The NHANES 2011–2016. *Environ. Sci. Pollut. Res.* **2023**, *30*, 31395–31407. [\[CrossRef\]](#)
- Liang, J.; Chen, F.; Fang, G.; Zhang, X.; Li, Y.; Ma, B.; Lin, S.; Pan, J.; Zhang, Z. Relationship Between Plasma Copper Concentration and Body Fat Distribution in Children in China: A Cross-Sectional Study. *Biol. Trace Elem. Res.* **2020**, *198*, 430–439. [\[CrossRef\]](#) [\[PubMed\]](#)
- Hernández-Mendoza, H.; Martínez-Navarro, I.; Hernández-Ochoa, E.; Espinoza-Ruiz, M.; Lugo-Trampe, A.; Trujillo-Murillo, K.d.C.; López-García, M.A.; Rios-Lugo, M.J.; Chang-Rueda, C. Serum zinc levels are associated with obesity and low-density lipoprotein cholesterol in Mexican adults. *J. Trace Elem. Med. Biol.* **2022**, *73*, 127002. [\[CrossRef\]](#)
- Jaksic, M.; Martinovic, M.; Gligorovic-Barhanovic, N.; Vujacic, A.; Djurovic, D.; Nedovic-Vukovic, M. Association between inflammation, oxidative stress, vitamin D, copper and zinc with pre-obesity and obesity in school children from the city of Podgorica, Montenegro. *J. Pediatr. Endocrinol. Metab.* **2019**, *32*, 951–957. [\[CrossRef\]](#)
- Müller, M.J.; Braun, W.; Pourhassan, M.; Geisler, C.; Bösby-Westphal, A. Application of standards and models in body composition analysis. *Proc. Nutr. Soc.* **2016**, *75*, 181–187. [\[CrossRef\]](#) [\[PubMed\]](#)
- Nöthlings, U.; Krawczak, M. PopGen. Eine populationsbasierte Biobank mit Langzeitverfolgung der Kontrollkohorte. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* **2012**, *55*, 831–835. [\[CrossRef\]](#)
- Wanek, S.; Di Giuseppe, R.; Esatbeyoglu, T.; Plachta-Danielzik, S.; Ratjen, I.; Jacobs, G.; Nöthlings, U.; Koch, M.; Schlesinger, S.; Rimbach, G.; et al. Vitamin E (α - and γ -Tocopherol) Levels in the Community: Distribution, Clinical and Biochemical Correlates, and Association with Dietary Patterns. *Nutrients* **2017**, *10*, 3. [\[CrossRef\]](#)
- Barbaresko, J.; Siebert, S.; Koch, M.; Aits, I.; Lieb, W.; Nikolaus, S.; Laudes, M.; Jacobs, G.; Nöthlings, U. Comparison of two exploratory dietary patterns in association with the metabolic syndrome in a Northern German population. *Br. J. Nutr.* **2014**, *112*, 1364–1372. [\[CrossRef\]](#)
- Koch, M.; Borggrefe, J.; Barbaresko, J.; Groth, G.; Jacobs, G.; Siebert, S.; Lieb, W.; Müller, M.J.; Bösby-Westphal, A.; Heller, M.; et al. Dietary patterns associated with magnetic resonance imaging-determined liver fat content in a general population study. *Am. J. Clin. Nutr.* **2014**, *99*, 369–377. [\[CrossRef\]](#)
- Ainsworth, B.E.; Haskell, W.L.; Herrmann, S.D.; Meckes, N.; Bassett, D.R., Jr.; Tudor-Locke, C.; Greer, J.L.; Vezina, J.; Whitt-Glover, M.C.; Leon, A.S. 2011 Compendium of Physical Activities: A second update of codes and MET values. *Med. Sci. Sports Exerc.* **2011**, *43*, 1575–1581. [\[CrossRef\]](#)
- DIN EN ISO 17294-2: 2017-01; Water Quality—Application of Inductively Coupled Plasma Mass Spectrometry (ICP-MS)—Part 2: Determination of Selected Elements Including Uranium Isotopes (ISO 17294-2:2016). ISO: Geneva, Switzerland, 2017.

23. Weber, K.S.; Ratjen, I.; Enderle, J.; Seidel, U.; Rimbach, G.; Lieb, W. Plasma boron concentrations in the general population: A cross-sectional analysis of cardio-metabolic and dietary correlates. *Eur. J. Nutr.* **2022**, *61*, 1363–1375. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Nöthlings, U.; Hoffmann, K.; Bergmann, M.M.; Boeing, H. Fitting portion sizes in a self-administered food frequency questionnaire. *J. Nutr.* **2007**, *137*, 2781–2786. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Dehne, L.I.; Klemm, C.; Henseler, G.; Hermann-Kunz, E. The German Food Code and Nutrient Data Base (BLS II.2). *Eur. J. Epidemiol.* **1999**, *15*, 355–358. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Waniek, S.; Di Giuseppe, R.; Plachta-Danielzik, S.; Ratjen, I.; Jacobs, G.; Koch, M.; Borggrefe, J.; Both, M.; Müller, H.-P.; Kassubek, J.; et al. Association of Vitamin E Levels with Metabolic Syndrome, and MRI-Derived Body Fat Volumes and Liver Fat Content. *Nutrients* **2017**, *9*, 1143. [\[CrossRef\]](#)
27. Fischer, K.; Moewes, D.; Koch, M.; Müller, H.-P.; Jacobs, G.; Kassubek, J.; Lieb, W.; Nöthlings, U. MRI-determined total volumes of visceral and subcutaneous abdominal and trunk adipose tissue are differentially and sex-dependently associated with patterns of estimated usual nutrient intake in a northern German population. *Am. J. Clin. Nutr.* **2015**, *101*, 794–807. [\[CrossRef\]](#)
28. Müller, H.-P.; Raudies, F.; Unrath, A.; Neumann, H.; Ludolph, A.C.; Kassubek, J. Quantification of human body fat tissue percentage by MRI. *NMR Biomed.* **2011**, *24*, 17–24. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Heil, W.; Ehrhardt, V. *Referenzbereiche für Kinder und Erwachsene: Präanalytik*; Boehringer: Mannheim, Germany, 2007.
30. Jiang, S.; Ma, X.; Li, M.; Yan, S.; Zhao, H.; Pan, Y.; Wang, C.; Yao, Y.; Jin, L.; Li, B. Association between dietary mineral nutrient intake, body mass index, and waist circumference in U.S. adults using quantile regression analysis NHANES 2007–2014. *PeerJ* **2020**, *8*, e9127. [\[CrossRef\]](#)
31. Gu, K.; Xiang, W.; Zhang, Y.; Sun, K.; Jiang, X. The association between serum zinc level and overweight/obesity: A meta-analysis. *Eur. J. Nutr.* **2019**, *58*, 2971–2982. [\[CrossRef\]](#)
32. Tungtrongchitr, R.; Pongpaew, P.; Phonrat, B.; Tungtrongchitr, A.; Viroonudomphol, D.; Vudhivai, N.; Schelp, F.P. Serum copper, zinc, ceruloplasmin and superoxide dismutase in Thai overweight and obese. *J. Med. Assoc. Thai* **2003**, *86*, 543–551.
33. Kerkadi, A.; Alkudsi, D.S.; Hamad, S.; Alkeldi, H.M.; Salih, R.; Agouni, A. The Association between Zinc and Copper Circulating Levels and Cardiometabolic Risk Factors in Adults: A Study of Qatar Biobank Data. *Nutrients* **2021**, *13*, 2729. [\[CrossRef\]](#)
34. Gu, K.; Li, X.; Xiang, W.; Jiang, X. The Relationship Between Serum Copper and Overweight/Obesity: A Meta-analysis. *Biol. Trace Elem. Res.* **2020**, *194*, 336–347. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Fukasawa, H.; Niwa, H.; Ishibuchi, K.; Kaneko, M.; Iwakura, T.; Yasuda, H.; Furuya, R. The Impact of Serum Zinc Levels on Abdominal Fat Mass in Hemodialysis Patients. *Nutrients* **2020**, *12*, 656. [\[CrossRef\]](#) [\[PubMed\]](#)
36. García, O.P.; Ronquillo, D.; Caamaño, M.d.C.; Camacho, M.; Long, K.Z.; Rosado, J.L. Zinc, vitamin A, and vitamin C status are associated with leptin concentrations and obesity in Mexican women: Results from a cross-sectional study. *Nutr. Metab.* **2012**, *9*, 59. [\[CrossRef\]](#)
37. Ghosh, C.; Yang, S.H.; Kim, J.G.; Jeon, T.-I.; Yoon, B.H.; Lee, J.Y.; Lee, E.Y.; Choi, S.G.; Hwang, S.G. Zinc-chelated Vitamin C Stimulates Adipogenesis of 3T3-L1 Cells. *Asian-Australas. J. Anim. Sci.* **2013**, *26*, 1189–1196. [\[CrossRef\]](#)
38. Chen, M.-D.; Lin, P.-Y.; Cheng, V.; Lin, W.-H. Zinc supplementation aggravates body fat accumulation in genetically obese mice and dietary-obese mice. *Biol. Trace Elem. Res.* **1996**, *52*, 125–132. [\[CrossRef\]](#)
39. Santoro, A.; McGraw, T.E.; Kahn, B.B. Insulin action in adipocytes, adipose remodeling, and systemic effects. *Cell Metab.* **2021**, *33*, 748–757. [\[CrossRef\]](#)
40. Smidt, K.; Pedersen, S.B.; Brock, B.; Schmitz, O.; Fisker, S.; Bendix, J.; Wogensens, L.; Rungby, J. Zinc-transporter genes in human visceral and subcutaneous adipocytes: Lean versus obese. *Mol. Cell. Endocrinol.* **2007**, *264*, 68–73. [\[CrossRef\]](#)
41. Yazihan, N.; Akdas, S. Serum zinc level and dietary zinc intake status in non-alcoholic fatty liver disease: A meta-analysis and systematic review. *Hepatol. Forum* **2020**, *1*, 59–67. [\[CrossRef\]](#)
42. Antonucci, L.; Porcu, C.; Iannucci, G.; Balsano, C.; Barbaro, B. Non-Alcoholic Fatty Liver Disease and Nutritional Implications: Special Focus on Copper. *Nutrients* **2017**, *9*, 1137. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Wang, Y.; Jia, X.-F.; Zhang, B.; Wang, Z.-H.; Zhang, J.-G.; Huang, F.-F.; Su, C.; Ouyang, Y.-F.; Zhao, J.; Du, W.-W.; et al. Dietary Zinc Intake and Its Association with Metabolic Syndrome Indicators among Chinese Adults: An Analysis of the China Nutritional Transition Cohort Survey 2015. *Nutrients* **2018**, *10*, 572. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Kim, M.-H.; Choi, M.-K. Seven dietary minerals (Ca, P, Mg, Fe, Zn, Cu, and Mn) and their relationship with blood pressure and blood lipids in healthy adults with self-selected diet. *Biol. Trace Elem. Res.* **2013**, *153*, 69–75. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Neggers, Y.H.; Bindon, J.R.; Dressler, W.W. The relationship between zinc and copper status and lipid levels in African-Americans. *Biol. Trace Elem. Res.* **2001**, *79*, 1–13. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Ma, J.; Xie, Y.; Zhou, Y.; Wang, D.; Cao, L.; Zhou, M.; Wang, X.; Wang, B.; Chen, W. Urinary copper, systemic inflammation, and blood lipid profiles: Wuhan-Zhuhai cohort study. *Environ. Pollut.* **2020**, *267*, 115647. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Tiber, A.M.; Sakhaei, M.; Joffe, C.D.; Ratnaparkhi, M.V. Relative value of plasma copper, zinc, lipids and lipoproteins as markers for coronary artery disease. *Atherosclerosis* **1986**, *62*, 105–110. [\[CrossRef\]](#)
48. Hininger-Favier, I.; Andriollo-Sanchez, M.; Arnaud, J.; Meunier, N.; Bord, S.; Graham, C.; Polito, A.; Maiani, G.; O'Connor, J.M.; Coudray, C.; et al. Age- and sex-dependent effects of long-term zinc supplementation on essential trace element status and lipid metabolism in European subjects: The Zenith Study. *Br. J. Nutr.* **2007**, *97*, 569–578. [\[CrossRef\]](#)

49. Nazem, M.R.; Asadi, M.; Jabbari, N.; Allameh, A. Effects of zinc supplementation on superoxide dismutase activity and gene expression, and metabolic parameters in overweight type 2 diabetes patients: A randomized, double-blind, controlled trial. *Clin. Biochem.* **2019**, *69*, 15–20. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Ranasinghe, P.; Wathurapatha, W.S.; Galappatthy, P.; Katulanda, P.; Jayawardena, R.; Constantine, G.R. Zinc supplementation in prediabetes: A randomized double-blind placebo-controlled clinical trial. *J. Diabetes* **2018**, *10*, 386–397. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Wang, S.; Wang, N.; Pan, D.; Zhang, H.; Sun, G. Effects of Copper Supplementation on Blood Lipid Level: A Systematic Review and a Meta-Analysis on Randomized Clinical Trials. *Biol. Trace Elem. Res.* **2021**, *199*, 2851–2857. [\[CrossRef\]](#)
52. Deutsche Gesellschaft für Ernährung. *Referenzwerte für die Nährstoffzufuhr*, 2nd ed.; Deutsche Gesellschaft für Ernährung: Bonn, Germany, 2015.
53. Biesalski, H.-K. (Ed.) *Ernährungsmedizin: Nach dem Curriculum Ernährungsmedizin der Bundesärztekammer*, 281 Tabellen, 2nd ed.; Thieme: Stuttgart, Germany, 1999.
54. Bawadi, H.; Akasheh, R.T.; Kerkadi, A.; Haydar, S.; Tayyem, R.; Shi, Z. Validity and Reproducibility of a Food Frequency Questionnaire to Assess Macro and Micro-Nutrient Intake among a Convenience Cohort of Healthy Adult Qataris. *Nutrients* **2021**, *13*, 2002. [\[CrossRef\]](#)
55. Saravia, L.; Miguel-Berges, M.L.; Iglesia, I.; Nascimento-Ferreira, M.V.; Perdomo, G.; Bove, I.; Slater, B.; Moreno, L.A. Relative validity of FFQ to assess food items, energy, macronutrient and micronutrient intake in children and adolescents: A systematic review with meta-analysis. *Br. J. Nutr.* **2020**, *125*, 792–818. [\[CrossRef\]](#) [\[PubMed\]](#)
56. Milanino, R.; Marrella, M.; Gasperini, R.; Pasqualicchio, M.; Velo, G. Copper and zinc body levels in inflammation: An overview of the data obtained from animal and human studies. *Agents Actions* **1993**, *39*, 195–209. [\[CrossRef\]](#) [\[PubMed\]](#)
57. Malavolta, M.; Piacenza, F.; Basso, A.; Giacconi, R.; Costarelli, L.; Mocchegiani, E. Serum copper to zinc ratio: Relationship with aging and health status. *Mech. Ageing Dev.* **2015**, *151*, 93–100. [\[CrossRef\]](#)
58. Chasapis, C.T.; Ntoupa, P.-S.A.; Spiliopoulou, C.A.; Stefanidou, M.E. Recent aspects of the effects of zinc on human health. *Arch. Toxicol.* **2020**, *94*, 1443–1460. [\[CrossRef\]](#)
59. Cousins, R.J.; Leinart, A.S. Tissue-specific regulation of zinc metabolism and metallothionein genes by interleukin 1. *FASEB J.* **1988**, *2*, 2884–2890. [\[CrossRef\]](#)
60. Lowe, N.M.; Fekete, K.; Decsi, T. Methods of assessment of zinc status in humans: A systematic review. *Am. J. Clin. Nutr.* **2009**, *89*, 2040S–2051S. [\[CrossRef\]](#) [\[PubMed\]](#)
61. Harvey, L.J.; Ashton, K.; Hooper, L.; Casgrain, A.; Fairweather-Tait, S.J. Methods of assessment of copper status in humans: A systematic review. *Am. J. Clin. Nutr.* **2009**, *89*, 2009S–2024S. [\[CrossRef\]](#)
62. Ouchi, N.; Parker, J.L.; Lugus, J.J.; Walsh, K. Adipokines in inflammation and metabolic disease. *Nat. Rev. Immunol.* **2011**, *11*, 85–97. [\[CrossRef\]](#)

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

Supplementary Materials

Association of Plasma Zinc and Copper with Body Composition, Lipids and Inflammation in a Cross-Sectional General Population Sample from Germany

Cara Övermöhle ^{1,*}, Gerald Rimbach ², Sabina Waniek ¹, Eike A. Strathmann ¹, Tatjana Liedtke ¹, Paula Stürmer ¹, Marcus Both ³, Katharina S. Weber ^{1,†} and Wolfgang Lieb ^{1,†}

¹ Institute of Epidemiology, Kiel University, 24105 Kiel, Germany;

katharina.weber@epi.uni-kiel.de (K.S.W.); wolfgang.lieb@epi.uni-kiel.de (W.L.)

² Institute of Human Nutrition and Food Science, Kiel University, 24118 Kiel, Germany

³ Department of Diagnostic Radiology, University Hospital Schleswig-Holstein, 24105 Kiel, Germany

* Correspondence: cara.oevermoehle@epi.uni-kiel.de; Tel.: +49(0)431-500-30223

† These authors contributed equally to this work.

Table S1: Main characteristics of participants, separate for those individuals included and excluded in analyses in participants from the first follow-up examination (2010–2012) in the popgen-cohort from Northern Germany.

	Participants included in overall analysis (n=841)	Participants excluded from overall analysis (n=88)	<i>P</i> ¹	Participants included in analysis of adipose tissue (n=534)	Participants excluded from analysis of adipose tissue (n=395)	<i>P</i> ²	Participants included in analysis of liver fat (n=538)	Participants excluded from analysis of liver fat (n=391)	<i>P</i> ³
<i>n</i> (% female) ^{a,d}	841 (42)	87 (52)	0.077	534 (41)	394 (45)	0.318	538 (42)	390 (43)	0.772
Plasma concentration of zinc (µg/L) ^b	695.0 ± 83.1	708.1 ± 132.2	0.187	699.6 ± 85.3	691.7 ± 93.4	0.180	698.6 ± 85.6	693 ± 93.3	0.347
Plasma concentration of copper (µg/L) ^b	1020.0 ± 903.4	1143.6 ± 991.7	<0.001	1014.6 ± 899.9	1052.0 ± 920.0	<0.001	1018.6 ± 906.0	1040.6 ± 914.9	0.001
Age (years) ^b	61 ± 12	56 ± 15	0.001	61 ± 12	60 ± 14	0.072	61 ± 12	59 ± 14	0.016
Body mass index (kg/m ²) ^b	27.2 ± 4.2	30.0 ± 8.7	<0.001	27.0 ± 3.9	28.1 ± 6.0	<0.001	27.0 ± 4.0	28.1 ± 5.9	<0.001
Current smokers (yes, (%)) ^{a,e}	108 (13)	22 (29)	<0.001	46 (9)	84 (22)	<0.001	49 (9)	81 (21)	<0.001
Alcohol intake (g/day) ^{c,f}	9.0 (3.2; 18.5)	7.6 (2.1; 18.8)	0.483	9.8 (4.0; 19.2)	7.6 (2.4; 17.2)	0.006	9.6 (3.5; 19.0)	8.1 (2.8; 18.0)	0.092
Physical activity (MET-hours/week) ^{c,f}	90.5 (59.0; 131.5)	71.8 (47.1; 103.3)	0.003	90.2 (59.4; 132.1)	87.5 (56.3; 126.8)	0.169	91.4 (59.5; 131.7)	85.6 (55.3; 125.8)	0.083
Education level (low [<i><</i> 10 years], medium [10 years], high [<i>≥</i> 11 years]) ^{a,g}	292 (35) 271 (32) 278 (33)	31 (37) 31 (37) 22 (26)	0.422	162 (30) 185 (35) 187 (35)	161 (41) 117 (30) 113 (29)	0.003	163 (30) 188 (35) 187 (35)	160 (41) 114 (29) 113 (29)	0.002

Values are ^a *n* (%) (categorical variables), ^b mean ± SD (continuous normally distributed variables), ^c median (IQR) (continuous skewed variables); data only available for ^d *n*=928, ^e *n*=917, ^f *n*=923, ^g *n*=925.

¹ *P* values based on chi-square test (categorical variables), Kruskal-Wallis test (continuous skewed variables) or general linear models (continuous normally distributed variables); ² *P*-value for comparison between participants included vs. excluded from overall analysis; ³ *P*-values for comparison between participants included vs. excluded from adipose tissue analysis; ⁴ *P*-values for comparison between participants included vs. excluded from analysis of liver fat.

Abbreviations: CI, confidence interval; IQR, interquartile range; MET, metabolic equivalent of task; SD, standard deviation; T, tertile.

Table S2: Associations of plasma zinc and copper concentrations with anthropometric, metabolic and inflammatory traits in participants not taking zinc supplements (n=784).

Anthropometric, metabolic and inflammatory outcome variables	Zinc		Copper	
	Estimates (95% CI) ^a	P values	Estimates (95% CI) ^a	P values
Body mass index (kg/m ²)				
Model A1	1.56 (0.49; 2.64)	0.004	0.30 (-0.75; 1.36)	0.582
Model A2	1.35 (0.30; 2.42)	0.012	1.44 (0.20; 2.69)	0.023
Model A3	1.22 (0.18; 2.28)	0.021	1.44 (0.20; 2.69)	0.023
Model A4	1.22 (0.18; 2.28)	0.022	1.45 (0.21; 2.71)	0.022
Waist circumference (cm)				
Model A1	1.43 (0.48; 2.38)	0.003	-1.89 (-2.79; -0.99)	<0.001
Model A2	0.94 (0.11; 1.79)	0.027	1.16 (0.18; 2.16)	0.020
Model A3	0.86 (0.04; 1.70)	0.040	1.07 (0.09; 2.06)	0.033
Model A4	0.88 (0.05; 1.71)	0.038	1.10 (0.12; 2.09)	0.028
Waist-to-hip ratio				
Model A1	1.16 (0.52; 1.82)	<0.001	-2.48 (-3.08; -1.87)	<0.001
Model A2	0.69 (0.21; 1.16)	0.005	0.63 (0.07; 1.19)	0.027
Model A3	0.70 (0.23; 1.16)	0.003	0.40 (-0.15; 0.96)	0.153
Model A4	0.70 (0.24; 1.17)	0.003	0.42 (-0.14; 0.97)	0.141
Plasma triglyceride concentration (mg/dL)				
Model A1	3.36 (0.13; 6.68)	0.041	-1.51 (-4.56; 1.64)	0.343
Model A2	2.63 (-0.53; 5.88)	0.103	1.35 (-2.30; 5.13)	0.474
Model A3	1.81 (-1.21; 4.92)	0.242	-0.94 (-4.30; 2.55)	0.593
Model A4	1.73 (-1.29; 4.84)	0.265	-1.07 (-4.44; 2.42)	0.544
Plasma HDL concentration (mg/dL)				
Model A1	-2.01 (-3.82; -0.17)	0.032	7.32 (5.42; 9.25)	<0.001
Model A2	-1.37 (-3.01; 0.31)	0.110	1.32 (-0.66; 3.35)	0.193
Model A3	-0.44 (-2.00; 1.14)	0.583	2.86 (1.03; 4.73)	0.002
Model A4	-0.44 (-2.00; 1.15)	0.586	2.87 (1.03; 4.74)	0.002
Plasma LDL concentration (mg/dL)				
Model A1	3.30 (1.39; 5.24)	<0.001	3.11 (1.22; 5.04)	0.001
Model A2	3.21 (1.30; 5.15)	<0.001	3.60 (1.36; 5.89)	0.002
Model A3	3.75 (1.88; 5.66)	<0.001	2.72 (0.58; 4.90)	0.012
Model A4	3.74 (1.87; 5.64)	<0.001	2.66 (0.53; 4.84)	0.014
C-reactive protein (mg/L)				
Model A1	-6.70 (-12.84; -0.13)	0.046	38.68 (30.13; 47.79)	<0.001
Model A2	-6.91 (-12.99; -0.40)	0.038	46.98 (36.40; 58.38)	<0.001
Model A3	-8.85 (-14.25; -3.11)	0.003	37.48 (28.28; 47.35)	<0.001
Model A4	-8.91 (-14.32; -3.16)	0.003	37.47 (28.24; 47.36)	<0.001

Model A1 unadjusted. Model A2 adjusted for age and sex. Model A3 additionally adjusted for BMI (not for BMI, waist circumference and waist-to-hip ratio as exposure variable), education, smoking habits, season, lipid-lowering medication (for triglycerides, HDL and LDL as independent variables), fasting status (for triglycerides, HDL and LDL as independent variables) and physical activity and alcohol consumption. Model A4 additionally adjusted for total fat intake and intake of saturated fatty acids.

^a Regression coefficients indicates the percentage change in outcome variables per 1-SD increment in plasma zinc and copper.

Abbreviations: BMI, body mass index; CI, confidence interval; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

Table S3: Associations of plasma Zn and Cu with subcutaneous and visceral fat (n=534) and with liver signal intensity in participants not taking zinc supplements.

MRI traits as outcome variables	Zinc		Copper	
	Estimates (95% CI) ^a	P values	Estimates (95% CI) ^a	P values
Subcutaneous adipose tissue (dm ³)				
Model B1	3.58 (-0.26; 7.56)	0.068	9.43 (5.51; 13.49)	<0.001
Model B2	4.71 (0.98; 8.58)	0.013	3.22 (-1.14; 7.78)	0.150
Model B3	4.21 (0.50; 8.06)	0.026	4.23 (-0.19; 8.85)	0.061
Model B4	4.05 (0.32; 7.91)	0.033	4.14 (-0.29; 8.76)	0.067
Model B5	-0.33 (-2.28; 1.67)	0.746	0.67 (-1.66; 3.07)	0.575
Visceral adipose tissue (dm ³)				
Model B1	10.29 (5.47; 15.32)	<0.001	-12.46 (-16.17; -8.59)	<0.001
Model B2	7.31 (3.36; 11.41)	<0.001	1.52 (-2.96; 6.21)	0.512
Model B3	6.77 (2.94; 10.75)	<0.001	2.62 (-1.81; 7.25)	0.250
Model B4	6.87 (3.01; 10.87)	<0.001	2.65 (-1.79; 7.30)	0.246
Model B5	2.98 (0.37; 5.66)	0.025	-0.35 (-3.35; 2.75)	0.823
Liver signal intensity				
Model B1	7.16 (3.34; 11.11)	<0.001	-2.32 (-5.80; 1.29)	0.205
Model B2	6.60 (2.87; 10.46)	<0.001	-4.21 (-8.21; -0.04)	0.048
Model B3	4.71 (2.24; 7.24)	<0.001	-1.12 (-3.94; 1.79)	0.447
Model B4	4.64 (2.16; 7.18)	<0.001	-1.14 (-3.96; 1.77)	0.440
Model B5	3.82 (1.42; 6.29)	0.002	-1.74 (-4.47; 1.06)	0.219

Model B1 unadjusted. Model B2 adjusted for age and sex. Model B3 additionally adjusted for education, smoking habits, season, lipid-lowering medication, prevalent fatty liver disease (for liver signal intensity as outcome variable), physical activity and alcohol consumption. Model B4 additionally adjusted for total fat intake and intake of saturated fatty acids. Model B5 additionally adjusted for BMI

Subcutaneous and visceral adipose tissue and liver signal intensity entered into the models as ln-transformed variables.

^a Regression coefficients indicates the %-change in subcutaneous and visceral adipose tissue and liver signal intensity per 1-SD increment in plasma zinc and copper concentrations

Abbreviations: BMI, body mass index; CI, confidence interval; MRI, magnet resonance imaging; SD, standard deviation.

4 General discussion

The main results of our analyses in the presented papers were as follows:

1. In our elderly community-based sample, almost 20.4% of the participants had elevated plasma ferritin concentrations. By contrast, alterations in zinc and copper concentrations were less common. Elevated concentrations of circulating zinc were not observed and copper concentrations were elevated in 2.1% of the participants. As expected, deficiencies of ferritin, zinc and copper were rare (4.4%, 3.8% and 1.9%, respectively).
2. Plasma ferritin, zinc and copper concentrations were not predominantly influenced by diet. However, 13% of the variation of circulating ferritin was explained by high intake of potatoes, certain vegetables, beef, pork, processed meat, fats (frying and animal fat), beer and low intake of snacks, representing elements of a traditional German diet.
3. Plasma ferritin, zinc and copper were directly associated with anthropometric measures like BMI (ferritin, zinc and copper), WHR (zinc) and waist circumference (ferritin, zinc and copper) in multivariable-adjusted models.
4. Plasma ferritin was directly associated with C-reactive protein (CRP). Associations of plasma ferritin with anthropometric measures were attenuated and rendered statistically non-significant upon further adjustment for CRP, suggesting a mediation of the observed associations by inflammation.
5. Plasma zinc and copper were not only associated with common anthropometric measures, but also with subcutaneous (copper), visceral (zinc) and liver fat (zinc), determined by MRI examination. The associations of plasma zinc concentrations with liver fat were independent of BMI.

The subsequent discussion delves deeper into the major findings, offering a broader perspective beyond the individual paper's scope. It highlights potential mechanisms underlying the observed associations and addresses methodological aspects critical for the interpretation of the research findings.

4.1 Plasma ferritin, zinc and copper status

While ferritin and zinc status are relatively frequently assessed in different cohorts, data on copper status in larger populations are relatively scarce. Particularly for ferritin, elevated concentrations have been reported, especially in elderly people. For example, the *Copenhagen City Heart Study*, a cohort which is geographically close to our smaller cohort from Northern Germany, observed elevated plasma ferritin concentrations (≥ 200 $\mu\text{g/L}$) in 16% of the 8,988 participants [1]. This agrees well with our observations in the *popgen controls*, where we found 20.4% of participants to have elevated concentrations of ferritin, using slightly different thresholds. In contrast to the *Copenhagen City Heart Study* (≥ 200 $\mu\text{g/L}$ for both sexes) we used sex-specific thresholds based on WHO criteria (< 15 $\mu\text{g/L}$ to > 150 $\mu\text{g/L}$ in females and < 15 $\mu\text{g/L}$ to > 200 $\mu\text{g/L}$ in males). Different thresholds (serum ferritin > 300 $\mu\text{g/L}$ in men and > 200 $\mu\text{g/L}$ in women) were also applied within the original cohort of the *Framingham Heart Study* ($n = 1,016$) [2]. Within this sample, 12.9% of participants had elevated ferritin concentrations [2]. Due to the slightly higher threshold, the prevalence was expectedly lower compared to our sample, the *popgen controls*. Interestingly, the *EPIC-EurGast study* ($n = 828$) reported an even higher prevalence of elevated serum ferritin, namely 35% in males and 20% in females, based on reference values of the WHO which were also used in the *popgen controls* [3]. Taken together, even though there is variation between different studies, elevated ferritin concentrations are relatively common in the general population with reported prevalences roughly between 15% and 30%.

Depleted iron stores, defined as serum ferritin < 12 $\mu\text{g/L}$ were reported in 3% of the original cohort of the *Framingham Heart Study* [2], matching the prevalence of 4.4% (defined as plasma ferritin < 15 $\mu\text{g/L}$) within *popgen controls*. In the *EPIC-EurGast study*, prevalence of iron deficiency ranges around concentrations from *popgen controls* with 2.8% in males and 6.2% in females [3]. Thus, the concentrations measured in the *popgen controls* cohort appear to be similar to those measured in comparable cohorts. It should

be noted that iron deficiency appears to play a smaller role in westernized countries than iron excess.

Studies conducted in Italy, France and Japan have provided valuable insights into the status of zinc and copper in diverse populations. Overall, concentrations of zinc and copper were within the normal range. There were no reports of relevant deficiencies or of elevated concentrations of these essential minerals in large epidemiological studies. Therefore, we compared the mean values and standard deviations ($96.21 \pm 88.89 \mu\text{g/L}$ for zinc and $1058.23 \pm 223.68 \mu\text{g/L}$ for copper) observed in the *popgen controls* to prior studies.

For instance, among apparently healthy, free-living elderly individuals aged 70 – 85 years in Italy, serum zinc concentrations were measured at $13.50 \pm 1.93 \mu\text{mol/L}$ ($= 882.9 \pm 126.2 \mu\text{g/L}$) in men and $13.45 \pm 1.94 \mu\text{mol/L}$ ($= 879.6 \pm 126.9 \mu\text{g/L}$) in women, being higher than the concentrations in *popgen controls* [4]. The *U.VI.MAX* study in France examined individuals aged 35 – 60 years and found slightly higher concentrations of serum zinc in men ($13.5 \pm 1.8 \mu\text{mol/L} = 882.9 \pm 117.7 \mu\text{g/L}$) compared to women ($12.9 \pm 1.8 \mu\text{mol/L} = 843.7 \pm 117.7 \mu\text{g/L}$) [5]. Furthermore, a hospital-based study focusing on adults aged 40 years and above with and without metabolic syndrome revealed mean concentrations of $941.91 \pm 333.63 \mu\text{g/L}$ for serum zinc and $1043.45 \pm 306.36 \mu\text{g/L}$ for serum copper [6]. In another investigation involving free-living elderly women in Japan, serum copper concentrations were notably higher at $109 \pm 15 \mu\text{g/dL}$ ($= 1090 \pm 150 \mu\text{g/L}$), while serum zinc concentrations averaged at $78 \pm 12 \mu\text{g/dL}$ ($= 780 \pm 120 \mu\text{g/L}$) [7]. Additionally, the *Mashhad stroke and heart atherosclerotic disorders* study showed that individuals without hypertension had mean serum concentrations of $84.71 \pm 18.96 \mu\text{g/dL}$ ($= 847.1 \pm 189.6 \mu\text{g/L}$) for zinc and $103.29 \pm 36.31 \mu\text{g/dL}$ ($= 1032.9 \pm 363.1 \mu\text{g/L}$) for copper [8]. In conclusion, most prior studies reported higher circulating zinc concentrations than in the *popgen controls* cohort.

However, the copper concentrations measured in the other studies matched our results well.

Zinc and copper concentrations were mainly within the reference values. Deficiencies are rare in the general population. On the contrary, ferritin concentrations are elevated in a larger proportion (20.4%) of participants. Compared with other cohorts, our circulating ferritin concentrations were within the expected range. Our zinc concentrations seemed to be slightly lower in comparison, while the copper concentrations fit in well with other reports. All points considered, the results underline the relevance of characterizing micronutrient supply, especially in westernized cohorts and the potential causes and consequences of elevated ferritin concentrations, a relatively common finding in the general population.

4.2 Dietary correlates of plasma ferritin, zinc and copper

For plasma ferritin as proxy for iron status, we derived an exploratory dietary pattern that explained 13% of the maximum variance in circulating plasma ferritin concentrations. This pattern was characterized by high intake of potatoes, vegetables, beef, pork, processed meat, fats, beer and low intake of snacks. The pattern fits well for an elderly German cohort, since the food groups matches the traditional German cuisine (*i.e.* potatoes, meat and beer). Furthermore, our dietary pattern agrees well with findings from other studies, like the *EPIC-EurGast study* ($n = 828$), which also identified daily alcohol intake and heme iron intake (contained in animal-based foods like meat) as determinants for serum ferritin [3]. Additionally, within 634 free-living elderly participants (67 – 93 years of age) of the original cohort of the *Framingham Heart Study* similar findings were reported [9]. Alcohol and heme iron intake were major determinants of serum ferritin status [9]. However, also intake of vitamin C and iron supplementation were directly associated with serum ferritin [9]. Within our analyses, iron supplementation was considered as a confounder, while vitamin C intake and supplementation was not included.

Dietary intake of zinc and copper were not described in such detail in our analyses.

However, we observed that the dietary intake of zinc and copper did not differ according to tertiles of circulating plasma zinc, suggesting that zinc intake does not influence plasma zinc substantially. The median dietary zinc intake was 11.1 mg/day with an interquartile range of 9.4 mg/day up to 13.2 mg/day, meeting the recommendations given by the DGE quite well (11 – 16 mg/day for men and 7 – 10 mg/day for women) [10]. However, DGE recommendations are sex-specific and dependent on phytate intake. We did not differentiate between sexes and have not evaluated phytate intake, therefore, we can only compare our estimated dietary zinc intake with the recommendations of the DGE to a limited extent.

Concerning dietary copper intake, in our sample, we observed a surprisingly high copper intake (median: 2.5 mg/day; interquartile range: 2.1 to 2.9 mg/day), exceeding the DGE recommendations (1.0 – 1.5 mg/day). However, our intake values are based on estimations from the FFQ. In general, the FFQ is a fairly good tool for assessing habitual food intake, but this method is more suitable for assessing general dietary behavior than for accurately assessing the intake of individual nutrients [11]. The validity of the FFQ is examined in more detail later in the thesis (chapter 4.4.1). Thus, a median copper intake which exceeds the recommendations by almost 100% may be overestimated. Despite the surprisingly high dietary copper intake, we did not detect any noticeably high plasma copper concentrations in our cohort, which supports the hypothesis of an imprecise estimate of the dietary copper intake in our cohort by the FFQ.

All three nutrients – iron, zinc and copper – are essential nutrients in the human diet.

However, the isolated intake data of these nutrients is only suitable as an exposure variable to a limited extent, as on the one hand the estimation of the intake by means of FFQ must be viewed with caution (see chapter 4.4.1) and on the other hand the bioavailability of the nutrients is influenced by other factors, as already described in the introduction. Therefore, although the diet was recorded in the studies, it was not used as

exposure or outcome variables in the analyses. Instead, we used circulating plasma concentrations of ferritin, zinc and copper in our analyses.

4.3 Plasma ferritin, zinc and copper and body composition

Our findings of a direct association of plasma ferritin concentrations with BMI and waist circumference are in line with the results of prior publications [12–15]. Within the cross-sectional *Irish National Adult Nutrition Survey* (2008 – 2010) (n = 1,120), elevated serum ferritin was associated with excessive body fat percentage, assessed by bioelectrical impedance analysis [12]. Among 1,854 participants of the *Transition and Health during Urbanisation of South Africans* study, serum ferritin was directly associated with BMI, waist circumference and WHR, percentage body fat and subscapular skinfold thickness [13]. Not only classical anthropometric measurements appear to be associated with serum ferritin, but also hepatic fat content, visceral adipose tissue and subcutaneous adipose tissue as assessed by computer tomography were found to be directly associated with circulating concentrations of ferritin [15].

An association between circulating ferritin with parameters of obesity is also discussed as part of the dysmetabolic iron overload syndrome which is characterized by a mild increase in both liver and body iron stores, associated with components of the metabolic syndrome [12, 16]. This condition encompasses elevated ferritin concentrations, normal or moderately increased transferrin saturation and metabolic abnormalities such as increased BMI, elevated blood pressure, dyslipidemia, abnormal glucose metabolism and steatohepatitis [16]. The alteration in iron metabolism within the dysmetabolic iron overload syndrome is considered a multifactorial and dynamic process, triggered by factors like overnutrition and insulin resistance [12, 15, 16].

Also, plasma zinc and copper were directly associated with anthropometric measurements (zinc and copper) and even with subcutaneous adipose tissue (copper), visceral adipose tissue (zinc) and liver fat (zinc) in the *popgen controls* cohort.

Within the *popgen controls*, especially zinc appears to be associated with abdominal obesity. We observed consistent direct associations of zinc with MRI-determined visceral adipose tissue and liver fat that persisted in several multivariable-adjusted statistical models. Plasma concentrations of zinc were also directly associated with higher BMI, waist circumference and WHR. Zinc is an essential trace element involved in various physiological processes, including metabolism. Some studies, mainly animal studies, suggest that zinc may play a role in adipose tissue function and the regulation of body fat, which includes the storage and breakdown of fats in adipose tissue [17–20]. Zinc may influence enzymes and pathways related to lipid synthesis and storage [19]. As an illustration, a study involving a 40-day zinc supplementation period demonstrated increased expression of various factors, including fatty acid synthase (FAS), stearoyl-CoA desaturase-1 (SCD1), acetyl-CoA carboxylase 1 (ACC1), peroxisome proliferator-activated receptor gamma (PPAR γ) and sterol-regulatory element binding protein (SREBP1) in piglets [19]. Additionally, zinc may exhibit insulin-like effects [20]. Typically, insulin has the role of suppressing lipolysis in adipocytes, resulting in the accumulation of fat depots [21]. Hence, it is speculated that the insulin-like effects of zinc may also contribute to the suppression of lipolysis [21].

In addition, some observational studies in humans support our findings of direct associations of zinc with obesity parameters and also with blood lipids [22–25]. The Korean NHANES (2010) ($n = 1,896$) reported higher serum zinc to be associated with larger waist circumference in male participants [22]. Moreover, in a study involving 87 hemodialysis patients, circulating zinc concentrations were found to be directly associated with visceral adipose tissue [23]. Similarly, a Chinese observational study ($n = 12,028$) unveiled a link between higher dietary zinc intake and elevated triglyceride concentrations [24]. An intervention involving 387 participants supplementing with either 15 mg/day or 30 mg/day of zinc over a period of six months revealed that the higher dose (30 mg/day) led to an increase in low-density lipoprotein cholesterol among middle-aged individuals [25]. These diverse findings underscore the complexity of zinc's role in modulating obesity

parameters and blood lipid concentrations, warranting further investigation to elucidate the underlying mechanisms and potential implications for health outcomes.

While zinc was very consistently associated with anthropometric measurements as well as MRI-derived body fat traits, the associations for copper with these parameters were less consistent. In our sample, copper was directly associated with BMI, waist circumference and subcutaneous adipose tissue, but not with the typical parameters of abdominal obesity such as WHR and visceral adipose tissue. Interestingly, within the US NHANES 2011 – 2016 (n = 7,285), serum copper was directly associated with total body fat, with a larger effect on adipose tissue of the limbs, supporting our finding that copper was mostly associated with subcutaneous adipose tissue [26]. The study was just recently published in 2023 and the authors suggest that copper may have effects on the expression of adipokines; these postulated effects still need to be elucidated [26]. Furthermore, an excess of copper has the potential to catalyze the generation of reactive oxygen species, leading to oxidative damage and triggering an inflammatory reaction [27]. This association of copper with oxidative stress and inflammation might also contribute to the development of obesity [27]. In addition, diets rich in fat are also capable of generating reactive oxygen species [28]. It is noteworthy that a diet rich in copper usually goes along with a diet rich in fat, since copper is mainly contained in fatty foods like offal, meat (pork and beef), fish and nuts [10, 29]. In addition to these findings indicating a connection between copper and fat, there is a lack of studies on causal relationships.

Taken together, these associations of ferritin, zinc and copper with body composition need to be further investigated. Interestingly, the association of ferritin with anthropometric measurements is attenuated upon adjustment for CRP in our cohort. This potential relation between ferritin, inflammation and obesity is described in more detail in chapter **4.3.1.**

While the observed association between ferritin and anthropometry in the *popgen controls* cohort agrees well with previous reports, the published literature on the association of zinc

and copper with body composition is much more heterogeneous and in part contradictory. Heterogeneity in various aspect of the study designs, but also to the tightly regulated homeostasis of zinc and copper might contribute to this heterogeneous literature. Two paragraphs below will take a close look at these two aspects: heterogeneous study designs and their impact on research results (chapter 4.3.2) and the homeostasis in circulating zinc and copper (chapter 4.3.3).

4.3.1 *Ferritin as marker of iron status and inflammation*

Ferritin is a crucial indicator of the body's iron status and plays a central role in iron metabolism. Its primary function involves storing iron in a non-toxic and soluble form within the cells [30, 31]. This stored iron can be readily released when required by the body for various physiological processes. By ensuring controlled and non-toxic iron storage, ferritin prevents the generation of harmful free radicals that could damage cells and tissues, thereby mitigating the risks associated with iron toxicity [30, 31]. Ferritin releases iron in situations where iron is needed for processes like the synthesis of hemoglobin in red blood cells [30–32]. The concentrations of ferritin in the bloodstream serve as a valuable diagnostic marker for assessing an individual's iron status [30, 31].

Although ferritin is typically linked to iron concentrations, it is also increased as part of the acute-phase response during inflammation, even when iron concentrations remain unchanged [2, 3, 33]. In our sample, plasma ferritin was directly associated with CRP and additionally, CRP attenuated the observed associations of ferritin with anthropometric measurements, suggesting that chronic systemic inflammation might in part mediate this association (**pages 27 ff.**). In our elderly sample, at least two factors might induce and maintain chronic low-grade inflammation: aging and overweight.

Other elderly cohorts reported elevated inflammatory markers in association with circulating ferritin [2, 3]. In the *EPIC-EurGast* study (n = 828, median age 58.7, 43% females), high sensitivity CRP was directly associated with serum ferritin, even when excluding participants with high sensitivity CRP ≤ 5 mg/L [3]. The *Framingham Heart Study*

(n = 1,016, aged 67 – 96) found a 1.5-fold higher prevalence of elevated iron stores in diseased individuals, suggesting a link between circulating ferritin and inflammation [2]. However, elevated ferritin is a non-specific indicator, being increased in various inflammatory conditions, including autoimmune, inflammatory, infectious diseases and cancer [33]. Inflammatory cytokines like interferon gamma, interleukin-6 and tumor necrosis factor-alpha prompt the release of ferritin into the blood stream upon inflammatory conditions [33, 34].

Aging is widely acknowledged to be linked to chronic low-grade inflammation, commonly referred to as "inflammaging" [35, 36]. This complex phenomenon is influenced by factors such as the accumulation of cellular damage from exposure to environmental toxins, ultraviolet radiation and metabolic byproducts [35, 36]. Aging is also characterized by immunosenescence, involving a diminishing function of immune cells, leading to a less efficient response to pathogens and increased vulnerability to chronic inflammation [35, 37, 38]. Additionally, the aging process often goes along with an increase in multimorbidity, sustaining a state of chronic inflammation [39, 40].

On a parallel note, obesity is also considered a state of chronic inflammation [41, 42]. Inflammation in adipose tissue is initiated by dysfunctional adipocytes releasing inflammatory adipokines, compounded by the infiltration of immune cells from the bone marrow producing cytokines and chemokines [42]. The visceral adipose tissue, being metabolically more active than the subcutaneous adipose tissue, is of particular concern [41, 42]. Excessive visceral adipose tissue is associated with various diseases and increases *e. g.* the risk of type 2 diabetes [41].

Highlighting the significance of ferritin as an inflammation indicator, it is crucial to emphasize that a thorough evaluation of iron status and inflammation should encompass the measurement of various biomarkers and a comprehensive clinical assessment. Interpreting ferritin concentrations requires consideration within the context of the individual's overall health and medical history.

4.3.2 Heterogeneity between studies as an important contributor to conflicting results for correlates of zinc and copper

As already discussed in chapter 4.3, it was observed that direct associations of zinc and copper with body composition and blood lipids were similar to associations described in the published literature. Other studies, however, reported partially conflicting results. Hernández-Mendoza *et al.* conducted research in Mexico involving 172 participants from the general population, revealing an inverse association between serum zinc concentrations and BMI, obesity, as well as low-density lipoprotein cholesterol concentrations [43]. Similarly, Jiang *et al.* investigated a larger cohort of 19,952 individuals from the general population in the USA. Their findings demonstrated an inverse relationship between the intake of zinc and copper and both, BMI and waist circumference [44].

Looking at the partially conflicting results around zinc and copper, discussing more broadly potential causes of discrepant results in epidemiological studies is necessary.

Table 1 contains the studies discussed in the publication on zinc and copper and gives an overview of the different exposure variables, outcome variables and results. There is relevant heterogeneity between studies, including relevant differences in how zinc and copper exposure was assessed (blood concentrations, urinary excretion, dietary intake) and in the definitions of the analyzed outcome variables, especially body composition (**Table 1**). Body composition, can be assessed in various ways, ranging from anthropometric measures like BMI, waist circumference and WHR up to complex imaging methods, like e. g. dual X-ray absorptiometry, computer tomography and MRI. While dual X-ray absorptiometry and computer tomography are also very reliable measurements for body composition, MRI may still be considered the gold standard [45].

Table 1: Variations in relevant study design features between different studies, cited in this thesis.

Study	Exposure	Outcome	Results
Studies on body composition			
Kim <i>et al.</i> ; 2016 [22] Korea, general population n = 1,896	Serum zinc	BMI, waist circumference and total body fat (DEXA)	Serum zinc is directly associated with waist circumference and total body fat in men
Hernández-Mendoza <i>et al.</i> ; 2022 [43] Mexico, general population n = 172	Serum zinc	BMI, obesity, total cholesterol, HDL, LDL and triglycerides	Serum zinc was inversely associated with BMI and obesity and LDL cholesterol
Fukasawa <i>et al.</i> ; 2020 [23] Japan, hemodialysis patients n = 87	Serum zinc	Abdominal VAT and SAT (CT)	Serum zinc was directly associated with VAT and SAT
García <i>et al.</i> ; 2012[46] Mexico, adult women n = 580	Plasma zinc	BMI, waist circumference, total body fat (DEXA)	Plasma zinc higher in women with higher BMI but was not directly associated with total body fat
Jaksic <i>et al.</i> ; 2019 [47] Montenegro, children n = 202	Serum zinc and copper	Obesity (defined by BMI)	Serum zinc and copper were not elevated in children with obesity
Jiang <i>et al.</i> ; 2020 [44] USA, general population n = 19,952	Dietary zinc and copper intake (24 h recall)	BMI and waist circumference	Intake of zinc and copper was inversely associated with BMI and waist circumference
Wu <i>et al.</i> ; 2022 [26] USA, general population n = 7,285	Serum copper	BMI, waist circumference, total body fat, VAT and SAT (DEXA)	Serum copper is directly associated with total body fat
Liang <i>et al.</i> ; 2020 [27] China, children n = 454	Plasma copper	Total body fat (DEXA)	Serum copper was directly associated with total body fat and the association was mediated by CRP
Chen <i>et al.</i> ; 2021 [48] USA, general population n = 3,211	Serum copper	Prevalence of NAFLD	Serum copper is directly associated with prevalence of NAFLD
Studies on blood lipids			
Wang <i>et al.</i> ; 2018 [24] China, general population n = 12,028	Dietary zinc intake (24 h recall)	Triglycerides	Higher dietary zinc intake was directly associated with triglycerides
Kim, Choi; 2013 [49] Korea, general population n = 258	Dietary zinc intake (24 h recall)	Total cholesterol, HDL, LDL and triglycerides	Dietary intake of zinc and copper was not associated with blood lipids
Neggers <i>et al.</i> ; 2001 [50] USA, African-Americans n = 600	Serum zinc and copper	Total cholesterol, HDL, LDL and triglycerides	Serum zinc and copper were not associated with blood lipids
Ma <i>et al.</i> ; 2020 [51] China, general population n = 4,812	Urinary copper	Total cholesterol, HDL, LDL and triglycerides	Urinary copper was directly associated with triglycerides but not with the other blood lipids
Hininger-Favier <i>et al.</i> ; 2007 [25] Europe, general population n = 387	Zinc supplementation	Total cholesterol, HDL, LDL and triglycerides	Zinc supplement over 6 months raised LDL in middle-aged individuals
Nazem <i>et al.</i> ; 2019 [52] Iran, type 2 diabetes patients n = 35 (+35 controls)	Zinc supplementation	Total cholesterol, HDL, LDL and triglycerides	Zinc supplementation reduced concentrations of triglycerides and total cholesterol

Abbreviations: BMI, body mass index; DEXA, dual X-ray absorptiometry; CRP, C-reactive protein; CT, computer tomography; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; NAFLD, non-alcoholic fatty liver disease; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue

Furthermore, if methodologically a separate assessment of subcutaneous and visceral adipose tissue is possible (by e. g. MRI), these two fat depots should be considered as distinct outcome variables, since they might have different health implications. For example, visceral fat is more metabolically active and considered the major driving force in adipose tissue inflammation and subsequent unfavorable changes in metabolism [41, 42]. Most studies mentioned in **Table 1** did not differentiate between different types of adipose tissue. Additionally, liver fat was not analyzed in these studies; only one observational study assessed the prevalence of non-alcoholic fatty liver disease as a proxy [48].

Therefore, the validation of our observations regarding the correlates of zinc and copper (**page 40 ff.**) in a similarly designed cohort using the same measurement methods would be desirable.

4.3.3 Homeostasis of zinc and copper

A lack of consistency in the described associations of zinc and copper with various traits of cardiometabolic risk and body composition may be promoted by the tight homeostasis of both trace elements, leading to only small variations that may not have clinical implications. Homeostasis of zinc and copper in humans is a carefully regulated process that ensures the balance of these essential trace elements within the body [53]. Both zinc and copper play crucial roles in various physiological functions and therefore their concentrations are tightly controlled to maintain optimal health [53].

Zinc homeostasis is primarily acquired within the gastrointestinal tract through absorption and excretion into the feces [53]. A study from 1978 in rats illustrates the highly adaptive mechanisms of zinc absorption. When rats were fed a diet almost zinc-free, absorption rates were estimated to be nearly 100% [54]. When the daily diet was shifted to a zinc-containing diet, absorption rates dropped to approximately 55% [54]. Simultaneously, the rate in zinc excretion via feces, increased along the amount of zinc contained in the diet [54]. Interestingly, while the excretion of zinc immediately responds to changes in zinc

intake, the absorption rates appear to respond slower but more effective [53]. However, absorption and excretion cannot be measured directly in humans and therefore, all estimations are based on theoretical models [53].

Later on, several mammalian zinc transporters were identified, including ZnT (zinc transporter) proteins and ZIP (Zrt/Irt-like protein) [55, 56]. Zinc transporters of the ZnT family reduce intracellular zinc concentrations by transporting zinc from the cytoplasm to the lumen of organelles or the extracellular space [55]. The ZIP family plays a role in elevating intracellular zinc concentrations either by transporting the metal from the extracellular space or organellar lumen into the cytoplasm [55]. Once absorbed, zinc is distributed throughout the body, concentrating in tissues such as the liver, pancreas and muscles [53, 55–57]. Intracellular storage occurs in metallothionein, a protein with a high affinity which can bind up to seven zinc atoms [56].

Similarly, copper is obtained from the diet and its absorption in the small intestine is facilitated by proteins such as CTR1 (copper transporter 1), while for example ATP7A (ATPase copper transporting alpha) is responsible for the efflux of copper in the portal blood stream [58]. After being absorbed in the intestines, 75% of copper in the portal circulation is assimilated by the liver, with the remaining 25% entering the peripheral circulation primarily bound to albumin [59–61]. Within the liver, 20% of the assimilated copper is subsequently re-excreted into the gastrointestinal tract, while 80% is transported to the periphery bound to ceruloplasmin [59, 61]. Considering copper concentrations in various gastrointestinal fluids, it has been estimated that around 2.5 mg of copper are eliminated daily through biliary flow [59, 60, 62]. Additionally, a comparable amount is excreted through other gastrointestinal secretions, including saliva, gastric juice, pancreatic secretion and intestinal fluid secretion [59, 62].

In summary, the homeostasis of zinc and copper involves processes of absorption, distribution, storage and excretion. Conjointly, these mechanisms play vital roles in maintaining a balance for optimal physiological function. This homeostasis may only allow

for small differences in plasma zinc and copper concentrations which means that strong effects on adiposity parameters may be harder to find within the homeostatic range, promoting conflicting results within the literature.

4.4 Methodological considerations and potential biases

Both research projects included in this thesis are conducted using cross-sectional approaches. This methodology allows for a snapshot of the *popgen controls* cohort at a specific moment, enabling the assessment of prevalent conditions and their associated factors. It is valuable for generating hypotheses and exploring associations between exposures and outcomes.

However, one important limitation of this study design lies in the inability to establish a cause-and-effect relationship due to the absence of a temporal sequence between exposure and outcome. Cross-sectional analyses focus on prevalence rather than incidence, making them less suitable for studying developmental changes over time. Beyond the cross-sectional design, the use of an FFQ to assess dietary intake and potential systematic error (biases) in the sample selection as well as in the applied measuring methods warrant consideration.

4.4.1 *Validity and limitations of food frequency questionnaires*

The FFQ is a common tool for assessing dietary intake in epidemiological studies [11]. Because of its limited cost and good time efficiency, it is suitable for large scale epidemiological studies [63]. In general, it is important in nutrition studies to find a good balance between the effort and benefit of different survey methods. Potential alternatives to an FFQ are 24 h recalls and weighing protocols or a combination of different methods (see below). A weighing protocol over several days or the use of double-labeled water are considered the most accurate methods for recording people's diets [11]. However, these methods are either very time-consuming and/or cost-intensive. In order to estimate dietary intake over a longer period of time, they would have to be repeated more frequently, which is generally impractical in large studies due to the high number of participants

(several hundreds or even thousands). In contrast, the FFQ assesses the usual diet over a defined period in the past and provides a sufficiently accurate overview of dietary exposure in epidemiological studies [11]. The barrier to participation in such a survey is significantly lower than with a weighing protocol. However, in the FFQ, specific statements on nutrient intake can only be made with greater uncertainty than in weighing protocols, for example [11].

Furthermore, FFQs are prone to significant error, encompassing both systematic and random inaccuracies [63]. One major issue is underreporting. On the one hand, underreporting may be linked to psychological aspects like social desirability [11, 63], but on the other hand, it may happen due to difficulties in remembering (recall bias, see below in chapter 4.4.2) [63]. Data from the *Observing Protein and Energy Nutrition* (OPEN) study, conducted between September 1999 and March 2000, indicate that as intake concentrations increase, underreporting tends to increase [63]. This suggests that accurately reporting consumption becomes more challenging with higher intake, possibly due to the difficulty in recalling a wider range of foods or larger portion sizes, or societal pressure to consume less [63]. Unfortunately, this is a general problem with nutrition surveys. In addition, it is repeatedly discussed whether FFQs are too imprecise as a survey method to accurately determine intake of individual nutrients.

For macro- and micronutrients, the correlation coefficient between FFQ and weighing protocols typically is 0.5 to 0.7, which is moderately high and equals the correlation coefficient between measurements of physical activity using questionnaires and actual physical activity in epidemiological studies [11]. For example, for iron, the UK arm of the EPIC study described a fairly good agreement between the intake derived from the weighing protocol and from the FFQ (no significant differences in intake, Spearman Correlation Coefficient = 0.43) [64]. Concerning data analysis, adjusting for total energy intake improves the validity of an FFQ [11], which has been done in both our research projects included in this thesis.

More recently, a combination of different dietary assessment tools has been used to increase validity and accuracy. In the UK biobank [65] and the German National Cohort [66], both landmark epidemiological studies, a combination of FFQ with 24 h recalls at different time points has been used [65, 66]. By combining both, not only habitual intake can be captured, but also seasonal fluctuations [65, 66].

Even though FFQ derived data appear to be fairly robust, neither of our projects focused on the estimated daily intake of iron, zinc and copper as exposure variable. We rather used circulating plasma concentrations of ferritin, zinc and copper as primary variables of interest to avoid discrepancies due to potentially inaccurate estimates of the FFQ. The intake of iron and zinc supplements, respectively, was adjusted for in the analyses.

4.4.2 *Potential biases*

Biases are systematic errors that occur in all epidemiological studies to a certain extent. The main form of systematic error are selection bias, information bias and (residual) confounding [67, 68].

Selection bias occurs when individuals in the selected study sample are not a true representation of the target population, which can result in an overestimation or underestimation of the true association between exposures and outcomes [67, 68]. This can affect the ability to generalize the study findings to the wider population [67, 68]. A certain form of selection bias has likely occurred in the initial establishment of *popgen controls*, as detailed below. Initially, out of approximately 23,000 individuals who were invited to participate and provide a blood sample, a total of 4,267 individuals consented to participate in the control population (18%). However, only 747 of these 4,267 individuals participated in the full examination program and were available for re-contact (20%) [69]. Even though we cannot quantify this bias, it is very likely that those who participated were systematically different from all invited participants. A second selection bias might have occurred when blood donors were purposely included in order to generate a counterbalance to the predominantly elderly persons who registered after random contact

via the residents' registration office sample [69]. A third selection bias has likely occurred in the context of the MRI-subsample. Participation in the MRI examination was offered to everyone in the sample, but only around 60% attended the MRI examination. Those attending the MRI examination were on average leaner, were less likely smokers and had a higher education than the overall sample (**page 27 ff.**). The described biases were unavoidable due to the voluntary nature of the study.

Information bias arises from systematic errors in the collection of the data or in the measurements used to characterize the participants [11, 67, 68]. One type of information bias is called recall bias [67, 68]. A recall bias may have occurred in the measurement of habitual diet and physical activity. Participants may not remember past exposures correctly, leading to errors. Another type of information bias is reporting bias [67, 68], which may also lead to wrong measurements of exposure. Participants may provide socially desirable responses or misreport certain behaviors [11]. This is a typical source of error in nutritional science [11]. To some extent this error is controlled by the exclusion of participants with implausible energy intake (**page 27 ff.**). Importantly, we believe that – if recall bias and reporting bias have occurred – it is very likely that these misclassifications of diet and physical activity were non-differential, which means that these errors did not differ by plasma ferritin, zinc and copper concentrations.

The third important source of error is confounding [67, 68]. Confounding arises when a third factor is linked to both, the exposure and the outcome, causing a distorted or misleading association [67, 68]. It is crucial to control for confounding variables in epidemiological studies to accurately isolate the true association of the exposure with the outcome. Adjustments were made for potential confounders in the included projects. The selection of confounders was based on the literature, in particular on earlier publications on data from the *popgen controls*. However, it cannot be ruled out that further confounders exist that were not considered in the analyses so that a certain residual

confounding remains. Nevertheless, the most important confounding factors were included in our statistical models.

These potential sources of error as mentioned above are often unavoidable and must therefore generally be considered when interpreting epidemiological data. In the research projects presented here, possible errors were disclosed and considered in the data analysis to the extent possible.

4.5 Future research

Our cross-sectional studies on the dietary, metabolic and anthropometric correlates of ferritin, zinc and copper form a starting point for further analyses, in particular for studies with a longitudinal design. It would be interesting to see whether and how the population status of ferritin, zinc and copper changes over time and which are the correlates of such a change. On a parallel note, it would be interesting to analyze the relation of baseline ferritin, zinc and copper concentrations with changes in body composition. Furthermore, it could be promising to carry out similar analyses in other cohorts in order to confirm the reproducibility of the results. This could involve cohorts with a similar age structure and geographical location, but also cohorts with other structures in order to see whether and to what extent our results can be generalized.

4.6 Conclusion

As expected, our elderly sample from Northern Germany had relatively normal concentrations of plasma ferritin, zinc and copper in the vast majority of participants. Pronounced deficiencies of iron (as assessed by low ferritin concentrations), zinc and copper were rare (4.4%, 3.8% and 1.9%, respectively). However, elevated concentrations of ferritin were observed in approximately 20% of our participants. Diet did not exert a predominant influence on plasma concentrations of ferritin, zinc and copper. However, high intake of potatoes, specific vegetables, beef, pork, processed meat, fats (from frying and animal sources) and beer, along with low consumption of snacks, which are characteristic elements of the traditional German diet, explain 13% of the variance in

circulating ferritin concentrations. Dietary zinc intake approximately met the DGE recommendation, while copper intake exceeded these recommendations without leading to considerably high prevalence of elevated copper concentrations (2.1%).

Furthermore, ferritin, zinc and copper were associated with obesity parameters. Zinc and copper were not only associated with classical anthropometric measurements (such as BMI, WHR or waist circumference), but also with MRI measurements of visceral, subcutaneous and liver fat.

The associations of ferritin with an increased BMI were rendered statistically non-significant upon adjustment for CRP, suggesting that low-grade inflammation might have a role in mediating this association. Finally, the associations of zinc and copper with subcutaneous and visceral adipose tissue and with liver fat, were very consistent within the *popgen controls* cohort. Especially zinc and copper should be further investigated for potential adipogenic effects in cohorts and possibly intervention studies.

4.7 References

1. Ellervik C, Marott JL, Tybjærg-Hansen A, Schnohr P, Nordestgaard BG. Total and cause-specific mortality by moderately and markedly increased ferritin concentrations: general population study and metaanalysis. *Clin Chem*. 2014;60:1419–28. doi:10.1373/clinchem.2014.229013.
2. Fleming DJ, Jacques PF, Tucker KL, Massaro JM, D'Agostino RB, Wilson PW, Wood RJ. Iron status of the free-living, elderly Framingham Heart Study cohort: an iron-replete population with a high prevalence of elevated iron stores. *The American Journal of Clinical Nutrition*. 2001;73:638–46. doi:10.1093/ajcn/73.3.638.
3. Iglesias-Vázquez L, Arijia V, Aranda N, Aglago EK, Cross AJ, Schulze MB, et al. Factors associated with serum ferritin levels and iron excess: results from the EPIC-EurGast study. *Eur J Nutr* 2021. doi:10.1007/s00394-021-02625-w.
4. Finamore A, Devirgiliis C, Panno D, D'Aquino M, Polito A, Venneria E, et al. Immune response in relation to zinc status, sex and antioxidant defence in Italian elderly population: the ZENITH study. *Eur J Clin Nutr*. 2005;59 Suppl 2:S68-72. doi:10.1038/sj.ejcn.1602302.
5. Arnaud J, Touvier M, Galan P, Andriollo-Sanchez M, Ruffieux D, Roussel A-M, et al. Determinants of serum zinc concentrations in a population of French middle-age subjects (SU.VI.MAX cohort). *Eur J Clin Nutr*. 2010;64:1057–64. doi:10.1038/ejcn.2010.118.
6. Lu C-W, Lee Y-C, Kuo C-S, Chiang C-H, Chang H-H, Huang K-C. Association of Serum Levels of Zinc, Copper, and Iron with Risk of Metabolic Syndrome. *Nutrients* 2021. doi:10.3390/nu13020548.
7. Tsuboi A, Terazawa Watanabe M, Kazumi T, Fukuo K. Serum copper, zinc and risk factors for cardiovascular disease in community-living Japanese elderly women. *Asia Pac J Clin Nutr*. 2014;23:239–45. doi:10.6133/apjcn.2014.23.2.04.
8. Darroudi S, Saberi-Karimian M, Tayefi M, Tayefi B, Khashyarmanesh Z, Fereydouni N, et al. Association Between Hypertension in Healthy Participants and Zinc and

- Copper Status: a Population-Based Study. *Biol Trace Elem Res*. 2019;190:38–44. doi:10.1007/s12011-018-1518-4.
9. Fleming DJ, Jacques PF, Dallal GE, Tucker KL, Wilson PW, Wood RJ. Dietary determinants of iron stores in a free-living elderly population: The Framingham Heart Study. *The American Journal of Clinical Nutrition*. 1998;67:722–33. doi:10.1093/ajcn/67.4.722.
 10. Deutsche Gesellschaft für Ernährung. Referenzwerte für die Nährstoffzufuhr. 2nd ed. Bonn: Deutsche Gesellschaft für Ernährung; 2016.
 11. Willett W. *Nutritional epidemiology*. 3rd ed. New York: Oxford University Press; 2013.
 12. Moore Heslin A, O'Donnell A, Buffini M, Nugent AP, Walton J, Flynn A, McNulty BA. Risk of Iron Overload in Obesity and Implications in Metabolic Health. *Nutrients* 2021. doi:10.3390/nu13051539.
 13. Aderibigbe OR, Pisa PT, Mamabolo RL, Kruger HS, Vorster HH. The relationship between indices of iron status and selected anthropometric cardiovascular disease risk markers in an African population: the THUSA study. *Cardiovasc J Afr*. 2011;22:249–56. doi:10.5830/CVJA-2011-015.
 14. Alam F, Memon AS, Fatima SS. Increased Body Mass Index may lead to Hyperferritinemia Irrespective of Body Iron Stores. *Pak J Med Sci*. 2015;31:1521–6. doi:10.12669/pjms.316.7724.
 15. Iwasaki T, Nakajima A, Yoneda M, Yamada Y, Mukasa K, Fujita K, et al. Serum ferritin is associated with visceral fat area and subcutaneous fat area. *Diabetes Care*. 2005;28:2486–91. doi:10.2337/diacare.28.10.2486.
 16. Deugnier Y, Bardou-Jacquet É, Lainé F. Dysmetabolic iron overload syndrome (DIOS). *Presse Med*. 2017;46:e306-e311. doi:10.1016/j.lpm.2017.05.036.
 17. Ghosh C, Yang SH, Kim JG, Jeon T-I, Yoon BH, Lee JY, et al. Zinc-chelated Vitamin C Stimulates Adipogenesis of 3T3-L1 Cells. *Asian-Australas J Anim Sci*. 2013;26:1189–96. doi:10.5713/ajas.2013.13179.

18. Huang X, Jiang D, Zhu Y, Fang Z, Che L, Lin Y, et al. Chronic High Dose Zinc Supplementation Induces Visceral Adipose Tissue Hypertrophy without Altering Body Weight in Mice. *Nutrients* 2017. doi:10.3390/nu9101138.
19. Zhang HB, Wang MS, Wang ZS, Zhou AM, Zhang XM, Dong XW, Peng QH. Supplementation dietary zinc levels on growth performance, carcass traits, and intramuscular fat deposition in weaned piglets. *Biol Trace Elem Res.* 2014;161:69–77. doi:10.1007/s12011-014-0078-5.
20. Chen MD, Lin PY, Cheng V, Lin WH. Zinc supplementation aggravates body fat accumulation in genetically obese mice and dietary-obese mice. *Biol Trace Elem Res.* 1996;52:125–32. doi:10.1007/BF02789454.
21. Santoro A, McGraw TE, Kahn BB. Insulin action in adipocytes, adipose remodeling, and systemic effects. *Cell Metab.* 2021;33:748–57. doi:10.1016/j.cmet.2021.03.019.
22. Kim H-N, Song S-W, Choi W-S. Association between serum zinc level and body composition: The Korean National Health and Nutrition Examination Survey. *Nutrition.* 2016;32:332–7. doi:10.1016/j.nut.2015.09.006.
23. Fukasawa H, Niwa H, Ishibuchi K, Kaneko M, Iwakura T, Yasuda H, Furuya R. The Impact of Serum Zinc Levels on Abdominal Fat Mass in Hemodialysis Patients. *Nutrients* 2020. doi:10.3390/nu12030656.
24. Wang Y, Jia X-F, Zhang B, Wang Z-H, Zhang J-G, Huang F-F, et al. Dietary Zinc Intake and Its Association with Metabolic Syndrome Indicators among Chinese Adults: An Analysis of the China Nutritional Transition Cohort Survey 2015. *Nutrients.* 2018;10:572. doi:10.3390/nu10050572.
25. Hininger-Favier I, Andriollo-Sanchez M, Arnaud J, Meunier N, Bord S, Graham C, et al. Age- and sex-dependent effects of long-term zinc supplementation on essential trace element status and lipid metabolism in European subjects: the Zenith Study. *Br J Nutr.* 2007;97:569–78. doi:10.1017/S0007114507432974.

26. Wu H, Li Q, Zhang K, Zhao J. The association between serum copper and obesity and all-cause mortality: the NHANES 2011-2016. *Environ Sci Pollut Res*. 2023;30:31395–407. doi:10.1007/s11356-022-24432-4.
27. Liang J, Chen F, Fang G, Zhang X, Li Y, Ma B, et al. Relationship Between Plasma Copper Concentration and Body Fat Distribution in Children in China: a Cross-Sectional Study. *Biol Trace Elem Res*. 2020;198:430–9. doi:10.1007/s12011-020-02105-y.
28. Fernández-Sánchez A, Madrigal-Santillán E, Bautista M, Esquivel-Soto J, Morales-González A, Esquivel-Chirino C, et al. Inflammation, oxidative stress, and obesity. *Int J Mol Sci*. 2011;12:3117–32. doi:10.3390/ijms12053117.
29. Schek A. Ernährungslehre kompakt: Kompendium der Ernährungslehre für Studierende der Ernährungswissenschaft, Medizin und Naturwissenschaften und zur Ausbildung von Ernährungsfachkräften. 5th ed. Sulzbach im Taunus: Umschau Zeitschriftenverlag; 2013.
30. Andrews NC. Disorders of iron metabolism. *N Engl J Med*. 1999;341:1986–95. doi:10.1056/NEJM199912233412607.
31. World Health Organization. WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations. Geneva, Switzerland: World Health Organization; 2020.
32. Beard JL. Iron biology in immune function, muscle metabolism and neuronal functioning. *The Journal of Nutrition*. 2001;131:568S-579S; discussion 580S. doi:10.1093/jn/131.2.568S.
33. Mahroum N, Alghory A, Kiyak Z, Alwani A, Seida R, Alrais M, Shoenfeld Y. Ferritin - from iron, through inflammation and autoimmunity, to COVID-19. *J Autoimmun*. 2022;126:102778. doi:10.1016/j.jaut.2021.102778.
34. Fahmy M, Young SP. Modulation of iron metabolism in monocyte cell line U937 by inflammatory cytokines: changes in transferrin uptake, iron handling and ferritin mRNA. *Biochem J*. 1993;296 (Pt 1):175–81. doi:10.1042/bj2960175.

35. Franceschi C, Bonafè M, Valensin S, Olivieri F, Luca M de, Ottaviani E, Benedictis G de. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci.* 2000;908:244–54. doi:10.1111/j.1749-6632.2000.tb06651.x.
36. Franceschi C, Garagnani P, Parini P, Giuliani C, Santoro A. Inflammaging: a new immune-metabolic viewpoint for age-related diseases. *Nat Rev Endocrinol.* 2018;14:576–90. doi:10.1038/s41574-018-0059-4.
37. Barbé-Tuana F, Funchal G, Schmitz CRR, Maurmann RM, Bauer ME. The interplay between immunosenescence and age-related diseases. *Semin Immunopathol.* 2020;42:545–57. doi:10.1007/s00281-020-00806-z.
38. Cisneros B, García-Aguirre I, Unzueta J, Arrieta-Cruz I, González-Morales O, Domínguez-Larrieta JM, et al. Immune system modulation in aging: Molecular mechanisms and therapeutic targets. *Front Immunol.* 2022;13:1059173. doi:10.3389/fimmu.2022.1059173.
39. Wawer AA, Jennings A, Fairweather-Tait SJ. Iron status in the elderly: A review of recent evidence. *Mech Ageing Dev.* 2018;175:55–73. doi:10.1016/j.mad.2018.07.003.
40. Fairweather-Tait SJ, Wawer AA, Gillings R, Jennings A, Myint PK. Iron status in the elderly. *Mech Ageing Dev.* 2014;136-137:22–8. doi:10.1016/j.mad.2013.11.005.
41. Kohlgruber A, Lynch L. Adipose tissue inflammation in the pathogenesis of type 2 diabetes. *Curr Diab Rep.* 2015;15:92. doi:10.1007/s11892-015-0670-x.
42. Kawai T, Autieri MV, Scalia R. Adipose tissue inflammation and metabolic dysfunction in obesity. *Am J Physiol Cell Physiol.* 2021;320:C375-C391. doi:10.1152/ajpcell.00379.2020.
43. Hernández-Mendoza H, Martínez-Navarro I, Hernández-Ochoa E, Espinoza-Ruiz M, Lugo-Trampe A, Del Trujillo-Murillo KC, et al. Serum zinc levels are associated with obesity and low-density lipoprotein cholesterol in Mexican adults. *J Trace Elem Med Biol.* 2022;73:127002. doi:10.1016/j.jtemb.2022.127002.
44. Jiang S, Ma X, Li M, Yan S, Zhao H, Pan Y, et al. Association between dietary mineral nutrient intake, body mass index, and waist circumference in U.S. adults using

- quantile regression analysis NHANES 2007-2014. *PeerJ*. 2020;8:e9127.
doi:10.7717/peerj.9127.
45. Müller MJ, Braun W, Pourhassan M, Geisler C, Bosy-Westphal A. Application of standards and models in body composition analysis. *Proceedings of the Nutrition Society*. 2016;75:181–7. doi:10.1017/S0029665115004206.
46. García OP, Ronquillo D, Del Caamaño MC, Camacho M, Long KZ, Rosado JL. Zinc, vitamin A, and vitamin C status are associated with leptin concentrations and obesity in Mexican women: results from a cross-sectional study. *Nutr Metab (Lond)*. 2012;9:59. doi:10.1186/1743-7075-9-59.
47. Jaksic M, Martinovic M, Gligorovic-Barhanovic N, Vujacic A, Djurovic D, Nedovic-Vukovic M. Association between inflammation, oxidative stress, vitamin D, copper and zinc with pre-obesity and obesity in school children from the city of Podgorica, Montenegro. *J Pediatr Endocrinol Metab*. 2019;32:951–7. doi:10.1515/jpem-2019-0086.
48. Chen C, Zhou Q, Yang R, Wu Z, Yuan H, Zhang N, et al. Copper exposure association with prevalence of non-alcoholic fatty liver disease and insulin resistance among US adults (NHANES 2011-2014). *Ecotoxicol Environ Saf*. 2021;218:112295. doi:10.1016/j.ecoenv.2021.112295.
49. Kim M-H, Choi M-K. Seven dietary minerals (Ca, P, Mg, Fe, Zn, Cu, and Mn) and their relationship with blood pressure and blood lipids in healthy adults with self-selected diet. *Biol Trace Elem Res*. 2013;153:69–75. doi:10.1007/s12011-013-9656-1.
50. Neggers YH, Bindon JR, Dressler WW. The relationship between zinc and copper status and lipid levels in African-Americans. *Biol Trace Elem Res*. 2001;79:1–13. doi:10.1385/BTER:79:1:01.
51. Ma J, Xie Y, Zhou Y, Wang D, Cao L, Zhou M, et al. Urinary copper, systemic inflammation, and blood lipid profiles: Wuhan-Zhuhai cohort study. *Environ Pollut*. 2020;267:115647. doi:10.1016/j.envpol.2020.115647.

52. Nazem MR, Asadi M, Jabbari N, Allameh A. Effects of zinc supplementation on superoxide dismutase activity and gene expression, and metabolic parameters in overweight type 2 diabetes patients: A randomized, double-blind, controlled trial. *Clin Biochem*. 2019;69:15–20. doi:10.1016/j.clinbiochem.2019.05.008.
53. King JC, Shames DM, Woodhouse LR. Zinc homeostasis in humans. *The Journal of Nutrition*. 2000;130:1360S–6S. doi:10.1093/jn/130.5.1360S.
54. Weigand E, Kirchgessner M. Homeostatic adjustments in zinc digestion to widely varying dietary zinc intake. *Nutr Metab*. 1978;22:101–12. doi:10.1159/000176203.
55. Lichten LA, Cousins RJ. Mammalian zinc transporters: nutritional and physiologic regulation. *Annu Rev Nutr*. 2009;29:153–76. doi:10.1146/annurev-nutr-033009-083312.
56. Hennigar SR, Kelley AM, McClung JP. Metallothionein and Zinc Transporter Expression in Circulating Human Blood Cells as Biomarkers of Zinc Status: a Systematic Review. *Adv Nutr*. 2016;7:735–46. doi:10.3945/an.116.012518.
57. Cousins RJ. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. *Physiological Reviews*. 1985;65:238–309. doi:10.1152/physrev.1985.65.2.238.
58. Prohaska JR. Role of copper transporters in copper homeostasis. *The American Journal of Clinical Nutrition*. 2008;88:826S–9S. doi:10.1093/ajcn/88.3.826S.
59. Bost M, Houdart S, Oberli M, Kalonji E, Huneau J-F, Margaritis I. Dietary copper and human health: Current evidence and unresolved issues. *J Trace Elem Med Biol*. 2016;35:107–15. doi:10.1016/j.jtemb.2016.02.006.
60. Luza SC, Speisky HC. Liver copper storage and transport during development: implications for cytotoxicity. *The American Journal of Clinical Nutrition*. 1996;63:812S–20S. doi:10.1093/ajcn/63.5.812.
61. Harvey LJ, Dainty JR, Hollands WJ, Bull VJ, Beattie JH, Venelinov TI, et al. Use of mathematical modeling to study copper metabolism in humans. *The American Journal of Clinical Nutrition*. 2005;81:807–13. doi:10.1093/ajcn/81.4.807.

62. Linder MC, Wooten L, Cerveza P, Cotton S, Shulze R, Lomeli N. Copper transport. *The American Journal of Clinical Nutrition*. 1998;67:965S-971S. doi:10.1093/ajcn/67.5.965S.
63. Subar AF, Kipnis V, Troiano RP, Midthune D, Schoeller DA, Bingham S, et al. Using intake biomarkers to evaluate the extent of dietary misreporting in a large sample of adults: the OPEN study. *Am J Epidemiol*. 2003;158:1–13. doi:10.1093/aje/kwg092.
64. Bingham SA, Gill C, Welch A, Cassidy A, Runswick SA, Oakes S, et al. Validation of dietary assessment methods in the UK arm of EPIC using weighed records, and 24-hour urinary nitrogen and potassium and serum vitamin C and carotenoids as biomarkers. *Int J Epidemiol*. 1997;26 Suppl 1:S137-51. doi:10.1093/ije/26.suppl_1.s137.
65. Bradbury KE, Young HJ, Guo W, Key TJ. Dietary assessment in UK Biobank: an evaluation of the performance of the touchscreen dietary questionnaire. *J Nutr Sci*. 2018;7:e6. doi:10.1017/jns.2017.66.
66. Knüppel S, Clemens M, Conrad J, Gastell S, Michels KB, Leitzmann M, et al. Design and characterization of dietary assessment in the German National Cohort. *Eur J Clin Nutr*. 2019;73:1480–91. doi:10.1038/s41430-018-0383-8.
67. Rothman KJ. *Modern epidemiology with access code*. [Place of publication not identified]: Wolters Kluwer; 2018.
68. Celentano D. *Gordis Epidemiology*: Elsevier; 2019.
69. Nöthlings U, Krawczak M. PopGen. Eine populationsbasierte Biobank mit Langzeitverfolgung der Kontrollkohorte. [PopGen. A population-based biobank with prospective follow-up of a control group]. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz*. 2012;55:831–5. doi:10.1007/s00103-012-1487-2.

5 Summary

This thesis aimed to assess the iron, zinc and copper status of *popgen controls*, an elderly cohort from Northern Germany and to investigate their relationship with dietary intake and body composition. While iron and zinc deficiencies are relatively common on a global scale, malnutrition in Europe primarily affects vulnerable groups like children, pregnant women or the elderly. However, in Germany, the population's trace element status remains poorly documented and it is largely unclear, how these trace elements relate to classic cardiometabolic risk factors and, in particular, to obesity. Understanding these relationships is crucial, given the rising global prevalence of overweight and obesity.

Within the first research project, based on data of 460 participants (66 ± 12 years, 43% females) of the second follow-up examination of *popgen controls*, plasma ferritin concentrations were determined using immunoturbidimetry. Reduced rank regression was applied to identify an exploratory dietary pattern explaining maximal variance in circulating ferritin concentrations. Cross-sectional associations between anthropometric and metabolic traits with plasma ferritin concentrations were evaluated using multivariable-adjusted linear regression analysis. Non-linear relationships were explored using restricted cubic splines regression. The exploratory dietary pattern was defined by a high consumption of potatoes, specific vegetables, beef, pork, processed meat, fats (from frying and animal sources) and beer, along with a low intake of snacks, reflecting elements of the traditional German diet, explaining 13% of variance in plasma ferritin. An increment per one standard deviation (SD) in BMI and waist circumference was associated to linear increases in plasma ferritin by 10.26% (95% confidence interval 1.96% – 19.23%) and 14.03% (4.70% – 24.18%), respectively. Additionally, age demonstrated a non-linear association with plasma ferritin concentrations ($P= 0.003$). However, after further adjustment for CRP, only the association between ferritin and age remained statistically significant.

For the second research project, data from the first follow-up examination of the *popgen controls* were used ($n = 841$, 61 ± 12 years, 42% females). Zinc and copper concentrations were measured using inductively coupled plasma-mass spectrometry. Subcutaneous and visceral adipose tissue, as well as liver fat, were quantified via MRI in 534 and 538 participants, respectively. Associations were assessed using multivariable-adjusted linear regression analysis. An increase of one SD in zinc was linked to linear increases in BMI by 1.17% (0.15% – 2.20%), waist circumference by 0.85% (0.04% – 1.67%) and WHR by 0.64% (0.18% – 1.09%). Similarly, a 1-SD increase in copper was associated with an increase in BMI by 1.64% (0.41%-2.88%) and waist circumference by 1.22% (0.25% – 2.20%), though not with WHR. Even after adjusting for fat intake, zinc exhibited direct associations with visceral adipose tissue by 5.73% (2.04% – 9.56%) and liver fat by 3.84% (1.49% – 6.25%), with the latter association remaining significant independently of BMI. Copper was directly linked to subcutaneous adipose tissue by 4.64% (0.31% – 9.15%) before BMI adjustment but did not show an association with visceral adipose tissue or liver fat.

In conclusion, elevated ferritin concentrations were linked with adherence to a traditional German dietary pattern and unfavorable anthropometric traits, with associations possibly being in part driven by inflammation. The observed relationships of zinc and copper highlight their potential role in adiposity. Further studies are needed to better understand the implications of ferritin, zinc and copper in the context of obesity amid its increasing global prevalence.

6 Zusammenfassung

Ziel dieser Doktorarbeit war es, den Eisen-, Zink- und Kupferstatus der sogenannten *popgen-Kontrollen*, einer älteren Kohorte aus Norddeutschland, zu erheben und zu bewerten und den Zusammenhang von Eisen, Zink und Kupfer mit der Ernährung und mit der Körperzusammensetzung zu untersuchen. Während Eisen- und Zinkmangel weltweit von Bedeutung sind, betrifft Mangelernährung in Europa vor allem gefährdete Gruppen wie Kinder, Schwangere und ältere Menschen. In Deutschland ist der Spurenelementstatus der Bevölkerung insgesamt schlecht dokumentiert und es ist weitgehend unklar, wie diese Spurenelemente mit klassischen kardiometabolischen Risikofaktoren und insbesondere mit Adipositas zusammenhängen. Das Verständnis dieser Zusammenhänge ist angesichts der weltweit steigenden Prävalenz von Übergewicht und Adipositas von entscheidender Bedeutung.

Im Rahmen des ersten Forschungsprojekts wurden auf der Grundlage der Daten von 460 Teilnehmenden (66 ± 12 Jahre, 43% weiblich) der zweiten Nachuntersuchung der *popgen-Kontrollen* die Ferritinwerte im Plasma mittels Immunoturbidimetrie bestimmt. Durch reduzierte Rangregression wurde ein exploratives Ernährungsmuster ermittelt, das die maximale Varianz der zirkulierenden Ferritinkonzentrationen erklärt.

Querschnittsassoziationen zwischen anthropometrischen und metabolischen Merkmalen und Plasmaferritinwerten wurden mit Hilfe einer multivariat-adjustierten linearen Regressionsanalyse untersucht. Nichtlineare Zusammenhänge wurden mittels kubischer Splines-Regression untersucht. Das explorative Ernährungsmuster war durch einen hohen Verzehr von Kartoffeln, bestimmten Gemüsesorten, Rind- und Schweinefleisch, verarbeitetem Fleisch, Fetten (Bratfett und andere Fette aus tierischen Quellen) und Bier sowie einen geringen Verzehr von Zwischenmahlzeiten definiert, was Elemente der traditionellen deutschen Ernährung sind. Das Ernährungsmuster erklärt 13% der Varianz des Plasmaferritins. Ein Anstieg des BMI und des Taillenumfangs um eine Standardabweichung war mit einem linearen Anstieg des Plasmaferritins um 10,26% (95% Konfidenzintervall 1,96% – 19,23%) bzw. 14,03% (4,70% – 24,18%) assoziiert.

Darüber hinaus zeigte sich ein nichtlinearer Zusammenhang zwischen Alter und Plasmaferritinkonzentration ($P=0,003$). Nach weiterer Anpassung für das C-reaktive Protein als Entzündungsmarker blieb jedoch nur der Zusammenhang zwischen Ferritin und Alter statistisch signifikant.

Für das zweite Forschungsprojekt wurden die Daten der ersten Nachuntersuchung der *popgen-Kontrollen* verwendet ($n = 841$, 61 ± 12 Jahre, 42% weiblich). Die Zink- und Kupferspiegel wurden mittels induktiv gekoppelter Plasma-Massenspektrometrie gemessen. Subkutanes und viszerales Fettgewebe sowie Leberfett wurden bei 534 bzw. 538 Teilnehmenden mittels Magnetresonanztomographie quantifiziert. Die Zusammenhänge wurden mit Hilfe einer multivariablen, adjustierten linearen Regressionsanalyse untersucht.

Ein Anstieg um eine Standardabweichung in Zink war mit einem linearen Anstieg des BMI um 1,17% (0,15% – 2,20%), des Taillenumfangs um 0,85% (0,04% – 1,67%) und des Taillen-Hüft-Verhältnisses um 0,64% (0,18% – 1,09%) assoziiert. In ähnlicher Weise war eine Erhöhung des Kupfers um eine Standardabweichung mit dem BMI um 1,64% (0,41% – 2,88%) und dem Taillenumfang um 1,22% (0,25% – 2,20%) assoziiert, nicht jedoch mit dem Taillen-Hüft-Verhältnis. Selbst nach Adjustierung für die Fettaufnahme zeigte Zink einen Zusammenhang mit viszeralem Fettgewebe von 5,73% (2,04% – 9,56%) und Leberfett von 3,84% (1,49% – 6,25%), wobei der letztgenannte Zusammenhang unabhängig vom BMI signifikant blieb. Kupfer stand vor der BMI-Anpassung in einem direkten Zusammenhang mit dem subkutanen Fettgewebe (4,64% (0,31% – 9,15%)), zeigte aber keinen Zusammenhang mit dem viszeralen Fettgewebe oder dem Leberfett.

Zusammenfassend wurden erhöhte Ferritinwerte mit einem traditionellen deutschen Ernährungsmuster und ungünstigen anthropometrischen Merkmalen in Verbindung gebracht, wobei die Assoziationen zum Teil vermutlich durch geringgradige Entzündungsprozesse bedingt waren. Die beobachteten Zusammenhänge von Zink und

Kupfer unterstreichen ihre potenzielle Rolle bei der Adipositas. Weitere Studien sind erforderlich, um die Bedeutung von Ferritin, Zink und Kupfer im Zusammenhang mit der weltweit zunehmenden Prävalenz von Adipositas besser zu verstehen.