Vegetarian Diets and Sports –

Nutritional Status and Exercise Performance in Recreational Runners

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Abstract

Background and aim: The proportion of vegetarian diets has increased in recent years. The beneficial effects of a lacto-ovo vegetarian diet on various diseases such as type 2 diabetes and cardiometabolic disorders are well documented. In contrast, there are also potentially critical nutrients such as vitamin B_{12}, D, and iron. The popularity of vegetarian diets is increasing in endurance sports as well. However, previous studies investigating the relationship between vegetarian diets and sports are outdated, questionnaire-based and did not include nutritional or sports medical diagnostics, had a very low sample size or included only vegetarians and omnivores. There are no significant data on vegan athletes. In addition, data on exercise-induced metabolic changes do not exist. The validity of the current knowledge is therefore low. As a result, the aim of this thesis was to study the nutrient intake, status of selected biomarkers, exercise capacity and exercise-induced changes in energy metabolism (sirtuins), oxidative stress (malondialdehyde), amino acid profile (AA profile), and nitric oxide (NO) (nitrate, nitrite).

Methods: In a cross-sectional study 81 ambitious male and female recreational runners (18-35 years, BMI 20-25 kg / m², 2-5 training sessions per week) with an omnivorous (OMN, n=27), lacto-ovo vegetarian (LOV, n=26) or vegan (VEG, n=28) diet were included in the study and were matched according to age and gender. Initially, anthropometric examinations, recording of nutrient intake (incl. supplements) based on 3-day dietary records as well as fasting blood samples to determine the status of vitamin B_{12}, D, and iron, and folic acid as well as of zinc, magnesium, and calcium, were carried out. In addition, performance diagnostics and measurement of the lactate and glucose concentration by means of an incremental step test until exhaustion took place. The short-term food intake was conducted on the day of the performance diagnostics using a 24-hours dietary recall. In addition, blood samples were taken before and after exercise to analyze exercise-induced changes in sirtuins, malondialdehyde, AA profile, nitrate, and nitrite.

Results: On average, dietary nutrient intake was adequate for all three groups, which was in part due to supplementation. Despite partially quantitatively different nutrient intake, a comparable and adequate status of vitamin B_{12}, D, and iron biomarkers could be determined. The performance diagnostics revealed no differences between the groups in maximum power output related to body weight (OMN: 4.15 ± 0.48, LOV: 4.20 ± 0.47, VEG: 4.16 ± 0.55 watt/kg BW, p=0.917) and lactate and glucose concentrations. In addition, the study showed that the groups differ in terms of oxidative stress, NO metabolism, and AA profile. Exercise induced an increase in MDA in all three groups, no significant changes in nitrate and nitrite, and comparable increases in alanine, while there were differences in AA metabolism as well.

Conclusion: Ambitious recreational runners with vegetarian diets have an adequate status even with otherwise critical nutrients and thus show a high health awareness. Especially in VEG, there seem to exist adaptational mechanisms in energy metabolism, as shown by sirtuin activity and AA metabolism. However, these only partially affect performance. Factors such as exercise and genetic aspects seem to have a greater impact on performance than avoiding meat. In addition, it can be assumed that increased antioxidant intake has no additive effects on exercise-induced oxidative stress. The study shows that both vegetarian and vegan diets are an adequate alternative for ambitious recreational athletes. Further studies are needed to examine the influence of vegetarian diets on health and physical performance.

Trial registration: German Register of Clinical Trials DRKS00012377

Key words: Vegetarian diets, nutritional status, exercise performance

Zusammenfassung

Hintergrund und Ziel: Der Anteil vegetarischer Ernährungsformen ist in den letzten Jahren gestiegen. Die günstigen Effekte einer lakto-ovo-vegetarischen Ernährung auf verschiedene Erkrankungen wie Typ-2-Diabetes und kardiometabolische Erkrankungen sind gut erforscht. Allerdings gibt es auch potentiell kritische Nährstoffe wie Vitamin B_{12}, D und Eisen. Die Popularität pflanzenbasierter Kost steigt auch im Ausdauersport. Allerdings sind bisherige Studien, welche den Zusammenhang zwischen vegetarischer Ernährung und Sport untersuchten, veraltet, fragebogenbasierend und beinhalteten keine ernährungs- oder sportmedizinische Diagnostik, umfassten eine sehr geringe Probandenzahl oder schlossen nur Vegetarier und Omnivore ein. Bei veganen Sportlern gibt es praktisch keine aussagekräftigen Daten. Auch Daten zu belastungsinduzierten metabolischen Veränderungen gibt es nicht. Die Aussagekraft der aktuellen Studienlage ist daher gering. Infolgedessen war es das Ziel dieser Arbeit, die Nährstoffaufnahme, den Status ausgewählter Biomarker, die sportliche Leistungsfähigkeit sowie belastungsinduzierte Veränderungen von Parametern des Energiemetabolismus (Sirtuine), des oxidativen Stresses (Malondialdehyd), des Aminosäureprofils (AS-Profil) sowie des Stickstoffmonoxid-(NO) Metabolismus (Nitrat, Nitrit) zu untersuchen.

Methodik: Im Rahmen einer Querschnittsstudie wurden 81 ambitionierte Freizeitläufer (18-35 Jahre, BMI 20-25 kg/m², 2-5 x Training pro Woche), welche sich omnivor (OMN, n=27), lakto-ovo-vegetarisch (LOV, n=26) oder vegan (VEG, n=28) ernährten, in die Studie aufgenommen und nach Alter und Geschlecht zugeordnet. Zunächst erfolgten anthropometrische Untersuchungen, die Erfassung der Nährstoffzufuhr (inkl. Supplemente) anhand von 3-Tage Ernährungsprotokollen sowie Nüchternblutabnahmen zur Statusbestimmung von Vitamin B_{12}, D, Eisen und Folsäure sowie Zink, Magnesium und Calcium. Außerdem fand eine Leistungsdiagnostik und Messung der Laktat- und Glukosekonzentration mittels Stufentests bis zur maximalen Ausbelastung statt. Die kurzfristige Nahrungsaufnahme wurde am Tag der Leistungsdiagnostik mittels 24h-Recall durchgeführt. Darüber hinaus erfolgten vor und nach Belastung Blutentnahmen zur Diagnostik belastungsinduzierter Veränderungen von Sirtuinen, Malondialdehyd, dem AS-Profil sowie Nitrat und Nitrit.

Ergebnisse: Die Nährstoffaufnahme war durchschnittlich bei allen drei Gruppen adäquat, was partiell von der Supplementierung abhing. Trotz teilweise quantitativ unterschiedlicher Nährstoffaufnahme konnte ein vergleichbarer und zudem adäquater Status von Biomarkern des Vitamin B_{12}-, D-, und Eisen-Stoffwechsels festgestellt werden. Die Leistungsdiagnostik ergab keine Unterschiede bezüglich maximaler Leistungsfähigkeit (OMN: 4.15±0.48, LOV: 4.20±0.47, VEG: 4.16±0.55 Watt/kg KG; p=0.917) sowie Laktat- und Glukosekonzentrationen zwischen den Gruppen. Darüber hinaus zeigte die Studie, dass sich die Gruppen in Bezug auf oxidativen Stress, NO-Metabolismus und AS-Profil voneinander unterscheiden. Die Belastung induzierte in allen drei Gruppen einen Anstieg von MDA, keine signifikanten Veränderungen von Nitrat und Nitrit und vergleichbare Anstiege des Alanins, während es ebenso Unterschiede im AS-Metabolismus gab.

Schlussfolgerung: Ambitionierte Freizeitläufer mit vegetarischen Ernährungsmustern weisen einen adäquaten Status auch bei sonst kritischen Nährstoffen auf und zeigen damit ein hohes Gesundheitsbewusstsein. Es scheint insbesondere bei VEG Anpassungsmechanismen im Energiestoffwechsel zu geben, was durch die Sirtuinaktivität und den AS-Metabolismus gezeigt wurde, welche allerdings nur partiell die Leistungsfähigkeit zu beeinflussen scheinen. Faktoren wie Training und Genetik scheinen einen größeren Einfluss auf die Leistungsfähigkeit zu haben, als das Meiden von Fleisch. Darüber hinaus kann angenommen werden, dass eine erhöhte Antioxidantienaufnahme keine additiven Effekte auf belastungsinduzierten oxidativen Stress hat. Die Studie verdeutlicht, dass sowohl eine vegetarische als auch vegane Ernährung adäquate Alternativen für ambitionierte Freizeitsportler darstellen. Weitere Studien sind notwendig, um den Einfluss vegetarischer Ernährungsmuster auf Gesundheit und sportliche Leistungsfähigkeit zu untersuchen.

Studienregistrierung: Deutsches Register Klinischer Studien DRKS00012377

Stichwörter: Vegetarische Ernährungsformen, Nährstoffstatus, sportliche Leistungsfähigkeit

Scientific publications derived from this thesis

Original research papers (peer-review)

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II. Josefine Nebl, Jan Philipp Schuchardt, Alexander Ströhle, Paulina Wasserfurth, Sven Haufe, Julian Eigendorf, Uwe Tegtbur and Andreas Hahn (2019). Micronutrient Status of Recreational Runners with Vegetarian or Non-Vegetarian Dietary Patterns. Nutrients, 11(5):1146.

III. Josefine Nebl, Sven Haufe, Julian Eigendorf, Paulina Wasserfurth, Uwe Tegtbur and Andreas Hahn (2019). Exercise capacity of vegan, lacto-ovo-vegetarian and omnivorous recreational runners. J Int Soc Sports Nutr, 16(1):23.

IV. Arne Björn Potthast*, Josefine Nebl*, Sven Haufe, Paulina Wasserfurth, Andreas Hahn and Anibh Das. The exercise-dependent changes in sirtuin activities in plasma from vegans differ from those in omnivores and lacto-ovo-vegetarians. *submitted in Molecular Nutrition & Food Research*

V. Josefine Nebl, Kathrin Drabert, Sven Haufe, Paulina Wasserfurth, Julian Eigendorf, Uwe Tegtbur, Andreas Hahn* and Dimitrios Tsikas* (2019). Exercise-induced Oxidative Stress, Nitric Oxide and Plasma Amino Acid Profile in Recreational Runners with Vegetarian and Non-vegetarian Dietary Patterns. Nutrients, 11(8):1875.

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Conference contributions

Presentation

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Poster

Arne Björn Potthast, Josefine Nebl, Andreas Hahn and Anibh Das (2019). Impact of nutritional habits on sirtuins in blood from recreational runners. Monatsschr Kinderheilkd, 167(4):374.

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List of Abbreviations

4-AD	4-androstenedione
ACSM	American College of Sports Medicine
ADP	adenosine diphosphate
AHS 2	Adventist Health Study 2
A_{tot}	total concentration of weak acids
ATP	adenosine triphosphate
CH	carbohydrates
CK	creatine kinase
COOH	carboxyl group
CP	creatine phosphate
CPS1	carbamoyl phosphate synthase
DHAS	dihydroepiandrosterone sulphate
DHT	dihydrotestosterone
EN%	energy %
f	female
F	fat
FFA	free fatty acids
FOXO	forkhead box protein
FSH	follicle-stimulating hormone
GDH	glutamate dehydrogenase
Gln	glutamine
Glu	glutamate
GOT	glutamate oxaloacetate transaminase
GPT	glutamic pyruvic transaminase
GPx	glutathione peroxidase
GTP	guanosine triphosphate
Hb	hemoglobin
HCO_3^-	bicarbonate
Hct	hematocrit
HDL	high density lipoprotein
HIF 1α	hypoxia-inducible factor
HMGCR	3-hydroxy-3-methyl-glutaryl-coenzyme A reductase
HNE	4-hydroxy-2-nonenal
HR	heart rate
IOC	International Olympic Committee
ISSN	International Society of Sports Nutrition

LCAD	long-chain acyl-CoA dehydrogenase
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
LOV	lacto-ovo vegetarian
LV	lacto vegetarian
m	male
MCH	mean corpuscular volume
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MDA	malondialdehyde
MVC	maximal voluntary contraction
NADH	reduced form of nicotinamide adenine dinucleotide
NF-κb	nuclear factor-κB
NH_4^+	ammonium
NO	nitric oxide
non-SU	non-supplement users
$O^{2-\cdot}$	superoxide anion
OH	hydroxy group
OMN	omnivorous
P	protein
pCO_2	partial pressure of carbon dioxide
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PPARα	peroxisome proliferator-activated receptor gamma
PRAL	potential renal acid load
PUFA	polyunsaturated fatty acids
RBC	red blood cell count
RPE	ratings of perceived exertion
SHBG	sex hormone-binding globulin
SID	strong ion difference
SIRT	sirtuin
SOD	superoxide dismutase
SU	supplement users
T	testosterone
TC	total cholesterol
TCA	tricarboxylic acid cycle
TG	triglycerides

TSH	thyroid-stimulating hormone
VEG	vegan
VO_{2max}	maximal oxygen consumption
α-KG	alpha-ketoglutarate

1. General introduction

1.1. Aim of this dissertation thesis

Vegetarian diets are gaining popularity in the western world. Nowadays, the health benefits of a lacto-ovo vegetarian diet are well documented. Large-scale studies such as the Adventist Health Study (AHS) and the European Prospective Investigation into Cancer and Nutrition (EPIC) Oxford Study clearly show that vegetarian dietary patterns may have beneficial effects on various diseases such as obesity, type 2 diabetes, cardiometabolic diseases, hypertension, and cancer [1–9], although healthy non-vegetarian diets can achieve the same effects [10]. Hence, several nutrition societies, such as the Academy of Nutrition and Dietetics (USA), the British Nutrition Foundation (GB), the National Program for the Promotion of a Healthy Diet (Portugal) and the National Health and Medical Research Council (Australia), recommend vegetarian nutrition in all life stages, including for athletes [11–14]. In contrast, a strict vegan diet is viewed as critical due to the risk for an undersupply with several nutrients such as protein, long-chain n3 fatty acids, riboflavin, vitamin B_{12}, vitamin D, calcium, iron, and zinc [15].

Although vegetarian diets are becoming increasingly popular in endurance sports [16] and the prevalence of ambitious recreational runners who practice vegetarianism or veganism is increasing [17], there is little evidence on health status and exercise capacity of vegetarian or vegan athletes. Only a few studies have reported the dietary intake (partly incomplete) of vegetarian athletes [18–20], whereas the nutrient intake and status of vegan athletes is rather unknown. The question of whether vegetarian and especially vegan athletes can meet their requirements is still unclear. A recent questionnaire-based study suggests that vegan and vegetarian endurance athletes have the same health status as omnivores [21,22]. However, in order to assess the health status, the examination of biochemical data is indispensable. There are only a few biochemical data on nutrient supply and on exercise capacity, which additionally are outdated [19,23]. Furthermore, studies did not differentiate between vegetarians and vegans [18]. In addition to exercise capacity, diet-related adaptations to exercise-induced physiological metabolic processes are also conceivable. However, there are no data available.

In addition to the lack of biochemical and performance-specific data, the differentiated consideration of vegetarianism and veganism in endurance sports is missing. Consequently, an investigation of the nutrient intake in combination with biochemical parameters and parameters of exercise capacity appears meaningful. Those scientific insights provide the foundation for the assessment of the relationship between vegetarian diets and health status as well as performance. These findings can help to formulate initial recommendations for vegetarian/vegan athletes to optimize their health status and performance.

Study objectives

In order to generate new information on the status of athletes practicing vegetarian diets, data were collected as part of a cross-sectional study from recreational athletes following a vegan or lacto-ovo vegetarian diet and compared with those of omnivores. Nutrient intake, nutritional status in the form of biochemical parameters and exercise capacity were analyzed. Additionally, parameters of the exercise-induced alterations of energy metabolism (sirtuins), oxidative stress (malondialdehyde, MDA), nitric oxide (NO) metabolism (nitrate, nitrite) and amino acid profile were investigated. The following research questions form the basis of this dissertation thesis, which are addressed in the respective scientific publication:

1. How is the nutrient supply of vegan and lacto-ovo vegetarian recreational athletes compared to omnivores and do they meet the recommendations? (Paper I, chapter 2.1.)
2. Do vegan, lacto-ovo vegetarian and omnivorous recreational athletes differ in terms of biochemical parameters in blood? (Paper II, chapter 2.2.)
3. Are there differences in exercise capacity between vegan, lacto-ovo vegetarian and omnivorous recreational athletes? (Paper III, chapter 2.3.)
4. Do vegetarian dietary patterns influence exercise-induced regulation of sirtuin activity? (Paper IV, chapter 2.4.)
5. Are there differences in the expression of parameters of the exercise-induced oxidative stress in the form of MDA, NO metabolism and amino acid profile between vegan, lacto-ovo vegetarian and omnivorous athletes? (Paper V, chapter 2.5.)

1.2. Vegetarian diets

1.2.1. Definitions, distribution, and motivation

As early as 500 before Christ, Pythagoras founded vegetarianism (“vegetare” – to grow) as a diet that in addition to the predominant proportion of foods derived from plants is characterized by abstaining from meat, fish and its products. Depending on the dietary intake, several subgroups exist [11] (**Table 1**).

Vegetarianism was defined as
“[…] a diet that uses only or predominantly plant-based foods such as grains, vegetables, fruits, legumes, nuts, and seeds. Depending on the form of vegetarianism, products from live animals such as milk, eggs and honey and all products made from them may also be included. Excluded are foods derived from dead animals, such as meat, fish (including other aquatic animals) and all products derived from them. On the basis of the consumed food, one differentiates lacto-ovo, lacto and ovo vegetarians as well as vegans, whereby the latter refuse all animal products, including honey and commodities from animal parts (wool, fur, leather, etc.).” [24]

In contrast to vegetarianism, veganism is understood as a way of life and thus defined as
“[…] a philosophy and way of living which seeks to exclude—as far as is possible and practicable—all forms of exploitation of, and cruelty to, animals for food, clothing or any other purpose; and by extension, promotes the development and use of animal-free alternatives for the benefit of humans, animals and the environment. In dietary terms, it denotes the practice of dispensing with all products derived wholly or partly from animals.” [25]

Table 1 Types of vegetarian diets.

Type	Exclusion of
lacto-ovo-vegetarianism	meat, fish and their products
lacto-vegetarianism	meat, fish, their products and eggs
ovo-vegetarianism	meat, fish, their products and dairy products
veganism	all animal-derived products
raw food	(almost) all animal-derived products + heated foods

The table was designed according to [11].

However, there are also many overlaps and mixed forms, so in reality, this classification is not always possible due to inhomogeneous diets.

Distribution

Vegetarianism is distributed differently worldwide, with high prevalence in India and low prevalence in Australia and the US (**Figure 1**). It is estimated that there are approximately 4.3 % up to 10 % vegetarians in Germany, whereas vegans are said to represent about 1.6% [16,26–28].

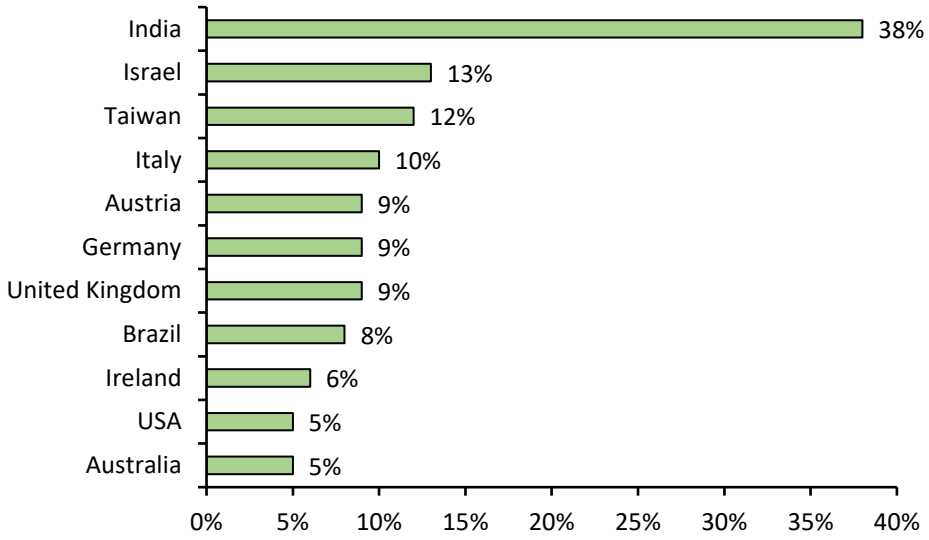

Figure 1 Proportion of vegetarians worldwide in 2016 (exemplary).

The figure was modified in accordance with [29] and in addition of [30].

The **motives** for following vegetarian diets are manifold and currently changing. Apart from ethical motives, health and performance aspects play an important role [31]. Additionally, religious and ecological aspects must be mentioned [32].

1.2.2. Nutrient intake and status

The difficulty in assessing the nutrient status is that there is not one vegetarian or vegan diet as described in chapter 1.2.1. Therefore, no general statement can be made about the nutritional status of vegetarians and vegans [24]. In addition, the nutritional evaluation is hampered by the fact that the local recommendations are defined for the healthy general population (Deutsche, Österreichische und Schweizerische Gesellschaften für Ernährung, D-A-CH [33]) and there are no separate recommendations for vegetarians and athletes (for details see chapter 1.3.4.).

Overall, the predominant or exclusive consumption of plant foods are reflected in the nutrient profile. Several studies investigated the dietary intake of vegetarians and vegans, including two large-scale studies (the EPIC Oxford Study and the Adventist Health Study, AHS I and II)

with more than 60.000 and 96.000 participants, respectively [34,35]. The **energy** intake of omnivores is typically higher compared to vegetarians and especially vegans [34,36–42]. Also, there are differences regarding the macro nutrient ratio, as vegetarians and vegans consume more (complex) **carbohydrates** than omnivores due to the high or exclusive proportion of vegetable foods [34,36–38,40,43]. In both omnivorous and vegetarian dietary patterns, the percentage of **protein** intake is above the recommendations [34,44]. Several studies found that vegetarians and especially vegans have a lower protein intake than omnivores [37–39,43] and thus are closer to the recommendations [34,44]. Considering the quality of dietary protein and the supply with essential amino acids, both, omnivores and lacto-ovo vegetarians can achieve an adequate biological value. But vegans can also achieve adequate amounts [45] if adequate energy intake is guaranteed. The **fat** intake of vegetarians and vegans is typically lower to that of omnivores [38]. However, lacto-ovo vegetarians typically consume significantly less saturated and larger amounts of monounsaturated and polyunsaturated fatty acids [34]. Since long-chain omega-3 polyunsaturated fatty acids (PUFA, > 20 C-atoms) are only present in marine sources, the intake of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) is lower in vegetarian diets than in omnivorous [46,47]. In addition, due to the lower conversion rate of alpha-linoleic acid (ALA), which occurs in plant oils, vegetarians and vegans have a lower status of EPA and DHA in blood [46–49]. In contrast, due to the high or exclusive intake of plant-based foods, vegetarians and especially vegans consume significantly more **fiber** compared to omnivores [34,38,43,50–52].

When considering the micronutrient supply, a differentiation between dietary intake and blood concentrations is crucial, since inhibiting or promoting substances and the bioavailability have an impact on the status [24]. Additionally, the assessment of the supply situation is hampered by the fact that clinical parameters have not been established for all nutrients. Furthermore, the minerals potassium, magnesium, calcium and zinc are subject to strict homeostatic regulations [53], whereby the dietary intake is not directly reflected.

A lacto-ovo vegetarian as well as a vegan diet per se, is associated with a **high intake** of β-carotene, vitamin C, E, B_1, folic acid, pantothenic acid, and biotin [34,38,41,42,50,52]. In addition, vegetarian diets generally provide adequate amounts of potassium, magnesium, vitamin K, B_6, copper, and selenium [34,38,41–43,50,51].

Due to the low alimentary intake, on the one hand, and the low bioavailability from vegetable sources, on the other hand, there are some **critical nutrients** for vegetarian dietary patterns. The most critical nutrient is vitamin B_{12}, which occurs in small amounts in milk and dairy products, while a vegan diet contains in purely arithmetic terms 0 µg [24]. Therefore, a supplementation of vitamin B_{12} or fortified foods is recommended [11]. Further, although the dietary intake of iron is characteristically higher in people with vegetarian diets, investigations

showed an undersupply of ferritin in especially female vegetarians and vegans [54]. Therefore, vegans are advised to consume 1.8 fold more iron than omnivores [14]. In fact, vegetarian diets contain high amounts of iron-absorption inhibiting substances such as fiber and phytic acid. However, the dietary intake of vitamin C as an iron absorption promoting substance is elevated as well [41,42]. Thus, a targeted choice of food can ensure an adequate supply of iron in vegetarian diets [11].

In addition, vitamin D is a critical nutrient for the general population, independent of diet, as even omnivores do not reach the recommended vitamin D intake [34,38]. Further, calcium (vegans), zinc, and iodine are considered critical nutrients in vegetarian diets [34,55–59].

1.2.3. Health status

The health status depends on a variety of factors, including genetics, but also **lifestyle** factors such as diet, physical exercise, mental health, smoking status, and alcohol abuse. In general, vegetarians have a healthy lifestyle compared to the general population, but also health-conscious non-vegetarians can achieve similar preventive effects [10,60]. In addition, vegetarians often show a higher socioeconomic status compared to the average population, which is associated with a decreased risk of obesity [34].
In general, people who practice vegetarian dietary patterns usually have a favorable **body composition**, meaning lower body mass index (BMI) compared to omnivores. The BMI decreases in the following order: omnivores > lacto-ovo vegetarians > vegans [51,61]. However, there are also vegetarian populations, which have a high proportion of overweight [51].

There is large evidence, that vegetarian diets based on a broad variety of foods have a beneficial effect on certain metabolic diseases:

- In the meantime, it has been shown that a health-conscious vegetarian diet can reduce the risk of **type 2 diabetes** by 40-50%, even after adjustment of the BMI [62,63]. Additionally, an association between meat consumption and diabetes risk was observed [64,65]. Further, the metabolic syndrome occurs less frequently [66].
- In addition, a lacto-ovo vegetarian but also vegan diet has a positive effect on blood pressure [9] and lipid profile [67], which in turn can reduce the risk of ischemic and **cardiovascular events** by 30% [1,2,4,5,7,68]. However, atherothrombotic events can be increased, if vitamin B_{12} deficiency occurs, which results in increased homocysteine levels [57]. In addition, vegetarians tend to have higher platelet hyperaggregability [69,70].
- While the **bone mineral density** of lacto-ovo vegetarians in comparison to omnivores hardly differs [71], vegans show diminished values by up to 4% [72,73]. There are no differences between lacto-ovo vegetarians and omnivores in terms of fracture risk, while

vegans have a 30% increased risk [74]. However, this increased risk is due to the low calcium intake and not due to the vegan diet *per se* [74,75].

• A meta-analysis (n=124 706) showed that vegetarians had an 18% reduced overall risk of developing **cancer** [76]. However, the risk for cancer depend on type of vegetarianism, as lacto-ovo vegetarians had a 10% reduced risk compared to omnivores [77]. Regarding mortality, there is only a minor difference between vegetarians and non-vegetarians, indicating a healthier lifestyle for non-vegetarians compared to the general population [2,78].

• Although various studies in vegetarians show a 38-48% lower **mortality** rate and an increased life expectancy by 9.4 years compared to the general population, the same mortality rate in vegetarians in comparison to health-conscious omnivores was observed [76,78,79].

1.3. Nutrient requirements, metabolic changes and adaptations as a result of physical activity

1.3.1. General aspects

In addition to genetic components and training, nutrition is a crucial factor in athletic performance [80]. Thus, a diet that is adapted to the respective sport type (e.g. sports of endurance vs. strength), duration and intensity (e.g. recreational vs. high-performance) is required [80]. Further, external factors such as climatic conditions and diet have a strong impact on the requirements of athletes. Therefore, specific nutritional strategies should be taken into account to optimize body composition, health, training adaptations, physical performance, and regeneration [81]. In this thesis, endurance sport is considered in more detail.

Overall, aerobic physical activities lead to positive metabolic effects. More specifically, these adjustments further result in **long-term adaptations**, such as an increase in heart and blood volume [82], increased capillarization and therefore improved oxygen supply [83], improved oxidative capacity through increased synthesis and enlargement of mitochondria [84], increased glucose uptake of the skeletal muscle [85], increased glycogen synthesis in muscle and liver [86], energy supply from fats and increased fatty acid oxidation [87,88] as well as higher occurrence of slow twitch fibers [89]. Further, intense endurance exercise induces **short-term effects** such as oxidative processes, which will be described in more detail in the context of this work (see chapter 1.3.4).

Since the exercise level of sports has a significant impact on the need for nutrients, a differentiated consideration should be made. The different exercise levels can be defined as follows:

Recreational sport: "*Recreational was defined as currently playing the sport in an organized competition at any grade and never having represented a sport at any grade at a regional or above level, including junior representation."* [90]

High-performance sport: "*A person who is a member of any national team or other high-level representative team in any sport organized by a National Sports Federation."* [91]

The characterization and classification of athletes are hindered by the fact that the professional societies published different definitions of exercise level in training (**Table 2**).

Table 2 Characterization of physical activity based on exercise level.

IOC and ACSM	ISSN
Low-intensity or skill-based activities	**General physical activity:** 30-40 min/day 3 times a week
Moderate exercise 1 h/day	**Moderate levels of intense training** 2-3 h/day 5-6 times a week
Moderate to high endurance program 1-3 h/day	**High-volume intense training** 3-6 h/day 1-2 sessions/day 5-6 times a week
Extreme commitment > 4-5 h/day	

The table was designed in accordance with [81,92,93].
Abbreviations: ACSM, American College of Sports Medicine; IOC, International Olympic Committee; ISSN, International Society of Sports Nutrition.

The task of sports nutrition is to provide the organism with adequate energy-providing macronutrients, micronutrients, and fluids to suit individual needs during training and competitive phases [94]. Therefore, in addition to optimal energy supply, it is necessary to consider exercise-induced adaptations as well as the associated increased needs for athletes. Adapted to the type of sport and intensity, professional societies have defined guidelines for the recommended nutrient intake.

In Germany, **dietary recommendations** for healthy adults are stated by the D-A-CH [33]. However, no recommendations for both recreational athletes and high-performance athletes are given. In general, recreational athletes are viewed to meet their needs through a mixed diet [81]. On an international level, expert societies such as the American College of Sports Medicine (ACSM) [81], the International Olympic Committee (IOC) [95], and the International Society of Sports Nutrition (ISSN) [93] have defined guidelines for the nutritional intake of athletes. However, these recommendations only apply to recreational athletes to a limited extent, while the International Society for Exercise Immunology issued guidelines for optimizing immunological changes during exercise, which also include recreational athletes [96]. Recreational athletes are a separate collective, as both the recommendations for the general population and the recommendations for competitive athletes are inadequate.

Although mainly high-performance athletes are affected by an **increased need for nutrients**, recreational athletes may also be concerned especially when exogenous factors such as heat

and high humidity are included [97,98]. Inadequate intake of micronutrients and the associated low blood levels have an adverse effect on physical performance, regeneration capacity, increased susceptibility to infections, chronic fatigue, and an increased risk of injury [99–101]. Beside exercise-induced metabolic changes, there are further aspects that may be reasonable for considerable losses and increased need for several micronutrients in intensive or long-lasting physical stress (**Table 3**). As part of this dissertation, energy metabolism (chapter 1.3.2.) and oxidative stress (chapter 1.3.4.) are examined in more detail.

Table 3 Possible reasons for increased nutrient requirements.

Reason		Possible affected nutrients
↑ **losses via**		
• **sweat**	- thermoregulation	Na, K, Mg, Ca, Fe, water-soluble vitamins
• **urine**	- ↑ aldosterone → ↑ glomerular filtration rate	
• **feces**	- micro bleedings in the intestine	Fe
• **foot strike hemolysis**	- capillary hemolysis especially in runners	Fe
↑ **requirements**		
• **oxidative stress**	- ↑ need of antioxidative enzymes (catalase, SOD, GPx) and coenzymes	Fe, Zn, Cu, Mn, Se, Vit C, E, B_2, β-carotene
• **blood formation**	- age of erythrocytes reduced from 120 to 70 days → improved oxygen transport	Fe, B_6, B_{12}, folate
↓ **dietary intake**	- diet low in wholegrain, meat, fruits, and vegetables	All nutrients, especially β-carotene, Vit C, B_6, D, folic acid, I, Mg, Ca, Fe, Zn
	- malnutrition, eating disorders	

The table was designed in accordance with [102–111].
Abbreviations: GPx, glutathione peroxidase; SOD, superoxide dismutase.

1.3.2. Energy metabolism

Beside the aforementioned aspects for increased requirements, an adequate energy supply is also essential for optimized physical performance [80]. In order to perform muscle work, energy must be provided by mobilizing, transporting, and degrading high-energy substrates with adenosine triphosphate (ATP) extraction. Depending on the duration and intensity of the endurance exercise, ATP and creatine phosphate (CP) are first used to generate energy, followed by muscle glycogen and fatty acids [112] (**Figure 2**).

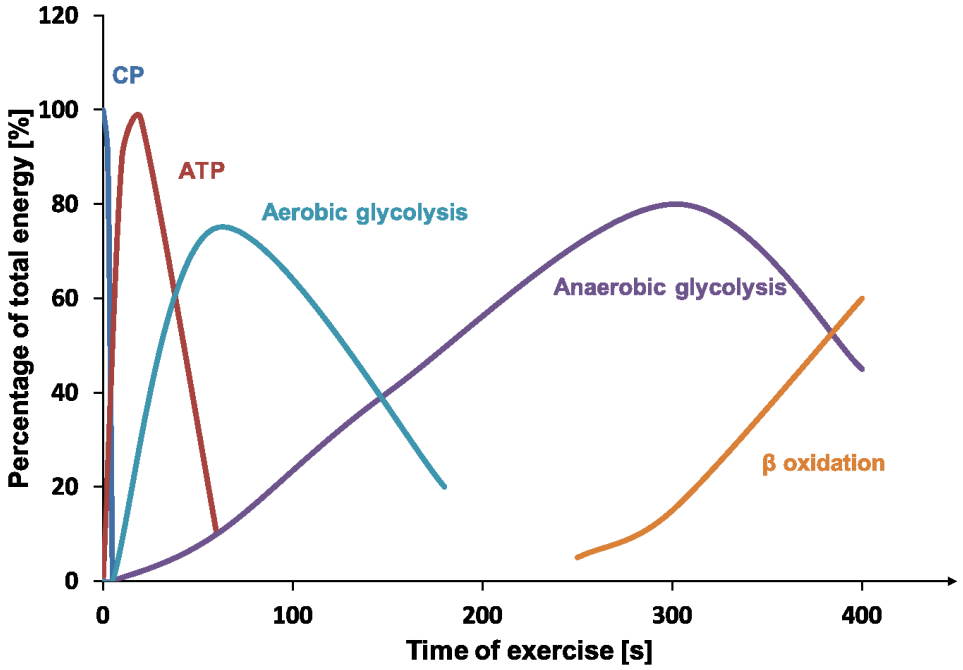

Figure 2 Share of energy supply processes under endurance exercise.

The figure was modified in accordance with [112].
Abbreviations: ATP, adenosine triphosphate; CP, creatine phosphate.

Although primarily carbohydrates and secondary fats are used for energy supply in endurance sports and intense physical activities, proteins additionally serve as energy substrates. Thus, glucogenic amino acids are transformed into intermediates of the Krebs cycle (**Figure 3**). Although amino acids underlie homeostatic regulatory processes, blood concentrations reflect a combination of dietary intake as well as anabolic and catabolic processes [113,114]. It could be demonstrated that both long- and short-term endurance exercises induce metabolic changes in amino acid profile, since studies observed a decrease of total amino acid concentration post-exercise by 15-30% [115–120]. Aromatic amino acids, on the other hand, were demonstrated to increase by 6-11% [121,122].

Overall, increased energy consumption requires increased activity of regulatory mechanisms and leads to increased oxidative processes [123].

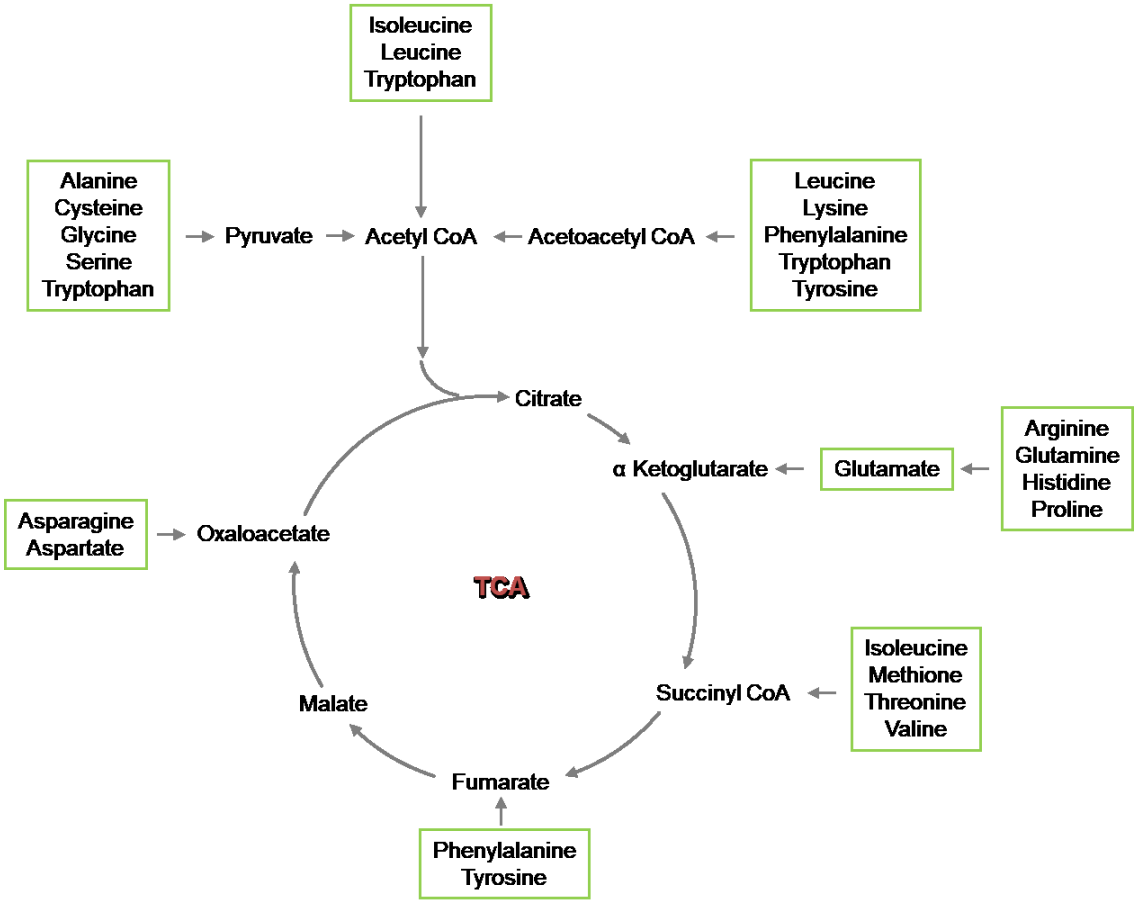

Figure 3 Amino acid metabolism.

The figure was modified in accordance with [112].
Abbreviations: TCA, tricarboxylic acid cycle.

1.3.3. Sirtuins

Increased energy demand during exercise requires an increased rate of glycolysis, Krebs cycle, fatty acid oxidation, and mitochondrial respiratory chain. These processes are closely connected and i.a. regulated by endocrine factors such as insulin and glucagon, but also by allosteric ligands such as ATP, citrate and NAD^+ [124,125].

Currently, another regulatory mechanism has gained attention, namely the NAD^+ dependent deacylases, named sirtuins (derived from the first explored sirtuin Sir2 [silent mating type information regulation 2] in *Saccharomyces cerevisiae*) [126–128].

Beside regulatory functions in energy metabolism, sirtuins seem to be associated with antioxidative defense [126,129] (**Figure 4**). Primarily, sirtuins catalyze the deacylation or ADP-ribosylation of lysine residues, which are always NAD^+ dependent [130,131]. There is evidence that sirtuins are involved in further metabolic processes such as gene expression, apoptosis [132], stress response [133], mitochondrial biogenesis [134], fatty acid oxidation [135], insulin response [136], inflammation [137] and aging processes [128,138] by deacetylating targets

such as peroxisome proliferator-activated receptor gamma (PPARα), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), fork-head box protein O (FOXO3), hypoxia-inducible factor (HIF) 1α, tumor suppressor p53 and nuclear factor-κB (NF-κb) [139]. Therefore, it has been demonstrated that blood concentrations of sirtuins correlate with several organ dysfunctions like coronary heart disease [140–143], diabetes type I and II [144] and cancer [145,146]. However, most studies are animal or in vitro studies and the investigation in humans is rare.

Until now, seven sirtuins (SIRT1-SIRT7) have been classified in mammals [147]. While SIRT1, 6 and 7 are located in the nucleolus and SIRT2 in the cytosol, SIRT3, 4 and 5 are mitochondrial enzymes [148]. However, a subcellular shift of SIRT1 and 3 into the cytosol appear to be possible [148].

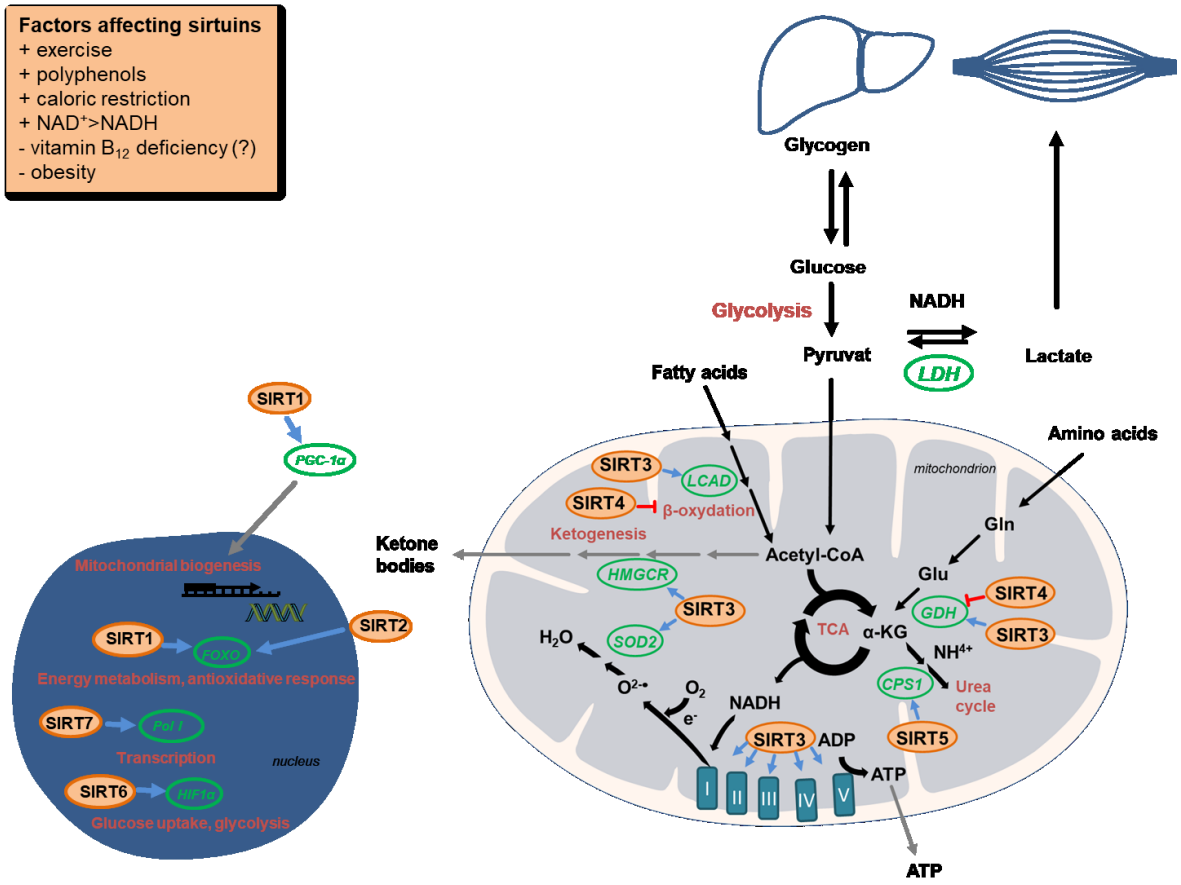

Figure 4 Influences of sirtuins in energy metabolism and oxidative stress response (exemplary).

The figure was modified in accordance with [149] in addition to [150–154]. Blue arrows represent increasing effects, red lines represent inhibiting effects. By regulating different co-substrates (e.g. PGC-1α, FOXO or several enzymes), sirtuins also influence metabolic processes such as the TCA, gluconeogenesis, and β-oxidation. Abbreviations: α-KG, alpha-ketoglutarate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; CPS1, carbamoyl phosphate synthase; e^-, electron; FOXO, forkhead box protein; HMGCR, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; GDH, glutamate dehydrogenase; Gln, glutamine; Glu, glutamate; LCAD, long-chain acyl-CoA dehydrogenase; LDH, lactate dehydrogenase; NAD^+, nicotinamide adenine dinucleotide; NADH, reduced form of NAD^+; NH_4^+, ammonium; $O^{2-\bullet}$, superoxide anion; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; SIRT, sirtuin; SOD, superoxide dismutase; TCA, tricarboxylic acid cycle.

Sirtuins as metabolic regulators are themselves subject to regulatory processes (e.g. via phosphorylation [155]), which implies an up- or downregulation of their activity and consequently an up- or downregulation of the respective function [148,156]. Further, sirtuins have partly contrary effects and thus a mutual regulation of sirtuin takes place. Few data on sirtuin activity in humans showed an increase of sirtuin activity after **physical exercise** [157–159], which indicate an activation of the aforementioned energy-providing pathways [157,160–168]. In addition, sirtuin activity possibly can be affected by **dietary factors**. First, caloric restriction was shown to be associated with an increase of SIRT1, which was associated with the reduction of aging processes and associated diseases [169,170]. Second, polyphenols, such as resveratrol, are described to encourage sirtuin activity [171,172]. In contrast, decreased bioavailability of vitamin B_{12} was shown to decrease SIRT1 expression, which in turn induces irreversible endoplasmic reticulum stress in a cell model [173].

The exploration of the role of sirtuins is still in its infancy, but may open up a new interesting area to better understand the energy metabolism and antioxidative defense in humans.

1.3.4. Oxidative stress

Increased energy consumption also leads to an increased incidence of oxidative processes, as the oxidation of carbon compounds serves as an important energy source [80]. This implies an imbalance between formation and degradation of reactive oxygen and nitrogen species (ROS and RNS, respectively). A higher occurrence of ROS or RNS can lead to either unfavorable or protective metabolic pathways [174,175]. On the one hand, free radicals are known to attack carbon double-bounds of PUFAs which leads to modification of lipids [176]. The main products of lipid peroxidation are lipid hydroperoxides, 4-hydroxy-nonenal (HNE) and the stress-sensitive malondialdehyde (MDA) (**Figure 5**), which is the most frequently detected biomarker of oxidative stress [176,177]. Due to its cytotoxic functions, studies showed a connection between MDA and the pathogenesis of atherosclerosis [178], Alzheimer´s disease [179], cancer [180], diabetes [181], endothelial dysfunction [182], and cardiovascular diseases [183].

Beside unfavorable effects, on the other hand, oxidative stress is also responsible for the activation of various health-promoting signaling pathways [184]. In fact, studies showed an association between the occurrence of ROS and muscular cytokines, leading to anti-inflammatory processes and training adaptations [184]. For example, the regulatory function of nitric oxide (NO) is stimulated by oxidative stress. NO is synthesized by nitric oxide synthase out of arginine [175] in endothelial cells and is involved in a wide range of metabolic processes, including vasodilatation, and therefore of great importance in exercise performance. However,

the unstable NO generally cannot be analyzed *per se* due to its short half time, whereby its metabolic products (nitrite and nitrate) can be detected [185].

Further possibilities to assess oxidative processes or antioxidative response are markers of oxidative damage of DNA (e.g. oxidized purine, pyrimidine), protein (e.g. oxidation products of lysine), and lipid damage (e.g. isoprostane) as well as markers of antioxidative capacity via endogenous (e.g. super oxide dismutase, gluthatione, gluthatione peroxidase) and dietary antioxidants (e.g. tocopherole, ascorbate) and total antioxidantive capacity (e.g. total peroxyl radical-trapping antioxidant parameter, TRAP) [186].

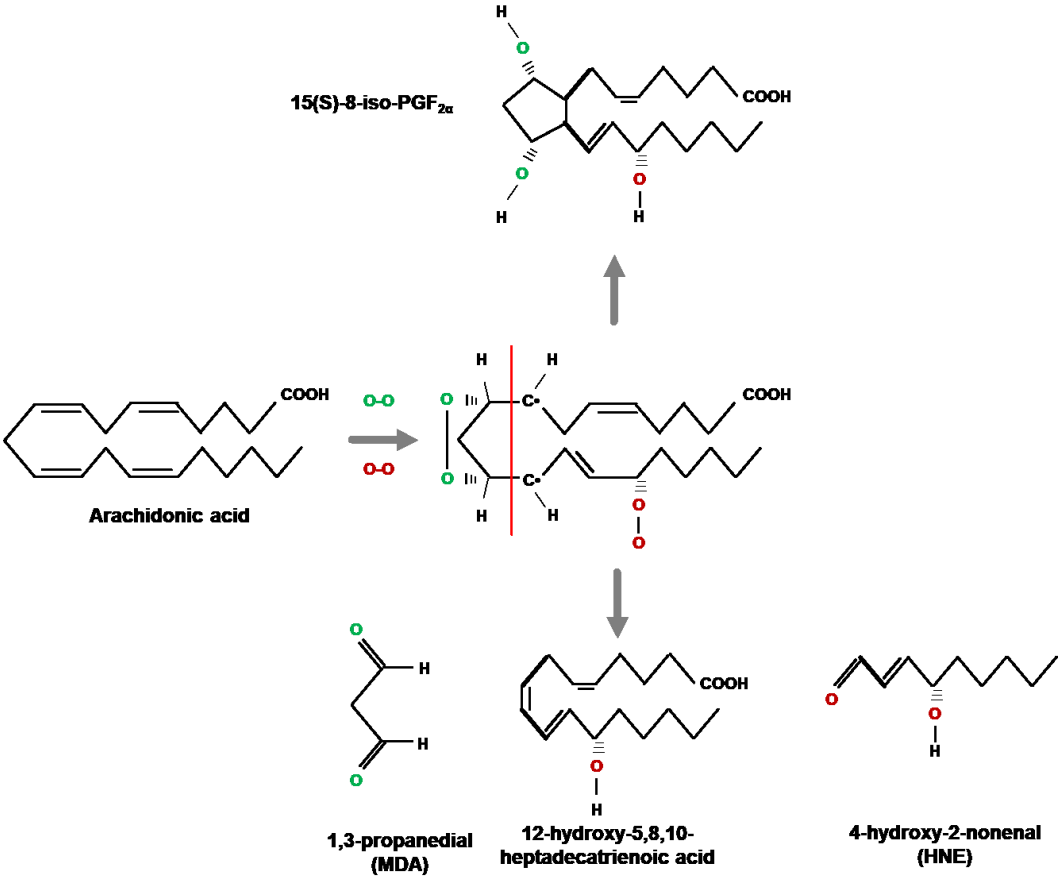

Figure 5 Simplified mechanisms of lipid peroxidation and the formation of MDA using the example of arachidonic acid.

The figure was modified in accordance with [177].
Abbreviations: COOH, carboxyl group; HNE, 4-hydroxy-2-nonenal; MDA, malondialdehyde; OH, hydroxy group; 15(S)-8-iso-PGF$_{2\alpha}$, F2-isoprostane 15(S)-8-iso-prostaglandin F$_{2\alpha}$.

1.4. Vegetarian diets and endurance performance

Various nutritional strategies were claimed to positively affect health, performance, training adaptation and regeneration. Already in the antique, the impact of diet on athletic performance has been described [187]. In contrast to earlier views, when meat was seen as a performance-enhancing food [188], vegetarian diets today have a high popularity and the interest of vegetarian nutrition and athletic performance is growing [16]. Since vegetarian diets show indisputable health benefits (see chapter 1.2.3.), one could hypothesize that parameters of physical performance can also be affected [189,190].

On the one hand, the characteristically high intake of carbohydrates is one favorable factor of vegetarian diets [18,38,42,191] (see chapter 1.2.2.). In addition, vegetarians and vegans consume typically higher amounts of antioxidative substances such as vitamin C and vitamin E (see chapter 1.2.2.), which could have favorable effects on exercise-induced oxidative stress. In fact, little literature exists, which summarizes the probable advantageous effect of higher intake of antioxidants on cardiovascular health in endurance sports [192]. However, whether this increased intake actually benefits athletic performance has not been studied.

On the other hand, the consumption of protein, creatine, and carnitine is typically lower in vegetarians and vegans compared to omnivores, which could be unfavorable for exercise performance [193,194].

So far, there are only a few studies examining the relationship between vegetarian diets and sport [195,196]. Among them are case studies of high-performance athletes practicing vegetarian nutrition, cross-sectional surveys comparing the performance of athletes who practice different diets, and intervention studies describing the direct or indirect impact of vegetarian nutrition on athletic performance (**Table 4 and 5**).

Case reports

In fact, a few case studies describe the exceptional performance of individual athletes (**Table 4**). For example, Leischik and Spelsberg characterized an ultra-triathlete who practiced a raw vegan diet and finished the Triple-Ironman in 41 hours and 18 minutes [197]. The echocardiography and spiroergometry revealed no differences between this ultra-triathlete and controls. Wirnitzer and Kornexl reported about a female vegan cyclist who participated in the 2004 Transalp Challenge, had a relative peak power output of 4.6 W/kg and finished the race in 41 hours, 59 minutes and 45 seconds and thus the 16th place in the mixed category [198]. Although the authors conclude, that a well-planned vegan diet can meet the requirements of individual competitive athletes and that this diet is compatible with ultra-endurance performance, results of case studies should be interpreted with caution.

Cross-sectional studies

In 1986, Hanne and colleagues carried out a cross-sectional survey on the athletic performance of 49 vegetarian (lacto-ovo /lacto vegetarians, vegans) and 49 omnivorous endurance athletes, matched according to age, sex, body size and type of athletic activity (**Table 4**) [23]. The researchers discovered adequate blood levels of selected parameters and comparable performance regarding aerobic (cycle ergometer stress test) and anaerobic capacity (Wingate test) in both groups.

In 2006, an Indian research team by Khanna (n=64) investigated athletic performance by recording the time to exhaustion via graded exercise until exhaustion on a treadmill in lacto-ovo and lacto vegetarians in comparison to omnivores. Overall, vegetarians had lower endurance times compared to omnivores, while lacto-ovo vegetarians had better endurance compared to lacto vegetarians (lacto vegetarians: 12.18±2.62 vs. lacto-ovo vegetarians: 14.63±2.34 min vs. non-vegetarians: 15.77±3.59 min; $p<0.01$). Recovery measured by heart rate after 2 and 3 min was fastest in lacto vegetarians compared to the two remaining groups (e.g. 2 min recovery heart rate in lacto vegetarians: 93.0±16.4 min vs. lacto-ovo vegetarians: 112.9±16.4 min vs. non-vegetarians: 104.6±14.8 min; $p<0.05$) [20].

Another recent cross-sectional by Lynch and colleagues (2016) discovered a comparable peak torque and maximum oxygen uptake (VO_{2max}) between male vegetarians and omnivores (62.6±15.4 vs. 55.7±8.4 ml/kg/min, respectively; $p=0.220$). However, the authors found a higher VO_{2max} in female vegetarians (53.0±6.9 vs. 47.1±8.6 ml/kg/min, respectively; $p<0.05$) [18].

Intervention studies

In order to examine the direct and indirect effects of vegetarian diets on athletic performance, various intervention studies were carried out (**Table 5**).

Since athletes are often more susceptible to infections than non-athletes, which can negatively impact athletic performance [99–101], Richter and colleagues investigated the influence of a lacto-ovo vegetarian diet on the **immune status** of male endurance athletes [199]. After 6 weeks intervention period, the number of CD3+, CD8+, CD4+, CD16+ and CD14+ and the activity of natural killer cells was identical for both the lacto-ovo vegetarian diet and the control [199]. The authors concluded that a lacto-ovo vegetarian diet does not influence parameters of immune function [199]. However, the parameters were not measured during or after exercise, but at rest, and the effects on immunological reactions under stress could bring different results.

Another work by Raben and colleagues investigated the endurance performance after changing to a lacto-ovo vegetarian diet (6 week intervention period) [200]. All tests showed equal performance in both groups [200].

A research team from Belgium investigated the influence of a vegetarian or omnivorous diet in combination with sprint training on the **muscle carnosine content** and **buffer capacity** [201]. The study revealed a significant group-training interaction in carnosine content of the soleus, whereby the values from the omnivorous diet non-significantly increased and those from the vegetarian group non-significantly decreased. However, muscle carnosine content and carnosine synthase mRNA were not affected by the vegetarian diet [201].

As **acid-based status** is also associated with athletic performance, Hietavala and colleagues investigated the effect of a vegetarian diet on blood acidity status [202]. Although submaximal oxygen uptake at 40, 60 and 80% of VO_{2max} after the vegetarian diet was higher than in controls, the intervention had no effect on maximum oxygen uptake. The authors concluded that this would mean a lower exercise economy [202]. However, as not only the protein source but also the amount of protein absorbed changed over the period of the intervention, a meaningful conclusion is not possible. In addition, another approach was to examine the impact of the high intake of basic substances in the form of plant foods on acid-base status, which probably have favorable effects on exercise performance [203]. But current data give no evidence for a benefit of a high intake of basic substances in exercise performance [204].

Since vegetarian diets are low in **creatine, carnosine, and carnitine**, Blancquaert and colleagues studied the effect of a lacto-ovo vegetarian diet with and without the supplementation of β-alanine and creatine on the muscle and plasma levels of the respective biomarkers [205]. Physical performance was comparable in both groups. Carnosine content of soleus ($p<0.001$) and gastrocnemius ($p=0.001$) increased after 6 month of vegetarian diet + supplementation. The creatine pool diminished after 3 months of vegetarian nutrition, which could be counteracted by supplementation, while the carnitine and carnosine levels remained the same [205].

For the sake of completeness, in addition to the studies mentioned above, there are a few other studies, which examined the influence of a vegetarian diet by **strength**. Three studies with similar study designs examined the impact of a lacto-ovo vegetarian diet on strength either with a beef containing supplement or a plant-based supplement [206–208]. All three interventions did not show any differences in the strength between the groups. In addition to the small number of subjects, Campbell´s study examined sedentary overweight subjects, while Wells and Haub's studies did not describe the athletic activity of the participants prior to the start of the studies.

Moreover, the influence of 6-day carnitine supplementation on exercise performance via a modified Wingate test was tested in physically active vegetarians compared to placebo-controlled omnivores (n=7 and 17, respectively) [209]. Overall, most parameters (body mass, plasma glucose lactate, creatine) did not differ between the groups after intervention. However, peak power output increased in controls, but not in vegetarians.

Currently, there is no evidence that vegetarian diets have any beneficial or unfavorable effects on athletic performance in endurance athletes [195]. However, the previous study situation is insufficient. First, data of nutritional and physical parameters are outdated [19,23]. Second, the latest data on nutrient supply and health are questionnaire-based but do not include biochemical parameters [21,22,210–212]. Third, several studies did not define the training status of the subjects or included athletes from different disciplines [18,20,207,208]. Forth, only one study examined a differentiated consideration of various types of vegetarianism [20]. Fifth, several studies, especially intervention studies, included only a small number of subjects [199,200,202,206–208]. Also, the intervention period of the intervention studies was partially short [202]. Lastly, study data about nutritional intake and both biochemical parameters and performance parameters of vegan athletes are missing. Since nutrient intake and status can vary between vegetarians and vegans (see chapter 1.2.2.), studies with differentiation of the vegetarian diets in athletes are needed.

Table 4 Case reports and cross-sectional studies examing the relationship between vegetarian dietary pattern and performance.

Reference	Study data	Diets	Nutritional intake	Biochemical parameters	Physical performance	Results of performance diagnostics
Case reports						
Leischik and Spelsberg 2014	n=1, m, 48 y, ultra-triathlete, Triple-Ironman (11.4 km swimming, 540 km cycling, 126 km running)	raw VEG	not reported	leu, ery, Hct, Hb, MCV, MCH, MCHC, tromb, alk. Ph., GTP, GOT, GPT, LDH, amylase, lipase, CK, P, TC, HDL, LDL, TAG, TSH, Fe, free testosterone, Vit B_{12}, ferritin, folic acid	spiroergometry, time to finish	time to finish: 41:18 min no differences compared to controls
Wirnitzer and Kornexl 2014	n=1, f, 30 y, amateur mountain biker, Transalp Challenge (8 days; altitude climbed, 22,500 m; total distance, 662 km)	VEG	24-hours dietary recall	iron, ferritin, Hb, Hct, vitamin B_{12}, Hcy	Incremental cycling test, HR, power, RPE, time to finish	time to finish: 41:59:45 min peak power: 4.6 W/kg
Cross-sectional studies						
Hanne et al. 1986	n=98, m+f, 17-60 y, endurance athletes (marathon, rowing, cycling, swimming, football, basketball and water-ball)	vegetarian, OMN	not reported	protein and glucose in urine, hemoglobin, hematocrit, uric acid, proteins and glucose in the blood	cycle ergometer stress test, 30-sec Wingate test: total power, peak power and percentage of fatigue, RPE	comparable performance regarding aerobic and anaerobic capacity
Khanna et al. 2006	n=64, f, 16-24 y, national athletes (no specific description)	LOV, LV, OMN	24-hours dietary recall	Hb	time to exhaustion via graded exercise till exhaustion on a treadmill, recovery HR	time to exhaustion highest in OMN, followed by LOV and then LV recovery HR after 2 and 3 min fastest in LV
Lynch et al. 2016	n=70, m+f, 21-58 y, competitive endurance athletes (running, cycling and triathlon)	vegetarian, OMN	7-day food log	not reported	spiroergometry, dynamometer testing	comparable peak torque and VO_{2max} higher VO_{2max} in f vegetarians

Abbreviations: CK, creatine kinase; FFQ, food frequency questionnaire; Frap, ferric reducing antioxidant power; GOT, glutamate oxaloacetate transaminase; GPT, glutamic pyruvic transaminase; GTP, guanosine triphosphate; HDL, high density lipoprotein; LDH, lactate dehydrogenase; LDL, low density lipoprotein; LOV, lacto-ovo vegetarians; LV, lacto vegetarians; MCH, mean corpuscular volume; MCHC; mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; NO, nitric oxide; OMN, omnivores; TG, triglycerides; TC, total cholesterol; TSH, thyroid-stimulating hormone; RPE, ratings of perceived exertion; TBARs, thiobarbituric acid reactive substance; VEG, vegans.

Table 5 Intervention studies examining the effect of vegetarian diets on performance-related outcomes.

Reference	Study data	Intervention	Biochemical parameters	Physical performance	Results
Richter et al. 1991	n=8, m, 21-28 y, well-trained athletes (cycling, running, rowing, mixed aerobic activities)	6 w cross-over (4 w wash-out), macro nutrient ratio (both): 57 EN% CH, 14 EN% P and 29 EN% F **Group 1**: meat-rich diet, 31% plant P **Group 2**: LOV, 82% plant P	immune parameters: number of CD3+, CD8+, CD4+, CD16+ and CD14+ and the activity of natural killer cells	not reported	identical number of CD3+, CD8+, CD4+, CD16+ and CD14+ and activity of natural killer cells
Raben et al. 1992	n=8, m, 21-28 y, well-trained athletes (cycling, running, rowing, mixed aerobic activities)	6 w cross-over (4 w wash-out), **Group 1** (LOV, n=4): 58 EN% CH, 15 EN% P and 27 EN% F, 5.7gxMJ fiber, 13gxMJ iron **Group 2** (OMN, n=4): 58 EN% CH, 14 EN% P and 28 EN% F, 2.7gxMJ fiber, 10gxMJ iron	Hb, serum iron, serum transferrin, T, free T, SHBG, DHT, 4-AD, DHAS, estrone, estradiol, estrone sulfate, LH, FSH, prolactin	spiroergometry, MVC and the isometric endurance at 35% of the MVC on quadriceps muscle and elbow flexors	comparable performance
Baguet et a. 2011	n=20, m+f, 21.5±1.7 y, physical active (2-3 hours per week)	5 w **Group 1** (OMN): beta-alanine-rich creatine monohydrate **Group 2** (LOV) **Both**: + 1 g/d creatine monohydrate + sprint training	muscle biopsy: carnosine content in soleus, gastrocnemius lateralis and tibialis anterior, non-bicarbonate buffering capacity, carnosine content and carnosine synthase mRNA expression	repeated sprint ability test (6x6s), lactate measurements	no effects on muscle buffering capacity and peak power output
Hietavala et al. 2012	n=9, m, 23.5±3.4 y, physical active (walking, jogging, cycling, resistance training)	4 d cross-over (16 d wash-out) **Group 1** (OMN, n=5) **Group 2** (LOV, n=4): low protein vegetarian diet (0.8 g/kg BW), designed by PRAL	venous blood pH, SID, A_{tot}, pCO_2, HCO_3^-, FFA, TG	incremental cycling test with spiroergometry, venous blood lactate, glucose	higher submaximal oxygen uptake at 40, 60 and 80% of VO_{2max} in group 2, no effect on VO_{2max}

Blancquaert et al. 2018	n=40, f, 25.6±7.3 y, comparable VO_{2max}: Group 1: 39.4±6.4 ml/min, Group 2: 36.6±6.3 ml/min, Group 3: 40.4±6.4 ml/min, p=0.504	6 m **Group 1** (n=15): LOV + placebo **Group 2** (n=14): LOV + 0.8-0.4 g/d β-alanine + 1 g creatine monohydrate/d **Group 3** (n=10): OMN control	CR, carnosine and carnitine (muscle biopsy and plasma), 25(OH)D, plasma guanidonoacetate,acetylcarnitine, 24 h urine: pi-methylhistidine, tau-methylhistidine and anserine	incremental cycling test with spiroergometry	no differences in VO_{2max} and time to exhaustion stable plasma alanine concentrations for group 1 and 3, in group 2 increased plasma - alanine concentration (in soleus and gastrocnemius muscle)

Abbreviations: 4-AD, 4-androstenedione; A_{tot}, total concentration of weak acids; BW, body weight; CH, carbohydrates; DHAS, dihydroepiandrosterone sulphate; DHT, dihydrotestosterone; EN%, energy percent; F, fat; FFA, free fatty acids; FSH, Follicle-stimulating hormone; Hb, hemoglobin; HCO_3^-, bicarbonate; Hct, hematocrit; HDL, high density lipoprotein; LDL, low density lipoprotein; LH, Luteinizing hormone; LOV, lacto-ovo vegetarian; MCH, mean corpuscular volume; MVC, maximal voluntary contraction; P, proteins; pCO_2, partial pressure of carbon dioxide; PRAL, Potential renal acid load; SHBG, sex hormone-binding globulin; SID, strong ion difference; T, testosterone; TC, total cholesterol; TG, triglycerides; VO_{2max}, maximal oxygen consumption.

2. Scientific publications

2.1. Paper I

Characterization, dietary habits and nutritional status of omnivorous, lacto-ovo-vegetarian and vegan recreational runners – a pilot study

Authors: Nebl J, Schuchardt JP, Wasserfurth P, Haufe S, Eigendorf J, Tegtbur U, Hahn A

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Chacterization, dietary habits and nutritional intake of omnivorous, lacto-ovo vegetarian and vegan runners – a pilot study

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Abstract

Background: The number of people preferring plant-based nutrition is growing continuously in the western world. Vegetarianism and veganism are also becoming increasingly popular among individuals participating in sport. However, whether recreationally active vegetarian and vegan populations can meet their nutritional needs is not clear.

Methods: The purpose of this cross-sectional study was to compare the nutrient intake of omnivorous (OMN, n = 27), lacto-ovo vegetarian (LOV, n = 26) and vegan (VEG, n = 28) recreational runners (two to five training sessions per week) with intake recommendations of the German, Austrian and Swiss Nutrition Societies (Deutsche, Österreichische und Schweizerische Gesellschaften für Ernährung, D-A-CH) for the general population. Lifestyle factors and supplement intake were examined via questionnaires; dietary habits and nutrient intake were determined based on 3-day dietary records.

Results: More than half of each group did not reach the recommended energy intake (mean OMN: 10.4, 95 % CI 8.70–12.1; LOV: 9.67, 8.55–10.8; VEG: 10.2, 9.12–11.3 MJ). Carbohydrate intake was slightly below the recommendations of > 50 energy percent (EN%) in OMN (46.7, 43.6–49.8 EN%), while LOV (49.4, 45.5–53.3 EN%) and VEG (55.2, 51.4–59.0 EN%) consumed adequate amounts (p = 0.003). The recommended protein intake of 0.8 g/kg body weight (D-A-CH) was exceeded in all three groups (OMN: 1.50, 1.27–1.66; LOV: 1.34, 1.09–1.56; VEG: 1.25; 1.07–1.42 g/kg BW; p =0.047). Only VEG (26.3, 22.7–29.8 EN%) did not achieve the recommended fat intake of 30 EN%. The supply of micronutrients, such as vitamin D and cobalamin, was dependent on supplement intake. Similarly, female OMN ($OMN_{non\text{-}SU}$: 11.2, 9.11–12.2 mg) and LOV ($LOV_{non\text{-}SU}$: 12.8, 9.47–16.1 mg) achieved the recommended daily intake of 15 mg iron only after supplementation, while VEG ($VEG_{non\text{-}SU}$: 19.8, 15.7–24.0 mg) consumed adequate amounts solely via food.

Conclusion: All three groups were sufficiently supplied with most nutrients despite the exceptions mentioned above. The VEG group even showed advantages in nutrient intake (e.g. carbohydrates, fiber and iron) in comparison to the other groups. However, the demand for energy and several macro- and micronutrients might be higher for athletes. Thus, it is also necessary to analyze the endogenous status of nutrients, including functional parameters, to evaluate the influence of a vegetarian and vegan diet on the nutrient supply of athletes.

Trial registration: German Clinical Trial Register (DRKS00012377), registered on April 28, 2017

Keywords: Recreational endurance athletes, plant-based diets; nutrient supply, vegetarianism, veganism, nutrient survey

Background

Plant-based diets, especially vegetarianism and veganism, are increasingly gaining popularity in the western world. These alternative diets are characterized by a predominance of foodstuffs derived from plants in varying amounts and range from abstaining from meat, meat products and fish and to complete rejection of animal products, as applicable for vegans (VEG) [1,2]. About 4.3 to 10 % of the population in Germany are estimated to be vegetarians, whereas the number of VEG is estimated at 1.6 % [3–5]. Switzerland, Italy, Austria and the UK have a similar number of vegetarians as Germany at 9–11 % [6]. In the United States, only 5 % of the population is considered vegetarian [7], however, this is still more than 16 million people.

It is undisputed that a lacto-ovo vegetarian (LOV) diet based on a broad variety of foods generally ensures the supply of (nearly) all nutrients in adults [1,8,9] and has favorable effects on the cardiometabolic risk compared to the usual mixed diet [10–14]. Moreover, plant-based diets show beneficial associations with obesity, type 2 diabetes, hypertension and cancer [15–18], although healthy omnivore (OMN) diets can achieve similar effects [19]. Consequently, several nutrition societies recommend LOV diets as a healthy diet for all stages of life [8,20–22]. By contrast, strict VEG nutrition is viewed as critical due to the risk for an undersupply with critical nutrients such as protein, long-chain n3 fatty acids, riboflavin, cobalamin, vitamin D, calcium, iron and zinc [23]. Thorough planning and engagement with a VEG diet are required to adjust the nutrient supply and meet the needs in different population groups.

A balanced diet also plays an important role for athletes. The impact of a plant-based diet on the health and performance of athletes is becoming a growing interest [4]. However, data on the prevalence of vegetarians or VEG as recreational and professional athletes are still sparse and only a few studies have investigated the nutritional status of vegetarian athletes [27–29]. Therefore, it is of great importance to investigate the nutritional status of athletes using data on dietary habits combined with analytical data on the nutrient status and functional outcomes.

Such findings enable an evaluation of whether athletes who follow plant-based diets can meet their nutritional needs or show nutrient imbalances. Furthermore, such data form the basis for assessing the relationship of a plant-based diet with the body composition, the antioxidant and immunological capacity and, ultimately, with the health and performance of athletes [24,25,27]. Present studies investigating the relationship between a vegetarian and VEG diet and exercise do not differentiate between vegetarians and VEG [24], are outdated [26], questionnaire-based [28–30] or do not contain nutritional assessment including biochemical markers [31,32].
The nutrient supply status of athletes consuming a balanced mixed diet including animal-based foods can usually be classified as safe, including critical nutrients. However, there is a lack of scientific data investigating the question of whether vegetarian and especially VEG athletes are undersupplied with critical nutrients, and whether this affects health and performance. To date, no data exist on the nutritional and athletic conditions of VEG recreational runners and there are no recommendations regarding nutrient intake for LOV and VEG athletes. Therefore, in order to fill the knowledge gap between nutrient intake, status and performance, the novel approach of this study is to compare the dietary habits, nutritional intake, body composition and performance diagnostics of VEG and LOV recreational runners with OMN runners. We present here a comparison of the nutritional supply status of these three groups and a comparison with reference values of the German, Austrian and Swiss Nutrition Societies for healthy adults (Deutsche, Österreichische und Schweizerische Gesellschaft für Ernährung: D-A-CH) [33]. These data may serve as a first basis to determine specific recommendations regarding the nutrient intake for vegetarian and vegan athletes in the future.

Methods

Participants

This cross-sectional study was conducted at the Institute of Food Science and Human Nutrition, Leibniz University Hannover, Germany. Ethical approval was provided by the Ethics Committee at the Medical Chamber of Lower Saxony (Hannover, Germany). The study was conducted in accordance with the Declaration of Helsinki. All subjects gave their written informed consent. The study was registered in the German Clinical Trial Register (DRKS00012377).

Eighty-one healthy recreational runners (mean age: 27.5 ± 4.14 yr., height: 1.75 ± 0.80 m, body mass: 67.7 ± 9.56 kg, BMI: 22.0 ± 1.94 kg/m^2, m = 31, f = 50) aged between 18 and 35 years were recruited from the general population in Hannover, Germany, via local running events, online running communities and online vegetarian and VEG communities.

The eligibility of subjects was assessed using questionnaires. Participants were selected based on the following inclusion criteria: OMN, LOV or VEG diet for at least half a year, body

mass index (BMI) between 18.5 and 25.0 kg/m^2 and run regularly two to five times per week for at least 30–60 min. Regular running sessions were documented via self-reporting data. The following criteria led to exclusion: Any cardiovascular, metabolic or malignant disease, diseases regarding the gastrointestinal tract, pregnancy, food intolerances and addiction to drugs or alcohol. Participants were allowed to take dietary supplements, but the use of performance-enhancing substances (e.g. alkaline salts, creatine) led to exclusion.

Methods and examination procedure

A questionnaire which included food groups the participants usually consume had to be completed to categorize subjects as OMN, LOV and VEG recreational athletes.

Participants were matched according to age and gender. Subjects who were included in the study collective were invited to an examination. Prior to the examination, subjects fulfilled a 3-day dietary record over three consecutive days, including two weekdays and one weekend day. The nutritional diaries were checked by nutritionists for completeness, readability and plausibility. Ambiguities were clarified with subjects if necessary. Seventy-nine out of eighty-one participants returned the completed record. The following food groups were analyzed: Meat, meat products and sausages, fish and seafood, milk and dairy products, eggs, fat and oil, whole grain products, cereal products, pastries, potatoes, vegetables, legumes, soy, fresh fruits, nuts and seeds, sweets, alcoholic drinks, alcohol, nonalcoholic beverages, coffee, tea and fast food.

Nutrient intake was depicted in comparison to the reference values of the German, Austrian and Swiss Nutrition Societies for healthy adults (D-A-CH) [33]. Amino acid intake was compared to the reference values of the World Health Organization (WHO) [34].

Participants completed a questionnaire regarding their supplement intake, status of health and running activity. Training frequency and duration were self-reported by the subjects. The determination of anthropometric data followed. The measurements of body weight (BW) and height were carried out lightly clothed and without shoes, respectively. Waist circumference was determined using a tape measure. The BMI was calculated using the standard formula:

$$BMI = \frac{\text{body mass [kg]}}{(\text{height [m]})^2} .$$

Data analysis and statistical methods

The nutrition organization software PRODI6.4® (Nutri-Science GmbH, Freiburg, Germany) was used to analyze dietary habits, energy and nutrient intake from the 3-day dietary record. The composition of foods, which were not available in PRODI6.4®, have been requested from the manufacturer and the results were integrated into the software. The intake of animal- and plant-based iron was also calculated with the software. The compositions of all supplementary products mentioned at the time of evaluation were researched and multiplied by the intake frequency (daily intake (factor *1), two times a week (factor *2/7, three times a week (factor *3/7), four times a week (factor *4/7), irregular intake (factor *12/365) to calculate the average daily intake of the respective nutrients via supplements. Based on the intake frequencies above, the average daily intake for each mineral and vitamin was calculated for each subject individually.

Statistical analyses were performed using SPSS software (IBM SPSS Statistics 24.0; Chicago, IL, USA). Results are presented as mean ± standard deviation (SD) or 95 % confidence interval (CI). Normal distribution was checked using the Kolmogorov-Smirnov test. If data were normally distributed, one-way analysis of variance (ANOVA) was used to evaluate differences in nutritional status and intake between the three diet groups. The Kruskal-Wallis test was performed to analyze data with non-normal distribution. If there were significant differences between the groups, the post hoc test with Bonferroni correction was conducted. The Mann-Whitney U test was used to examine differences between supplement users (SU) and non-supplement users (non-SU) within the groups. The chi-square test was used to compare the differences between the frequencies of the three groups. Associations between parametric data were computed via Pearson and nonparametric data via Spearman's rho correlation. P values ≤ 0.05 were interpreted as statistically significant.

Results

Characterization of the study population

Twenty-seven of the 81 runners followed OMN nutrition, 26 were LOV and 28 were VEG (Figure 1). Men and women were equally distributed and there were no differences in the mean age and anthropometric data (Table 1). Only one female of the LOV had a waist circumference slightly over 80 cm; all other participants had values in the reference range of < 80 cm for women and < 94 cm for men. All but one of the 27 participants of the OMN group had followed the diet for > 3 years. By contrast, 4 out of 26 participants of the LOV group and 6 out of 28 of the VEG group had switched to their current diet for 0.5–1 year.

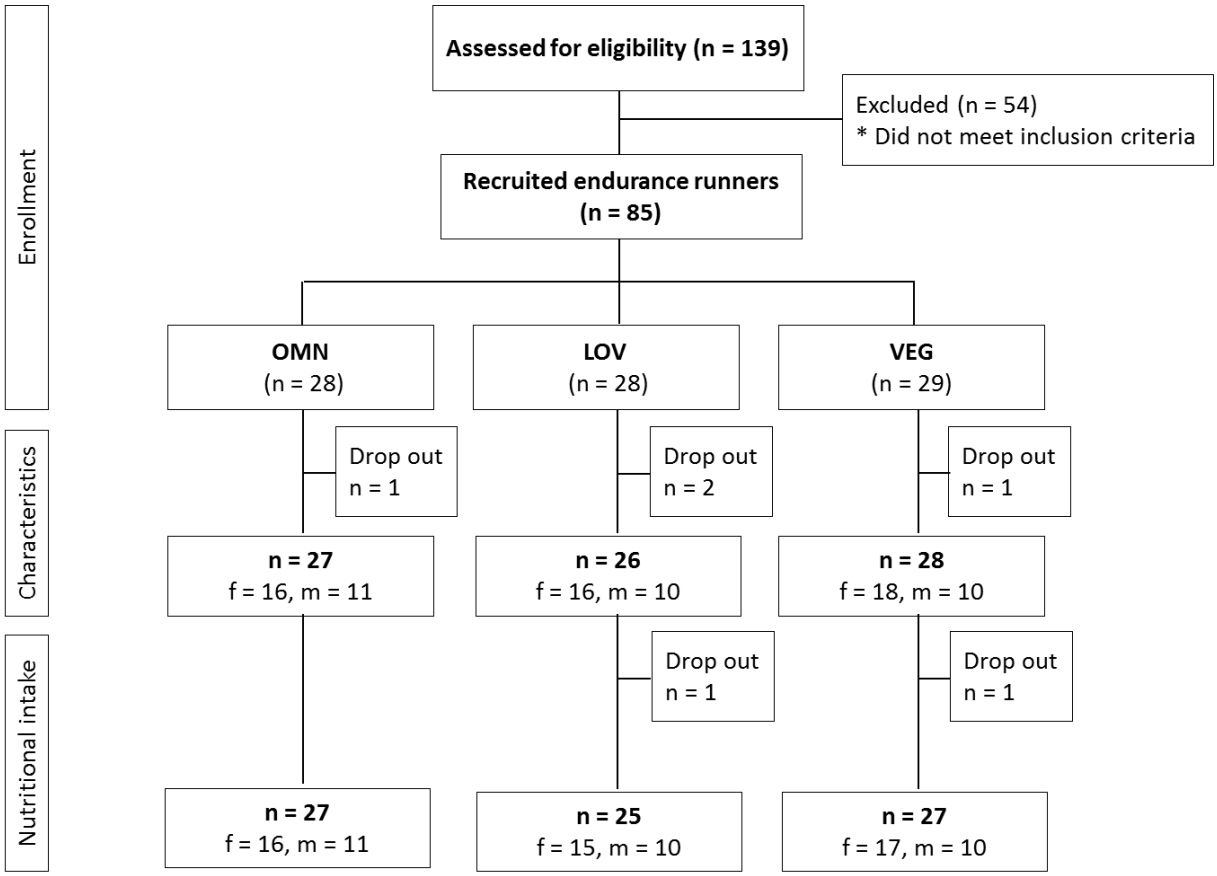

Figure 1 Flow chart of the study population.

Several subjects took dietary supplements. More precisely, 18 out of 28 participants (64.3 %) of the VEG, 10 out of 27 (37.0 %) of the OMN and 9 out of 26 (34.6 %) of the LOV group took supplements. Although considerably more subjects of the VEG group consumed supplements, there were no statistically significant differences between the groups. Magnesium, calcium, iron, cobalamin and vitamin D were commonly consumed supplements (Table 1). Magnesium and vitamin D were most commonly supplemented in the OMN group (22.2 % and not significant [n.s.], respectively), magnesium in LOV (17.9 %; n.s.), and cobalamin in VEG (53.9 %; $p = 0.005$, χ^2). Total nutrient intake of SU compared to non-SU was investigated (Figure 2 and 3). Statistically significantly higher cobalamin intake in SU compared to non-SU was found in both male and female VEG ($p = 0.019$ and 0.003, respectively) as well as in female OMN ($p = 0.027$) and LOV ($p = 0.026$). Magnesium ($p = 0.036$), vitamin D ($p = 0.018$) and iron ($p = 0.018$) intake was statistically significantly higher in female LOV SU compared to non-SU. Male SU in OMN also showed higher iron intakes than non-SU ($p = 0.004$). The analysis of fortified food products revealed only one subject who consumed a small amount (15 mg) of calcium-enriched soy drink, which can be neglected.

None of the subjects regularly consumed tobacco. The participants showed no differences in training frequency or duration (Table 1).

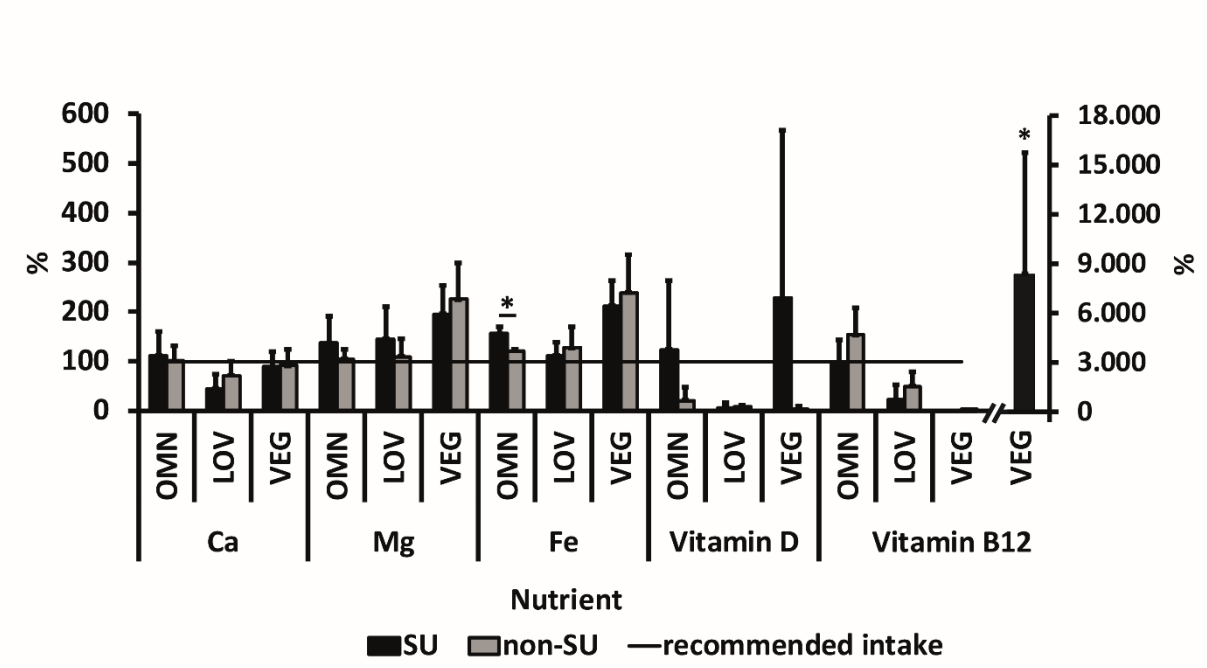

Figure 2 Nutrient intake in relation to the reference range: Supplement users vs. non-supplement users (males; mean+SD). OMN = omnivores, LOV = lacto-ovo vegetarians, VEG = vegans, SU = supplement users, non-SU = non-supplement users, recommended intake of the German, Austrian and Swiss Nutrition Societies (Deutsche, Österreichische und Schweizerische Gesellschaften für Ernährung, D-A-CH) [33]. The error bars represent the standard deviations of the average daily nutrient intake. Differences between SU and non-SU were analyzed using the Mann-Whitney U test. *p ≤ 0.05.

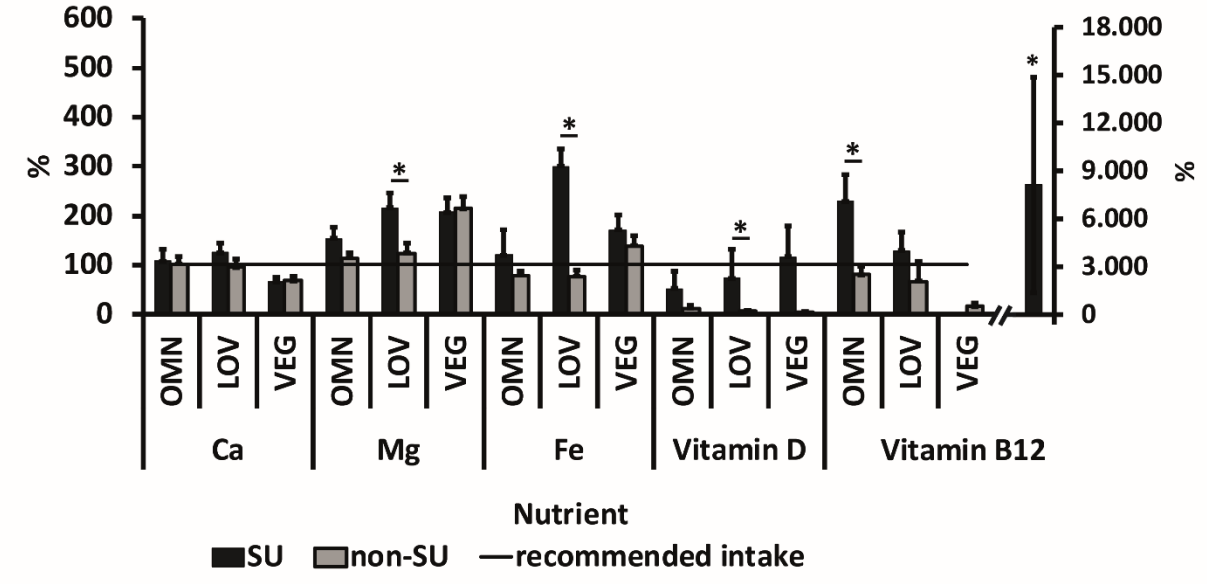

Figure 3 Nutrient intake in relation to the reference range: SU vs. non-SU (females; mean+SD). Recommended intake of the D-A-CH [33]. The error bars represent the standard deviations of the average daily nutrient intake. Differences between SU and non-SU were analyzed using the Mann-Whitney U test. *p ≤ 0.05.

Dietary habits

According to their diet, LOV and VEG consumed neither meat, meat products, fish nor seafood (Table 2). The VEG additionally waived milk, dairy products and eggs. The three groups consumed similar amounts of fat and oil, whole grain and cereal products as well as pastries. Moreover, there were no significant differences in the dietary intake of sweets, alcoholic drinks, coffee and tea. The VEG consumed significantly higher amounts of potatoes, vegetables and fresh fruit compared to LOV ($p_{LOV-VEG}$ = 0.013, 0.031 and 0.041, respectively) and OMN ($p_{OMN-VEG}$ = 0.017, 0.001 and 0.015, respectively). Legumes were consumed mainly in the VEG group ($p < 0.001$), while OMN consumed the highest amounts of fast food ($p = 0.016$) (Table 2).

Nutritional intake

None of the three groups differed in terms of energy consumption (Table 3); men (OMN: 12.3, 8.36–16.1; LOV: 10.3, 8.96–11.7; VEG: 11.5, 8.97–1 3.9 MJ; n.s.) had a higher energy intake than women (OMN: 9.11, 7.96–10.3; LOV: 9.22, 7.51–10.9; VEG: 9.47, 8.47–10.4 MJ; n.s.), which was statistically significant for OMN ($p = 0.023$). In comparison to the recommended values for people who perform sport several times a week (age group 19–25 and 25–51, physical activity level was classified at 1.7; [33]), only the average of female VEG and male OMN reached the recommendations. Low levels of energy intake were evident in 59.3 % of OMN, 52.0 % of LOV and 51.9 % of VEG, with no differences in frequency distribution. No significant associations were found between energy intake and age, BMI and frequency of training.

Regarding the **macronutrient** intake, there were significant differences between OMN and VEG. The VEG consumed a higher percentage of carbohydrates (55.2, 51.4–59.0 energy percent, EN%) compared to OMN (46.7, 43.6–49.8 EN%; $p_{OMN-VEG}$ = 0.002) (Table 3). Most subjects of the OMN group (70.4 %) and 50.2 % of the LOV group had low levels (< 50 EN%) of carbohydrates. By contrast, most subjects (66.7 %) of the VEG group had higher levels of carbohydrates (> 55 EN%). These differences were statistically significant ($p = 0.035$, χ^2). The absolute intake of carbohydrates differed only slightly.

Regarding the absolute dietary protein intake, there were only minor differences between the groups (Table 3). On average, all the groups were above the reference range of 0.8 g/kg BW; only one subject of the OMN group (3.70 %), two subjects of LOV (8.00 %) and two subjects of VEG (7.41 %) did not reach the recommendations (data not shown). All three groups were adequately supplied with all essential amino acids (see Additional file 1).

Considering the average relative fat intake, subjects in the OMN group ($p_{OMN-VEG}$ = 0.021) and LOV (n.s. compared to VEG) consumed higher amounts compared to VEG, who were below the recommendation of 30 EN% (Table 3). A low-fat intake (< 30 EN%) was observed in

70.4 % of the VEG, 44.0 % of the LOV and 25.9 % of the OMN group. These differences were significant ($p = 0.004$, χ^2). Differences in fatty acid intake patterns were observed. The highest intake of saturated fatty acids was observed in the OMN group (8.70, 7.13–10.3 EN%) followed by LOV (7.86, 6.17–9.55 EN%; n.s. compared to OMN) and VEG (4.57, 3.55–5.59 EN%; $p_{OMN-VEG} < 0.001$) (see Additional file 2). Monounsaturated fatty acids were least consumed by the VEG group (3.96, 3.02–4.91 EN%) compared to LOV (5.45, 3.77–7.13 EN%; n.s. compared to LOV) and OMN (5.95, 4.86–7.03 EN%; $p_{OMN-VEG} = 0.019$). No differences were observed in polyunsaturated fatty acid (PUFA) intake. On average, none of the three groups reached the recommended intake values of monounsaturated fatty acids (> 10 EN%) and PUFA (7–10 EN%). The intake of linoleic acid (LA) was 4.33 (3.44–5.21) EN% in the VEG group, 3.52 (2.57–4.46) EN% in LOV and 2.96 (2.50–3.42) EN% in OMN. Similarly, the intake of alpha-linolenic acid (ALA) was highest in the VEG group (0.80, 0.55–1.05 EN%) compared to LOV (0.68, 0.33–1.03 EN%, n.s. compared to VEG) and OMN (0.37, 0.27–0.48 EN%, $p_{OMN-VEG} = 0.005$). The ratio LA:ALA did not differ significantly between the groups, although OMN showed a less favorable ratio (1:8.04) (see Additional file 2). The PUFAs, eicosapentaenoic acid (EPA, 20:5n3) and docosahexaenoic acid (DHA, 22:6n3), were supplemented by two subjects of the OMN group, two subjects of the VEG and one of the LOV group. We observed the highest sum of EPA + DHA intake in the OMN group (0.54, 0.23–0.85 g), followed by LOV (0.08, 0.37–0.12 g; $p_{OMN-LOV} = 0.003$) and VEG (0.09, 0.01–0.17 g; $p_{OMN-VEG} < 0.001$).

Fiber intake was significantly higher in the VEG group (51.7, 44.1–59.4 g) compared to LOV (33.4, 28.6–38.2 g; $p_{OMN-LOV} = 0.006$) and OMN (27.0, 22.8–31.1 g; $p_{OMN-VEG} < 0.001$). The latter did not reach the minimum reference value of 30 g per day.

Micronutrient intakes also showed several differences between the groups (Table 4). Several participants did not reach the recommended intake for all the micronutrients examined (see Additional file 3). There were variations regarding the minerals, sodium, potassium and magnesium, while calcium and phosphorus values were similar. More precisely, lower sodium intake was observed in the LOV group ($p_{OMN-LOV} = 0.004$) and VEG ($p_{OMN-VEG} = 0.005$) compared to OMN (Table 4, p values of total intake are not shown). By contrast, the VEG group had significantly higher intake levels of potassium and magnesium compared to LOV ($p_{LOV-VEG} = 0.005$ and 0.001, respectively) and OMN ($p_{OMN-VEG} = 0.014$ and < 0.001, respectively) (Table 4, p values of total intake are not shown). On average, the LOV and VEG groups had calcium intakes < 1000 mg per day [33], and OMN consumed sufficient amounts (1026, 846–1207 mg) due to supplementation. A total of 64.0 % of the LOV group, 51.9 % of OMN and 44.4 % of VEG were below the recommendations for calcium (see Additional file 3).

There were also group differences regarding trace elements, except for the zinc values, which did not vary between the groups. All three groups had adequate dietary zinc intakes, however,

the male LOVs were slightly low (9.89, 5.33–14.5 mg). Female subjects reached the recommendations and so did the non-SU (OMN 8.46, 6.30–10.6 mg; LOV 9.44, 6.77–12.1 mg; VEG 9.89, 7.63–12.1 mg). We observed a high iron intake, particularly in the VEG group (Table 4). The mean iron intake was within the recommended area (10 mg/day [33]) in all three groups when only men were compared, and in both male SU and non-SU (Figure 2). The highest iron intake via food in women was found in the VEG group (19.8, 15.7–24.0 µg), followed by LOV (12.8, 9.47–16.1 µg; $p_{LOV\text{-}VEG} = 0.037$) and OMN (11.2, 9.01–13.2 µg; $p_{OMN\text{-}VEG} = 0.005$). Only the female SU in both the LOV and OMN groups reached the reference range (15 mg/day [33]) (Figure 3). The iron sources in the diet of the VEG group were exclusively plant-based food. However, the LOV and OMN groups consumed predominantly plant-based iron as well (Table 4). The worst supply was observed for iodine. Only 3.7 % of the OMN group and none of the subjects in LOV and VEG had values in a reference range of 200 µg per day (see Additional file 3) [33].

Variations were also observed in the vitamin intake between the groups (Table 5). On average, all three groups reached the recommended amounts for thiamine, pyridoxine and folate, while the reference value for vitamin D was not achieved, and the ascorbic acid intake was exceeded in all groups. Due to the supplementation, the highest average intake of cobalamin was observed in the VEG group (207, 102–313 µg), followed by OMN (4.97, 3.70–6.25 µg; n.s. compared to VEG) and LOV (2.96, 1.69–4.24 µg; n.s. compared to VEG) (Table 5). Riboflavin intake was low in 44.4 % of VEG subjects, 44.0 % of LOV and 22.2 % of OMN (see Additional file 3). We found the highest vitamin D intake in the VEG group (19.9, 2.75–37.0 µg), followed by OMN (8.29, 2.22–14.37 µg; n.s. compared to VEG) and LOV (4.52, -1.34–10.39 µg; n.s. compared to VEG) (Table 5). Only 22.2 % of the VEG group, 14.8 % of OMN and 4.00 % of LOV had vitamin D intakes within the recommendations (20 µg/day [33]).

Discussion

The aim of this study was to investigate for the first time the dietary habits and nutritional intake of German recreational runners practicing a VEG diet in comparison to a LOV or OMN diet.

Organizations such as *The American College of Sports Medicine* (ACSM), *The International Society for Sports Nutrition* (ISSN) and the *International Olympic Committee* (IOC) have defined guidelines for athletes [35–37]. As these few existing recommendations for mainly high-performance athletes were only partially applicable to this study collective, the nutrient intake was compared with intake recommendations of the D-A-CH for the general population. However, the D-A-CH does not specify any certain reference values for ambitious recreational athletes and recommends only the percentage of carbohydrate and fat intake, while there are absolute recommendations regarding protein intake [33].

The literature considers an adequate supply of athletes with all micronutrients through a balanced mixed diet, but it is unknown whether a vegetarian and especially VEG diet can provide all the important nutrients for athletes.

The type, duration and intensity of sport determine the energy requirements. The ISSN recommends an energy intake from 7.5–10.0 MJ for athletes with general physical activity levels of 30-40 min three to four times a week [35]. In order to assess the energy demand, the ACSM recommends various options (e.g. based on the daily recommended intake, the basal metabolic rate and a factor of physical activity or metabolic equivalents) [37]. The IOC refers to the fat-free mass (30–45 kcal/kg FFM/day) [38]. Our subjects trained an average of three times a week for about 60 min, which corresponds to an estimated physical activity level value of about 1.7 [33]. More than half of each group did not reach the recommended energy intake, which is not uncommon in endurance athletes [39]. There were no differences among the groups, which agrees with the results of Lynch and colleagues, who compared 35 vegetarian athletes with 35 omnivores [24].

Macronutrients

Carbohydrates are the most important sources of energy and endurance athletes strive to consume carbohydrates to benefit from full glycogen stores [40]. Depending on the intensity and type of training or competition, gender and external influences, an absolute amount of 3–7 g/kg BW is recommended. Thus, participants in the present study achieved the recommendations for carbohydrate intake. Similar to previous studies with non-athletes [41–44], the VEG group had the highest intake of carbohydrates (55.2, 51.4–59.0 EN%) compared to OMN (46.7, 43.6–49.8 EN%; $p_{OMN\text{-}VEG}$ = 0.002) and LOV (49.4, 45.5–53.3 EN%; n.s. compared to VEG), which can be explained by the increased intake of potatoes and fruit, since the intake of whole grain and cereal products, pastries and sweets were similar for all groups.

The protein needs of athletes have been widely discussed [45–47]. The ACSM and IOC recommend a range of 1.2–2.0 g/kg BW for (endurance) athletes [37,38] and pay no attention to ambitious recreational athletes. By contrast, the ISSN recommends 0.8–1.0 g/kg BW for general fitness [35]. According to the IOC and ACSM, the recommended amount also applies to vegetarians. The average protein intake of all three groups was within the reference ranges of the ACSM and IOC, but above the reference range of the ISSN. In addition to absolute protein intake, it is important to consider the quality of the proteins. Protein sources were mainly meat, meat products and sausages, fish and dairy products for the OMN group, milk, dairy products and eggs for LOV, and cereal products and soybeans for VEG. The biological value of animal proteins is slightly higher compared to plant-based proteins. Compared to the reference values of the WHO, on average, all groups met the reference range for amino acid intake [34]. Hence, it can be assumed that all three groups – including VEG – had an adequate

protein and amino acid supply. This is consistent with the literature, which has shown that non-athlete LOV and VEG appear to be within the range of recommendations for protein intake [44,48].

Dietary fats are valuable energy sources and have structural and regulatory functions. Regarding adequate fat intake, the recommendations vary strongly between the sports societies. While the ACSM recommends a daily intake of 20–35 EN% but not less than 20 EN% fat [37], the IOC advises an intake of ≥ 15–20 EN% fat, depending on the type of sport [49]. By contrast, both D-A-CH and ISSN recommend a fat intake of 30 EN% [33,35]. Most subjects in the three groups reached the recommendations of the D-A-CH [33], ISSN and ACSM. In addition, it is important to evaluate the PUFA intake of athletes, which was below the reference value in all three groups [33]. The PUFAs play a pivotal role in health due to their precursor function as regulatory lipid mediators. The International Society for the Study of Fatty Acids and Lipids recommends a daily sum EPA + DHA intake of 0.5 g, which was achieved by the OMN group (0.54, 0.23–0.85 g), but not by LOV (0.08, 0.04–0.12 g; $p_{OMN\text{-}LOV} = 0.003$) or VEG (0.09, 0.01–0.17 g; $p_{OMN\text{-}VEG} < 0.001$) [50]. The supply situation of LOV and VEG in the study collective can be classified as inadequate, which is consistent with other studies regarding non-athlete vegetarians and VEG [51]. The EPA/DHA supplements were only consumed occasionally in the VEG and LOV groups. The resulting LA:ALA ratios in the VEG (1:5.71) and LOV groups (1:5.30) were within the reference range [33]. The OMN group showed higher LA:ALA ratios (1:8.04), which are consistent with the results of the German Nutrition Survey [52].

Micronutrients

It is generally thought that athletes consume high amounts of micronutrients via dietary supplements due to their increased health awareness [53]. However, several studies have shown insufficient micronutrient intake in athletes [54,55]. For this specific group, there are no clear recommendations for micronutrient intake. However, in the view of the ACSM, ISSN and IOC, an adequate supply of micronutrients is assured with a balanced mixed diet. A possible insufficient supply to vegetarians of zinc, iron, riboflavin, cobalamin and vitamin D is described in the ACSM and IOC guidelines [36,37], while the ACSM additionally mentions calcium, pyridoxine and folate. A specific risk of an insufficient micronutrient supply with a VEG diet is not mentioned.

In the present study, magnesium, calcium, iron, vitamin D and cobalamin were the most frequently supplemented nutrients. Cobalamin intake was strongly dependent on supplementation, especially for both female and male VEG. Half of the VEG group supplemented cobalamin and, thus, had an adequate intake compared to the D-A-CH reference values of 4 µg per day [33]. As expected, subjects of the VEG group who did not

take cobalamin supplements had a marginal intake. Additionally, the dietary intake of the LOV group was insufficient, especially for males, who had cobalamin intakes below the recommendations, regardless of supplementation. However, although consuming cobalamin-rich foods such as meat, meat products and fish, its intake was still inadequate in one-third of the OMN group. Cobalamin is considered critical for VEG, but adequate intake should be ensured for every diet.

Due to high riboflavin levels in animal products, it was not surprising that the OMN group consumed the highest amounts, although, on average, VEG and female LOV reached the recommendations, which agrees with previous studies in non-athletes [56,57]. In contrast to Eisinger et al., who showed high intakes of riboflavin in LOV endurance runners [58], only female LOV achieved the reference values. Pyridoxine intake exceeded the recommendations in the VEG group due to the high consumption of vegetables, legumes, nuts and seeds, which has already been shown by other studies with non-athletes [56,59]. The VEG group showed a high folate intake due to the high amount of folate in green vegetables, yeast and nuts, while the folate intake of most OMN subjects was insufficient. These results are consistent with the German Nutrition Survey [52] and studies with athletes [54].

Similar to cobalamin, vitamin D intake was strongly dependent on the use of supplements. This becomes clear by comparing the vitamin D intake between SU and non-SU. On average, the VEG group (19.9, 2.75–37.0 µg) was closest to the recommendations of 20 µg per day compared to OMN (8.29, 2.21–14.4 µg) and LOV (4.52, -1.14–10.4 µg). However, the intake of vitamin D was considerably higher in SU compared to non-SU. Hence, the mean values for the vitamin D intake in the VEG group (including SU and non-SU) should be treated with caution. This also applies to the OMN and LOV group, although not quite as strongly pronounced. However, it is worth mentioning that an adequate vitamin D status can only be evaluated with the endogenous 25-hydroxyvitamin D status in the blood.

Similar to other studies with non-athletes [42,56], the highest iron intake from food (excluding supplements) was observed in VEG subjects compared to LOV and OMN. In addition, the VEG group had the highest iron intake via supplements compared to the other two groups. A total of more than 85 % of VEG subjects achieved the recommendations compared to only ~ 50 % in OMN and LOV. Male subjects of all groups were above the recommendations with more than 10 mg per day, independent of supplementation. Female OMN and LOV subjects achieved the recommendation of 15 mg daily only after supplementation. Interestingly, the VEG group reached the iron intake recommendations solely via food and not via supplements. The literature on the iron supply of athletes is inconsistent. Some studies found an adequate [60,61] and others an inadequate iron intake in athletes [62]. High-performance athletes might have increased requirements due to increased iron losses via sweat, urine and feces, which

results in a higher risk of iron deficiency anemia [61]. In addition to absolute amounts, the bioavailability of different iron species should be considered. Despite the exclusive consumption of plant-based iron of the VEG group, LOV and OMN also consumed predominantly plant iron sources. While plant-based foods contain non-heme iron, mainly in trivalent form (Fe^{3+}), which has a poor bioavailability of 1–5 %, meat and fish contain about 70 % of the total iron in the form of heme iron, which can be absorbed much better at 10–20 % [63,64]. Hence, the lower iron intake in OMN subjects compared to LOV and VEG does not necessarily result in a lower status. Moreover, further influences on bioavailability (promoting substances such as ascorbic acid or lactic acid and inhibiting substances such as phytic acid or oxalic acid, which occurs in vegetable foods) must be taken into account (the same applies to zinc, magnesium and calcium). Therefore, only functional parameters, such as transferrin and ferritin, indicate an adequate supply status.

The present results show that calcium is still a critical nutrient [55]. As expected, calcium intake was highest among OMN subjects, although more than half were below the reference range. The highest number of subjects with an intake below the reference range for calcium was found in the LOV group (64.0 %), although they consumed milk and dairy products. The calcium supply of athletes should be improved independently of dietary habits due to the importance of bone health, and normal nerve and muscle function. The mean intake of zinc was in the reference range for all groups, although male LOV subjects were slightly below. Female subjects and non-SU reached the recommendations. Interestingly, the zinc supply was similar in OMN and VEG subjects, although animal-based foods are rich in zinc and the zinc supplement intake in the VEG group was considerably lower than OMN. These results reveal that zinc-rich plant-based foods can secure adequate zinc supply. The literature on zinc supply is inconsistent. Some studies observed a slightly lower but adequate intake of zinc in vegetarians and VEG compared to OMN [43,48,56], other studies found no differences between vegetarian and OMN endurance athletes [24].

The fact that the data of dietary intake relied on self-reported data by subjects should be considered. Both under- and over-reporting are further sources of error in dietary records. Since the use of iodized salt is voluntary in Germany and a precise indication about the dietary intake is critical, the values of iodine intake should be considered with caution. Furthermore, there are limitations regarding the nutrition software which shows data gaps, especially regarding VEG products. We did not consider the water intake of the subjects, which might also influence nutrient (e.g. mineral) supply.

Conclusion

In summary, all three groups were adequately supplied with most nutrients. As expected, the intake of carbohydrates and fiber was highest in the VEG group, while the recommended

amount of fat was not reached. Moreover, all three groups exceeded the recommendations for absolute protein intake. The mean intake of micronutrients was partly dependent on supplementation, especially for vitamin D and cobalamin. Only female VEG achieved the recommended amounts for iron intake solely via food and not via supplements. However, the demand for several micronutrients might be higher for athletes due to increased losses. Recommendations of current guidelines for an adequate micronutrient intake of recreational athletes are sparse due to a lack of data.

Abbreviations

ACSM, The American College of Sports Medicine; ALA, alpha-linolenic acid; ANOVA, one-way analysis of variance; BMI, body mass index; bpm, beats per minute; BW, body weight; CI, confidence interval; D-A-CH, Deutsche, Österreichische und Schweizerische Gesellschaften für Ernährung, German, Austrian and Swiss Nutrition Societies; DHA, docosahexaenoic acid; EN%, energy percent; EPA, eicosapentaenoic acid; FFM, fat free mass; IOC, International Olympic Committee; ISSN, The International Society for Sports Nutrition; LA, linoleic acid; LOVs, lacto-ovo vegetarians; n.s., not significant; non-SU, non-supplement users; OMNs, omnivores; PUFA, polyunsaturated fatty acid; SD, standard deviation; SU, supplement users; VEGs, vegans; WHO, World Health Organization

Declarations

Ethics approval and consent to participate

The Ethics Committee at the Medical Chamber of Lower Saxony (Hannover, Germany) granted ethical approval for this research. The study was conducted in accordance with the Declaration of Helsinki. All participants gave their written informed consent.

Consent of publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Author´s contribution

JN, SH, UT and AH co-designed the study and survey materials. JN, PW, JE and SH were responsible for data acquisition. JN drafted the manuscript and conducted the statistical analyses. JPS and AH revised the manuscript critically for important content. All authors provided critical revisions and read and approved the final manuscript.

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Additional files

Additional file 1: Dietary intake of essential amino acids (mg/kg BW) according to dietary pattern.

Additional file 2: Dietary intake of fatty acids according to dietary pattern.

Additional file 3: Proportion of participants who did not reach the recommended dietary intake of minerals and vitamins. Dietary intake is depicted in addition to supplement intake.

Table 1 Characterization of the study population (mean±SD).

		OMN (n = 27)	p value OMN-LOV	LOV (n = 26)	p value LOV-VEG	VEG (n = 28)	p value OMN-VEG	p value 3 groups
Age (years)		27.4 ± 4.03	-	27.6 ± 4.31	-	27.5 ± 4.24	-	0.968[b]
Sex		m = 11, w = 16	-	m = 10, w = 16	-	m = 10, w = 18	-	0.929[d]
BMI (kg/m^2)		22.3 ± 1.74	-	21.6 ± 1.98	-	22.1 ± 2.09	-	0.436[b]
Waist (cm)	female	71.0 ± 4.3	-	70.1 ± 3.8	-	69.5 ± 5.0	-	0.057[a]
male		79.5 ± 4.3	-	76.4 ± 3.0	-	80.6 ± 4.1	-	0.591[a]
Systolic blood pressure (mm Hg)		121 ± 11.1	-	121 ± 13.4	-	116 ± 12.6	-	0.201[b]
Diastolic blood pressure (mm Hg)		74.0 ± 6.00	-	72.0 ± 4.00	-	72.0 ± 9.00	-	0.457[b]
Pulse rate (bpm)		66.0 ± 9.00	-	61.0 ± 8.00	-	65.0 ± 10.00	-	0.188[b]
Duration of diet								**0.001[d]**
< 0.5 years (%)		0		0		0		
0.5–1 year (%)		0		15.4		21.4		
1–2 years (%)		3.70		11.5		14.3		
2–3 years (%)		0		7.69		25.0		
> 3 years (%)		96.3		65.4		39.3		
Magnesium supplement user (%)		22.2		17.9		23.1		0.710[d]
Calcium supplement user (%)		11.1		3.85		7.14		0.210[d]
Iron supplement user (%)		11.1		15.4		19.3		0.689[d]
Vitamin B12 supplement user (%)		18.5		15.4		53.9		**0.005[d]**
Vitamin D supplement user (%)		22.2		3.85		23.1		0.078[d]
Smoker (%)		0		0	-	0	-	-
Training frequency per week		3.04 ± 0.98	-	3.24 ± 0.88	-	3.00 ± 0.85	-	0.502[b]
Running time per week (h)		2.72 ± 1.11	-	3.38 ± 1.43	-	2.65 ± 1.38	-	0.079[b]

OMN = omnivores, LOV = lacto-ovo vegetarians, VEG = vegans, BMI = body mass index, bpm = beats per minute.
[a] One-way ANOVA, [b] Kruskal Wallis test, [c] Post hoc test, [d] Chi-square test.

Table 2 Mean daily intake of different food categories calculated from a 3-day dietary record.

Food group (g/day)	OMN (n = 27)	p value OMN-LOV	LOV (n = 25)	p value LOV-VEG	VEG (n = 27)	p value OMN-VEG	p value 3 groups
Meat	85.8 ± 58.8	0.000[c]	-	1.000[c]	-	0.000[c]	**0.000[b]**
Meat products and sausages	29.6 ± 32.1	0.000[c]	-	1.000[c]	-	0.000[c]	**0.000[b]**
Fish and seafood	28.7 ± 39.9	0.000[c]	-	1.000[c]	-	0.000[c]	**0.000[b]**
Milk and dairy products	290 ± 183	1.000[c]	279 ± 311	0.000[c]	-	0.000[c]	**0.000[b]**
Eggs	23.8 ± 37.4	1.000[c]	15.8 ± 25.0	0.003[c]	-	0.000[c]	**0.000[b]**
Fat and oil	9.85 ± 14.8	-	10.3 ± 12.1	-	12.0 ± 10.8	-	0.228[b]
Whole grain products	33.2 ± 48.7	-	50.6 ± 58.8	-	51.0 ± 59.0	-	0.294[b]
Cereal products	208 ± 141	-	188 ± 130	-	220 ± 120	-	0.678[a]
Pastries	58.8 ± 50.0	-	58.0 ± 100	-	37.4 ± 73.8	-	0.067[b]
Potatoes	44.1 ± 79.3	1.000[c]	37.5 ± 62.3	0.013[c]	118 ± 130	0.017[c]	**0.005[b]**
Vegetables (except potatoes, legumes)	265 ± 237	0.511[c]	324 ± 187	0.031[c]	521 ± 258	0.000[c]	**0.000[b]**
Legumes (except soybeans)	3.70 ± 8.08	0.054[c]	27.7 ± 39.7	0.092[c]	66.4 ± 68.1	0.000[c]	**0.000[b]**
Soybeans	-	0.007[c]	54.4 ± 95	0.031[c]	151 ± 179	0.000[c]	**0.000[b]**
Fresh fruit	266 ± 160	1.000[c]	288 ± 171	0.041[c]	518 ± 404	0.015[c]	**0.009[b]**
Nuts and seeds	4.57 ± 8.30	0.044[c]	19.7 ± 23.7	0.578[c]	26.0 ± 29.3	0.000[b]	**0.001[b]**
Sweets	37.0 ± 39.3	-	38.9 ± 44.4	-	20.2 ± 33.6	-	0.148[b]
Alcoholic drinks	131 ± 210	-	101 ± 198	-	63.0 ± 146	-	0.184[b]
Alcohol	5.50 ± 8.64	-	3.89 ± 6.91	-	2.26 ± 5.57	-	0.345[b]
Nonalcoholic beverages (except coffee and tea)	1103 ± 1095	-	794 ± 1098	-	1246 ± 1258	-	0.339[b]
Coffee	170 ± 164	-	279 ± 238	-	148 ± 198	-	0.051[b]
Tea	257 ± 398	-	181 ± 310	-	221 ± 339	-	0.999[b]
Fast food	57.1 ± 75.2	0.063[c]	32.7 ± 87.2	1.000[c]	16.6 ± 38.1	0.025[c]	**0.016[b]**

All nutrients excluding dietary supplements. OMN = omnivores, LOV = lacto-ovo vegetarians, VEG = vegans.
Data are presented as mean±SD. [a] One-way ANOVA, [b] Kruskal Wallis test, [c] Post hoc test.

Table 3 Absolute and relative daily energy and macronutrient intake of the study population calculated from a 3-day dietary record.

Nutrient intake	OMN (n = 27)	p value OMN-LOV	LOV (n = 25)	p value LOV-VEG	VEG (n = 27)	p value OMN-VEG	p value 3 groups	Reference values (m/f)
Energy								
Energy intake (MJ)	10.4 (8.70, 12.1)	-	9.67 (8.55, 10.8)	-	10.2 (9.12, 11.3)	-	0.989[b]	11.9–12.3/9.41–9.83
Macronutrients								
Carbohydrate (EN%)	46.7 (43.6, 49.8)	0.824[c]	49.4 (45.5, 53.3)	0.067[c]	55.2 (51.4, 59.0)	0.002[c]	**0.003[a]**	> 50
Carbohydrate (g/kg BW)	4.31 (3.45, 5.17)	1.000[c]	4.22 (3.52, 4.91)	0.094[c]	5.01 (4.40, 5.62)	0.111[c]	**0.049[b]**	
Protein (EN%)	16.7 (15.1, 18.9)	0.540[c]	15.9 (13.6, 18.2)	0.295[c]	13.8 (12.5, 15.0)	0.007[c]	**0.009[b]**	
Protein (g/kg BW)	1.50 (1.27, 1.66)	0.159[c]	1.34 (1.09, 1.56)	1.000[c]	1.25 (1.07, 1.42)	0.063[c]	**0.047[b]**	0.8
Fat (EN%)	32.5 (30.5, 34.5)	0.432[c]	30.8 (26.8, 34.8)	0.708[c]	26.3 (22.7, 29.8)	0.021[c]	**0.026[b]**	30
Fiber (g)	27.0 (22.8, 31.1)	0.176[c]	33.4 (28.6, 38.2)	0.006[c]	51.7 (44.1, 59.4)	0.000[c]	**0.000[b]**	≥ 30

OMN = omnivores, LOV = lacto-ovo vegetarians, VEG = vegans, MJ = mega joule, EN% = energy percent, BW = body weight, reference values of the German, Austrian and Swiss Nutrition Societies (Deutsche, Österreichische und Schweizerische Gesellschaften für Ernährung, D-A-CH) [33]. Data are presented as mean (95 % CI). [a] One-way ANOVA, [b] Kruskal Wallis test, [c] Post hoc test.

Table 4 Dietary mineral intake of the study population calculated from a 3-day dietary record (nutrient intake via food and supplements).

		OMN (n = 27)	p value OMN-LOV	LOV (n = 25)	p value LOV-VEG	VEG (n = 27)	p value OMN-VEG	p value 3 groups	Reference values (m/f)*
Na (g)	food	2.65 (2.17, 3.12)	0.004[b]	1.72 (1.44, 2.00)	1.000[b]	1.72 (1.46, 1.99)	0.005[b]	**0.001[a]**	1.5
supplement		0	-	0	-	0	-	-	
K (g)	food	3.16 (2.88, 3.50)	1.000[b]	3.04 (2.55, 3.52)	0.005[b]	4.65 (3.85, 5.50)	0.014[b]	**0.002[a]**	4[c]
supplement		0	-	0.00 (0.00, 0.01)	-	0.00 (0.00, 0.01)	-	0.372[a]	
Ca (mg)	food	981 (813, 1149)	-	901 (716, 1085)	-	730 (614, 846)	-	0.115[a]	1000
supplement		45.1 (-32.0, 122)	-	0	-	6.37 (-2.22, 15.0)	-	0.214[a]	
P (g)	food	1.43 (1.26, 1.60)	-	1.34 (1.08, 1.61)	-	1.33 (1.15, 1.52)	-	0.495[a]	0.7
supplement		0	-	0	-	0	-	-	
Mg (mg)	food	346 (310, 382)	0.990[b]	388 (324, 452)	0.001[b]	599 (518, 679)	0.000[b]	**0.000[a]**	350/300
supplement		36.7 (0.44, 73.0)	-	53.2 (-5.58, 112)	-	54.3 (-7.09, 116)	-	0.910[a]	
Fe (mg)	food (total)	11.9 (10.6, 13.2)	1.000[b]	12.8 (10.8, 14.7)	0.001[b]	19.6 (16.8, 22.4)	0.000[b]	**0.000[a]**	10/15
plant-based iron		7.44 (6.33, 8.54)	0.105[b]	10.7 (8.95, 12.5)	0.000[b]	19.6 (16.8, 22.4)	0.000[b]	**0.000[a]**	
animal iron		4.45 (3.67, 5.24)	0.013[b]	2.02 (1.41, 2.61)	0.000[b]	0	0.000[b]	**0.000[a]**	
supplement		1.70 (-1.36, 4.77)	-	1.52 (-1.19, 4.24)	-	3.74 (-0.64, 8.12)	-	0.675[a]	
Zn (mg)	food	9.74 (8.32, 11.2)	-	8.88 (7.30, 10.5)	-	10.7 (9.21, 12.2)	-	0.214[a]	10/7
supplement		2.23 (-1.59–6.04)	-	0.90 (-0.70–2.49)	-	0.47 (-0.48–1.41)	-	0.648[a]	
Cu (mg)	food	1.63 (1.43, 1.84)	0.819[b]	1.85 (1.56, 2.13)	0.001[b]	2.93 (2.51, 3.34)	0.000[b]	**0.000[a]**	1.0-1.5
supplement		0	-	0	-	0	-	-	
Mn (mg)	food	4.75 (3.87, 5.62)	0.188[b]	6.29 (5.05, 7.54)	0.067[b]	8.48 (7.10, 9.85)	0.000[b]	**0.000[a]**	2.0-5.0
supplement		0	-	0	-	0	-	-	
I (µg)	food	88.8 (64.1, 114)	0.190[b]	61.6 (49.4, 73.7)	1.000[b]	57.7 (48.4, 67.0)	0.060[b]	**0.048[a]**	200
supplement		0	-	0	-	0	-	-	

OMN = omnivores, LOV = lacto-ovo vegetarians, VEG = vegans, reference values of the German, Austrian and Swiss Nutrition Societies (Deutsche, Österreichische und Schweizerische Gesellschaften für Ernährung, D-A-CH) [33].
Data are presented as mean (95 % CI). [a] Kruskal Wallis test, [b] Post hoc test, [c] Estimated values.

Table 5 Dietary vitamin intake of the study population calculated from a 3-day dietary record (nutrient intake via food and supplements).

		OMN (n = 27)	p value OMN-LOV	LOV (n = 25)	p value LOV-VEG	VEG (n = 27)	p value OMN-VEG	p value 3 groups	Reference values (m/f)*
A [retinol equ.] (mg)	food	1.45 (0.81, 2.10)	-	1.26 (0.91, 1.61)	-	1.72 (1.27, 2.16)	-	0.221[a]	1.0/0.8
	supplement	0	-	0	-	0	-	-	
D (µg)	food	2.61 (1.34, 3.89)	1.000[b]	1.67 (1.02, 2.32)	0.037[b]	1.04 (0.46, 1.62)	0.003[b]	**0.002[a]**	20
	supplement	5.68 (-0.12, 11.5)	-	2.75 (-2.91, 8.40)	-	18.8 (1.61, 36.1)	-	0.086[a]	
E (mg)	food	9.66 (7.85, 11.5)	0.851[b]	11.4 (9.03, 13.7)	0.280[b]	16.4 (12.5, 20.4)	0.015[b]	**0.018[a]**	14/12[c]
	supplement	1.12 (-0.47–2.71)	-	0.15 (-0.16–0.47)	-	0.04 (-0.37–0.11)	-	0.411[a]	
K (µg)	food	92.5 (63.5, 122)	0.119[b]	181 (96.6, 266)	0.058[b]	261 (164, 359)	0.000[b]	**0.000[a]**	70/60
	supplement	0	-	0	-	0	-	-	
B_1 [thiamine] (mg)	food	1.38 (1.21, 1.55)	0.502[b]	1.20 (0.98, 1.43)	0.003[b]	1.86 (1.56, 2.16)	0.143[b]	**0.004[a]**	1.2/1.0
	supplement	0.56 (-0.58, 1.70)	-	0.17 (-0.16, 0.50)	-	0.09 (-0.08, 0.26)	-	0.888[a]	
B_2 [riboflavin] (mg)	food	1.57 (1.34, 1.79)	-	1.54 (1.12, 1.96)	-	1.38 (1.16, 1.59)	-	0.278[a]	1.4/1.1
	supplement	0.56 (-0.58, 1.70)	-	0.01 (-0.01, 0.03)	-	0.11 (-0.98, 0.33)	-	0.896[a]	
Niacin (mg)	food	21.4 (18.5, 24.3)	0.033[b]	15.8 (12.3, 19.3)	1.000[b]	17.3 (12.3, 22.3)	0.021[b]	**0.010[a]**	15/12
	supplement	0.62 (-0.52, 1.77)	-	0.09 (-0.09, 0.27)	-	1.31 (-1.12, 3.74)	-	0.645[a]	
Pantothenic acid (mg)	food	5.23 (4.38, 6.07)	-	5.36 (4.04, 6.68)	-	6.39 (4.96, 7.81)	-	0.461[a]	6[c]
	supplement	0.95 (-0.95, 2.85)	-	0	-	0.04 (-0.19, 0.11)	-	0.374[a]	
B_6 [pyridoxine] (mg)	food	1.91 (1.61, 2.20)	0.670[b]	1.59 (1.27, 1.91)	0.002[b]	2.63 (2.10, 3.16)	0.087[b]	**0.003[a]**	1.5/1.2
	supplement	0.47 (-0.31, 1.25)	-	0.46 (-0.11, 1.04)	-	0.16 (-0.07, 0.40)	-	0.497[a]	
Biotin (µg)	food	50.9 (44.9, 56.9)	-	56.7 (43.4, 69.9)	-	64.5 (51.4, 77.6)	-	0.573[a]	30–60[c]
	supplement	6.10 (-5.33, 17.5)	-	0	-	0.70 (-0.44, 1.90)	-	0.373[a]	
Folate (µg)	food	307 (249, 364)	1.000[b]	327 (265, 389)	0.024[b]	478 (402, 572)	0.001[b]	**0.001[a]**	300
	supplement	11.3 (-5.01, 27.6)	-	2.20 (-2.33, 6.72)	-	41.9 (-20.2, 104)	-	0.261[a]	
B_{12} [cobalamin] (µg)	food	4.02 (3.12, 4.92)	0.057[c]	2.49 (1.49, 3.48)	0.002[b]	0.79 (0.47, 1.12)	0.000[b]	**0.000[a]**	4
	supplement	0.96 (-0.21, 2.13)	0.002[b]	0.84 (-0.20, 1.89)	1.000[b]	206 (101, 312)	0.004[b]	**0.001[a]**	
C [ascorbic acid] (mg)	food	153 (110, 196)	1.000[b]	143 (107, 179)	0.003[b]	293 (222, 365)	0.001[b]	**0.000[a]**	110/95
	supplement	3.16 (-1.07, 7.38)	-	0.17 (-0.18, 0.51)	-	7.80 (-1.26, 13.7)	-	0.126[a]	

OMN = omnivores, LOV = lacto-ovo vegetarians, VEG = vegans, retinol equ. = retinol equivalent, reference values of the German, Austrian and Swiss Nutrition Societies (Deutsche, Österreichische und Schweizerische Gesellschaften für Ernährung, D-A-CH) [33].
Data are presented as mean (95 % CI). [a] Kruskal Wallis test, [b] Post hoc test, [c] Estimated values.

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2.2. Paper II

Micronutrient status of recreational runners with vegetarian and non-vegetarian dietary patterns

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Micronutrient Status of Recreational Runners with Vegetarian or Non-Vegetarian Dietary Patterns

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Abstract: Vegetarian diets have gained popularity in sports. However, few data exist on the status of micronutrients and related biomarkers for vegetarian and vegan athletes. The aim of this cross-sectional study was to compare the micronutrient status of omnivorous (OMN, n = 27), lacto-ovo-vegetarian (LOV, n = 26), and vegan (VEG, n = 28) recreational runners. Biomarkers of vitamin B_{12}, folate, vitamin D, and iron were assessed. Additionally, serum levels of calcium, magnesium, and zinc were examined. Lifestyle factors and supplement intake were recorded via questionnaires. About 80% of each group showed vitamin B_{12} adequacy with higher levels in supplement users. Mean red blood cell folate exceeded the reference range (>340 nmol/L) in all three groups (OMN: 2213 ± 444, LOV: 2236 ± 596, and VEG: 2354 ± 639 nmol/L; not significant, n.s.). Furthermore, vitamin D levels were comparable (OMN: 90.6 ± 32.1, LOV: 76.8 ± 33.7, and VEG: 86.2 ± 39.5 nmol/L; n.s.), and we found low prevalence (<20%) of vitamin D inadequacy in all three groups. Less than 30% of each group had depleted iron stores, however, iron deficiency anemia was not found in any subject. Our findings suggest that a well-planned, health-conscious lacto-ovo-vegetarian and vegan diet, including supplements, can meet the athlete's requirements of vitamin B_{12}, vitamin D and iron.

Keywords: vegetarianism; veganism; recreational athletes; nutrient supply; nutrient status

1. Introduction

Micronutrients such as vitamins as well as major and trace minerals are involved in various metabolic processes important to physical performance [1]. Regular physical activities are associated with several biochemical training adaptations like an increased expression of antioxidant enzymes or increased blood formation, which, as a result, cause higher micronutrient requirements. Moreover, studies dealing with competitive athletes observed insufficient intake of energy-supplying macronutrients but also micronutrients, especially in athletes with unfavorable or restricted food choices [2,3]. Additionally, due to increased physical stress, through increased sweating and losses via urine, feces, and foot-strike hemolysis, athletes might have increased requirements of several micronutrients like iron and zinc. There is well-defined research that underlines the fact that an inadequate micronutrient status compromises physical performance and regeneration capacity, whereas the risk of upper respiratory tract infections (URTI) increases [4–6].

Currently, plant-based nutrition is a topical issue in sports medicine. There is debate about whether a plant-based diet can provide all the required nutrients in adequate amounts for an athlete. However, several nutrition societies have concluded that well-planned vegetarian dietary patterns, including a wide variety of plant foods, can be adequate for athletes [7–12], and numerous sportspeople from various disciplines have already shifted to a plant-based diet [13].

Previous studies have examined the nutritional status of vegans compared to vegetarians and omnivores, but these studies were largely with non-athletes [14]. In general, individuals following a plant-based diet showed adequate supply with most nutrients. However, it is generally assumed that limiting animal products in the diet increases the risk of certain micronutritional deficiencies. Actually, "the more foods eliminated from any diet, the greater the risk of deficiency" [15]. Undoubtedly, animal-derived foods like lean red meat, fish, and eggs are excellent sources of vitamin B_{12} and provide high amounts of bioavailable zinc, iron, and vitamin D [16–18]. Furthermore, dairy products are rich in calcium and other minerals [19]. Consequently, calcium, zinc, iron, vitamin B_{12}, and vitamin D are described as critical nutrients in vegetarian and especially vegan dietary patterns [20].

However, up to now, only few data on the micronutrient biomarker status of vegetarian athletes exist [21,22]. Furthermore, most studies did not differentiate the various types of vegetarianism. Thus, the micronutrient status of athletes consuming a vegetarian and vegan dietary pattern is rather unknown.

To fill this knowledge gap, the approach of this study was to determine the nutritional status of selected parameters of vegan recreational runners in comparison to lacto-ovo-vegetarians and omnivores. In addition, we ascertained the influence of dietary supplement use on micronutrient biomarker status. It was hypothesized that micronutrient status differed between the groups.

2. Materials and Methods

2.1. Study Design and Participants

This cross-sectional study was conducted according to the guidelines laid down in the Declaration of Helsinki. The ethics committee at the medical chamber of Lower Saxony (Hannover, Germany) approved all procedures. All subjects gave their written informed consent. The study was registered in the German Clinical Trial Register (DRKS00012377).

The study was conducted at the Institute of Food Science and Human Nutrition, Leibniz University Hannover, Germany.

Eighty-one healthy omnivorous (OMN), vegetarian (LOV), and vegan (VEG) recreational runners (men and women) aged between 18 and 35 years were recruited from the general population in Hannover, Germany, via local running events, online running communities, as well as online vegetarian and vegan communities.

Eligibility of subjects was assessed using questionnaires. Subjects were preselected via screening questionnaires according to the following inclusion criteria: omnivorous, lacto-ovo-vegetarian, or vegan diet for at least half a year, body mass index (BMI) between 18.5 and 25.0 kg/m^2, and regularly running (2 to 5 times per week) for at least 30–60 min. Regular running sessions were documented via self-reporting data. The following criteria led to exclusion: any cardiovascular, metabolic, or malignant disease; diseases regarding the gastrointestinal tract; pregnancy; food intolerances; and addiction to drugs or alcohol. The use of dietary supplements did not lead to exclusion except if they were performance-enhancing substances (e.g., alkaline salts, creatine).

The categorization of omnivorous, lacto-ovo-vegetarian, and vegan was based on questionnaires, which initially included a question about the current diet. Secondly, consumed food groups were queried to make sure that the participants classified themselves correctly. Subjects were classified as "omnivorous" if they consumed grains, plant foods, legumes, milk and dairy products, and eggs as well as fish, meat, and meat products. "Lacto-ovo-vegetarians" were characterized by the consumption of grains, plant foods, legumes, milk and dairy products, and eggs. The consumption of grains, plant

foods, and legumes characterized "vegans". Participants that were included in the study population were matched according to age and gender. They were invited to the Institute of Food Science and Human Nutrition of the Leibniz University Hannover for a comprehensive examination. Participants completed a questionnaire regarding their supplement intake (frequency and dosage), health status, and running activity. Training frequency and duration were self-reported by the subjects.

2.2. Analytical Methods

After overnight fasting (≥10 h fasting period), blood samples were collected between 06:00 and 10:00 a.m. Blood samples were obtained by venipuncture of an arm vein using Multiflyneedles (Sarstedt, Nümbrecht, Germany) into serum, EDTA, or special monovettes for tHcy (Sarstedt). All samples were stored at ~5 °C and were transferred to the laboratory on the same day.

All micronutrient parameters described below were determined in an accredited and certified laboratory (Laborärztliche Arbeitsgemeinschaft für Diagnostik und Rationalisierung e.V., in Hannover, Germany).

Briefly, vitamin B_{12} and holotranscobalamin (Holo-TC) in serum were determined with the use of the electrochemiluminescence immunoassay method (ECLIA) on cobas®test systems (Roche Diagnostics GmbH, Mannheim, Germany) according to [23,24] (pp. 281–283). Liquid chromatography with mass spectrometry coupling (LC-MS/MS) was applied to assess methylmalonic acid (MMA) in serum [25]. Plasma homocysteine (tHcy) was determined by HPLC with a fluorescence detector in accordance with [26]. The four marker combined vitamin B-12 indicator (4cB12) was computed from concentrations of vitamin B_{12} in serum, Holo-TC, MMA, and homocysteine according to published formula [27]:

$$4\text{cB12} = \log_{10}\left(\frac{\text{HoloTC} * \text{B}_{12}}{\text{MMA} * \text{tHcy}}\right) - (\text{age factor}).$$

Vitamin D status (25-hydroxyvitamin D, 25(OH)D) was measured in serum by ECLIA (Roche Diagnostics GmbH, Mannheim, Germany) according to [28].

Folate was analyzed in red blood cells (RBCs) as a reliable biomarker for tissue folate status [29] via ECLIA on cobas®8000 modular analyzer series (Roche Diagnostics GmbH, Mannheim, Germany) [30].

Calcium status [31] and magnesium [32] status were assessed in serum by a photometric method (Beckman Coulter®, Krefeld, Germany). Atomic absorption spectrometry (AAS, PerkinElmer, Inc., Waltham, Massachusetts) was used to measure serum zinc concentrations [33]. Iron in serum was assessed using a photometric method according to [34]. To determine iron stores, ferritin was measured via immunoturbidimetric assay (Beckman Coulter®, Krefeld, Germany) as well as transferrin in serum [35,36]. A photometric method was used to measure hemoglobin (Hb) [37–39]. Transferrin saturation [40], hematocrit (Hct) [41], and mean corpuscular volume (MCV) [42] were calculated using standard formulas.

2.3. References Values

According to a WHO Technical Consultation on vitamin B_{12} deficiencies, a cutoff value for serum vitamin B_{12} was set at <150 pmol/L [43] to indicate deficiency, and cutoffs for related parameters were set as the following: MMA > 271 mmol/L [44,45], Holo-TC < 35 pmol/L [46], and homocysteine > 10 µmol/L [47,48].

The following five categories for the dimensionless unit score 4cB12 were classified: probable vitamin B_{12} deficiency (<−2.5), possible vitamin B_{12} deficiency (−2.5 to −1.5), low vitamin B_{12} (−1.5 to −0.5), vitamin B_{12} adequacy (−0.5 to 1.5), and elevated vitamin B_{12} (>1.5) [27].

Vitamin D status was assessed according to the following 25(OH)D thresholds: <25.0 (deficiency), 25.0 to 49.9 (insufficiency), 50.0 to 74.9 (sufficiency), and ≥75.0 nmol/L (optimal) [49–51].

According to the WHO Consultation, RBC folate levels <340 nmol/L were regarded as deficient [43].

The following cutoff points were used to define adequate biomarker status: calcium 2.2–2.6 mmol/L [52] (pp. 231–234), magnesium 0.65–1.05 mmol/L [53] (pp. 339–340), and zinc 12–15 µmol/L [54]. According to the WHO, cutoffs for parameters of iron status were set as: serum iron <10 µmol/L,

ferritin <15 µg/L (depleted iron stores), Hb <13 or <12 g/dL (men and women, respectively, anemia), MCV <80 fl (indication for iron deficiency anemia), transferrin ≥47.7 g/L (increased iron requirement), and transferrin saturation <16% (insufficient iron supply) [41].

2.4. Data Analysis and Statistical Methods

Data are shown as mean ± standard deviation (SD) or frequency and percent. SPSS software (IBM SPSS Statistics 24.0; Chicago, IL, USA) was used for statistical analyses. The Kolmogorov–Smirnov test was used to control distribution. If data were normally distributed, a one-way analysis of variance (ANOVA) was used to evaluate differences in nutritional status between the three diet groups. In contrast, the Kruskal Wallis test was performed to analyze data with non-normal distribution. Afterwards, a post hoc test with Bonferroni correction was conducted to analyze differences between the individual groups. In order to examine differences between supplement users (SU) and non-SU or men and women within the groups, the t-test (for parametric data) and the Mann–Whitney U test (for nonparametric data) were used. Moreover, to compare the differences between the frequencies of the three groups, a chi-square test was used. In addition, Pearson correlation was computed to calculate correlations between parametric data. Finally, to assess associations between nonparametric data, Spearman´s rho correlation was used. Statistical significance was set at the 0.05 level.

3. Results

In total, 27 OMN, 26 LOV, and 28 VEG met the inclusion criteria and were included in the study. Between the three groups, there were no differences regarding gender distribution as well as mean in age and BMI (Table 1). All three groups consumed comparable frequencies of dietary supplements, except vitamin B_{12}, which was the most commonly used supplement among VEG. LOV and VEG followed their diet for a shorter period compared to OMN ($p = 0.001$, χ^2). All participants were nonsmokers and had similar training habits (Table 1).

Table 1. Participant characteristics by dietary patterns of the study population.

Measure	Omnivores (*n* = 27)	Lacto-Ovo (*n* = 26)	Vegan (*n* = 28)	*p* value
Age, years	27.4 ± 4.03	27.6 ± 4.31	27.5 ± 4.24	0.968 [a]
Sex	m = 11, f = 16	m = 10, f = 16	m = 10, f = 18	0.929 [b]
BMI, kg/m^2	22.3 ± 1.74	21.6 ± 1.98	22.1 ± 2.09	0.436 [a]
Duration of diet				
0.5–1 year, *n* (%)	0 (0)	4 (15)	6 (21)	
1–2 years, *n* (%)	1 (4)	3 (12)	4 (14)	0.001 [b]
2–3 years, *n* (%)	0 (0)	2 (8)	7 (25)	
>3 years, *n* (%)	26 (96)	17 (65)	11 (39)	
Vitamin B_{12} SU, *n* (%)	4 (19)	4 (15)	15 (54)	0.005 [b]
Vitamin D SU, *n* (%)	5 (22)	1 (4)	7 (25)	0.078 [b]
Folate SU, *n* (%)	3 (11)	1 (4)	5 (18)	0.262 [b]
Iron SU, *n* (%)	3 (11)	4 (15)	5 (18)	0.689 [b]
Calcium SU, *n* (%)	3 (11)	1 (4)	2 (7)	0.210 [b]
Zinc SU, *n* (%)	4 (15)	3 (12)	2 (7)	0.662 [b]
Magnesium SU, *n* (%)	5 (22)	4 (15)	5 (18)	0.770 [b]
Training frequency per week	3.04 ± 0.98	3.24 ± 0.88	3.00 ± 0.85	0.502 [a]
Running time per week, h	2.72 ± 1.11	3.38 ± 1.43	2.65 ± 1.38	0.079 [b]

SU = supplement users. Values are given as means ± SD or *n* (%). [a] Kruskal Wallis test and [b] chi-square test.

3.1. Biomarkers of Vitamin B_{12} Status

Overall, all three groups showed an adequate biomarker status of vitamin B_{12}-related parameters (Table 2), even when considering only men or women as subgroups (Table S1). However, the vitamin B_{12} status of supplement users of VEG and OMN was higher compared to non-SU, and a higher proportion of the non-SU had B_{12} parameters outside the reference range.

Table 2. Biomarkers of Vitamin B_{12} status.

Parameter	Supplementation	Omnivores $n = 27$	p value Omnivores vs. Lacto-Ovo	Lacto-Ovo $n = 26$	p value Lacto-Ovo vs. Vegan	Vegan $n = 28$	p value Omnivores vs. Vegan	p value
	n_{SU}	5		4		15		
	$n_{non\text{-}SU}$	22		22		13		
Vitamin B_{12}, pmol/L		323 ± 121	-	316 ± 146	-	320 ± 247	-	0.586 [b]
	SU	350 ± 112	-	261 ± 149	-	396 ± 318	-	0.590 [b]
	non-SU	316 ± 124	-	326 ± 148	-	244 ± 115	-	0.118 [b]
Deficient (<150 pmol/L), *n* (%)		1 (4)		2 (8)		3 (11)		0.349 [d]
	SU	0 (0)		1 (4)		1 (4)		
	non-SU	1 (4)		1 (4)		2 (7)		
Holo-TC, pmol/L		80.4 ± 30.1	-	85.9 ± 36.9		67.8 ± 39.4	-	0.168 [a]
	SU	92.4 ± 37.7	-	80.5 ± 53.5		82.0 ± 37.9	-	0.871 [a]
	non-SU	76.1 ± 28.9	n.s.	86.8 ± 34.7	0.013 [c]	52.1 ± 37.9	n.s.	0.016 [a]
Deficient (<35 pmol/L), *n* (%)		1 (4)		2 (8)		6 (21)		0.043 [d]
	SU	0 (0)		1 (4)		1 (4)		
	non-SU	1 (4)		1 (4)		5 (18)		
MMA, nmol/L		264 ± 174	-	266 ± 176	-	363 ± 570	-	0.693 [b]
	SU	261 ± 177	-	400 ± 362	-	216 ± 161	-	0.186 [b]
	non-SU	264 ± 177	-	234 ± 123	-	535 ± 801	-	0.226 [b]
Deficient (>271 nmol/L), *n* (%)		6 (22)		7 (27)		8 (29)		0.720 [d]
	SU	1 (4)		2 (8)		1 (4)		
	non-SU	5 (19)		5 (19)		7 (25)		
tHcy, µmol/L		12.2 ± 2.93	-	13.2 ± 6.47	-	12.8 ± 4.26	-	0.920 [b]
>10 µmol/L, *n* (%)		19 (82)		15 (58)		22 (79)	-	0.266 [d]
4cB12		0.91 ± 0.50	-	0.91 ± 0.75	-	0.70 ± 0.76	-	0.442 [a]
	SU	1.12 ± 0.56	-	0.52 ± 1.37	-	1.10 ± 0.67	-	0.490 [a]
	non-SU	0.86 ± 0.49	n.s.	0.98 ± 0.60	0.020 [c]	0.35 ± 0.75	n.s.	0.021 [a]

SU = supplement users, non-SU = non-supplement users, Holo-TC = holotranscobalamin, MMA = methylmalonic acid, 4cB12 = four marker combined vitamin B-12 indicator [27], n.s. = not significant, and tHcy = total homocysteine. Values are given as means ± SD or *n* (%) of the study population in the different cutoff values. [a] One-way ANOVA, [b] Kruskal Wallis test, [c] post hoc test, and [d] chi-square test.

Regarding 4cB12, on average, all three groups had an adequate status (Figure 1). Most subjects of each group (~80%) showed vitamin B_{12} adequacy, while 19% of LOV, 16% of OMN, and 7% of VEG had an elevated vitamin B_{12} status (>1.5). Again, the vitamin B_{12} biomarker status was higher in SU (statistically significant within the VEG group), and an overall tendency (significant for non-SU in LOV and VEG) towards lower levels in VEG compared to OMN and LOV was observed (Figure 1).

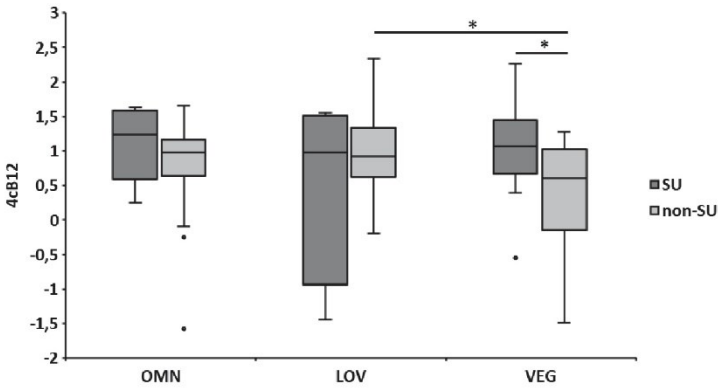

Figure 1. Vitamin B_{12} indicator (4cB12) of the dietary patterns according to supplementation. Categories of vitamin B_{12} status: <−2.5 = probable B_{12} deficiency, −2.5 to −1.5 = possible B_{12} deficient, −1.5 to −0.5 = low vitamin B_{12}, −0.5 to 1.5 = B_{12} adequacy, and >1.5 = elevated B_{12} [27]. The error bars represent the standard errors of the average 4cB12. Differences between groups were analyzed using one-way ANOVA, while differences between SU and non-SU were computed by Student´s t-test; * $p \leq 0.05$. OMN = omnivores, LOV = lacto-ovo-vegetarians, VEG = vegans, SU = supplement users, non-SU = non-supplement users, and 4cB12 = four marker combined vitamin B-12 indicator.

An inverse association was observed for homocysteine and Holo-TC in all three groups (r = −0.505, $p = 0.007$; r = −0.720, $p < 0.001$; and r = −0.400, $p = 0.035$ for OMN, LOV, and VEG, respectively). Also, inverse associations were found in the VEG group between homocysteine, vitamin B_{12} in serum (r = −0.577, $p = 0.002$), and MMA (r = 0.430, $p = 0.028$). The 4cB12 level was positively correlated with the average daily vitamin B_{12} supplement intake in the VEG group (r = 0.422, $p = 0.025$) but not in the other two groups. Further, there was a tendency of lower 4cB12 in non-SU of the VEG group, who had been vegan for a longer period compared to those who practiced a vegan diet for a shorter period ($p = 0.039$).

3.2. Biomarkers of Folate Status

RBC folate status exceeded the reference range (>340 nmol/L) in all three groups (Table 3). Additionally, in LOV, RBC folate levels were positively associated with serum vitamin B_{12} (r = 0.494, $p = 0.010$) and Holo-TC levels (r = 0.629, $p = 0.001$), while no correlations could be observed in the VEG and OMN groups. No significant differences were found in SU compared to non-SU in OMN ($p = 0.865$) and LOV ($p = 0.198$), but significance was found for VEG ($p = 0.030$).

Table 3. Biomarkers of folate and vitamin D status.

Parameter		Omnivores $n = 27$	Lacto-Ovo $n = 26$	Vegan $n = 28$	p value
	n_{SU}	3	1	5	
	$n_{non\text{-}SU}$	24	25	23	
RBC folate, nmol/L		2213 ± 444	2236 ± 596	2354 ± 639	0.577 [a]
	SU	2254 ± 776	1456 ± 0	2903 ± 494	0.134 [a]
	non-SU	2207 ± 413	2246 ± 586	2233 ± 609	0.966 [a]
Deficient (<340 nmol/L), *n* (%)		0	0	0	-
	n_{SU}	6	1	7	
	$n_{non\text{-}SU}$	21	25	21	
25(OH)D, nmol/L		90.6 ± 32.1	76.8 ± 33.7	86.2 ± 39.5	0.354 [a]
	SU	120 ± 40.4	152 ± 0	117 ± 26.3	0.619 [a]
	non-SU	82.2 ± 24.6	73.8 ± 30.6	73.8 ± 37.3	0.592 [a]
Optimal		18 (67)	11 (42)	16 (57)	0.219 [b]
(≥75 nmol), *n* (%)	SU	5 (19)	1 (4)	7 (25)	
	non-SU	13 (48)	10 (39)	9 (32)	
Sufficient		6 (22)	10 (39)	5 (18)	
(50–74.9 nmol/L), *n* (%)	SU	1 (4)	0 (0)	1 (4)	
	non-SU	5 (19)	10 (39)	4 (14)	
Insufficient		3 (11)	5 (19)	5 (18)	
(25–49.9 nmol/L), *n* (%)	SU	0 (0)	0 (0)	0 (0)	
	non-SU	3 (11)	5 (19)	5 (18)	
Deficient		0 (0)	0 (0)	2 (7)	
(<25 nmol/L), *n* (%)	SU	0 (0)	0 (0)	0 (0)	
	non-SU	0 (0)	0 (0)	2 (7)	

n_{SU} = number of supplement users, $n_{non\text{-}SU}$ = number of non-supplement users, RBC folate = red blood cell folate, and 25(OH)D = 25-hydroxyvitamin D. Values are given as means ± SD or *n* (%) of the study population at the different cutoff values. [a] One-way ANOVA, and [b] Chi-square test.

3.3. Biomarkers of Vitamin D Status

Average values of 25(OH)D were in the reference range in all three groups without any differences between the dietary groups (Table 3) and also according to gender (Table S2). However, with 76.8 nmol/L the 25(OH)D levels of LOV were in the lower reference range. An inadequate vitamin D biomarker status (<50 nmol/L) was found in <25% of all three groups. Again, vitamin D biomarker status was dependent on supplementation. In detail, all participants who took vitamin D supplements had values >50 nmol/L (Table 3). Furthermore, 25(OH)D concentrations of the total non-SU population were significantly higher in summer (83.1 ± 30.7 nmol/L) than in winter (66.0 ± 28.0 nmol/L; $p = 0.021$) and, regardless of the season, they were higher in SU (124 ± 30.1 nmol/L) compared to non-SU (76.9 ± 30.7 nmol/L; $p < 0.001$). The 25(OH)D level was positively associated with the average daily vitamin D supplement intake in OMN (r = 0.400, $p = 0.039$) and VEG (r = 0.576, $p = 0.001$) but not in LOV (n.s.).

3.4. Biomarkers of Iron Status and Hematological Parameters

Hematological and iron status parameters are shown in Table 4. On average, ferritin concentrations were in the reference range for all three dietary groups and both genders. Considering only men, OMN showed significantly higher ferritin concentrations (115 ± 44.8 µg/L) compared to LOV (64.3 ± 40.8 µg/L, $p = 0.024$) and VEG (64.6 ± 36.7 µg/L, $p = 0.028$) (Table 4). There were no differences among OMN, LOV, and VEG women (25.9 ± 23.0 vs. 23.9 ± 12.9 vs. 32.1 ± 22.8 µg/L, n.s., respectively). In all three groups, significantly higher levels of most parameters were found in men. Depleted iron stores (ferritin <15 µg/L) were observed only in women (26% of OMN, 23% of LOV, and 18% of VEG) without significant differences between the groups ($p = 0.619$). Biomarkers of iron status were not associated with iron supplement intake in any group.

Table 4. Biomarkers of iron status and hematological parameters.

Parameter		Omnivores $n = 27$,	p value Omnivores vs. Lacto-Ovo	Lacto-Ovo $n = 26$,	p value Lacto-Ovo vs. Vegan	Vegan $n = 28$	p value Omnivores vs. Vegan	p value
	n_{SU} / $n_{non\text{-}SU}$	3 / 24		4 / 22		5 / 23		
Iron serum, µmol/L	f	14.5 ± 7.91	-	16.7 ± 7.03	-	15.7 ± 6.00	-	0.671 [a]
	m	22.2 ± 6.37	-	20.0 ± 8.76	-	18.4 ± 6.80	-	0.493 [a]
Deficiency (<10 µmol/L), *n* (%)	f	7 (26)		4 (15)		2 (7)		0.353 [d]
	m	0 (0)		1 (4)		0 (0)		-
Ferritin, µg/L	f	25.9 ± 23.0		23.9 ± 12.9		32.1 ± 22.8		0.441 [b]
	m	115 ± 44.8	-	64.3 ± 40.7	-	64.6 ± 36.7	-	0.010 [b]
Depleted iron stores (<15 µg/L), *n* (%)	f	7 (26)	0.024 [c]	6 (23)	n.s.	5 (18)	0.028 [c]	0.619 [d]
	m	0 (0)		0 (0)		0 (0)		-
Transferrin, µmol/L	f	46.4 ± 12.9		41.6 ± 7.79		40.2 ± 7.54		0.316 [b]
	m	34.7 ± 4.27	-	38.2 ± 4.40	-	39.0 ± 6.16	-	0.092 [b]
Increased iron requirement (≥47.7 µmol/L), *n* (%)	f	6 (22)	-	3 (12)	-	3 (11)	-	0.306 [d]
	m	0 (0)		1 (4)		0 (0)		-
Transferrin saturation	f	17.2 ± 12.6	-	21.0 ± 9.97	-	20.4 ± 8.93	-	0.543 [a]
	m	32.5 ± 10.2	-	26.8 ± 12.2	-	24.8 ± 11.6	-	0.288 [a]
Insufficient iron supply (<16%), *n* (%)	f	10 (37)		5 (19)		7 (25)		0.184 [d]
	m	0 (0)		1 (4)		3 (11)		0.261 [d]
Hb, g/dL	f	13.0 ± 1.08	-	13.6 ± 0.78	-	13.4 ± 1.20	-	0.260 [a]
	m	15.1 ± 0.64	-	14.8 ± 1.00	-	15.2 ± 0.84	-	0.661 [a]
Anemia (<12.0 g/dL), *n* (%)	f	3 (11)		0 (0)		4 (14)		0.198 [d]
(<13.0 g/dL), *n* (%)	m	0 (0)		0 (0)		0 (0)		-
Hct, L/L	f	0.39 ± 0.03	-	0.41 ± 0.03	-	0.41 ± 0.04	-	0.083 [a]
	m	0.44 ± 0.03	-	0.43 ± 0.02	-	0.44 ± 0.03	-	0.730 [a]
< 0.36 (f)/0.39 (m), *n* (%)		0 (0)		0 (0)		0 (0)		-
MCV, fl	f	87.7 ± 3.34	-	89.3 ± 5.06	-	89.5 ± 3.59	-	0.410 [b]
	m	87.2 ± 2.78	-	88.8 ± 3.42	-	87.3 ± 4.05	-	0.597 [b]
Iron deficiency anemia (<80 fl), *n* (%)		0 (0)		0 (0)		0 (0)		-

MCV = Mean corpuscular volume. Values are given as means ± SD or *n* (%) of the population at the different cutoff values. [a] One-way ANOVA, [b] Kruskal Wallis test, [c] post hoc test, and [d] chi-square test.

Intake of oral contraceptives was observed in 63% of female OMN, 31% of LOV, and 11% of VEG, but no associations to parameters of iron metabolism were found (n.s.). In addition, no significant differences were observed between iron SU and non-SU (Table S3).

3.5. Serum Levels of Calcium, Zinc, and Magnesium

Independent of supplementation, calcium (OMN: 2.45 ± 0.09, LOV: 2.45 ± 0.07, and VEG: 2.45 ± 0.10 mmol/L), zinc (OMN: 14.1 ± 1.82, LOV: 13.5 ± 2.50, and VEG: 12.3 ± 2.17 µmol/L), and magnesium (OMN: 0.83 ± 0.05, LOV: 0.83 ± 0.05, and VEG: 0.86 ± 0.06 mmol/L) serum levels were in the reference range in all groups, and no subject had calcium or magnesium levels below the reference range. Regarding zinc, 50.0% of VEG, 23.1% of LOV, and 11.1% of OMN had low levels (<12 µmol/L).

4. Discussion

This is the first cross-sectional study evaluating the biomarker status of several vitamins and minerals of recreational runners practicing vegetarian and vegan diets compared to an omnivorous diet. Since there were no comparable values of vegan recreational runners in the literature, our study results were compared with data of vegan/vegetarian nonathletes as well as with omnivorous athletes.

4.1. Vitamin B_{12}

There are different biomarkers for assessing the vitamin B_{12} status including vitamin B_{12} and Holo-TC in serum or plasma as well as methylmalonic acid and homocysteine in serum. In addition, several cutoff values were defined to assess the vitamin B_{12} status [55]. Plasma vitamin B_{12} concentration by itself does not reliably unveil vitamin B_{12} deficiency and should, therefore, be determined in combination with functional vitamin B_{12} parameters such as MMA [56]. In contrast to vitamin B_{12} levels in plasma, circulating Holo-TC represents the most sensitive parameter for diagnosing early vitamin B_{12} deficiency [57]. Elevated MMA and tHcy levels in plasma are also sensitive metabolic markers for low vitamin B_{12} levels [20]. However, the specificity of MMA and tHcy as biomarkers for vitamin B_{12} status is limited [58]. As a novel score to determine the vitamin B_{12} status, the 4cB12 indicator combines serum vitamin B_{12}, serum Holo-TC, plasma tHcy, and serum MMA [27]. The formula for calculating 4cB12 was established with a database of 5211 subjects from various nations, ages, and health status [27].

In our study, all vitamin B_{12} biomarkers showed an adequate to optimal supply when compared to reference values. The findings were independent of the respective dietary group but dependent on supplementation. Considering the non-SU, it was obvious that VEG had the lowest vitamin B_{12} supply markers although in an adequate supply area. Interestingly, there were only marginal differences between the non-SU of the OMN and LOV groups, suggesting that a lacto-ovo-vegetarian diet, containing milk and dairy products, provides certain amounts of vitamin B_{12}. Considering the duration of the diet, no impact on the vitamin B_{12} supply in OMN and LOV was found. Nevertheless, our results should be considered with caution, as these findings are in contrast to most studies showing an inadequate B_{12} status in the majority of vegans and vegetarians, as summarized in [56]. For example, in systematic reviews, an inadequate vitamin B_{12} serum status was observed in up to 87% of vegetarians and vegans, and this was combined with elevated MMA levels (32%–83%) and decreased Holo-TC levels (72%–93%) [56,59]. However, some studies included only non-SU or did not differentiate regarding vitamin B_{12} supplementation [56,59]. But, the Adventist Health Study 2 clearly showed an association between vitamin B_{12} supplementation and serum B_{12} and Holo-TC levels [60].

Despite the adequate supply, increased tHcy levels (>10 µmol/L) were found in >50% of all three dietary groups. The observed tHcy levels (OMN: 12.2 ± 2.93, LOV: 13.2 ± 6.47, and VEG: 12.8 ± 4.26 µmol/L) partly agreed with data from a meta-analysis, which found average tHcy levels of 16.4 µmol/L in vegans, but, in contrast to the present results, this study found significantly higher tHcy in vegans compared to omnivores and vegetarians [48].

Homocysteine is an independent risk factor for ischemic heart disease and ischemic stroke. By increasing the homocysteine concentration by 5 µmol/L, the risk of coronary events increases by 18%, and the risk of stroke increases by 19% [58]. Therefore, recreational athletes adopting a vegan diet should be encouraged to take dietary vitamin B_{12} supplements.

4.2. Red Blood Cell (RBC) Folate

Red blood cell folate concentration is a sensitive biomarker for the folate status, and concentrations <340 nmol/L are indicative of folate deficiency in healthy adults [43]. In contrast, cutoff values of ≥906 nmol/L have been estimated for women of reproductive age for the prevention of neural tube effects. In the present study population, high RBC folate concentrations were observed, and the mean levels of folate concentrations in the erythrocytes were similar in all three dietary groups (OMN: 2213 ± 444, LOV: 2236 ± 596, and VEG: 2354 ± 639 nmol/L; p = 0.577). Results of non-SU of the three groups revealed a very homogenous picture. Since in Germany foods are not fortified with folate (with the exception of salt), the results suggest a high intake of folate-rich foods, like green leafy vegetables, because of the high health compliance in all three groups.

As the values appeared to be extraordinarily high, the applied method was tested and internally validated on the basis of several laboratories (LabA, LabB, and LabC). The results of the comparisons of the laboratories showed a clear correlation between the present values and the values of other laboratories ($r_{LabA\text{-}LabB} = 0.656$, $p = 0.039$; $r_{LabA\text{-}LabC} = 0.902$, $p < 0.001$). Currently, other studies have found comparably high RBC folate levels. A recent study by Gallego-Narbón found average RBC folate levels of 1704 nmol/L in Spanish lacto-ovo-vegetarians and vegans [61]. Also, a recent publication of the Adventist Health Study 2 showed RBC folate levels >2000 nmol/L [62].

4.3. 25-Hydroxyvitamin D (25(OH)D)

The serum 25(OH)D concentration reflects the amount of vitamin D attained from both dietary sources and endogenous synthesis and is a sensitive biomarker for the vitamin D status. However, there is no final consensus about the concentration of serum 25(OH)D that would achieve the greatest benefit for health. While 25(OH)D levels of >75 nmol/L are considered as optimal, levels ≥50 nmol/L are regarded as adequate, levels of 25–49.9 nmol/L as inadequate, and levels <25 nmol/L as deficient [51,63]. The difficulty in assessing the vitamin D status is more due to the methodology, as ECLIA was discussed to overestimate the 25(OH)D levels [64]. Consequently, in addition to ECLIA, 25(OH)D levels were analyzed via liquid chromatography tandem-mass spectrometry (LC-MS/MS) [65] for validation as well. The variability was <10%, which was considered tolerable.

In our study, all three dietary groups had comparable average 25(OH)D values of >75 nmol/L and showed a similar low prevalence (<20%) of vitamin D inadequacy (<50 nmol/L), while vitamin D deficiency (<25 nmol/L) was present in only two VEG subjects. Nevertheless, the vitamin D supply of the present population is to be regarded as exceptional, since there is a high prevalence of vitamin D deficiency in the general population [63]. Adequate vitamin D supply in the present subjects could be explained by the high proportion of SU (specific vitamin D supplements as well as multivitamin supplements). Interestingly, all subjects who consumed vitamin D (containing) supplements had 25(OH)D levels >50 nmol/L and, therefore, had an adequate supply, while inadequate to deficient supply concerned only non-SU. Overall, in the total study population the vitamin D status was dependent on vitamin D supplement intake.

Our findings are consistent with results of the Adventist Health Study 2, which showed that the vitamin D status depends only to a small extent on nutritional factors and much more on supplementation [66]. Additionally, a meta-analysis showed that only about 56% of 2313 athletes (22.5 ± 5 years old) had inadequate vitamin D levels (defined by the authors with <80 nmol/L), which was influenced by season, type of sport (indoor/outdoor), and latitude [67]. These results are largely consistent with the present findings, where 33% of OMN, 43% of VEG, and 58% of LOV were not optimal supplied (<75 nmol/L).

There are many factors which could influence vitamin D status. Presumably, the adequate supply status is due to the relatively long stay outdoors, because other studies also showed an adequate supply status of female runners [68]. All subjects were recruited from Hanover or the surrounding area, which has a latitude of 52°N. In contrast to the present results, a German nationwide (latitude of 52°–54°) study with almost 7000 subjects (age 18–79 years) showed 25(OH)D levels of 45.1 and 45.3 nmol/L for men and women, respectively [69]. Since the present study population was about 27 years old, it could be assumed that there was sufficient intrinsic synthesis [69]. Additionally, our examinations took place from summer to winter months (May to December 2017), so a high endogenous synthesis could be expected [70]. Additional influences of vitamin D status such as recent holidays, individual sun exposure, as well as sun protection habits (e.g., the use of sun cream or sun-protecting clothes) [71] were not examined.

4.4. Iron

There is wide consensus that serum ferritin is the most sensitive parameter to detect depleted iron stores and, therefore, isolated iron deficiency [40,72,73]. Depleted iron stores are present if ferritin concentrations are <15 µg/L [72]. However, in addition to depleted iron stores, iron deficiency anemia can only be diagnosed in the presence of decreased serum iron, transferrin saturation, Hb, and MCV levels [40].

Considering the ferritin levels of the present subjects, <30% of each group had depleted iron stores, but iron deficiency anemia was not found in any subject. Surprisingly, female VEG had the highest mean ferritin levels (32.1 ± 22.8 µg/L), while among men the OMN group showed the highest levels (115 ± 44.8 µg/L). Again, high interindividual variations were observed. In contrast to parameters of vitamin B_{12} and vitamin D metabolism, supplementation was not crucial for an adequate iron supply—also found for LOV and VEG. Since the iron bioavailability is higher in animal-based foods compared to plant-based foods (10%–20% vs. 1%–5%), a similar status could be only achieved through a high intake of iron-containing plant-based foods such as whole grain and legumes as well as availability-enhancing food ingredients (e.g., vitamin C). Therefore, our data indicate that a targeted choice of plant foods can also ensure an adequate iron supply.

Our results are largely consistent with the literature, since vegetarians and vegans, in general, show hemoglobin and serum iron levels in the reference range. Also, iron deficiency is not more common than in omnivores [14,74,75], whereas ferritin levels, in contrast to our results, are often low [76,77]. For example, Elorinne and colleagues observed significantly lower ferritin levels in vegans (median of 26 µg/L) compared to nonvegetarians (median of 72 µg/L) [75]. Moreover, even 8% of female vegans of the German Vegan Study had iron deficiency anemia [77]. Therefore, especially in female recreational athletes who consume (almost) only plant-based foods, the risk of insufficient iron supply is increased [78,79].

4.5. Calcium, Zinc, and Magnesium

Average values of all three groups showed adequate serum levels of all three minerals. Most subjects of VEG had low levels of zinc, which was consistent with previous findings [14]. However, in contrast to the previously mentioned parameters, serum levels of calcium, zinc, and magnesium are not directly linked to dietary intake as a result of the tight homeostatic regulation of blood levels.

4.6. Limitations

In addition to the small sample size, only a certain age group (18–35 y) was examined. Also, the study took place mainly in summer months, which possibly influenced study results. Further, the current training phase could have an effect on the results.

4.7. Future Research Directions

Future research is needed to examine the nutrient status of vegetarian/vegan athletes. Also, data of various disciplines and different levels (e.g., professional level) would be of great interest. In addition, intervention studies are needed to investigate the influence of a vegetarian/vegan diet on various biomarkers.

5. Conclusions

In summary, our data suggest that a well-planned, health-conscious lacto-ovo-vegetarian and vegan diet, including supplements, can meet the recreational athlete's requirements of vitamin B_{12}, vitamin D, and iron.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/11/5/1146/s1, Table S1: Biomarkers of iron status and hematological parameters according to gender, Table S2: Biomarkers of vitamin D status according to gender, Table S3: Biomarkers of iron status and hematological parameters according to supplement intake.

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2.3. Paper III

Exercise capacity of vegan, lacto-ovo vegetarian and omnivorous recreational runners

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RESEARCH ARTICLE Open Access

Exercise capacity of vegan, lacto-ovo-vegetarian and omnivorous recreational runners

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Abstract

Background: In search of the right nutrition for the athlete, numerous nutritional strategies and diets were discussed over time. However, the influence of plant-based diets, especially veganism, on exercise capacity has not been clarified.

Methods: We conducted a cross-sectional study to compare the exercise capacity of vegan (VEG, $n = 24$), lacto-ovo-vegetarian (LOV, $n = 26$) and omnivorous (OMN, $n = 26$) recreational runners. To determine maximal exercise capacity, participants performed an incremental exercise test on a bicycle ergometer until voluntary exhaustion. During the test capillary blood samples were taken at several time points for the measurement of arterial lactate [lac] and glucose [glc] concentrations. To determine nutrient intake, a 24 h dietary recall was conducted.

Results: The groups showed comparable training habits in terms of training frequency (mean 3.08 ± 0.90 time/wk., $p = 0.735$), time (mean 2.93 ± 1.34 h/wk., $p = 0.079$) and running distance (mean 29.5 ± 14.3 km/wk., $p = 0.054$). Moreover, similar maximum power output (P_{maxBW}) was observed in all three groups (OMN: 4.15 ± 0.48 W/kg, LOV: 4.20 ± 0.47 W/kg, VEG: 4.16 ± 0.55 W/kg; $p = 0.917$) and no differences regarding [lac] throughout the exercise test and maximum lactate could be observed between the groups (OMN: 11.3 ± 2.19 mmol/l, LOV: 11.0 ± 2.59 mmol/l, VEG: 11.9 ± 1.98 mmol/l; $p = 0.648$).

Conclusion: The data indicate that each examined diet has neither advantages nor disadvantages with regard to exercise capacity. These results suggest that a vegan diet can be a suitable alternative for ambitious recreational runners.

Trial registration: German Clinical Trials Register (DRKS00012377). Registered on 28 April 2017

Keywords: Recreational runners, Vegan, Vegetarian, Plant-based diets, Exercise capacity

Background

Most endurance athletes are interested in diets that positively affect exercise capacity and health, reduce body fat and promote the development of lean muscle mass [1]. Already thousands of years ago, the diet of athletes was seen as an important mean to increase performance [2]. While in the past meat was seen as an irreplaceable performance-enhancing food [3], today the trend is developing in the opposite direction: From partial exclusion (lacto−/ovo−/lacto-ovo-vegetarians) to the total elimination (veganism) of animal products from the diet. Since the prevalence of ambitious runners following plant-based diets is increasing [4, 5],the impact of those diets with regard to athletes performance and health is becoming of growing interest [6].

Due to the favorable impact on health [7–12] it could be assumed that performance parameters are also influenced by plant-based diets based on a broad variety of foods. Parameters to analyze exercise capacity include maximum power output and lactate concentration, with the latter particularly important with increasing exercise intensities and the associated increased in lactate production by anaerobic energy supply. Since vegetarian diets are characterized by higher intake of carbohydrates one could hypothesize that there are favorable effects on exercise capacity [13–16]. Also, the increased intake of

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antioxidants in plant-based diets might have positive effects on exercise-induced oxidative stress [15, 16]. On the other hand, it has been shown that vegetarians and especially vegans have lower ferritin levels whereas hemoglobin levels and the prevalence of iron deficiency anemia are generally indistinguishable from omnivores [15, 17, 18]. In addition, especially a vegan diet is usually characterized by low intake of protein, creatine and carnitine, which could negatively impact performance [19, 20].

To date, the impact of a plant-based diet on athletic performance is not clearly understood. Recent case reports showed that even vegan athletes can reach top athletic performances [21, 22]. Other studies dealing with a vegetarian and vegan diet related to sport are questionnaire-based and do not include nutritional or sports medical diagnostics [4, 5, 23, 24]. Further studies assessing nutritional and sports medical parameters are outdated [25] or did not differentiate between vegetarians and vegans [14]. A recent cross-sectional study described the oxidative status of male vegan, vegetarian and omnivorous recreational athletes but did not examine exercise capacity [26]. In addition to cross-sectional studies, there are also a few intervention studies that examine the effect of a vegetarian diet on athletic performance. However, their impact is low due to the low number of subjects and short intervention periods [27–31].

As a consequence, we conducted a study to test the hypothesis that there are no differences in exercise performance of omnivorous, lacto-ovo-vegetarian and vegan recreational runners.

Subjects and methods

Participants

Seventy-six healthy omnivorous (OMN, $n = 26$), lacto-ovo-vegetarian (LOV, $n = 24$) and vegan (VEG, $n = 24$) recreational runners between 18 and 35 years conducted laboratory physical exercise tests (for details see Table 1).

Subjects were recruited from the general population in Hannover, Germany, via local running events, online running communities as well as online vegetarian and vegan communities. To avoid seasonal influences, the recruitment happened batchwise from May until December 2017. Participants were matched according to age and gender.

Participants were categorized upon enrolling for the study. To categorize subjects as omnivorous, lacto-ovo-vegetarian and vegan, a questionnaire which included questions about their current diet had to be completed. Additionally, usually consumed food groups were queried, to avoid subjectively wrong classifications. Subjects were "omnivorous", if they consumed cereals, plant-based foods, legumes, milk and dairy products, eggs, as well as fish, meat and meat products. "Lacto-ovo-vegetarians" were defined as they consumed cereals, plant-based foods, legumes, milk and dairy products, and eggs. "Vegans" were characterized by consumption of cereals, plant-based foods, and legumes.

Subjects were selected based on the following inclusion criteria: omnivorous, lacto-ovo-vegetarian or vegan diet for at least half a year, body mass index (BMI) between 18.5 and 25.0 kg/m^2 and regular run training 2 to 5 times per week. Training duration, distance and time of a typical exercise training week were documented via self-reporting data. The following criteria led to exclusion: any cardiovascular, metabolic or malignant disease, diseases regarding the gastrointestinal tract, pregnancy, nutrient intolerances as well as addiction to drugs or alcohol. The use of dietary supplements in physiological doses did not lead to exclusion, except performance-enhancing substances (e.g. creatine).

Ethical approval was provided by the Ethics Committee at the Medical Chamber of Lower Saxony (Hannover, Germany). The study was conducted in accordance with the Declaration of Helsinki. All subjects gave their written informed consent. This study is registered in the German Clinical Trial Register (DRKS00012377).

Study procedure

First of all, the measurement of body weight (seca®, Hamburg, Germany) was carried out lightly clothed and without shoes. Second, an electrocardiogram in rest and a short medical examination were carried out and evaluated by an experienced cardiologist to make sure that the participants could join the exhaustion test. After the medical examination, a 24 h dietary recall was conducted by qualified personnel before the exercise test started. To analyze the nutrient and energy intake of the 24 h recall, the nutrition organization software PRODI® (Nutri-Science GmbH, Freiburg, Germany) was used.

The primary outcome maximum exercise capacity was measured as maximum power related to body weight (P_{maxBW}) reached in the graded exercise test (GXT). Secondary outcomes included maximum power output related to lean body mass (P_{maxLBM}), maximal and submaximal lactate [lac] and glucose [glc] concentrations during the GXT. The GXT was performed until voluntary exhaustion on a bicycle ergometer (Excalibur, Lode B.V., Groningen, Netherlands). Prior to physical performance test, participants were asked not to do any strenuous activities 24 h prior the performance diagnostics. Subjects were requested to maintain their usual diet. After a warm-up period of 6 min at 50 W, the workload increased by 16.7 W per minute. Heart rate (HR) was measured continuously beat-to-beat throughout all testing sessions with an HR-monitor (RS800 CX Polar, Finland). To ensure that the subjects achieve their maximum

Table 1 Characterization of the study population

	OMN (*n* = 26)	*P* value OMN-LOV	LOV (n = 26)	*P* value LOV-VEG	VEG (*n* = 24)	*P* value OMN-VEG	*P* value 3 groups
Age, years	27.2 ± 4.05	–	27.6 ± 4.31	–	27.5 ± 4.26	–	0.937[a]
Sex	m = 10, w = 16	–	m = 10, w = 16	–	m = 9, w = 15	–	0.997[c]
BMI, kg/m^2	22.2 ± 1.73	–	21.6 ± 1.98	–	22.0 ± 2.23	–	0.559[a]
Duration of diet							0.001[d]
< 0.5 years (%)	0		0		0		
0.5–1 year (%)	0		15.4		20.8		
1–2 years (%)	3.8		11.5		12.5		
2–3 years (%)	0		7.7		29.2		
> 3 years (%)	96.2		65.4		37.5		
Training habits							
Training frequency per week	3.04 ± 0.98	–	3.19 ± 0.90	–	3.00 ± 0.85	–	0.735[a]
Running distance per week, km	28.03 ± 14.66	–	34.41 ± 14.53	–	25.53 ± 12.30	–	0.054[a]
Running time per week, h	2.72 ± 1.11	–	3.38 ± 1.43	–	2.65 ± 1.38	–	0.079[a]
Heart rate during training, bpm	159.91 ± 8.89	–	151.99 ± 12.29	–	156.46 ± 12.52	–	0.173[b]
Body composition							
TBW, L	39.3 ± 6.74	–	38.5 ± 6.40	–	38.9 ± 8.20	–	0.864[a]
LBM, kg	53.7 ± 9.21	–	52.6 ± 8.75	–	53.2 ± 11.2	–	0.866[a]
Body fat, %	21.5 ± 5.91	–	21.8 ± 6.19	–	20.7 ± 5.79	–	0.797[b]
BCM, %	54.5 ± 3.33	0.043[c]	52.3 ± 3.25	n.s.	52.5 ± 2.76	n.s.	0.029[b]

OMN = omnivorous athletes, *LOV* = lacto-ovo-vegetarian athletes, *VEG* = vegan athletes, *n.s.* = not significant, *TBW* = total body water, *LBM* = lean body mass, *BCM* = body cell mass. Data are presented as mean (SD)
[a]Kruskal Wallis test
[b]One-way ANOVA
[c]Post hoc test
[d]Chi square test

performance, they were verbally motivated by personnel, but they were not allowed to get out of the saddle. During the test, arterialized capillary blood samples were taken from the earlobe at rest, every 50 W and at termination of the test. Samples were immediately transferred into a glucose/lactate hemolysis solution (EKF-diagnostics GmbH, Barleben, Germany). Lactate and glucose concentrations were directly analyzed by a lactate/glucose biosensor (Biosen S-Line Lab+, EKF-diagnostics GmbH, Barleben, Germany).

On a separate day (at least 48 h apart), lean body mass (to a nearest of 100 g), total body water, body cell mass and relative body fat (%) were measured using a bipolar bioelectrical impedance analyzer (BIA) (Nutriguard M, Data Input Company, Darmstadt, Germany) as well as the relative software NutriPlus© 5.4.1 (Data Input Company, Darmstadt, Germany). BIA measurements were carried out in a fasting state. The participants were in lying position for 5 min before the measurement to ensure a uniform distribution of body fluids. In order to guarantee an accurate measurement, the subjects were instructed previously to lie relaxed and steady during the measurement and slightly bend their limbs from the torso. The measurement was carried out by a professional nutritionist.

Data analysis and statistical methods

Statistical analyses were performed using SPSS software (IBM SPSS Statistics 24.0; Chicago, IL, USA). Results are shown in mean ± standard deviation (SD). First, normal distribution was checked by using the Kolmogorov-Smirnov test. If data were normally distributed, one-way analysis of variance (ANOVA) was used to evaluate differences between the three diet groups. Further, to analyze data with non-normally distribution, Kruskal Wallis test was performed. Additionally, if there were significant differences between the groups, post hoc test with Bonferroni correction was conducted. Moreover, the chi-square test was used to compare differences between the frequency distribution of the three groups. Associations between parametric data were computed via Pearson, non-parametric data via Spearman's rho correlation. *P* values ≤0.05 were set as statistically significant.

Results

From a total of 76 runners 26 were included in the OMN, 26 in the LOV and 24 in the VEG group. Men and women were equally distributed ($p = 0.997$, Table 1). Mean age (27.4 ± 4.16 y) and BMI (21.9 ± 1.97 kg/m^2) did not differ significantly between the groups. Additionally, all three groups did not differ in their training frequency, running time and running distance (Table 1). Moreover, none of the subjects consumed tobacco on a regular basis.

Exercise capacity

For P_{maxBW} (OMN: 4.15 ± 0.48, LOV: 4.20 ± 0.47, VEG: 4.16 ± 0.55 W/kg BW) and P_{maxLBM} (OMN: 5.29 ± 0.48, LOV: 5.39 ± 0.52, VEG: 5.26 ± 0.58 W/kg LBM), there were no significant differences between the groups ($p = 0.917$ and $p = 0.696$ for P_{maxBW} and P_{maxLBM}, respectively). When comparing total men and women, men showed higher P_{maxBW} (4.41 ± 0.45 W/kg vs. 4.02 ± 0.47 W/kg, $p = 0.001$). Additionally, there were no differences between performance-related parameters when comparing only women (P_{maxBW} women: OMN: 3.99 ± 0.46, LOV: 4.06 ± 0.44, VEG: 4.02 ± 0.53 W/kg, $p = 0.910$) or men (P_{maxBW} men: OMN: 4.41 ± 0.41 W/kg, LOV: 4.43 ± 0.46, VEG: 4.39 ± 0.52, $p = 0.979$) between the three study groups. Training frequency, running time and distance were not associated with P_{maxBW} in any group. In all three groups, training frequency, running time and distance were significantly correlated. Both, the maximum ($p = 0.648$) and the submaximal [lac] revealed no differences between the groups (Fig. 1). Similarly, we found no differences in maximum ($p = 0.960$) and submaximal [glc] (Fig. 2).

Dietary intake

The 24 h dietary recall revealed some differences in nutrient intake between the groups (Table 2). While total energy and protein intake were comparable in all three groups, VEG consumed significantly higher amounts of carbohydrates, fiber, magnesium, iron, folate and vitamin E compared to OMN and also LOV. However, consumption of dietary fat and vitamin B_{12} was significantly lower in VEG compared to the two other groups.

Discussion

To the best of our knowledge, this was the first investigation providing a differential analysis of exercise capacity and lactate/glucose concentrations of vegan, lacto-ovo-vegetarian and omnivorous recreational runners. Our findings that VEG, OMN and LOV show no significant differences in maximum exercise capacity as measured by P_{maxBW} indicate that the evaluated diets do not have detrimental effects on exercise performance in recreational runners. In this regard the evaluation of the 24 h dietary recalls showed a sufficient supply in most nutrients.

Previous studies focused on the comparison between vegetarian and omnivorous athletes and observed no differences regarding physical performance [32]. An earlier study examining vegetarians and omnivores, who underwent a cycle ergometer stress test to determine the aerobic capacity and a Wingate test to estimate anaerobic capacity found no differences in performance parameters [25]. A recent study testing the physical performance of 35 vegetarian and 35 omnivorous endurance athletes, observed a 13% greater maximal oxygen consumption ($VO_{2\ max}$) in female vegetarians than in omnivores, while no differences were found in males [14]. Our study cannot directly be compared as Lynch and colleagues examined VO_{2max} and performed their exercise tests on a treadmill. Notably, previous studies did not focus on vegans and no lactate/glucose measurements as markers of anaerobic metabolism were carried out. Our study extends existing knowledge as we could show that vegan and vegetarian runners did not differ from omnivores in terms of exercise capacity and glucose utilization from low to maximum effort.

Few studies investigating the effect of a short-term lacto-ovo-vegetarian diet on performance revealed different results. A 6 and 5 weeks defined lacto-ovo-vegetarian diet did not have a significant influence on aerobic capacity or repeated sprint ability, respectively, compared to controls [27, 33]. In contrast, Hietavala et al. examined the effect of a 4-day low-protein vegetarian diet compared to a mixed diet (0.8 ± 1.11 g/kg BW vs. 1.59 ± 0.28 g/kg BW) in recreationally active men. They observed significantly increased oxygen uptake at different exercise intensities, suggesting that submaximal cycling economy was poorer after a low-protein vegetarian diet [28]. Since the protein intake was restricted and no typical vegetarian diet was studied, the results may not be evident. Moreover, research of Hietavala indicated that a food selection with a high proportion of plant foods may favorably affect the acid-base status and thus potentially positively impact performance [34]. However, a recent review showed no impact on exercise capacity through a diet rich in basic substances [35]. To date, long-term intervention studies are lacking in order to be able to make clear statements about the effect of a vegetarian/vegan diet on exercise capacity.

With increasing intensity of physical activity, an anaerobic energy supply predominates with increasing lactate production. Although vegans had a higher dietary carbohydrate intake in comparison to the other two groups (VEG: 4.66 ± 1.79 vs. OMN: 3.87 ± 1.34 vs. LOV: 3.76 ± 1.55 g/kg BW) no differences regarding submaximal and maximal lactate as well as glucose values were observed between the groups, suggesting no significant influence

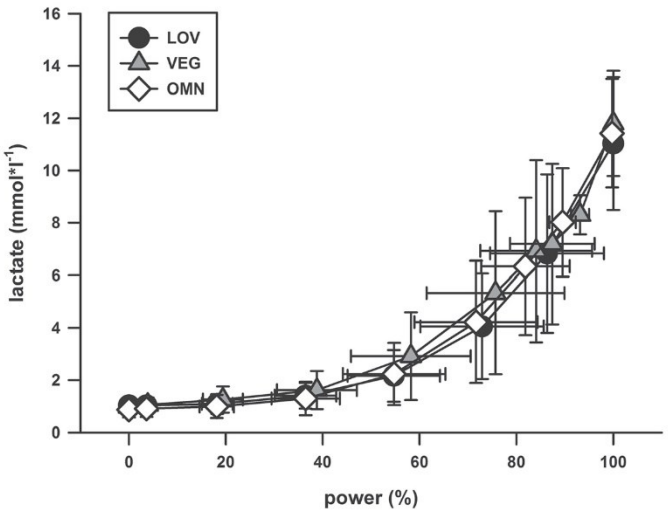

Fig. 1 Lactate concentrations in relation to relative power output. No differences were found between the groups in either the submaximal or the maximal [lac] values. OMN = omnivorous athletes, LOV = lacto-ovo-vegetarian athletes, VEG = vegan athletes. Data are presented as mean (SD)

of diet on glucose utilization. Several factors can affect lactate kinetics during incremental exercise like previous exercise activities, water balance and caffeine consumption [36]. Furthermore, if the intramuscular glycogen stores are emptied, the rate of glycolysis is severely impaired and consequently, lactate production is reduced. It could be suggested that the individual response to exercise training have a stronger impact on exercise capacity than the consumption of meat or animal products, a phenomenon partly attributable to the sex and genetic background of an individual but still incompletely understood [37, 38].

We found a comparable BMI and body composition in all three groups. In contrast, Hanne et al. found a higher body fat mass in female omnivorous athletes compared to vegetarians [25]. However, so far there are no comparative data of vegan athletes. Results of the 24 h dietary recalls did not agree with a study by Lynch et al.

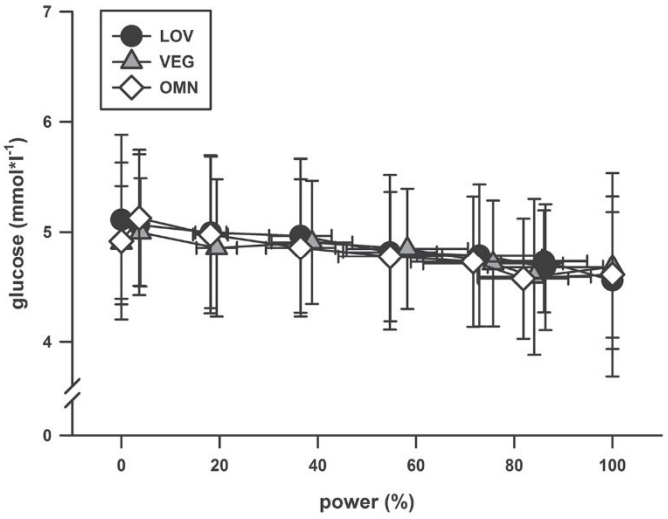

Fig. 2 Glucose concentrations in relation to relative power output. No differences were found between the groups in [glu] values. OMN = omnivorous athletes, LOV = lacto-ovo-vegetarian athletes, VEG = vegan athletes. Data are presented as mean (SD)

Table 2 Nutrient intake of the study population determined via 24 h dietary recall

	OMN ($n = 26$)	*P* value OMN-LOV	LOV ($n = 26$)	*P* value LOV-VEG	VEG ($n = 24$)	*P* value OMN-VEG	*P* value 3 groups	Reference values* (m/f)
Energy intake, MJ	9.49 ± 3.52	–	9.04 ± 3.73	–	9.17 ± 3.53	–	0.898[a]	
Macronutrients								
Carbohydrate, EN%	49.4 ± 10.7	n.s.	48.7 ± 9.96	0.008[c]	58.9 ± 14.3	0.016[c]	0.004[a]	50–55
Carbohydrate, g/kg BW	3.87 ± 1.34	–	3.76 ± 1.55	–	4.66 ± 1.79	–	0.095[a]	
Protein, EN%	17.0 ± 6.13	–	16.5 ± 7.42	–	13.9 ± 3.97	–	0.202[b]	12–15
Protein, g/kg BW	1.37 ± 0.65	–	1.29 ± 0.81	–	1.10 ± 0.57	–	0.252[b]	0.8
Fat, EN%	32.2 ± 11.1	n.s.	32.7 ± 9.63	0.026[c]	24.8 ± 10.6	0.043[c]	0.015[a]	30–35
Fiber, g	29.6 ± 15.0	n.s.	31.6 ± 12.9	< 0.001[c]	52.1 ± 23.6	< 0.001[c]	< 0.001[a]	≥ 30
Minerals								
Sodium, g	2.85 ± 1.89	n.s.	2.23 ± 1.23	0.036[c]	1.40 ± 1.00	0.003[c]	0.003[b]	1.5
Potassium, g	3.03 ± 1.14	n.s.	3.07 ± 1.14	n.s.	4.38 ± 2.08	0.041[c]	0.031[b]	4[d]
Calcium, mg	1102 ± 619	n.s.	1252 ± 546	0.035[c]	903 ± 554	n.s.	0.042[b]	1000
Magnesium, mg	429 ± 144	n.s.	443 ± 161	0.014[c]	639 ± 294	0.008[c]	0.004[b]	350/300
Iron, mg	15.3 ± 11.9	n.s.	12.7 ± 5.35	0.029[c]	18.4 ± 7.86	n.s.	0.018[b]	10/15
Zinc, mg	12.0 ± 6.16	–	10.1 ± 3.93	–	10.4 ± 4.99	–	0.752[b]	10/7
Phosphorus, mg	1444 ± 674	–	1458 ± 685	–	1341 ± 634	–	0.871[b]	700
Copper, mg	2.12 ± 1.55	n.s.	2.15 ± 0.81	n.s.	2.90 ± 1.25	0.002[c]	0.002[b]	1.0–1.5
Vitamins								
Thiamine, mg	1.42 ± 0.80	n.s.	1.39 ± 1.22	0.036[c]	1.82 ± 0.85	n.s.	0.037[b]	1.2/1.0
Riboflavin, mg	1.58 ± 1.17	–	1.80 ± 1.58	–	1.23 ± 0.66	–	0.346[b]	1.4/1.1
Niacin, mg	35.4 ± 23.5	–	30.7 ± 20.1	–	31.1 ± 14.1	–	0.677[b]	15/12
Pyridoxine, mg	2.00 ± 1.84	n.s.	1.71 ± 1.62	0.034[c]	2.32 ± 1.23	n.s.	0.033[b]	1.5/1.2
Cobalamin, µg	5.05 ± 5.44	n.s.	3.61 ± 3.12	< 0.001[c]	0.76 ± 0.34	< 0.001[c]	< 0.001[b]	4
Biotin, µg	53.7 ± 39.6	n.s.	63.2 ± 44.5	n.s.	72.5 ± 31.4	0.017[c]	0.021[b]	30-60[d]
Pantothenic acid, mg	5.73 ± 5.21	–	6.15 ± 6.13	–	5.92 ± 3.23	–	0.298[b]	6[d]
Folate, µg	303 ± 196	n.s.	346 ± 244	0.025[c]	452 ± 177	0.002[c]	0.002[b]	300
Retinol equivalents, mg	1.41 ± 1.53	–	1.74 ± 1.50	–	2.21 ± 2.68	–	0.314[b]	1.0/0.8
Ascorbic acid, mg	140 ± 151	n.s.	148 ± 142	n.s.	237 ± 165	0.024[c]	0.018[b]	110/95
Vitamin D, µg	1.97 ± 3.30	–	2.07 ± 1.87	–	1.32 ± 1.84	–	0.129[b]	20
Vitamin E, mg	11.7 ± 6.44	n.s.	13.3 ± 10.8	0.032[c]	21.1 ± 13.6	0.018[c]	0.009[b]	14/12[d]

OMN = omnivorous athletes, *LOV* = lacto-ovo-vegetarian athletes, *VEG* = vegan athletes, *MJ* = mega joule, *BW* = body weight, *n.s.* = not significant
*Reference values of the German, Austrian and Swiss Nutrition Societies [39]. Nutrient intake excluding supplement intake. Data are presented as mean (SD)
[a]One-way ANOVA
[b]Kruskal Wallis test
[c]Post hoc test
[d]Estimated values

[14] since the nutrient intake data of the present LOV and OMN in our study were comparable. Only the VEG group consumed the typically higher amounts of carbohydrates, fiber, magnesium, iron and folate, and less fat and vitamin B_{12}.

As a limitation, the present study did not determine oxygen uptake, which would be an interesting parameter assessing the efficiency of cardiorespiratory fitness during physical activity until exhaustion. Further, 24 h dietary recalls may represent not the usual, but the current nutrient intake and have disadvantages regarding rare foods and subjective influences on the stated amounts of consumption. We performed tests on a bicycle as a standardized and save method for assessing exercise capacity and lactate kinetics during exhaustive exercise testings. However,

the use of a bicycle instead of a treadmill for runners is a potential limitation of our study.

Conclusion

Taken into account the aforementioned limitations, the results suggest that there are no differences in exercise capacity between vegan, lacto-ovo-vegetarians and omnivorous recreational runners. Given current data we conclude, that a lacto-ovo-vegetarian and also vegan diet might be suitable alternatives for recreational athletes. Further long-term intervention studies are needed to clarify the influence of a vegetarian and especially vegan diet on an individual's exercise capacity.

Abbreviations

[glc]: glucose concentration; [lac]: lactate concentrations; ANOVA: one-way analysis of variance; BCM: body cell mass; BMI: body mass index; BW: body weight; FFM: fat free mass; GXT: graded exercise test; HR: heart rate; LBM: lean body mass; LOV: lacto-ovo-vegetarian athletes; n.s.: not significant; OMN: omnivorous athletes; P_{maxBW}: maximum power related to body weight; P_{maxLBM}: maximum power output related to lean body mass; SD: standard deviation; TBW: total body water; VEG: vegan athletes; $VO_{2\ max}$: maximal oxygen consumption

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Consent of publication

Not applicable.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author's contributions

JN, SH, UT, and AH co-designed the study and survey materials. JN, PW, JE, and SH were responsible for data acquisition. JN drafted the manuscript and conducted the statistical analyses. AH, JE and SH revised the manuscript critically for important content. All authors provided critical revisions, read and approved the final manuscript.

Ethics approval and consent to participate

The Ethics Committee at the Medical Chamber of Lower Saxony (Hannover, Germany) granted ethical approval for this research. The study was conducted in accordance with the Declaration of Helsinki. All participants gave their written informed consent.

Competing interests

The authors declare that they have no competing interests.

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2.4. Paper IV

The exercise-dependent changes in sirtuin activities in plasma from vegans differ from those in omnivores and lacto-ovo-vegetarians

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The exercise-dependent changes in sirtuin activities in plasma from vegans differ from those in omnivores and lacto-ovo-vegetarians

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Abbreviations: BIA – bioelectrical impedance analysis, BW - body weight, FOXO3 – forkhead box protein O 3, LBM – lean body mass, LOV – lacto-ovo-vegetarian, OMN – omnivorous, PGC-1α – Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, p53 – protein 53, qRT-PCR – quantitative reverse transcription-polymerase chain reaction, ROS – reactive oxygen species, SIRT – sirtuin, SOD2 – mitochondrial manganese superoxide dismutase, U – enzyme units, VEG – vegan

Keywords: metabolic regulation, recreational runners, sirtuins, vegan, vegetarian

Abstract: Nutrition affects metabolic regulation in humans. Sirtuins are essential regulators of cellular energy metabolism: SIRT1, SIRT3, and SIRT4 have a direct effect on glycolysis, oxidative phosphorylation and fatty acid oxidation. This cross-sectional study investigated the effect of different diets on sirtuin regulation after exercise. SIRT1 and SIRT3 - SIRT5 were measured in blood from omnivorous, lacto-ovo-vegetarian and vegan recreational runners (21-25 subjects, respectively) before and after exercise at the transcript, protein, and enzymatic levels. Analyses showed a significant correlation of all sirtuins with antioxidative substances ascorbate and tocopherol. SIRT1, SIRT3, and SIRT5 enzyme activities increased after exercise in omnivores and lacto-ovo-vegetarians, commensurate with increased energy demand during exercise. However, activities were reduced in vegans. Reduced SIRT1, SIRT3, and SIRT5 enzyme activities in vegans may have a negative impact on energy metabolism

and markers of antioxidative response. Decreased sirtuin activities in vegan participants may be due to an increased intake of the antioxidative substances tocopherol and ascorbate.

Introduction

Sirtuins are NAD+ dependent deacylases that regulate mitochondrial energy metabolism as well as cellular response to stress.[1,2] In mammals, seven different sirtuins (SIRT1-SIRT7) with specific subcellular localization and enzymatic reactions are known.[3] SIRT1, SIRT6, and SIRT7 are nuclear enzymes, SIRT3, SIRT4 and SIRT5 are located in mitochondria, while SIRT2 is the only cytosolic sirtuin. Additionally, a subcellular shift of SIRT1 and SIRT3 into the cytosol was described under specific conditions.[4] The enzymatic reactions catalyzed by sirtuins are either a NAD+-dependent deacylation of lysine residues or a NAD+-dependent ADP-ribosylation of lysine residues.[5] The most common enzymatic reaction of sirtuins is deacetylation while SIRT5 is predominantly a desuccinylase, demalonylase, and deglutarylase.[6] The acetylation level of the mitochondrial proteome is 65%[7] which makes these proteins amenable to regulation by sirtuins. Blood levels of sirtuins were shown to correlate with several organ dysfunctions like coronary heart disease in obese patients[8–11] or type 1 and type 2 diabetes.[12]

Nutritional factors may influence sirtuin activity. Caloric restriction has been linked to longevity and protection from age-related diseases via sirtuins.[13,14] A promoting effect of polyphenols on sirtuin activity[15,16] and an activating effect of resveratrol have been described.[16] As polyphenols are phytochemicals it can be hypothesized that a plant-based diet positively affects sirtuin activity. Plant-based diets such as vegetarian (predominant consumption of plant-based foods) and vegan (exclusive consumption of plant-based foods) nutrition are high in a variety of polyphenols. A lacto-ovo-vegetarian diet based on a broad variety of foods may protect from obesity, type 2 diabetes, hypertension, cardio metabolic disorders and cancer.[17–22] On the other hand, plant-based diets are low in vitamin B_{12}, which could have also modulating effects on sirtuin activity.[23] Ghemrawi and colleagues described a decreased SIRT1 expression due to lack of vitamin B_{12}.[23] These observations prompted us to compare sirtuins in blood from vegans, lacto-ovo-vegetarians, and omnivores.

During physical exercise, energy demand increases, especially in skeletal and cardiac muscle, the energy demand of the heart may vary 10-fold. In order to maintain metabolic homeostasis, energy production must increase commensurate with the increased energy demand during exercise. Mitochondrial energy production is regulated passively via substrate (ADP) saturation and actively via activation of ATP synthase (complex V of the respiratory chain) as previously described.[24] Furthermore, the Krebs cycle enzymes are under the control of calcium[25] which increases in response to exercise. Sirtuins are regulators of energy

production. SIRT3 activates complex 1 of the respiratory chain[26,27] complex 2[28], complex 3[27], complex 4[29] and complex 5[30] and regulates mitochondrial biogenesis.[31] Also, SIRT1 stimulates mitochondrial biogenesis[32] and oxidative phosphorylation via PGC-1α[32], while fatty acid oxidation is inhibited by SIRT4.[33] Hence, a better understanding of the regulation of human energy metabolism by sirtuins may offer a new approach to exercise physiology. To the best of our knowledge, literature regarding the influence of exercise performance on sirtuin activity in humans is scarce. For example, an investigation by Villanova and colleagues showed that sirtuin activity might be upregulated by physical exercise.[34] Suwa et al. as well as Covington et al. also described up-regulation of sirtuins after exercise.[35,36] A similar result was observed in rats with exercise training in treadmills resulting in activation of SIRT1 signaling pathways.[37] Zhuang et al. additionally showed transactivation of SIRT1 by FOXO3 and p53 in response to exercise.[38]

As diet may have an effect on sirtuins, we hypothesize that diet may not only influence basal sirtuin levels but the sirtuin response to exercise performance as well. We, therefore, studied sirtuins in blood from omnivores, lacto-ovo vegetarians and vegans before and after physical exercise.

Materials and methods

Participants

Participants were recruited from the general population in Hannover, Germany, by advertisements. Participants were pre-selected via screening questionnaires according to the following inclusion criteria: Omnivorous, lacto-ovo-vegetarian or vegan diet for at least half a year, BMI between 18.5 and 25.0 kg/m^2 and regular running exercise 2 to 5 times per week. The following criteria led to exclusion: Any cardiovascular, metabolic or malignant disease, diseases regarding the gastrointestinal tract, pregnancy, nutrient intolerances as well as addiction to drugs or alcohol. Participants were matched according to age and gender.

Ethical approval was granted by the Ethics Committee at the Medical Chamber of Lower Saxony (Hannover, Germany; 12/2017). The study was conducted in accordance with the Declaration of Helsinki. All participants gave their written informed consent prior to recruitment. This study is registered in the German Clinical Trial Registry (DRKS00012377).

Methods

All subjects underwent a sports-medical examination. First, a 24 h dietary recall was conducted (food and beverages consumed in the last 24 hours, including antioxidants, polyphenols, caffeine, vitamin B_{12}). Subsequently, an incremental exercise test was performed on a bicycle ergometer (Excalibur, Lode B.V., Groningen, Netherlands) until voluntary exhaustion.

Participants were asked not to perform any strenuous activities one day before and on the same day of the exercise test, and to maintain their usual diet. After a warm-up period of 6 minutes at 10 Watt (W), the test started at 50 W and increased by 16.7 W per minute (50 W per 3 min). For maximum performance, the body weight-related power output (W/kg BW) and time to exhaustion (s) were determined. To ensure that the subjects achieve their maximum performance, they were verbally motivated by staff, but they were not allowed to get out of the saddle. Before and after the exercise test (pre and post), venous blood samples were collected, aliquoted immediately and stored at -80° C. On a separate day, body composition was estimated using a bipolar bioelectrical impedance analyzer (BIA) (Nutriguard M, Data Input Company, Darmstadt, Germany) as well as the relative software NutriPlus© 5.4.1 (Data Input Company, Darmstadt, Germany).

Sample preaparation

2 ml EDTA-blood from every survey participant were taken. 500 µl of the blood sample was transferred into RNAprotect Animal Blood Tubes (Qiagen, DE). These samples were used for RNA isolation. The remaining 1.5 ml of blood were centrifuged at 3,300x g for 3 minutes. We collected the blood plasma and used it for analyses of sirtuins.

RNA isolation and qRT-PCR

RNA was isolated with RNeasy Protect Animal Blood Kit (Qiagen, DE) according to the product protocol. The isolated RNA was reverse transcribed to cDNA with the Omniscript RT Kit (Qiagen, DE). Real-time PCR of different cDNA samples was carried out with SYBR green on a 7900 HT fast real-time PCR system (Applied Biosystems, DE). Used primers are shown in supplementary data Table S1. Relative changes in the mRNA expression were calculated according to Vandesompele et al..[39]

Sirtuin activity assay

SIRT1 and SIRT3 deacetylase activities and SIRT5 desuccinylase activity were determined by using SIRT1, SIRT3 and SIRT5 fluorometric drug discovery assay kits (Enzo Life Science, CH). To ensure that enzyme capacity was measured we added a surplus of NAD+ to our assays. We followed the manufacturer's protocol with serum samples diluted 1:5 in HEPES buffer (110 mM NaCl, 2.6 mM KCl, 1.2 mM $KH2PO_4$, 1.2 mM MgSO4x7H2O, 1.0 mM CaCl2, 25 mM HEPES) and lysed by sonification for 10 seconds with 20 kHz at an amplitude of 75%. For normalization of the determined SIRT-activity signals, we measured total protein concentration of the analyzed samples lysed in HEPES buffer with the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, US).

Data analysis and statistical methods

Statistical analyses were performed using SPSS software (IBM SPSS Statistics 24.0; Chicago, IL, US) and GraphPad Prism 7.02 (GraphPad Software Inc., US). Results are shown as mean ± SD. First, normal distribution was checked by using the Kolmogorov-Smirnov test. If data were normally distributed, one-way ANOVA was used to evaluate differences between the three diet groups. For data with non-parametric distribution, a Kruskal Wallis test was performed. Additionally, if there were significant differences between the groups, a Post Hoc test with Bonferroni correction was conducted (Dunn´s multiple comparison test). Moreover, the Chi-square test was used to compare differences between the frequency distribution of the three groups. Associations between parametric data were computed via Pearson, non-parametric data via Spearman´s rho correlation. P values ≤ 0.05 were regarded as statistically significant.

To analyze the nutrient intake of the 24h dietary protocol, the nutrition organization software PRODI® (Nutri-Science GmbH, Freiburg, Germany) was used.

Results

In total, seventy-six healthy male and female omnivorous (OMN), lacto-ovo-vegetarian (LOV) and vegan (VEG) recreational runners aged between 18 and 35 years were included in the study. However, in five subjects, no analyses could be performed due to failure to collect blood or (pre-) analytical errors (Figure S1). Details of the study population are summarized in Table 1.

Maximum power output during the exercise test (OMN: 4.15±0.48, LOV: 4.20±0.47, VEG: 4.16±0.55 Watt/kg BW) and time to exhaustion (OMN: 1199 ± 177, LOV: 1197 ± 183, VEG: 1187 ± 237 s) did not differ significantly between the study groups, no gender-differences could be found.

Sirtuin activity

We analyzed the sirtuin capacity (under substrate saturation) in vitro in blood of 71 participants before and after exercise (Table 1). There were no significant differences in basal sirtuin activity levels of all sirtuins between the 3 nutritional groups, no gender-differences were observed. We detected a significant increase of SIRT1 enzyme capacity in omnivores (Figure 1 A) with an activity of 4.1 U/mg protein (±1.5) before exercise and 5.7 U/mg (±3.6) afterward. The induction of the SIRT1 capacity in response to exercise in lacto-ovo-vegetarians was not significant with activity levels of 4.9 U/mg (±1.7) before and 6.3 U/mg (±2.9) after exercise. A similar result was observed for samples from vegan participants. Here, the values before and

after exercise were unchanged with SIRT1 capacity levels of 4.3 U/mg (±2.1) before and 4.2 U/mg (±1.4) after exercise (Figure 1 A).

The results were similar for omnivores in case of SIRT3 (before [12.4 U/mg (±5.3)] and after [15.8 U/mg (±7.8)] exercise, $p<0.05$) and SIRT5 (before [0.7 U/mg (±0.3)] and after [0.9 U/mg (±0.3)] exercise, $p<0.05$), as well as for lacto-ovo-vegetarians with insignificant changes from 10.7 U/mg (±3.2) before to 13.4 U/mg (±4.9) after exercise for SIRT3 (Figure 1 B) and a change from 0.5 U/mg (±0.2) before to 0.6 U/mg (±0.2) after exercise for SIRT5 (Figure 1 C). SIRT3, as well as SIRT5 levels of sirtuin capacity, decreased in samples of vegan participants. For SIRT3 a reduction by ~10% to 11.5 U/mg (±4.8) after exercise and from 0.68 U/mg (±0.4) before to 0.66 U/mg (±0.4) after exercise was observed for SIRT5.

Since we observed an altered result in participants with a vegan diet, we reanalyzed our data for sirtuin capacity with a paired analysis approach to detect intraindividual alterations within single participants. Therefore, we subtracted the sirtuin capacity before exercise from the sirtuin capacity after exercise.

Also, the SIRT1 capacity was reduced in response to exercise in vegan participants. While there was an induction of 1-2 U/mg protein in omnivores and lacto-ovo-vegetarians, the SIRT1 capacity in vegans was reduced by ~0.7 U/mg protein. This was a significant difference compared to omnivores and lacto-ovo-vegetarians as well (Figure 2 A).

For SIRT3, a similar result was observed (Figure 2 B). In omnivores, we detected an induction by 3 U/mg protein after exercise and an increase of 2 U/mg protein in lacto-ovo-vegetarians. For samples of vegan participants, we observed a slight decrease by 0.5 U/mg protein. The vegan group differed again significantly from the omnivorous and lacto-ovo-vegetarian group.

SIRT5 showed a slightly different result (Figure 2 C). Similar to SIRT1 and SIRT3 omnivores showed an increase in enzyme activity by 0-16 U/mg protein. For vegan participants, a significantly different reduction by 0.004 U/mg protein was observed. In contrast to the results of SIRT1 and SIRT3, we detected only a small induction by 0.02 U/mg protein in the lacto-ovo-vegetarian group, resulting in no significant difference between vegan and lacto-ovo-vegetarian participants in SIRT5.

Although the change in sirtuin capacity was likely caused by altered posttranslational modifications we examined possible changes at gene expression levels of the analyzed sirtuins.

We measured the relative expression levels of SIRT1, SIRT3, SIRT4, and SIRT5. Basal levels before exercise were not different between groups and there were no gender differences. The changes in expression levels were calculated similarly to the changes in sirtuin capacity (under

substrate saturation) before and after exercise. For none of the analyzed sirtuin expression levels a significant change in gene expression was detectable (Figure 3A-C). The overall distribution of relative expression levels of vegan participants was similar to the omnivore and vegetarian groups.

A subset of each group was tested and in the samples examined no differences between the groups were detectable.

Correlations

In an attempt to find possible explanations for the differences between vegan participants and the omnivorous and lacto-ovo-vegetarian groups we correlated the capacities of SIRT1, SIRT3, and SIRT5 with different parameters from the 24 h dietary recall. We tested potential correlations between sirtuin capacities and several substances potentially having an influence on sirtuin activities. We did not detect correlations of sirtuin capacity with caffeine intake, blood insulin levels, blood glucose levels and active and total vitamin B_{12}-concentrations in blood. Additionally, we tested possible correlations of polyphenolic and flavonoid substances from the dietary recall, also without significant correlations. Furthermore, we checked if there were correlations between sirtuin capacities and the exercise intensity of probands and since sirtuins are responding to caloric restriction, correlations of sirtuin capacities with the caloric intake. All of these correlations were not significant (p-values > 0.05) (Table S2).

We found significant inverse correlations of sirtuin activities with the intake of the antioxidative substances tocopherol and ascorbate. Tocopherol showed a significant correlation ($p<0.05$, $R=0.27$) with all three analyzed sirtuin enzyme activities (Figure 4 A-C). For ascorbate, the correlations were similar but only in case of SIRT1 statistically significant ($p=0.042$, $R=0.28$) (Figure 4 D) while the correlations showed low but not significant p-values for SIRT3 ($p=0.061$, $R=0.25$) (Figure 4 E) and SIRT5 ($p=0.148$ $R=0.17$) (Figure 4 F).

We calculated ascorbate and tocopherol uptake within the 24 h recall in the study groups OMN, LOV and VEG. In vegan participants, we found increased levels of ascorbate as well as tocopherol (Figure 5), resulting in previously described correlations.

Several subjects took dietary supplements (OMN: 38.5, LOV: 34.6, VEG: 62.5%). Commonly consumed supplements were magnesium (OMN: 23.1, LOV: 15.4, VEG: 16.7%), iron (OMN: 7.69, LOV: 11.5, VEG: 16.7%), vitamin B12 (OMN: 19.2, LOV: 15.4, VEG: 50%) and vitamin D (OMN: 23.1, LOV: 3.85, VEG: 20.8%).

Discussion

To the best of our knowledge, this is the first investigation on sirtuins in humans with different diets at basal level and after physical exercise.

Sirtuins are known to be linked to nutrition. The first evidence of sirtuins as functional markers in blood was published by Tarantino et al.[8] with SIRT4 showing an inverse correlation to obesity. Alterations of sirtuins were described for caloric restriction with an induction of sirtuin expression and activity, reviewed by Kapahi et al..[40] Furthermore, it was reported that sirtuin activities can be altered by glucose supply.[41] We are aware that blood sirtuins may not necessarily reflect sirtuin-function in tissue, however we think that blood levels are a reasonable surrogate parameter for tissue levels.

In our study, we compared omnivores to lacto-ovo vegetarians and vegans. For sirtuins 1, 3, 4 and 5 in blood, no differences could be observed at gene expression level prior to exercise. At basal enzyme level, no differences could be observed for sirtuins 1, 3 and 5. For sirtuin 4, no enzyme assay was available; therefore measurement was done only at the gene expression level. In a pilot study, basal sirtuin activities at recruitment were compared to enzyme activities just prior to exercise (n=5), no differences were found. We hypothesized that sirtuins are altered in omnivores, lacto-ovo-vegetarians, and vegans as previously observed in different animals.[37,38]

Increased energy demand during exercise has to be met by increased flux at the levels of glycolysis, the Krebs cycle, fatty acid oxidation and the mitochondrial respiratory chain (oxidative phosphorylation). Increased capacities of sirtuins 1 and 3 as observed in our study in omnivores and to a lesser (non-significant) extent in lacto-ovo vegetarians during physical exercise result in activation of these pathways.[27,29–34,42–44] Previous studies in different animals showed induction of SIRT1 levels after exercise as well.[35,38] In our study, the capacity of sirtuin 4, an important regulator of fatty acid oxidation[33], could not be measured in the absence of an adequate assay. In vegans, sirtuin capacities decreased or remained unchanged during exercise. This may possibly result in energy deficiency in skeletal and heart muscle during exercise though we only measured sirtuins in blood and not in muscle. Furthermore, only in vitro enzyme capacities under substrate saturation were measured which do not necessarily reflect actual in vivo activities. Whether this leads to clinical symptoms or subclinical energy deficiency in tissues is still unknown.

Basal levels of enzyme capacities did not differ between the different groups (Figure 1) which may suggest a similar nutrition level for all study participants. SIRT5 capacity was somewhat lower in lacto-ovo-vegetarians compared to the other group, though not significantly, this may indicate reduced protein intake in vegetarians, since SIRT5 is an important regulator of the

urea cycle, where toxic ammonia from protein degradation is converted to urea. Actually, protein intake in the 24-hour recall was slightly reduced in vegetarians in a non-significant manner (p=0.22).

We have previously reported a correlation between ROS levels after treatment with antioxidants and sirtuin activities.[45] This prompted us to correlate the dietary intake of the antioxidants tocopherol and ascorbate assessed by the dietary recall with sirtuin levels. We found a negative correlation of these compounds with sirtuin capacities in all probands.

Increasing evidence suggests that sirtuins play an important role regarding stress responses.[1] Especially SIRT3 is involved in the cellular response to oxidative stress by deacetylating and activating the SOD2.[46,47] Furthermore, SIRT3 affects ROS detoxification by inducing the glutathione system as well as the thioredoxin system.[48] This may protect organs from exercise-induced mitochondrial ROS production.[49]

Reduced SIRT1 and SIRT3 enzyme capacities have a variety of different cellular consequences. Energy metabolism, especially the mitochondrial respiratory chain, is downregulated[50] as well as antioxidative response. SOD2 is a target of SIRT1 as well as SIRT3 and is the main ROS detoxification enzyme in mitochondria.[47,51] Only a few publications give evidence for a direct effect of antioxidative substances in humans. Some studies suggested that altered ROS levels act as effectors on sirtuin activity in response to high levels of antioxidants during exercise.[52]

We measured sirtuins in blood which is obviously a limitation. However, it has previously been shown that sirtuin levels in blood correlate with different organ dysfunctions like coronary heart disease in obese patients[8–11] or type 1 and type 2 diabetes.[12] Furthermore, exercise leads to metabolic stress in different organs and it would not ethically be sound to biopsy multiple organs in humans. Therefore, blood levels of sirtuins were used as surrogate parameters of sirtuin function in tissues.

In conclusion, we show in this study that sirtuins can be measured in human blood at enzyme, protein and gene expression levels. Basal enzyme capacities of sirtuins 1, 3 and 5 were not influenced by dietary habits (omnivores, lacto-ovo vegetarians and vegans); at gene expression and protein levels, no impact of diet on sirtuins 1, 3, 4 and 5 was found. While enzyme capacities of sirtuins 1 and 3 were up-regulated during exercise in omnivores and to a lesser extent in lacto-ovo vegetarians as a reflection of increased energy demand, enzyme capacities of sirtuins 1, 3 and 5 were down-regulated in blood from vegans. This may be related to the higher intake of the antioxidants tocopherol and ascorbate as judged by dietary recalls. Whether these changes are of clinical relevance, remains to be elucidated.

Supplementary Materials: Figure S1: Flow chart of the study population; Table S1: Sequences of qRT-PCR-Primers; Table S2: Correlations of SIRT-activities with different parameters

Author Contributions: The author´s responsibilities were as follows: JN, AP, SH, AH, and AD designed the study; JN, AP, SH and PW were responsible for research conduction; AP and JN conducted statistical analyses and wrote the manuscript; AD, AH and SH revised the manuscript critically for important content; all authors provided critical revisions, read and approved the final manuscript.

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Figure legends

Figure 1. Absolute enzyme capacities (under substrate saturation) of sirtuins SIRT1, SIRT3 and SIRT5. The figure shows the enzyme activities before (pre) and after (post) exercise in the three analyzed study groups omnivores (OMN), lacto-ovo-vegetarians (LOV) and vegans (VEG) for the sirtuins SIRT1 (A), SIRT3 (B) and SIRT5 (C). Data are shown as median ± quartiles and extrema; n=21-25; Statistical analysis with Kruskal Wallis test and Dunn´s multiple comparison test; * = p<0.05.

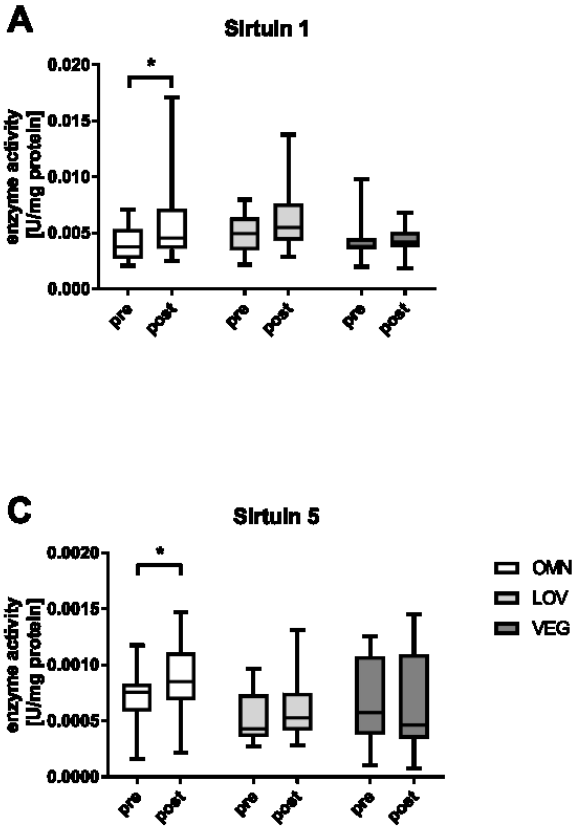

Figure 2. Changes of enzyme capacity (under substrate saturation) of sirtuins SIRT1, SIRT3 and SIRT5 after exercise. The response of sirtuins to exercise was calculated as the difference of enzyme capacities before (pre) and after (post) exercise. Sirtuins in the three study groups omnivores (OMN), lacto-ovo-vegetarians (LOV) and vegans (VEG) are shown: SIRT1 (A), SIRT3 (B) and SIRT5 (C). Data are shown as mean difference ± SD; n=21-25; Statistical analysis with Kruskal Wallis test and Dunn´s multiple comparison test; * = p<0.05.

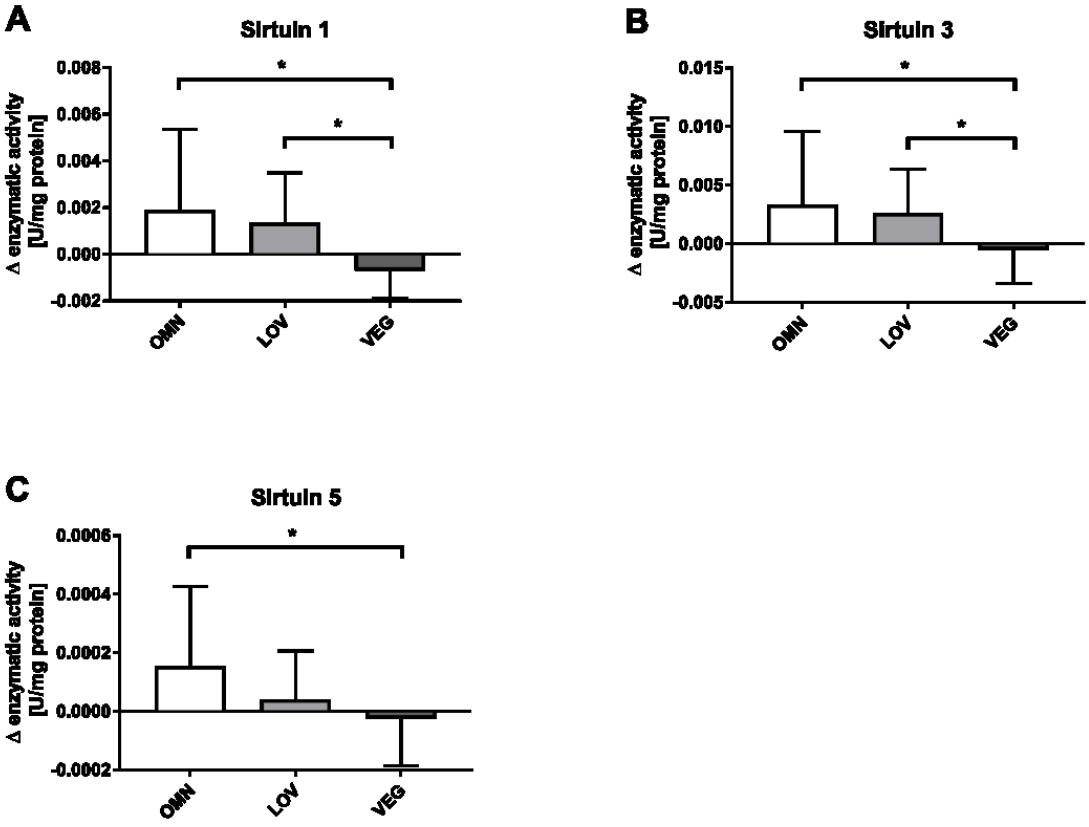

Figure 3. Changes in the relative expression of sirtuins SIRT1, SIRT3, SIRT4 and SIRT5 after exercise. The figure shows the differences of relative expression before (pre) and after (post) exercise in the three analyzed study groups omnivores (OMN), lacto-ovo-vegetarians (LOV) and vegans (VEG) for the analyzed sirtuins SIRT1 (A), SIRT3 (B) and SIRT5 (C). Data are shown as median ± quartiles and extrema; n=21-25; Statistical analysis with Kruskal Wallis test; * = $p<0.05$.

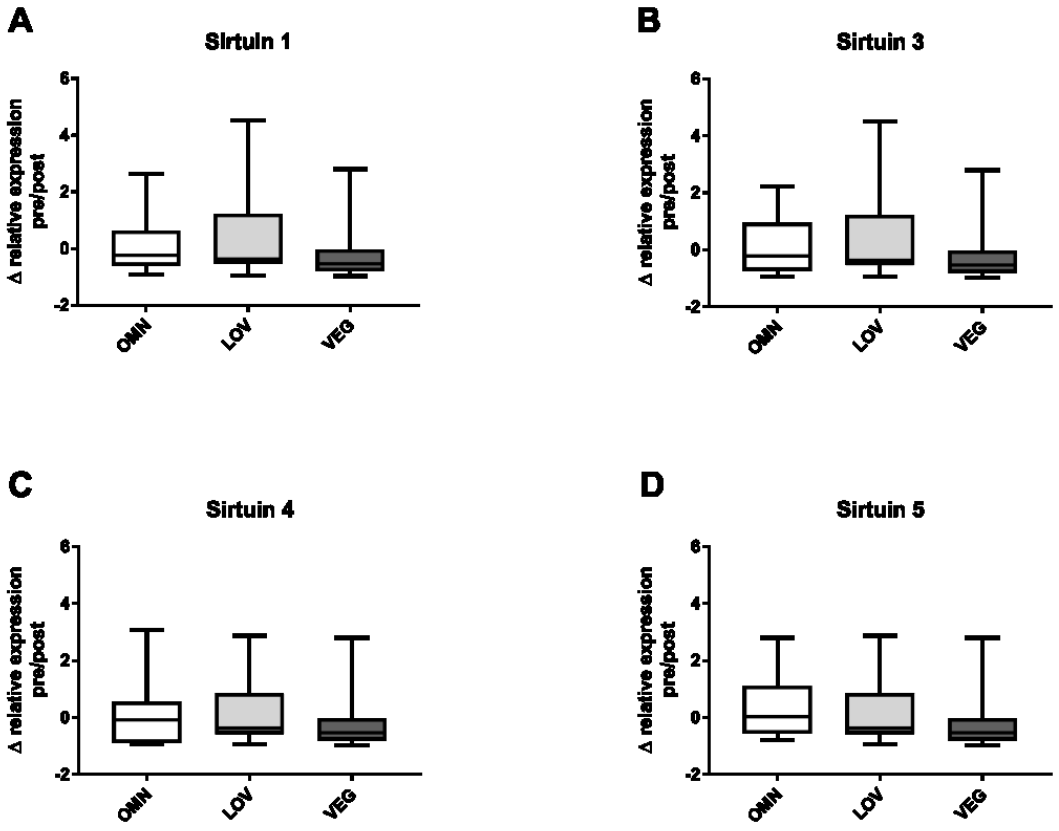

Figure 4. Correlations of calculated tocopherol and ascorbate intake with SIRT1, SIRT3, and SIRT5 capacity levels. The figure shows the correlations of changes in enzyme capacities (post-pre exercise) with either tocopherol (A-C) or ascorbate (D-F) for all analyzed sirtuins. For correlation analyses, all study groups were pooled (n=71). Correlations for tocopherol (A-C) and ascorbate with SIRT1 were statistically significant; Statistical analysis with Spearman correlation test; * = $p<0.05$, $R=0.27$.

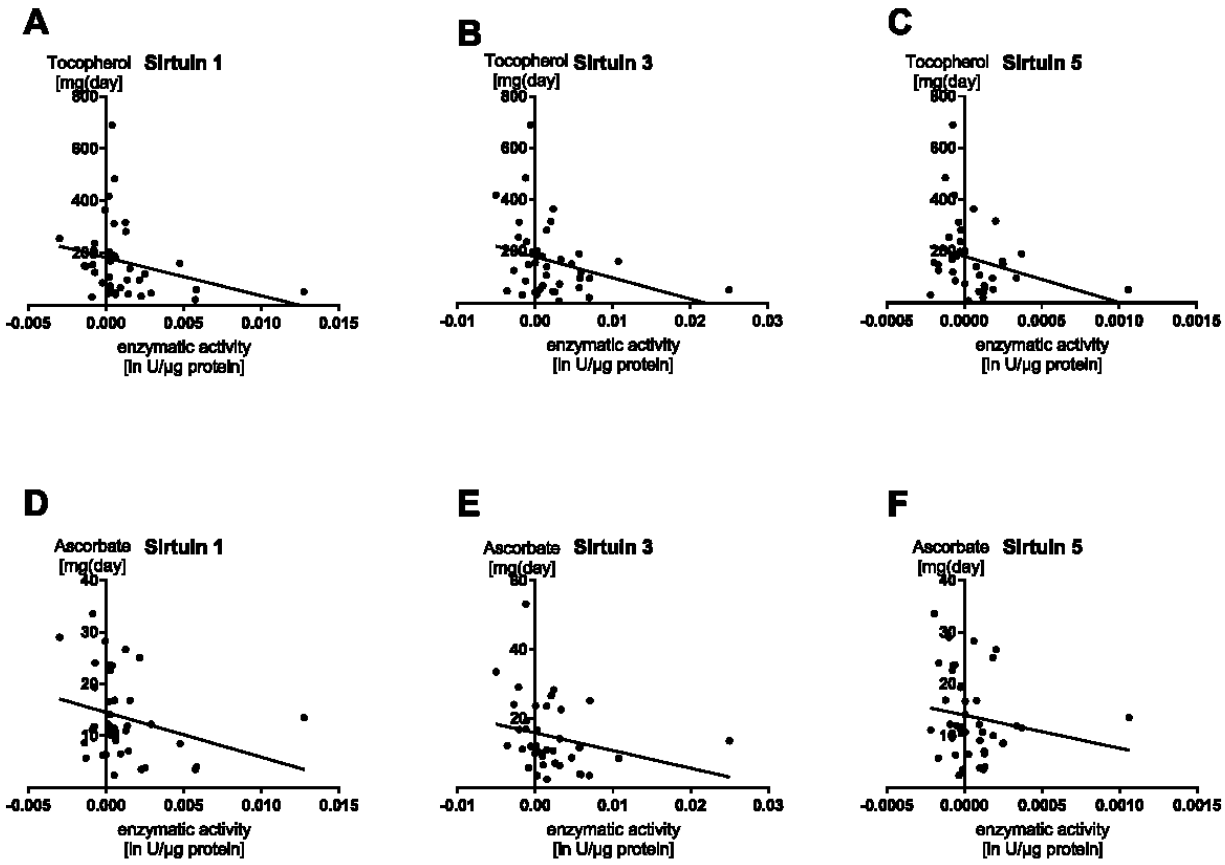

Figure 5. Intake of ascorbate (A) and tocopherol (B) in the three analyzed study groups omnivores (OMN), lacto-ovo-vegetarians (LOV) and vegans (VEG) during 24 h dietary recall. Data are shown as median ± quartiles and extrema; n=21-25; Statistical analysis with Kruskal Wallis test and Dunn´s multiple comparison test; * = p<0.05.

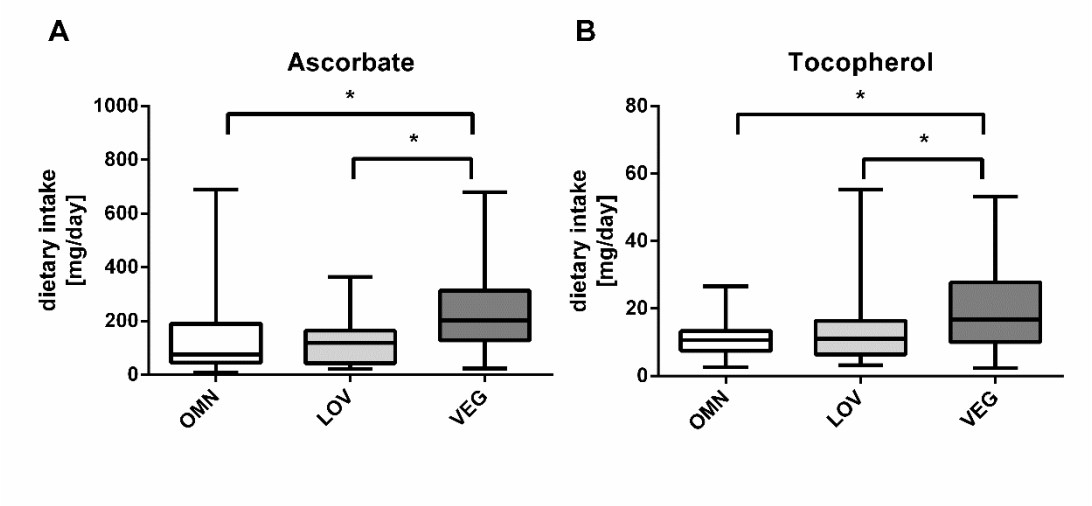

Table 1. Participant characteristics by dietary patterns of the study population.

	OMN (n=25)	**LOV (n=25)**	**VEG (n=21)**	**p-value**
Age (y)	27.2±4.1	27.6±4.4	27.2±4. 4	0.888[a]
Sex	m=10, w=15	m=10, w=15	m=9, w=12	0.975[b]
BMI (kg/m^2)	22.3±1.74	21.6±1.98	22.1±2.09	0.426[a]
LBM (kg)	54.1±9.2	52.7±8.9	54.6±11.3	0.869[a]
Body fat (%)	21.4±6.0	21.2±5.6	20.2±5.3	0.752[c]
Duration of diet				**0.001[b]**
< 0.5 years, n (%)	0 (0)	0 (0)	0 (0)	
0.5 - 1 year, n (%)	0 (0)	4 (16)	5 (24)	
1 - 2 years, n (%)	1 (4)	3 (12)	3 (14)	
2 - 3 years, n (%)	0 (0)	2 (8)	7 (33)	
> 3 years, n (%)	24 (96)	16 (64)	6 (29)	
Smoker (%)	0	0	0	-
Training frequency per week	3.0±0.9	3.2±0.9	2.9±0.8	0.469[a]
Running time per week (h)	2.7±1.1	3.3±1.3	2.6±1.5	0.237[a]

OMN = omnivores, LOV = lacto-ovo-vegetarians, VEG = vegans, SU = supplement users, n.s. = not significant. Values are given as means ± SD or n (%). [a] Kruskal Wallis test, [b] Chi-square test, [c] One-way ANOVA.

2.5. Paper V

Exercise-induced oxidative stress and amino acid profile in recreational runners with vegetarian and non-vegetarian dietary patterns

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Exercise-Induced Oxidative Stress, Nitric Oxide and Plasma Amino Acid Profile in Recreational Runners with Vegetarian and Non-Vegetarian Dietary Patterns

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Abstract: This study investigated the exercise-induced changes in oxidative stress, nitric oxide (NO) metabolism and amino acid profile in plasma of omnivorous (OMN, $n = 25$), lacto-ovo-vegetarian (LOV, $n = 25$) and vegan (VEG, $n = 23$) recreational runners. Oxidative stress was measured as malondialdehyde (MDA), NO as nitrite and nitrate, and various amino acids, including homoarginine and guanidinoacetate, the precursor of creatine. All analytes were measured by validated stable-isotope dilution gas chromatographic-mass spectrometric methods. Pre-exercise, VEG had the highest MDA and nitrate concentrations, whereas nitrite concentration was highest in LOV. Amino acid profiles differed between the groups, with guanidinoacetate being highest in OMN. Upon acute exercise, MDA increased in the LOV and VEG group, whereas nitrate, nitrite and creatinine did not change. Amino acid profiles changed post-exercise in all groups, with the greatest changes being observed for alanine (+28% in OMN, +21% in LOV and +28% in VEG). Pre-exercise, OMN, LOV and VEG recreational runners differ with respect to oxidative stress, NO metabolism and amino acid profiles, in part due to their different dietary pattern. Exercise elicited different changes in oxidative stress with no changes in NO metabolism and closely comparable elevations in alanine. Guanidinoacetate seems to be differently utilized in OMN, LOV and VEG, pre- and post-exercise.

Keywords: diet; exercise; malondialdehyde; plasma; nitric oxide; vegan; vegetarian

1. Introduction

Intense exercise induces oxidative stress. The majority of the reactive oxygen and nitrogen species including free radicals cannot be determined in biological samples such as blood, because of their high chemical reactivity, their low concentration and extremely short half-life. Instead of, their stable and analytically accessible metabolites are measured. Biological malondialdehyde (MDA) is a product of lipid-peroxidation and one of the most widely used and generally accepted biomarkers of oxidative stress [1]. Numerous human studies revealed associations between oxidative stress and the pathogenesis of atherosclerosis, endothelial dysfunction and cardiovascular diseases [2–5]. On the other hand, however, oxidative stress has been associated with various signalling pathways including nitric oxide (NO). It is assumed that oxidative stress may exert health-promoting effects on physical activity and, consequently, on training adaptations and human physiology, such as insulin sensitivity [6,7]. For these reasons, the equivocal role of exercise-induced oxidative stress needs further clarification [8].

In addition to the endogenous defence systems, dietary antioxidant intake (e.g., vitamin C and vitamin E) is crucial to avoid adverse effects of oxidative stress. Antioxidant supplements are widely consumed in everyday life and in sports. In general, a balanced mixed diet can provide the athlete with adequate amounts of antioxidants. However, dietary intake of antioxidants in athletes is often insufficient [9,10]. Plant-based diets, such as those consumed in vegetarianism and veganism, are rich in antioxidants and are assumed to prevent from oxidative damage. In fact, plant-based diets seem to be advantageous over average mixed diets in various diseases, such as diabetes, hypertension and cancer [11–13]. Several groups reported on positive effects of plant-based diets on exercise-induced oxidative stress and inflammatory parameters [14,15]. However, these data are based solely on the assumption that vegetarians have a higher intake of antioxidants compared to omnivores.

A recent study investigated the oxidative status in moderately active vegan, vegetarian and omnivorous men [16]. In that study, a higher antioxidant capacity was measured in omnivores compared to both vegans and vegetarians. This in vitro study found that incubation of the rat cardiac myoblastic cell line H9c2 with vegan serum samples elevated thiobarbituric acid-reactive substances and induced cell death [16]. Yet, it is unclear whether oxidative burden depends on the dietary pattern under physical stress in vivo. Thus far, there are no studies to demonstrate that the assumed increased intake of antioxidants indeed provides protection against exercise-induced oxidative stress [15].

Amino acids undergo tightly controlled homeostatic regulation. Their concentrations in blood reflect a balanced interaction between dietary intake, endogenous synthesis, catabolic and anabolic processes [17,18]. Exercise-induced adaptations have been observed in amino acid metabolism [19]. Long-lasting endurance events showed an overall decrease by 15–30% in total circulating amino acid concentration [20–22], while the concentration of aromatic amino acids often increased by 6–11% [23,24]. Results of previous studies show that the plasma amino acid profile differs between male omnivorous, vegetarian and vegan non-athletes [25–31]. However, there are no such data from athletes. Additionally, no data on exercise-induced changes in circulating amino acids and their metabolites have been reported thus far.

We have hypothesized that there are differences between omnivorous, lacto-ovo-vegetarian and vegan recreational runners with respect to exercise-induced oxidative stress, NO metabolism and circulating amino acids. The present study was conducted to prove this hypothesis by determining the concentrations of circulating MDA as a measure of lipid peroxidation, nitrite and nitrate as the major NO metabolites, and several amino acids and their metabolites in plasma samples collected in a previously reported study on healthy omnivorous, lacto-ovo-vegetarian and vegan recreational runners before and after laboratory physical exercise tests [32]. The flow chart of the study is reported as supplementary information (Figure S1). All analytes were measured by using fully validated and clinically proven gas chromatographic-mass spectrometric methods and stable-isotope labelled analogs as internal standards [33,34]. The method used for the measurement of the amino acids in the plasma samples provides the sum concentration for Ile and Leu (Ile+Leu), Asp and Asn (Asp+Asn), Glu and Gln (Glu+Gln), and for Orn and Cit (Orn+Cit). Thus, for these amino acid pairs their summed concentration is reported and considered in statistical analyses. The global arginine bioavailability ratio was calculated by dividing the plasma Arg concentration by the sum of the concentrations of Orn and Cit.

2. Materials and Methods

2.1. Study Design, Participants, Physical Exercise and Blood Sampling

The cross-sectional study on which the present work is based on has been previously reported in detail [32] (Figure S1). Subjects were recruited via advertisements from the general population in Hannover (Germany). The main inclusion criteria were: Omnivorous (OMN), lacto-ovo-vegetarian (LOV) or vegan (VEG) diet for at least half a year, body mass index (BMI) between 18.5 and 25.0 kg/m^2 and regular running exercise two to five times per week. Exclusion criteria were: Any cardiovascular,

metabolic or malignant disease, gastrointestinal diseases, pregnancy or lactation, nutrient intolerances, drug and alcohol dependency, concurrent participation in another clinical study, participation in a study in the last 30 days and retraction of the consent by the subject. Inclusion and exclusion criteria were queried using a screening questionnaire. Participants were matched according to age and gender. Omnivores consumed both plant and animal foods, lacto-ovo vegetarians excluded meat and fish from their diet and vegans only consumed plant-based foods. Food and beverages consumed in the last 24 h were recorded via 24 h dietary recall [32]. Seventy-six healthy, non-smoking OMN, LOV and VEG recreational runners conducted incremental 20–30 min lasting stress on a bicycle ergometer (Excalibur, Lode B.V., Groningen, Netherlands). Venous blood samples (7.5-mL EDTA monovettes, Sarstedt®, Nümbrecht, Germany) were collected from 73 subjects before and after the exercise load. No blood samples could be taken from three subjects, either before or/and after exercise test (Table S1). There were no statistically significant differences regarding age, gender, BMI and training habit between the groups (Table 1). Dietary intake of antioxidants, amino acids and fatty acids by diet group is reported in Table 2.

Table 1. Characterization of the study populations.

	OMN (n = 25)	LOV (n = 25)	VEG (n = 23)	p Value
Age (years)	27.2 ± 4.1	27.6 ± 4.4	27.3 ± 4.3	0.917[a]
Gender (n, males/females)	10/15	10/15	8/15	0.913[b]
BMI (kg/m^2)	22.3 ± 1.8	21.5 ± 1.9	21.9 ± 2.2	0.412[a]
Weekly training frequency	3.0 ± 0.9	3.2 ± 0.9	3.0 ± 0.9	0.757[a]
Weekly running (h)	2.7 ± 1.1	3.3 ± 1.3	2.7 ± 1.4	0.122[a]

OMN = omnivores; LOV = lacto-ovo-vegetarians; VEG = vegans. Data are presented as mean ± SD. [a] Kruskal-Wallis test, [b] Chi square test.

Table 2. Dietary intake of antioxidants and amino acids (mean ± SD) by diet group.

Parameters	OMN	p Value OMN vs. LOV	LOV	p Value LOV vs. VEG	VEG	p Value OMN vs. VEG	p Value
Antioxidants (mg)							
Vitamin C	143 ± 153	n.s.	148 ± 145	n.s.	218 ± 138	n.s.	0.037
Vitamin E	12.0 ± 6.36	n.s.	13.1 ± 11.0	0.040	19.7 ± 12.0	n.s.	0.021
Amino acids (g)							
Arg	4.57 ± 2.60	-	3.75 ± 2.21	-	4.53 ± 2.72	-	0.442
Thr	3.60 ± 2.40	-	2.95 ± 1.86	-	2.47 ± 1.25	-	0.185
Val	4.93 ± 3.04	-	4.38 ± 2.73	-	3.51 ± 1.87	-	0.213
Leu+Ile	11.4 ± 7.10	-	10.2 ± 6.60	-	8.05 ± 4.51	-	0.200
Met	1.90 ± 1.24	n.s.	1.56 ± 1.17	n.s.	0.99 ± 0.61	0.004	0.005
Phe	4.02 ± 2.23	-	3.68 ± 2.24	-	3.29 ± 1.80	-	0.507
Tyr	3.27 ± 1.89	-	3.04 ± 2.08	-	2.29 ± 1.33	-	0.134
Lys	5.64 ± 3.85	-	4.57 ± 3.21	-	3.36 ± 1.97	-	0.062
Trp	1.07 ± 0.64	-	0.91 ± 0.54	-	0.81 ± 0.39	-	0.346
Fatty acids							
ALA (g)	1.43 ± 1.58	-	1.85 ± 2.27	-	2.16 ± 1.65	-	0.115
PUFA (EN%)	4.87 ± 2.30	-	4.76 ± 2.34	-	6.26 ± 2.94	-	0.085

ALA, alpha-linolenic acid; PUFA, polyunsaturated fatty acids; EN% = energy percent; n.s., not significant. Statistical analysis was performed with Kruskal-Wallis test to examine group differences. Post-hoc test was conducted for statistically significant differences.

Ethical approval was provided by the Ethics Committee at the Medical Chamber of Lower Saxony (Hannover, Germany; 12/2017). In accordance with the Declaration of Helsinki, written informed consent was obtained from all subjects prior to their participation in the study. This study is registered in the German Clinical Trial Register (DRKS00012377).

2.2. Sample Preparation and Biochemical Analyses

EDTA-anticoagulated blood was centrifuged (4 °C, 1620× *g*), 500 µL plasma aliquots were immediately aliquoted in 1.5 mL Eppendorf Tubes® (Eppendorf AG, Hamburg, Germany) and frozen at –80 °C until analysis. The plasma samples were transferred frozen on dry-ice to the Institute of Toxicology at Hannover Medical School and stored in frozen state at −20 °C until analysis within the next few days. On each day of analysis, a certain number of plasma samples left thaw at room temperature. Aliquots of 100 µL of the thawed samples were taken for the simultaneous analysis of MDA, nitrite, nitrate and creatinine and transferred into 1.5 mL glass vials (Macherey-Nagel, Düren, Germany). For the analysis of amino acids, further 10 µL plasma aliquots were taken and transferred into 1.5 mL glass vials as well.

Plasma MDA, nitrate, nitrite and creatinine were analysed simultaneously as reported elsewhere [33]. Aliquots (10 µL) of a mixture of the internal standards containing 400 µM [^{15}N]nitrate, 40 µM [^{15}N]nitrite and 1 mM d_3-creatinine in distilled water were added to the 100-µL plasma aliquots. Aliquots (15.4 µL) of the internal standard d_2-MDA solution in 10 mM HCl (65 µM) were also added. The final concentrations of the stable-isotope labelled internal standards were 40 µM for [^{15}N]nitrate, 4 µM for [^{15}N]nitrite, 100 µM d_3-creatinine and 10 µM d_2-MDA with respect to the plasma volume. After addition of acetone (400 µL) and the derivatization reagent pentafluorobenzyl bromide (10 µL) to the samples, the glass vials were tightly closed and then heated for 60 min at 50 °C. After cooling to room temperature, acetone was removed under a gentle stream of nitrogen. Extraction of excess pentafluorobenzyl bromide and the reaction products from the remaining aqueous phase was carried out by adding ethyl acetate (1 mL) and by vortex-mixing the mixtures for two minutes at the highest speed using a Heidolph vortex mixer model Reax 2000 (Schwabach, Germany). After centrifugation (5 min, 3350× *g*) the upper organic phase was decanted and dried over anhydrous Na_2SO_4 (about 10 mg per sample). Subsequently, the samples were centrifuged again (5 min, 3350× *g*) and 750 µL aliquots of the organic phase were transferred into 1.8 mL autosampler glass vials (Macherey-Nagel; Düren, Germany) for gas chromatography-mass spectrometry analysis.

Plasma amino acids were analyzed simultaneously by gas chromatography-mass spectrometry in 10 µL aliquots using trideutero-methyl esters of the individual amino acids as the internal standards after preparation of the methyl ester pentafluoropropionyl derivatives as reported elsewhere [34].

2.3. Gas Chromatographic-Mass Spectrometric Analyses

Gas chromatographic-mass spectrometric analyses were performed on a single quadrupole mass spectrometer model ISQ directly interfaced with a Trace 1310 series gas chromatograph equipped with an autosampler AS 1310 from ThermoFisher (Dreieich, Germany). Different oven temperature programs were used for the separation of the derivatives of nitrate, nitrite, creatinine and MDA, on the one hand, and of the derivatives of the amino acids and their metabolites, on the other hand. Selected-ion monitoring of specific anions for unlabelled nitrate, nitrite, creatinine, MDA and for their stable-isotope labelled analogues was performed [33]. Amino acids were analysed by selected-ion monitoring of specific anions for endogenous and their stable-isotope labelled analogues as reported previously [34].

2.4. Data Analysis and Statistical Methods

Data are presented as mean ± standard deviation (SD). To control distribution, the Kolmogorov–Smirnov test was used. To evaluate differences between the three diet groups, a one-way analysis of variance was used for parametric data. For non-parametric data the Kruskal–Wallis test was performed. For statistically significant differences a post hoc test with Bonferroni correction was conducted to analyse differences between the individual groups. To examine differences between pre- and post-exercise within a group, the *t*-test (for parametric data) and the Mann–Whitney U test (for non-parametric data) were used. Further, to calculate correlations between parametric data,

the Pearson correlation was computed. Finally, to assess associations between non-parametric data, Spearman's rho correlation was used. Values of $p \leq 0.05$ were regarded as statistically significant. All statistical analyses were conducted using SPSS software (IBM SPSS Statistics 24.0; Chicago, IL, USA) and GraphPad Prism 7.02 (GraphPad Software Inc., San Diego, CA, USA).

3. Results

The pre-exercise and post-exercise plasma concentrations of the biochemical markers measured in the present study are summarized in Table 3.

Table 3. Plasma concentrations (mean ± SD; mM for creatinine; μM for the other analytes) and mean percentage changes (Δ, %) of the biochemical parameters of the three groups pre-exercise (pre) and post-exercise (post).

Parameters	OMN			LOV			VEG		
	Pre	Post	Δ (%)	Pre	Post	Δ (%)	Pre	Post	Δ (%)
Oxidative Stress/NO Metabolism	(*n* = 25)			(*n* = 25)			(*n* = 23)		
MDA	0.52 ± 0.09	0.56 ± 0.10	+9.2	0.50 ± 0.07	0.62 ± 0.15 †	+24	0.57 ± 0.13	0.68 ± 0.15 †	+15
Nitrate	70.7 ± 15.9	70.5 ± 17.8	−0.3	91.9 ± 43.8	94.9 ± 50.8	+3.3	120 ± 146	102 ± 41.9	−5.7
Nitrite	1.93 ± 0.26	1.85 ± 0.21	−4.4	2.67 ± 0.61	2.56 ± 0.53	−4.2	2.50 ± 1.09	2.36 ± 0.42	−18
Kidney function									
Creatinine	94.1 ± 20.7	93.4 ± 17.3	−0.7	90.0 ± 32.8	92.4 ± 40.2	+2.7	86.4 ± 37.6	84.7 ± 20.7	−2.1
Amino acids	(*n* = 24)			(*n* = 25)			(*n* = 22)		
Ala	411 ± 100	530 ± 112 ‡	+28	400 ± 85.9	485 ± 111 ‡	+21	439 ± 104	557 ± 117 ‡	+28
Thr	194 ± 75.0	167 ± 63.4 ‡	−15	161 ± 47.9	148 ± 66.6 †	−8.0	158 ± 41.7	157 ± 62.9*	−0.1
Gly	249 ± 68.4	231 ± 57.6 *	−7.4	248 ± 77.5	227 ± 63.8 †	−8.3	324 ± 79.5	312 ± 70.8	−4.2
Val	373 ± 150	332 ± 104 †	−12	332 ± 116	302 ± 99.9 *	−8.9	298 ± 106	284 ± 75.8	−4.2
Ser	204 ± 152	235 ± 220	+16	168 ± 59.9	147 ± 78.6 *	−17	185 ± 91.5	150 ± 22.3 *	−19
Sar	2.24 ± 0.99	2.22 ± 1.00	−1.6	1.84 ± 0.55	2.05 ± 0.64 †	+11	1.66 ± 0.46	1.95 ± 0.51 †	+17
Leu +Ile	300 ± 125	251 ± 76.5 †	−17	231 ± 102	209 ± 74.7 *	−9.4	227 ± 75.9	254 ± 127	+12
GAA	3.79 ± 1.24	3.25 ± 0.89	−13	2.63 ± 0.64	3.01 ± 0.94	+9.9	3.42 ± 0.74	3.88 ± 1.02	+12
Asp+Asn	104 ± 28.9	94.7 ± 32.2	−9.7	75.8 ± 17.0	66.1 ± 18.7 †	−13	107 ± 22.9	97.0 ± 17.3 *	−9
Pro	213 ± 75.3	188 ± 59.9 †	−12	220 ± 73.2	199 ± 66.6 †	−9.6	214 ± 59.1	209 ± 67.9	−0.8
Met	65.5 ± 13.0	62.6 ± 12.7 *	−4.4	60.2 ± 7.03	57.7 ± 7.88	−4.2	65.5 ± 6.94	65.4 ± 6.78	+0.2
Glu+Gln	802 ± 236	787 ± 235	−1.6	682 ± 106	654 ± 116	−4.1	766 ± 107	773 ± 117	+1.6
Orn+Cit	59.8 ± 21.2	50.1 ± 15.8 ‡	−17	55.1 ± 13.7	48.0 ± 12.1 ‡	−13	59.0 ± 19.6	56.2 ± 18.3	−3.8
Phe	80.6 ± 20.6	72.0 ± 17.5 †	−12	68.1 ± 17.4	61.8 ± 13.9 †	−9.2	73.7 ± 16.6	70.7 ± 12.9	−3.3
Tyr	75.3 ± 34.1	66.2 ± 25.3 †	−13	65.9 ± 25.4	60.6 ± 21.2 †	−9.4	58.3 ± 18.0	58.1 ± 15.8	−0.4
Lys	206 ± 69.1	184 ± 51.0 †	−12	109 ± 37.5	120 ± 39.9 *	+9.3	153 ± 47.2	149 ± 33.4	−1.9
Arg	91.8 ± 29.7	77.4 ± 21.0 †	−12	69.1 ± 19.1	66.5 ± 18.2 *	−6.0	93.5 ± 25.9	90.3 ± 23.6	−2.9
hArg	1.89 ± 0.93	1.64 ± 0.93	−11	1.09 ± 0.39	1.10 ± 0.38	+1.2	1.51 ± 0.81	1.58 ± 0.75	+5
Trp	34.5 ± 11.6	45.8 ± 68.8	+33	22.9 ± 9.28	17.2 ± 5.43 ‡	−25	22.7 ± 4.70	18.6 ± 5.45	−18
GAA/hArg	2.26 ± 0.91	2.53 ± 1.22	+11	2.62 ± 1.25	2.98 ± 1.49 *	+12	2.79 ± 1.34	2.86 ± 1.21	+2.5
GABR	1.57 ± 0.33	1.58 ± 0.30	+6.3	1.27 ± 0.27	1.41 ± 0.31	+17	1.65 ± 0.34	1.67 ± 0.32	+1.9

+ indicates increase, − indicates decrease. Asterisks indicate statistical differences (* $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$). GAA, guanidinoacetate; hArg, homoarginine; GABR, global arginine bioavailability ratio.

3.1. Pre-Exercise Concentrations

The highest pre-exercise plasma MDA ($p_{LOV} = 0.020$) and plasma nitrate ($p_{OMN} = 0.049$) concentrations were observed in VEG, suggesting higher pre-exercise oxidative stress and NO synthesis in this group. The highest pre-exercise plasma nitrite concentration was found in LOV ($p_{OMN} < 0.001$), suggesting higher pre-exercise endothelial NO synthesis in this group. Plasma creatinine levels tended to be higher in OMN compared to LOV and especially to VEG. Expectedly, men had higher plasma creatinine concentrations compared to women (106 ± 34.5 vs. 80.5 ± 17.2 μM, $p < 0.001$).

Regarding the pre-exercise plasma amino acids, VEG had the highest concentrations of Gly ($p_{omn} = 0.002$, $p_{LOV} = 0.002$), Asp+Asn ($p_{LOV} < 0.001$, $p_{OMN\text{-}LOV} = 0.001$) and Arg ($p_{LOV} = 0.004$, $p_{OMN\text{-}LOV} = 0.011$). In contrast, OMN had the highest basal levels of Leu+Ile ($p_{LOV} = 0.033$), guanidinoacetate ($p_{LOV} = 0.001$, $p_{LOV\text{-}VEG} = 0.006$), Glu+Gln ($p = 0.041$), Lys ($p_{LOV} < 0.001$, $p_{LOV\text{-}VEG} = 0.006$), homoarginine ($p_{LOV} < 0.001$), and Trp ($p_{LOV} < 0.001$, $p_{VEG} = 0.005$) before exercise test. The global arginine bioavailability ratio was lowest in LOV compared to the other groups

($p_{OMN} = 0.005$, $p_{VEG} < 0.001$). These observations indicate considerable differences in the plasma amino acids profile in the study groups pre-exercise.

3.2. Exercise-Induced Changes

MDA plasma concentrations increased post-exercise in all groups, yet statistically significant increases were observed in the LOV and VEG groups. No significant group-differences in changes of MDA were observed. Plasma nitrate, nitrite and creatinine did not change statistically significantly post-exercise. These observations suggest exercise-induced elevation of oxidative stress in the LOV and VEG groups, but no changes in NO metabolism and kidney function.

With respect to the plasma amino acids and their metabolites, the concentration of the majority decreased statistically significantly upon exercise, suggesting exercise-induced consumption of these amino acids. Yet, considerable increases were seen for Ala: +28% in OMN, +21% in LOV and +28% in VEG. The plasma concentrations of Thr, Gly and Val decreased upon exercise in OMN and LOV. Upon exercise, the plasma concentration of Ser increased (+16%) in OMN, but decreased in LOV (−17%) and VEG (−19%). The highest post-exercise increase was observed for the plasma concentration of Trp (+33%) in the OMN group. Analogous to Ser, the greatest post-exercise decrease was observed for the plasma concentration of Trp (−25%) in the LOV group.

Upon exercise, the plasma concentration of Sar, which is the *N*-methylated glycine, increased in the LOV (+11%) and VEG (+17%) groups, but decreased slightly in the OMN group (−1.6%). The plasma concentration of Asp+Asn decreased significantly in LOV and VEG after exercise. Exercise-induced changes were observed for the plasma concentrations of Orn + Cit and Tyr in OMN (highest) and in VEG (lowest changes; $p = 0.045$ and $p = 0.004$, respectively). The plasma concentrations of Phe and Tyr decreased upon exercise to almost the same degree in OMN and LOV, but did decrease only to a small extent in VEG.

The plasma Lys concentration changed upon exercise in OMN (decrease) and LOV (increase); these changes differed significantly between OMN and VEG ($p = 0.001$). The plasma Arg concentration decreased statistically significantly upon exercise in OMN and LOV. Yet, this did not result in significant changes of the global arginine bioavailability ratio in all three groups. The plasma concentrations of the Arg metabolites homoarginine and guanidinoacetate did not change significantly in all groups post-exercise. The guanidinoacetate/homoarginine molar ratio increased in all groups upon exercise, yet the increase (+12%) was only in LOV significant. The plasma Glu+Gln concentration did not change upon exercise in all groups. Exercise-induced significant decreases in the plasma concentration of Leu+Ile were found in the OMN (−17%) and LOV (−9.4%) groups.

Although all three groups showed lower pre-exercise concentrations of Pro, the largest difference was observed in OMN, who differed significantly from VEG ($p = 0.015$).

Regarding homoarginine, the highest exercise-induced reduction was obtained in OMN, who differed significantly from VEG ($p = 0.010$); the latter had elevated levels after exercise.

The above mentioned observations indicate that plasma amino acids are differently managed by the groups during exercise.

3.3. Correlations

In all three groups, plasma nitrate concentration pre-exercise correlated with plasma nitrate concentration post-exercise ($r_{omn} = 0.808$, $p_{omn} < 0.001$; $r_{LOV} = 0.704$, $p_{LOV} < 0.001$; $r_{VEG} = 0.639$, $p_{VEG} = 0.001$). Plasma nitrite ($r = 0.470$, $p = 0.018$) and creatinine ($r = 0.723$, $p < 0.001$) concentrations pre- and post-exercise correlated only in OMN. Plasma MDA concentrations pre- and post-exercise correlated only in VEG ($r = 0.518$, $p = 0.011$). The plasma concentrations of the following amino acids correlated in all three groups pre- and post-exercise: Ala, Thr, Gly, Val, Sar, Leu+Ile, Asp+Asn, Pro, Met, Glu+Gln, Orn + Cit, Phe, Tyr, Lys, Arg, and homoarginine (Table S1). These findings suggest that exercise changes distinctly different lipid peroxidation, NO metabolism and plasma amino acid profile in the groups.

Correlation data of oxidative stress (MDA) and NO metabolism (nitrite and nitrate) with plasma amino acids are reported in Table 4. We found inverse correlations for MDA with guanidinoacetate pre-exercise and with Trp post-exercise. The inverse correlation of guanidinoacetate with both, MDA and nitrite, may suggest that guanidinoacetate is consumed during lipid peroxidation and endothelial NO synthesis. All significant correlations of nitrate were positive. Except for Trp pre-exercise all significant correlations of nitrite were negative. The global arginine bioavailability ratio correlated positively with MDA only post-exercise.

Table 4. Spearman correlation coefficients (r) and p values of circulating amino acids with MDA, nitrate and nitrite in the combined groups.

Parameter at the Respective Time	MDA (r, p)		Nitrate (r, p)		Nitrite (r, p)	
Pre-Exercise						
Ala	n.s.	n.s.	0.290	0.013	n.s.	n.s.
Gly	n.s.	n.s.	0.391	0.001	n.s.	n.s.
GAA	−0.247	0.041	n.s.	n.s.	−0.251	0.038
Lys	n.s.	n.s.	n.s.	n.s.	−0.361	0.002
hArg	n.s.	n.s.	n.s.	n.s.	−0.469	<0.001
Trp	n.s.	n.s.	n.s.	n.s.	0.237	0.048
GABR	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Post-exercise						
Gly	n.s.	n.s.	0.495	<0.001	n.s.	n.s.
Met	n.s.	n.s.	0.242	0.040	n.s.	n.s.
Orn+Cit	n.s.	n.s.	0.264	0.025	n.s.	n.s.
Ser	n.s.	n.s.	n.s.	n.s.	−0.274	0.030
Leu+Ile	n.s.	n.s.	n.s.	n.s.	−0.286	0.015
Asp+Asn	n.s.	n.s.	n.s.	n.s.	−0.308	0.008
Lys	n.s.	n.s.	n.s.	n.s.	−0.442	<0.001
Trp	−0.246	0.045	n.s.	n.s.	−0.299	0.014
GABR	0.275	0.029	n.s.	n.s.	n.s.	n.s.

GAA, guanidinoacetate; hArg, homoarginine; GABR, Global Arg bioavailability ratio; n.s., not significant.

3.4. Dietary Intake

In the whole study population, plasma MDA concentration pre-exercise (MDA_{pre}) was positively associated with vitamin E intake ($r = 0.258$, $p = 0.027$). Plasma MDA concentration post-exercise (MDA_{post}) was positively associated with α-linolenic acid intake ($r = 0.271$, $p = 0.020$), as well as with percentage of polyunsaturated fatty acids ($r = 0.258$, $p = 0.027$). Correlations between dietary intake (see Table 2) and plasma concentrations of amino acids pre- and post-exercise are summarized in Table 5. All found correlations were positive. The highest correlations were found for Val and Leu+Ile both pre- and post-exercise.

3.5. Associations with Exercise Capacity

MDA_{post} was positively correlated with maximum blood lactate concentration [Lac_{max}] ($r = 0.245$, $p = 0.040$). A positive correlation was found between the plasma nitrate concentration post-exercise and maximum blood glucose concentration [Glc_{max}] ($r = 0.244$, $p = 0.040$). These positive correlations suggest that lipid peroxidation and NO metabolism are associated with glucose metabolism. The plasma guanidinoacetate concentration pre-exercise was inversely correlated with body weight related maximum power output ($r = -0.261$, $p = 0.030$). Further, guanidinoacetate plasma concentrations pre- and post-exercise were inversely correlated with [Glc_{max}] (pre: $r = -0.304$, $p = 0.012$; post: $r = -0.264$, $p = 0.040$) and with the lean body mass-related maximum power output P_{maxLBM} (pre: $r = -0.321$, $p = 0.007$; post: $r = -0.299$, $p = 0.017$). In contrast to lipid peroxidation and NO metabolism,

guanidinoacetate seems to be utilized for energy generation, presumably serving as creatine precursor in skeletal muscles.

Table 5. Spearman correlation coefficients (*r*) and *p* values of dietary intake and plasma concentrations of amino acids in the whole cohort.

	Pre-Exercise		Post-Exercise	
Amino Acids	*r*	*p*	*r*	*p*
Arg	0.317	0.007	0.282	0.025
Thr	0.256	0.030	0.190	0.109
Val	0.560	<0.001	0.571	<0.001
Leu+Ile	0.459	<0.001	0.421	<0.001
Met	0.157	0.188	0.145	0.224
Phe	0.351	0.003	0.321	0.006
Tyr	0.341	0.003	0.380	0.001
Lys	0.335	0.004	0.342	0.003
Trp	0.129	0.286	0.324	0.007

4. Discussion

4.1. Pre-Exercise Status of Plasma Oxidative Stress, NO Metabolism and Amino Acids Profile

Plant-based nutrition is constantly gaining popularity. Even though more and more athletes pursue vegetarianism or veganism, exercise-induced oxidative stress and metabolism are not well understood. To our knowledge, the sole reported work on oxidative stress among recreational athletes with vegetarian pattern is from Vanacore and colleagues [16]. This group found higher levels of thiobarbituric acid-reactive substances and lower nitrite levels after incubation of untreated and H_2O_2-treated H9c2 cells with serum in vegans, vegetarians and omnivores. Vanacore et al. concluded that restrictive vegan diet had minor antioxidant capacity compared to the other diets. Yet, the major limitation of that study is its in vitro nature. Based on a study on previously untrained and pre-trained healthy young men, Ristow and colleagues concluded that exercise-induced oxidative stress is responsible for health-promoting effects, such as insulin sensitization [7]. Basically based on the concept of mitohormesis it was assumed that supplementation with antioxidants may preclude health-promoting effects of exercise in humans [7]. It could, therefore, be assumed that higher circulating concentrations of MDA, the prominent thiobarbituric acid-reactive substance and measure of lipid peroxidation, in vegans may be associated with health effects and could explain the comparably low prevalence of diabetes in subjects who practice a plant-based diet [11].

The study we report here is the first investigation examining changes in physical exercise-induced oxidative stress measured as plasma MDA, and in NO and amino acids metabolism in three groups of recreational runners, i.e., omnivorous (OMN), lacto-ovo-vegetarian (LOV) and vegan (VEG). The study participants did not differ in age and gender distribution, had closely comparable BMI values and no statistically different training frequency (Table 1). The highest dietary intake of the antioxidant vitamins C and E was recorded in the VEG group (Table 2). The daily dietary intake of α-linolenic acid intake and polyunsaturated fatty acids in terms of percentage energy was numerically, but not statistically significantly higher in the VEG group. Pre-exercise, the subjects of the three groups of our study had closely comparable plasma MDA concentrations. Based on the currently generally accepted assumption that higher circulating MDA concentrations indicate higher oxidative stress, the results of our study suggest that healthy young VEG subjects are not stronger prevented from exercise-induced oxidative stress by the reportedly higher intake of antioxidants.

In our study, the pre-exercise mean plasma nitrate and nitrite concentrations were considerably higher in the subjects of the LOV group (+23%) and of the VEG group (+41%) compared to those of the OMN group. This is most likely due to the higher nitrate content of the vegetarian dietary patterns [33]. Methionine (Met) is considered to possess antioxidative properties, in part due to its

sulphur atom. Reportedly, the subjects of the VEG group had a lower daily dietary intake of Met (Table 2). However, the pre-exercise concentrations of Met measured in the plasma samples of the VEG subjects were closely comparable to those of the OMN and LOV groups (Table 3). Unfortunately, our gas chromatographic-mass spectrometric method for amino acids does not allow measurement in plasma of the Met-relatives, each a thiol group-containing cysteine and glutathione [34]. With respect to the plasma concentrations of the other amino acids and their metabolites, we found rather moderate differences among the three groups pre-exercise (Table 3). The plasma concentrations of Gly, Asp+Asn and Arg were highest in the VEG group, in contrast to the energy-related Leu+Ile, and to Trp, which were the lowest concentrations in the VEG group. The highest plasma levels of Leu+Ile, guanidinoacetate, Glu+Gln, Lys, homoarginine, and Trp were measured in the OMN group. The plasma levels of Asp+Asn, guanidinoacetate, Glu+Gln, Lys, Arg and homoarginine were lower in the LOV group compared to the VEG group, which partly agrees with findings of the EPIC Oxford cohort [25]. For Val, Leu+Ile, Phe, Tyr, and Lys we found correlations to the dietary intake, which is largely consistent with previous findings [25]. Interestingly, the global arginine bioavailability ratio, i.e., the concentration ratio of plasma Arg to the sum of Cit and Orn concentration, did not differ between the groups pre-exercise suggesting no substantial differences in Arg-involving pathways including the urea cycle and the L-Arg/NO pathway.

The subjects of the VEG group had borderline lower plasma creatinine concentrations, presumably due to the creatine-poor or creatine-free vegan diet.

4.2. Exercise-Induced Effects on Oxidative Stress, NO Metabolism and Amino Acids Profile

We observed multiple and complex effects of physical exercise on oxidative stress, NO metabolism and amino acids profiling in the plasma of the subjects of the three groups.

Exercise led to increases in plasma MDA concentration, with the highest percentage increases being seen in the LOV (+24%) and VEG (+15%) groups, suggesting higher exercise-induced elevation of oxidative stress in these groups compared to the OMN group. MDA is produced by free radicals and enzyme-catalyzed lipid peroxidation of PUFAs including arachidonic acid and ALA [1]. Previous results [1] suggest that the higher dietary intake of ALA and PUFAs may have, at least in part, contributed to the higher plasma MDA concentrations measured in the LOV and VEG groups.

Exercise is generally considered to increase NO formation in the vasculature due to shear force-induced elevation of endothelial NO synthase. In our study, physical exercise did not cause statistically significant changes in plasma nitrate and nitrite concentrations, suggesting no appreciable effects of exercise on NO synthesis and metabolism. The lack of appreciable changes in plasma nitrite concentrations in the three groups suggests that the exercise (incremental stress test on a bicycle ergometer) did not alter the metabolism (e.g., reduction of nitrate to nitrite) or excretion/reabsorption of nitrite. The latter is supported by the lack of changes in plasma creatinine concentrations in all groups, suggesting unaffected renal function in terms of glomerular filtration rate.

The plasma concentration of the majority of the amino acids decreased upon exercise. This could be explained by catabolic processes such as transamination, oxidation, and gluconeogenesis, rather than due to changes in their renal filtration and reabsorption. It is worth mentioning that in gluconeogenesis up to 19% of energy can be obtained from Ala [21,35]. This concurs with the increase in the plasma concentration of Ala seen in all groups of our study post-exercise. Ala is likely to be provided by other organs and cells via cross-talk [33].

Regarding Pro, significant decreases were observed from pre- to post-exercise in OMN and LOV. These findings are consistent with the literature and may reflect the transformation to glutamate (Glu) and further to glutamine (Gln) [19,21]. Pro provides the highest energy capacity of non-essential amino acids (33 mol ATP per mol Pro). Interestingly, while Sar decreased post-exercise in OMN, an increase in LOV and VEG was observed. Since the latter groups consumed lower Met compared to OMN, these results suggest that LOV and VEG compensated the energy supply via Sar.

Guanidinoacetate is the direct precursor of creatine, which, in turn, is the direct precursor of the energy-related creatine phosphate (i.e., phospho-creatine). Creatine is present in large amounts in meat and fish, while vegetarian and vegan food contain very low amounts of creatine. The plasma concentration of guanidinoacetate did not increase significantly in the LOV (+10%) and VEG (+12%) groups upon exercise, while it decreased non-significantly (−13%) in the OMN group. Although not statistically significant, these changes may suggest that exercise induced consumption of guanidinoacetate in the OMN group to form creatine mainly in the kidney, liver and pancreas, on the one hand, and elevated enzymatic synthesis of guanidinoacetate in the LOV and VEG groups, on the other hand.

In fact, guanidinoacetate is produced from L-arginine and glycine by the catalytic action of L-arginine:glycine amidinotransferase (AGAT), which is abundantly expressed in the kidney [36]. AGAT also catalyses the synthesis of L-homoarginine, the methylene homolog of L-arginine, from L-arginine and L-lysine (Lys) [36]. Exercise induced similar, yet non-significant changes in the plasma concentrations of homoarginine: −11% in the OMN group, +1.2% in the LOV group and +5% in the VEG group. The relatively uniform changes in guanidinoacetate and homoarginine plasma concentrations in all groups may suggest that exercise increased the AGAT activity in the LOV group and more strongly in the VEG group. Presumably, this is required to come up to the higher demand on energy in skeletal muscles of the LOV and VEG runners, who are very likely to intake less creatine by their diets compared to the OMN runners.

4.3. Associations with Exercise Capacity

As previously reported, the cohorts of the present study were adequately supplied with iron, vitamin B_{12} and vitamin D [37], and had comparable maximum power output levels (P_{maxBW}) (OMN: 4.15 ± 0.48 Watt/kg, LOV: 4.20 ± 0.47 Watt/kg, VEG: 4.16 ± 0.55 Watt/kg; $p = 0.917$) as well as lactate concentrations (OMN: 11.3 ± 2.19 mM, LOV: 11.0 ± 2.59 mM, VEG: 11.9 ± 1.98 mM; $p = 0.648$) [32]. The correlations of the post-exercise plasma concentrations of MDA, nitrate and guanidinoacetate with the blood concentrations of lactate and glucose suggest that oxidative stress, NO metabolism and AGAT activity/expression are associated with lactate and glucose metabolism, albeit in opposite direction: positive for MDA and nitrate, and inverse for guanidinoacetate. However, oxidative stress was not associated with exercise capacity in the form of body weight- and lean body mass-related maximum power output, suggesting that high levels of oxidative stress may not affect exercise capacity. But the causality and the effect of higher plasma MDA levels remain to be elucidated. We found a correlation of $nitrate_{post}$ and $[Glc_{max}]$, which may reflect NO-dependent regulation of glucose uptake in skeletal muscle [38]. Thus, local NO increase during exercise might have favorable effects on glucose uptake. Previous studies found positive associations of a high dietary intake of nitrate-rich foods, such as beetroot, and athletic performance [39]. Bacterial nitrate reductase, which is present in mouth and gut flora, converts orally taken inorganic nitrate into nitrite, which in turn can be further converted to NO under certain conditions such as hypoxia. It would be interesting to examine oxygen consumption in future studies. The AGAT-catalysed synthesis of guanidinoacetate is the rate-limiting step in creatine production. The consistently inverse correlation of plasma guanidinoacetate concentration with body weight- and lean body mass-related maximum power output may suggest that the AGAT activity/expression may be a limiting factor in physical exercise.

4.4. Strengths and Limitations of the Study

A strength of our study is the use of fully validated and clinically proven gas chromatographic-mass spectrometric methods for the measurement of MDA, nitrate, nitrite and the majority of amino acids in plasma samples. Potential limitations of our study may be the relatively small number of participants included in the three groups and the methodological inability to discriminate between Orn and Cit, and to measure cysteine and glutathione, two important endogenous antioxidants. Nevertheless, the relatively constancy of the global arginine bioavailability ratio in the groups pre-exercise and

post-exercise suggest that major Arg-involving pathways are not affected by diet and physical exercise. Our study may also be limited by the fact that the analysis of the above mentioned parameters may have been affected by pre-analytical factors. The nutrient intake via a 24 h dietary recall reflects the actual but not necessarily the usual daily consumption; deviations due to subjective estimation of the stated amounts of nutrients are possible. We used nutrition-specific software for calculations which cannot accurately estimate the dietary intake of each of the amino acids examined in plasma.

4.5. Future Research Directions

It would be interesting to investigate the effects of dietary pattern and exercise intensity and endurance on oxidative stress, NO and amino acids metabolism in the general population and in other sport disciplines.

5. Conclusions

Pre-exercise, OMN, LOV and VEG recreational athletes had different states of oxidative stress, when measured as plasma MDA concentration, different pre-exercise plasma amino acid profiles, but comparable NO metabolism. The greatest concentration of plasma guanidinoacetate, the direct precursor of the energy-related creatine, was highest in the OMN athletes. In all groups, physical exercise induced elevation of oxidative stress, but caused different changes in amino acids metabolism. The plasma concentration of Ala, the most important amino acid in gluconeogenesis, increased uniformly in all groups. The changes observed are related in part to glucose metabolism and in part to the different management of creatine homeostasis. Guanidinoacetate is differently utilized by OMN, LOV and VEG.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/11/8/1875/s1, Figure S1: Flow chart of the previous study from which the collected plasma samples were analyzed in the present study for MDA, nitrate, nitrite, creatinine, and the amino acids (AA)., Table S1: Spearman correlation coefficients (r) and p values of the biochemical parameters pre- and post- exercise.

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3. General discussion

The aim of this dissertation thesis was to investigate the nutrient intake and body status, as well as exercise capacity and exercise-induced metabolic adaptations in the form of sirtuin activity, oxidative stress, NO metabolism, and amino acid profile of German recreational athletes with vegetarian diets in comparison to omnivores. A cross-sectional study was carried out with 81 recreational runners (18 – 35 years), who practiced an omnivorous (OMN), lacto-ovo vegetarian (LOV) or vegan (VEG) diet. The examination included both the examination of nutrient intake and biochemical status as well as the examination of performance-related and exercise-induced parameters. The results of the dissertation thesis are presented in 5 scientific publications (chapter 2).

3.1. Nutrient intake and nutritional status of recreational athletes with vegetarian diets compared to omnivores

Results of ***Paper I*** and ***II*** demonstrate that LOV and VEG, as well as OMN recreational runners, reached the recommendations of the D-A-C-H for most nutrients, which were partly reflected by the related biomarkers.

The assessment of the results was hampered by the absence of national recommendations for recreational athletes, on the one hand, and for vegetarians and vegans on the other hand. The existing guidelines of the ACSM, the IOC and the ISSN mainly focus on high-performance athletes [81,93,95] and were therefore only to some extent applicable to the present collective. As a consequence, the nutrient intake was compared to the recommendations of the D-A-C-H for healthy adults, which, however, do not include recommendations for recreational athletes [33].

Overall there is large consensus that a mixed diet can provide the requirements for athletes and that vegetarian athletes are also able to meet their dietary needs [11–14]. Interestingly, Turner McGrievy and colleagues found higher diet quality scores even in vegan and vegetarian runners compared to omnivores (n=422) [211]. So far, there are only a few studies on nutrient intake of vegetarian athletes [18,20], whereas data on vegan athletes are missing. Therefore, it is rather unknown whether recreational athletes practicing veganism meet their requirements.

Energy intake

Considering energy intake, more than 50% of the present study collective had energy intakes below the recommendations, which often occurs in endurance sports [213]. All three groups had comparable energy intake, which aligns with the results of previous studies comparing vegetarian and omnivorous endurance athletes [18,20].

Macronutrients

Carbohydrate requirements are dependent on various factors such as type and intensity of sports and vary from 3-7 g/kg BW [214]. In the present study, VEG had highest carbohydrate intake (5.01, 4.40-5.62 g/kg BW) compared to OMN (4.31, 3.45-5.17 g/kg BW) and LOV (4.22, 3.52-4.91 g/kg BW), which is in accordance with previous findings in non-athletes [38,42–44], while studies dealing with vegetarian athletes found various amounts of carbohydrate intake [18,20]. The results reflect a higher intake of potatoes and fruits in VEG, but no significant differences in whole grain and cereal products, pastries and sweets intake between the groups.

Further, the protein intake of the three study groups (OMN: 1.50, 1.27-1.66; LOV: 1.34, 1.09-1.56; VEG: 1.25; 1.07-1.42 g/kg BW) reached the reference range of the sports societies (1.2-2.0 g/kg BW [81,93,95]), which is higher than recommended by the D-A-C-H (0.8 g/kg BW) [33]. Moreover, the amino acid intake of all three groups was within the recommendations of the World Health Organization (WHO) [215]. The analysis of the recorded food groups showed that meat, meat products, and sausages, as well as fish and dairy products, were main protein sources for OMN, while milk, dairy products, and eggs were main sources for LOV and cereal products as well as soy for VEG. The findings are in accordance with the literature since previous studies also found that non-sportive lacto-ovo vegetarians and vegans [44,216] and vegetarian endurance athletes generally meet the recommended values [18].

In contrast to carbohydrate and protein intake, the professional societies defined diverse recommendations for fat intake in a margin of 15 – 35 EN% [33,81,93,105]. Mean fat intake of the present study collective was between 26 EN% (VEG) and 32 EN% (OMN) and therefore within the recommendations of the D-A-CH, ISSN, and ACSM and comparable to findings from Lynch and colleagues [18,33,81,93]. In contrast, the LA:ALA ratio of VEG (1:5.71) and LOV (1:5.30) was within the recommended ratio [33]. OMN had a higher LA:ALA ratio (1:8.04), which is in agreement with findings for the general population in Germany [217]. Further, the sum of EPA and DHA was below the recommendations of the International Society for the Study of Fatty Acids and Lipids (0.5 g) for LOV (0.08, 0.04-0.12 g) and VEG (0.09, 0.01-0.17 g), while OMN achieved the recommendations (0.54, 0.23-0.85 g) [218]. In comparison, the intake of men and women in the National Nutrition Survey II (0.16 g) was between the groups [219].

Micronutrients

The professional societies assume that athletes practicing a balanced omnivorous diet are adequately supplied with micronutrients [81,93,105]. In addition, due to their health awareness, athletes were shown to have a high dietary supplement intake [220], which was also observed in the present study collective where magnesium, calcium, iron, vitamin D and B_{12} were most frequently consumed. For athletes practicing a vegetarian diet, the ACSM and IOC named

zinc, iron, riboflavin, cobalamin and vitamin D as critical micronutrients [81,95] and additionally calcium, pyridoxine, and folate, which are mentioned by the ACSM [81].

Dietary intake and biomarkers of vitamin B_{12}

In the present study, the **vitamin B_{12}** intake and status of the VEG group was dependent on supplement intake (***Paper I*** and ***II***). Since about 50% of the VEG group consumed vitamin B_{12} supplements, the average dietary intake was higher (207, 102-313 µg) compared to the other groups (OMN: 4.97, 3.70-6.25 µg; LOV: 2.96, 1.69-4.24 µg). Therefore, the recommended intake of 4 µg/day [33] was on average achieved by OMN and VEG, while those vegan subjects who did not take supplements, had a marginal intake. Considering the LOV group, vitamin B_{12} intake was inadequate and independent of supplementation. In addition, about 30% of the OMN group had insufficient vitamin B_{12} intake although they consumed B_{12} containing foods such as meat, meat products, and fish. Lynch and colleagues found an adequate vitamin B_{12} intake of vegetarian athletes [18], which did not include dietary supplementation.

Although dietary intake of vitamin B_{12} differed between the groups, the respective biomarkers showed an adequate supply of all three groups. As expected, when comparing non-supplement users (non-SU), VEG had the lowest but still adequate vitamin B_{12} supply, reflected in the 4 markers combined vitamin B-12 indicator (4cB12) [221]. Surprisingly, non-SU of the OMN and LOV group differed only slightly, although LOV consumed less vitamin B_{12} than recommended, vitamin B_{12} status was apparently sufficient, since the duration of the diet had no influence on vitamin B_{12} supply. These findings are in contrast with previous findings, where up to 87% of vegetarians and vegans had insufficient vitamin B_{12} concentration in serum with elevated MMA (32-83%) and decreased holo-TC levels (72-93%) as well [222,223]. However, several studies did not differentiate between SU and non-SU and did not examine the impact of supplementation, although the correlation of vitamin B_{12} supplement intake and serum B_{12} as well as supplementation and holo-TC were previously described in the AHS 2 [224].

Dietary intake and biomarker of vitamin D

Comparable to vitamin B_{12}, vitamin D intake and status were depended on supplementation (***Paper I*** *and* ***II***). Highest total dietary intake was found in VEG (19.9, 2.75-37.0 µg), followed by OMN (8.29, 2.21-14.4 µg) and LOV (4.52, -1.14-10.4 µg). However, due to large differences in supplementation, these average results should be treated cautiously. The dietary intake of vitamin D was not reflected by the 25-hydroxy vitamin D (25(OH)D) concentration in blood. However, 25(OH)D represents both the dietary intake and the endogenous synthesis. All three present study groups showed sufficient blood levels of > 75 nmol/l and comparably low prevalence (20%) of vitamin D inadequacy (< 50 nmol/l). Furthermore, vitamin D deficiency was observed in two subjects of the VEG group. As vitamin D deficiency is a common problem

in the general population [225], the results of the 25(OH)D analysis should be interpreted as exceptional. The adequacy of vitamin D supply might be linked to supplementation, since all subjects consuming vitamin D supplements had 25(OH)D levels > 50 nmol/l and only non-SU had inadequate or deficient 25(OH)D values. These results are in accordance with the results of the AHS 2, where the authors found that vitamin D supply is dependent on supplement intake [226].

Vitamin D supply can be affected by various factors. First, a high endogenous synthesis could be expected since the investigation took place from May to December [227] and as the subjects were between 18 and 35 years old [228]. Additionally, present subjects were recreational runners who usually stay relatively long outside, which could explain high 25(OH)D levels, as an investigation with female runners revealed similar results [229]. Second, the latitude affects endogenous vitamin D synthesis, but present results stand in contrast to a German nationwide study, which found 25(OH)D concentrations of 45.1 and 45.3 nmol/l in males and females, respectively [228]. Further factors, such as current holidays in sunny regions, sun exposure, and sun protection habits [230] were not recorded.

Dietary intake and biomarkers of iron

In contrast to the aforementioned vitamins, iron intake and status were only partially dependent on supplementation (***Paper I*** and ***II***). Highest iron intake was observed in VEG, which is consistent with previous findings [38,231]. Further, about 85% of VEG had a sufficient iron intake, while it was about 50% of OMN and LOV. Beside male subjects of each group, female VEG achieved the reference values solely via food intake (19.8, 15.7-24.0 µg), while female OMN (11.2, 9.01-13.2 µg) and LOV (12.8, 9.47-16.1 µg) were dependent on supplement intake. The dietary iron intake was only partially reflected in iron status. Less than one-third of each group had low ferritin levels (< 15 µg/l) and no subject had iron deficiency anemia. The highest dietary iron intake in female VEG was reflected in high ferritin levels as well (32.1±22.8 µg/l). Indeed, the VEG group exclusively consumed plant iron sources, but LOV and OMN predominantly consumed plant-based iron as well. Plant-based iron has a bioavailability of about 1–5%, while animal-based sources contain about 70% heme iron, which has a bioavailability of about 10-20% [232,233]. The explanation for comparable iron status could be the high intake of iron bioavailability promoting substances such as vitamin C [234]. Further, as VEG and LOV consumed high amounts of legumes (OMN: 3.70±8.08 g, LOV: 27.7±39.7 g, VEG: 66.4±68.1 g) and soy (OMN: 0 g, LOV: 54.4±95 g, VEG: 151±179 g), the intake of phytoferritin, which has a higher bioavailability, in these groups can be assumed [235]. As the iron status was independent of supplementation, the results suggest that both a balanced vegetarian and omnivorous diet can provide adequate amounts of iron. Previous studies reported similar results, as people practicing vegetarian diets had adequate hemoglobin and

serum iron levels [38,40,236]. However, earlier investigations also found low ferritin levels [40,54,59], which are in contrast to the present results. Further, exercise-related increased requirements of iron should be taken into account [237–239].

Further nutrients

The obviously different amounts of folate intake were not reflected by red blood cell (RBC) folate since all three study groups had comparably high RBC folate levels (OMN: 2213±444 nmol/l, LOV: 2236±596 nmol/l, VEG: 2354±639 nmol/l; p=0.577). These findings are to be interpreted as exceptional, since folate deficiency in healthy adults is described < 340 nmol/l and present subjects are far from this cut off [240]. Recent findings of the AHS 2 agree with present results since the authors found RBC folate levels of > 2000 nmol/l in meat-eaters, fish-eaters, lacto-ovo vegetarians and vegans as well [241].

Although the dietary intake of calcium, magnesium, and zinc partly differed between the groups, the respective serum levels were on average adequate and not directly associated with dietary intake due to strict homeostatic regulations [53].

Further data on health status of vegan and vegetarian endurance athletes was provided by the Nutrition and Running High Mileage (NURMI) Study (n=245), determining health-related indicators and health-related behavior [21]. The authors found a lower body weight in vegetarians and vegans compared to omnivores and a lower prevalence of allergies in vegans [21]. The health status was further comparable between males and females [22]. Moreover, Boldt and colleagues investigated the WHO Quality of Life Brief questionnaire in 281 recreational runners and found a comparably high quality of life score in omnivores, lacto-ovo vegetarians, and vegans **[212]**.

3.2. Exercise performance of recreational athletes practicing vegetarian diets in comparison to omnivores

Results of ***Paper III*** demonstrate that LOV and VEG recreational runners of the present study appear to have the same maximum exercise capacity in the form of maximum power output compared to omnivorous counterparts (OMN: 4.15±0.48 Watt/kg BW, LOV: 4.20±0.47 Watt/kg BW, VEG: 4.16±0.55 Watt/kg BW; p=0.917). Additionally, similar submaximal and maximum lactate and glucose concentrations were observed. Although vegetarian diets lead to differences regarding dietary intake of nutrients such as carbohydrate, iron, and vitamin B_{12} (***Paper I***), those differences did not seem to affect exercise capacity. An insufficient supply of vitamin D and iron were shown to decrease physical performance [242,243] and recent evidence suggests that endurance athletes may have an altered vitamin B_{12} metabolism as

well [244]. Since the respective biomarkers of vitamin B_{12}, vitamin D, and iron were on average in the normal range in the present collective (***Paper II***), a comparable exercise capacity could be expected [242,243].

An earlier investigation comparing vegetarian and omnivorous athletes also found similar physical performance [23]. A more recent investigation showed a 13% greater VO_{2max} score in female vegetarians compared to omnivores [18]. However, the findings of the present study are only partly comparable with previous findings, since the authors did not differentiate between vegetarians and vegans and the subjects underwent an exercise test on a treadmill. Moreover, Lynch and colleagues examined only aerobic capacity and exercise tests were not carried out until exhaustion [18]. Further, previous investigations did not examine the anaerobic metabolism in the form of the submaximal and maximum lactate and glucose concentrations [18,23]. Therefore, the present study contributes to the understanding of glucose utilization in recreational athletes with vegetarian diets.

Besides cross-sectional studies, the effect of vegetarian diets on physical performance was investigated in intervention studies as well [200–202]. Those studies reported controversial findings. While both a 5- and 6-week intervention with a lacto-ovo vegetarian diet did not affect aerobic capacity and repeated sprint ability [200,201], a 4-day low-protein vegetarian diet was observed to have a disadvantageous influence on submaximal cycling economy, since submaximal oxygen uptake at 40, 60, and 80% of VO_{2max} increased after the intervention period [202]. However, the authors found no effect on maximum oxygen uptake and did not examine the effect of a lacto-ovo vegetarian diet *per se*, but on restricted protein intake, whereby the evidence of the results is questionable [195].

Besides the typically high intake of carbohydrates, vegetarian diets are also characterized by a high intake of basic substances [216]. This fact let Hietavala and colleagues to suggest that diets rich in plants may have an advantageous effect on physical performance [203]. However, the analysis of previous findings showed no effect of a high intake of basic substances on performance-related parameters [204].

The anaerobic energy supply increases with exercise intensity. In the present collective, VEG had the highest carbohydrate intake compared to LOV and OMN, however submaximal and maximum lactate and glucose concentrations were comparable between all three groups. Consequently, a higher carbohydrate intake, which is characteristic for vegetarian diets, did not appear to affect glucose utilization. Also, there are various factors such as glycogen storage, previous exercises, water balance and caffeine consumption, which influence lactate metabolism [245]. It seems that the individual sex and genetic background, as well as training habits, have a stronger effect on physical performance than the consumption or avoidance of animal products [246,247].

Further, the dietary intake 24 hours before exercise did not affect maximum power output nor glucose or lactate concentrations.

With regard to body composition and BMI, all three groups demonstrated comparability, which is contrary to previous findings, where female omnivorous athletes had a higher body fat mass compared to vegetarians [23].

3.3. Exercise-induced metabolic changes

To investigate metabolic changes, several biomarkers of energy metabolism, oxidative stress, NO and amino acid metabolism were analyzed before and after the exercise test.

Considering **sirtuins** as a reflection of energy supply and antioxidative defense [149,248], gene expression of SIRT 1, 3, 4 and 5 were comparable in all three groups pre-exercise (***Paper IV***). Additionally, no differences were observed at the basal enzyme level. Since comparable data in humans are missing, our results were compared with previous findings in a mouse model, which did not find differences in animals with different diets [249,250].

Increased physical activity requires an increased rate of energy-yielding processes such as glycolysis, the Krebs cycle, and the respiratory chain, for which reason increased sirtuin activity has also been suggested [149,151,251,252]. Since the capacities of SIRT1 and 3 were found to be increased after exercise, activation of those metabolic processes can be assumed [157,160–164,166–168]. This increase was observed in OMN and not significantly in LOV, which is in agreement with a previous animal study that found an increased capacity of SIRT1 after exercise [158,250]. Interestingly, sirtuin capacities in vegans decreased or did not change after exercise. To identify reasons for the group specific differences, the dietary intake data of the last 24 hours before exercise test, fasting blood levels and exercise-related parameters (***Paper II*** and ***III***) were correlated with sirtuin capacity and revealed an inverse correlation between sirtuin activity (SIRT1, 3 and 5) and tocopherol and ascorbate (SIRT1). There is evidence that exercise-induced ROS levels act as effectors on sirtuin activity in response to high levels of antioxidants [253].

Nevertheless, since sirtuin capacities were measured in blood, no direct associations can be made to skeletal and muscle content. Additionally, enzyme capacities were analyzed *in vitro* under substrate saturation and might therefore not directly reflect *in vivo* capacities. It would be highly interesting to analyze sirtuin activity in tissue, but as exercise induces metabolic stress in several organs, a multiple organ biopsy would be necessary, which is ethically unacceptable. However, this diverse response to exercise seems not to affect physical

performance (***Paper III***). As SIRT5 regulates the urea cycle, the slightly lower dietary protein intake, which was found in the 24 hours dietary recall, could be reasonable in LOV [254]. But, since the sirtuin metabolism in humans has hardly been studied so far, the significance of present results needs to be explored in future studies.

Although sirtuin capacities are associated with cellular response to stress [129], inverse correlations were found between dietary antioxidant intake of ascorbate and tocopherol. However, a few studies suggested that increased levels of ROS reflect sirtuin activity in response to high levels of antioxidants [253].

Interestingly, **MDA** concentrations pre-exercise were highest in VEG and exercise-induced changes of MDA were highest in LOV and VEG, suggesting that the higher intake of antioxidants in the VEG group had no additional effect on oxidative stress (***Paper V***). Further, the dietary PUFA and especially ALA intake was associated with MDA, which reflects a higher rate of lipid peroxidation [177]. Only one study exists by Vanacore and colleagues, which treated H9c2 and H-H9c2 cells (cardiomyoblast cell line) with serum of vegan, vegetarian and omnivorous recreational athletes [255]. They found higher concentrations of thiobarbituric acid reactive substances and decreased levels of nitrite and concluded that a restrictive vegan diet had minor antioxidant capacity compared to the other groups, which is doubtful since vegans are characterized by a high intake of antioxidants [255]. However, since the investigation was *in vitro*, the results should be treated cautiously. Further, since oxidative stress is responsible for many health-promoting effects such as insulin sensitizing [174], higher oxidative stress in LOV and VEG may explain the diabetes-preventive effect of vegetarian diets [61]. MDA concentrations post-exercise were associated with maximum lactate and glucose concentrations, but not with maximum power output, suggesting that higher levels of oxidative stress did not affect exercise capacity. Nevertheless, future studies should examine further markers of oxidative stress or antioxidative response (e.g. super oxide dismutase, gluthatione, gluthatione peroxidase) in endurance athletes with vegetarian diets in order to gain clarification of its significance [186].

Since plant foods contain high amounts of nitrate [256], the higher plasma nitrate and nitrite concentrations in LOV and VEG compared to OMN pre-exercise could be expected. Contrary to the expectations, no significant exercise-induced changes of nitrate and nitrite concentrations could be observed, suggesting no exercise-induced increase in **NO** synthesis [257,258]. Plasma concentrations of nitrate were positively associated with maximum lactate and glucose concentrations, indicating the NO-dependent glucose uptake in the skeletal muscle [259,260].

Furthermore, the study demonstrated an exercise-induced change in the **amino acid profile**. Although the present VEG group consumed lower amounts of methionine (results of the 24 h dietary recall), plasma concentrations were comparable between the three groups. Additionally, even the amino acid intake was within the WHO recommendations for all groups [215], both pre-exercise plasma concentrations and exercise-induced changes partly differed between the groups. Interestingly, the LOV group had lower plasma levels of Asp+Asn, GAA, Glu+Gln, Lys, Arg, and hArg pre-exercise compared to the VEG group, which partly agrees with findings of the EPIC-Oxford cohort [261]. Further agreements with the literature were found considering the correlation of plasma concentrations and dietary intake of Val, Leu+Ile, Phe, Tyr, and Lys [23]. The nutritional software limited the analysis, since it was not possible to detect the whole amino acid spectrum from the dietary intake.

Overall, amino acid concentrations decreased post-exercise, which could be explained by exercise-induced catabolic processes such as transamination, oxidation, and gluconeogenesis [116,262,263]. Interestingly, the low dietary intake of methionine in LOV and VEG seem to be compensated by utilization of sarcosine, since sarcosine plasma concentrations decreased in OMN, but increased in LOV and VEG. Further, while plasma guanidinoacetate (GAA) levels in OMN decreased post-exercise, an increase was observed in LOV and VEG. This may be explained because creatine as a product of GAA occurs in animal products such as meat and fish [205]. It is assumed that OMN utilizes GAA during exercise, while creatine-poor diets may result in increased synthesis via L-arginine:glycine amidinotransferase [264] to ensure energy supply. Additionally, GAA was inversely correlated with lactate and glucose levels as well as maximum power output, suggesting a higher GAA utilization leads to higher exercise capacity [265].

4. General conclusion and perspectives

This thesis contributes to a better understanding of nutrient supply, exercise capacity and exercise-induced metabolic adaptations in vegan, lacto-ovo vegetarian and omnivorous recreational athletes. It was demonstrated that all three groups were adequately supplied with most nutrients, had comparable exercise capacity and partly differed in terms of oxidative stress, nitric oxide metabolism and amino acid profile. The results indicate that a lacto-ovo vegetarian and vegan diet are suitable alternatives for recreational athletes.

It is important to point out that the present study collective was highly health compliant, which was expressed in the high level of supplement intake and the targeted choice of food. This, in turn, was reflected by the total intake of certain nutrients and status of biomarkers in the blood, where it was shown that vitamin B_{12} and vitamin D supply were dependent on supplementation. Further, the data indicates that a balanced vegetarian diet, based on a broad variety of foods, can meet iron requirements in recreational athletes without supplementation.

Since the collective had comparable age, BMI, training habits and showed an adequate supply, a comparable exercise capacity could be explained. Interestingly, although the carbohydrate intake of vegans was higher compared to the other groups, lactate and glucose concentrations were comparable at submaximal and maximum levels of exercise.

The investigation of exercise-induced metabolic adaptations suggests a diverse regulation of energy metabolism in athletes with vegetarian diets compared to omnivores. It was demonstrated that basal sirtuin capacities, as regulatory substances for energy metabolism and anti-oxidative response, did not differ pre-exercise. However, changes were observed post-exercise, since an up-regulation of SIRT1 and 3 were demonstrated in omnivores to a larger extent than lacto-ovo vegetarians, while the enzyme capacities were decreased in vegans. A down-regulation of SIRT1, 3 and 4 may consequently result in reduced energy metabolism, especially the mitochondrial respiratory chain, and anti-oxidative response. However, malondialdehyde as biomarker for oxidative stress was highest in vegans at baseline and increased significantly post-exercise in vegetarians and vegans. Also considering amino acid and NO metabolism, vegetarians and especially vegans appear to have different regulatory mechanisms, which in part were related to glucose metabolism.

Although the present investigation provided first data on a differentiated consideration of omnivorous, lacto-ovo vegetarian and vegan recreational athletes, the present collective may not be representative and the results should be considered cautiously. The study was limited by the sample size and only included an age-specific group (18-35 y). Also, an interesting future approach would be to examine the aerobic capacity (VO_{2max}) to generate data on the aerobic metabolism of vegetarian/vegan athletes. Additionally, further studies should clarify

the significance of those metabolic adaptations, since sirtuins may offer a new approach for the understanding of the energy metabolism. The examination of various types and intensities of sports would contribute to a better understanding of the role of vegetarian diets in sports. Further, there is great necessity to conduct long-term intervention studies to examine the effects of vegetarian diets on biochemical parameters of health as well as performance-related ones. Overall, future studies should contribute to the current state of knowledge by defining specific dietary intake recommendations for recreational athletes and athletes with vegetarian diets, which currently are missing.

5. References

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Appendix Paper I

Additional file 1 Dietary intake of essential amino acids (mg/kg BW) according to dietary pattern.

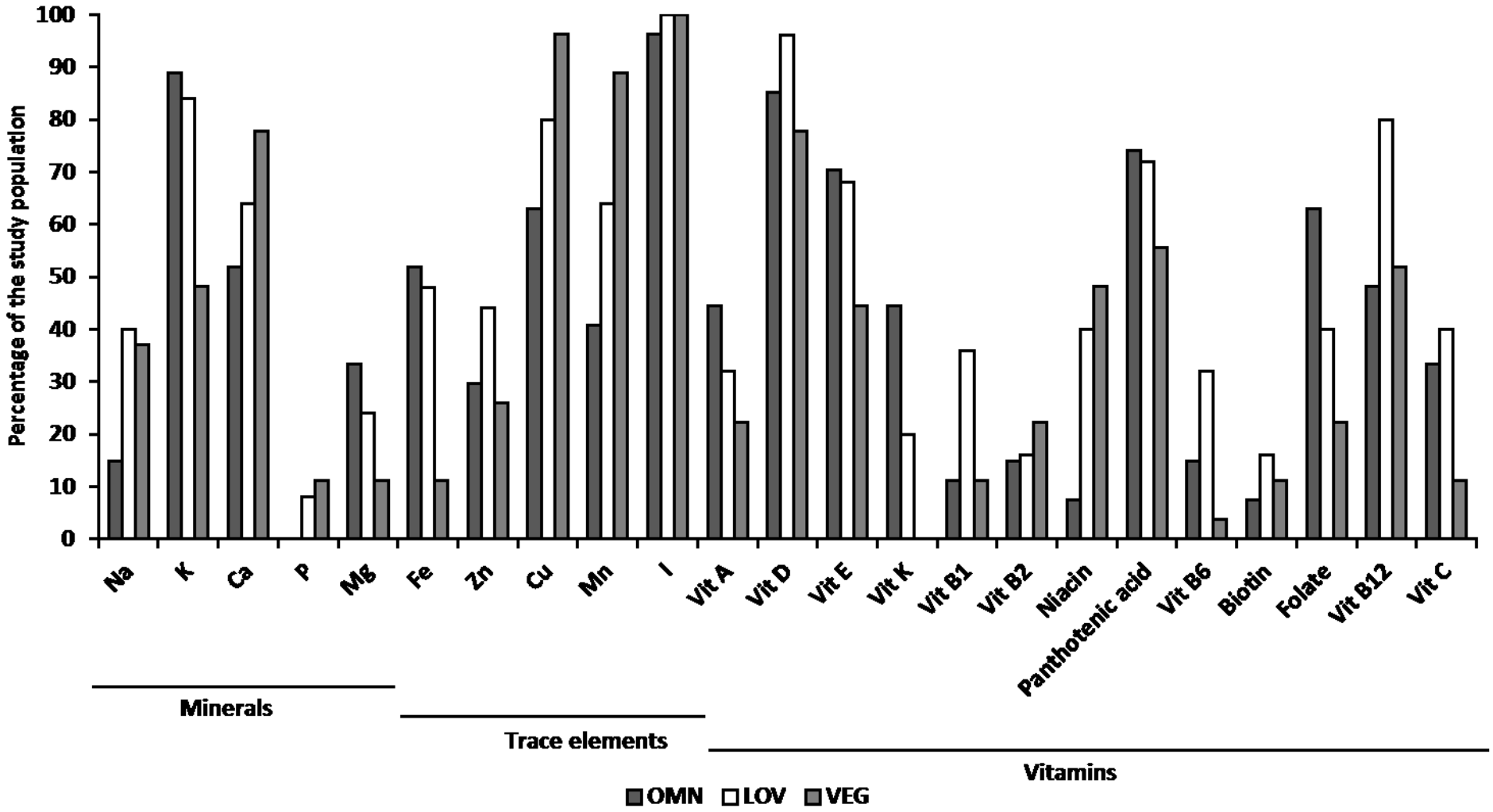
Amino acid	OMN (n=27)	P value OMN-LOV	LOV (n=26)	P value LOV-VEG	VEG (n=28)	P value OMN-VEG	P value 3 groups	Reference values (m/f)*
Isoleucine	54.9 (48.1, 61.6)	n.s.	50.0 (32.7, 67,2)	n.s.	41.0 (32.4, 50.0)	0.008	**0.007**[a]	20
Leucine	92.0 (80.8, 103)	0.047	83.2 (55.1, 111)	n.s.	68.9 (54.8, 83.0)	0.012	**0.008**[a]	39
Lysine	75.8 (64.7, 86.9)	0.007	58.8 (36.7, 80.8)	n.s.	44.1 (35.4, 52.9)	0.000	**0.000**[a]	30
Methionine	25.8 (21.9, 29.6)	0.015	20.5 (12.6, 28.4)	n.s.	15.2 (11.6, 18.8)	0.000	**0.000**[a]	10
Phenylalanine	52.8 (47.2, 58.5)	n.s.	48.6 (33.6, 63.7)	n.s.	44.4 (34.6, 54.2)	0.044	**0.022**[a]	25
Threonine	46.2 (40.2, 52.1)	0.020	38.7 (26.0, 51.4)	n.s.	34.2 (27.6, 40.9)	0.015	**0.006**[a]	15
Tryptophan	12.8 (11.2, 14.4)	n.s.	11.5 (7.90, 15.2)	n.s.	10.9 (8.70, 13.1)	n.s.	**0.037**[a]	4
Valine	64.2 (56.6, 71.7)	n.s.	58.0 (39.2, 76.8)	n.s.	48.7 (39.0, 58.5)	0.012	**0.009**[a]	26
Histidine	31.7 (27.8, 35.6)	0.011	26.0 (17.5, 34.5)	n.s.	22.9 (18.6, 27.2)	0.009	**0.003**[a]	10

OMN = omnivores, LOV = lacto-ovo-vegetarians, VEG = vegans, * reference values of the World Health Organization [215].
Data are presented as mean (95% KI). [a] Kruskal Wallis test, [b] Post Hoc Test.

Additional file 2 Dietary intake of fatty acids according to dietary pattern.

Fatty acid		OMN (n=27)	P value OMN-LOV	LOV (n=26)	P value LOV-VEG	VEG (n=28)	P value OMN-VEG	P value 3 groups	Reference values (m/f)*
SFA (EN%)		8.70 (7.13, 10.3)	n.s.	7.86 (6.17, 9.55)	0.006[b]	4.57 (3.55, 5.59)	0.000[b]	**0.000[a]**	7-10
MUFA (EN%)		5.95 (4.86, 7.03)	n.s.	5.45 (3.77, 7.13)	n.s.	3.96 (3.02, 4.91)	0.019[b]	**0.024[a]**	> 10
PUFA (EN%)		2.81 (2.29, 3.32)	-	3.21 (2.14, 2.97)	-	3.39 (2.63, 4.14)	-	0.513[a]	7-10
EPA (g)	food	0.19 (0.32, 0.35)	0.000[b]	0.01 (0.00, 0.01)	n.s.	0.00 (0.00, 0.00)	0.000[b]	**0.000[a]**	-
	supplement	0.08 (-0.03, 0.19)	-	0.01 (-0.01, 0.03)	-	0.04 (-0.03, 0.10)	-	0.823[a]	
DHA (g)	food	0.25 (0.14, 0.35)	0.031[b]	0.06 (0.03, 0.87)	n.s.	0.03 (0.01, 0,06)	0.000[b]	**0.000[a]**	-
	supplement	0.03 (-0.02, 0.08)	-	0.01 (-0.01, 0.02)	-	0.01 (-0.01, 0.04)	-	0.821[a]	
LA (EN%)		2.96 (2.50, 3.42)	n.s.	3.52 (2.57, 4.46)	n.s.	4.33 (3.44, 5.21)	n.s.	**0.049[a]**	2.5
ALA (EN%)		0.37 (0.27, 0.48)	n.s.	0.68 (0.33, 1.03)	n.s.	0.80 (0.55, 1.05)	0.005[b]	**0.007[a]**	0.5
LA:ALA ratio		1:8.04	-	1:5.30	-	1:5.71	-	0.481[a]	1:5

OMN = omnivores, LOV = lacto-ovo-vegetarians, VEG = vegans, SFA = saturated fatty acids, PUFA = polyunsaturated fatty acids, MUFA = monounsaturated fatty acids, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, ALA= alpha linolenic acid, LA= linoleic acid, EN% = energy percent, n.s. = not significant, * reference values of the German, Austrian and Swiss Nutrition Societies (Deutsche, Österreichische und Schweizerische Gesellschaften für Ernährung, D-A-C-H) [33].
Data are presented as mean (95% KI). [a] Kruskal Wallis test, [b] Post Hoc Test.



Additional file 3 Proportion of participants who did not reach the recommended dietary intake of minerals and vitamins. Dietary intake is depicted in addition to supplement intake. OMN = omnivores, LOV = lacto-ovo-vegetarians, VEG = vegans, Vit = vitamin.

Appendix Paper II

Table S1. Biomarkers of iron status and hematological parameters according to gender.

Biomarker		**Omnivores** n=27	**p value Omnivores *vs.* Lacto-Ovo**	**Lacto-ovo** n=26	**p value Lacto-Ovo *vs.* Vegan**	**Vegan** n=28	**p value Omnivores *vs.* Vegan**	**p value**
Vitamin B_{12}, pmol/l	f	302±116	-	345±134	-	324±301	-	0.273[b]
	m	353±127	-	281±188	-	311±145	-	0.317[b]
Deficient (< 150 pmol/l), n (%)	f	1 (4)		0 (0)		2 (7)		
	m	0 (0)		2 (8)		1 (4)		
Holo-TC, pmol/l	f	80.8±32.3	n.s.	79.8±28.0	0.042[c]	67.9±39.6	n.s.	0.047[a]
	m	79.8±28.0	-	68.1±34.4	-	67.7±41.1	-	0.662[a]
Deficient (< 35 pmol/l), n (%)	f	1 (4)		1 (4)		4 (14)		
	m	0 (0)		1 (4)		2 (7)		
MMA, nmol/l	f	270±181	-	253±171	-	448±703	-	0.687[b]
	m	253±171	-	331±227	-	209±73	-	0.062[b]
Deficient (> 271 nmol/l), n (%)	f	3 (11)		2 (8)		5 (18)		
	m	2 (7)		4 (15)		2 (7)		
tHcy, µmol/l	f	11.5±3.37	-	13.2±1.85	-	12.0±3.52	-	0.514[b]
	m	13.2±1.85	-	16.5±8.37	-	14.4±5.20	-	0.462[b]
> 10 µmol/l, n (%)	f	1 (4)		3 (12)		3 (11)		
	m	2 (7)		6 (23)		3 (11)		
4cB12	f	0.90	-	1.15	-	0.66	-	0.148[a]
	m	0.92	-	0.53	-	0.77	-	0.359[a]

f = female, m = male, Holo-TC = holotranscobalamin, MMA = methylmalonic acid, 4cB12 = 4 markers combined vitamin B-12 indicator [266], n.s. = not significant, tHcy = total homocysteine. Values are given as means ± SD or n (%) of the study population in the different cut-off values. [a] One-way ANOVA, [b] Kruskal Wallis test, [c] Post Hoc test.

Table S2. Biomarkers of vitamin D status according to gender.

Biomarker		**Omnivores** n=27	**Lacto-ovo** n=26	**Vegan** n=28	**p value**
25(OH)D, nmol/l	f	98.2±30.9	85.0±39.5	87.4±40.0	
	m	79.5±31.9	65.5±21.4	88.0±41.0	0.516[a]
Optimal	f	12 (44)	8 (31)	9 (32)	0.308[a]
(≥75 nmol), n (%)	m	6 (22)	3 (12)	7 (25)	
Sufficiency	f	4 (15)	4 (15)	5 (18)	
(50-74.9 nmol/l), n (%)	m	2 (7)	5 (19)	0	
Insufficiency	f	0	3 (12)	2 (7)	
(25-49.9 nmol/l), n (%)	m	3 (11)	2 (8)	2 (7)	
Deficiency	f	0	0	1 (4)	
(<25 nmol/l), n (%)	m	0	0	1 (4)	

f = female, m = male, 25(OH)D = 25-hydroxyvitamin D. Values are given as means ± SD or n (%) of the study population in the different cut-off values. [a] One-way ANOVA.

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Table S3. Biomarkers of iron status and hematological parameters according to supplement intake.

Biomarker		**Omnivores** n=27	**Lacto-ovo** n=26	**Vegan** n=28	**p value**
Iron serum, µmol/l	SU	19.4±4.74	12.6±3.58	13.1±4.99	0.277[a]
	non-SU	17.5±8.43	18.7±7.91	17.5±6.38	0.839[a]
Deficiency (<10 µmol/l), n (%)	SU	0 (0)	1 (4)	1 (4)	
	non-SU	7 (26)	4 (15)	1 (4)	
Ferritin, µg/l	SU	59.0±24.0	32.7±19.5	40.8±22.4	0.408[b]
	non-SU	62.6±57.5	40.4±34.7	45.7±33.7	0.706[b]
Depleted iron stores (< 15 µg/l), n (%)	SU	0 (0)	0 (0)	1 (4)	
	non-SU	7 (26)	6 (23)	3 (11)	
Transferrin, µmol/l	SU	36.4±1.78	44.8±4.04	36.4±1.78	0.061[b]
	non-SU	42.1±12.2	39.7±6.88	40.4±7.53	0.957[b]
Increased iron requirement (≥ 47.7 µmol/l), n (%)	SU	0 (0)	1 (4)	0 (0)	
	non-SU	6 (22)	3 (12)	3 (11)	
Transferrin saturation	SU	26.5±4.95	14.0±4.36	17.8±6.65	0.129[a]
	non-SU	23.2±14.3	24.4±11.1	23.1±10.4	0.913[a]
Insufficient iron supply (< 16%), n (%)	SU	0 (0)	1 (4)	2 (7)	
	non-SU	10 (37)	5 (19)	7 (25)	
Hb, g/dl	SU	13.9±1.48	14.9±0.76	13.5±0.59	0.166[a]
	non-SU	13.9±1.42	14.0±1.04	14.1±1.47	0.825[a]
Anemia (< 12.0/13.0 g/dl), n (%)	SU	3 (11)	0 (0)	4 (14)	
	non-SU	0 (0)	1 (4)	0	
Hct, l/l	SU	0.41±0.04	0.45±0.02	0.40±0.02	0.087[a]
	non-SU	0.41±0.04	0.41±0.03	0.43±0.04	0.263[a]
< 0.36 (f)/0.39 (m), n (%)		0 (0)	0 (0)	0 (0)	
MCV, fl	SU	87.9±4.03	91.0±2.80	88.0±4.00	0.543[a]
	non-SU	87.4±3.79	88.8±4.59	89.0±3.91	0.365[a]
Iron deficiency anemia (< 80 fl), n (%)		0 (0)	0 (0)	0 (0)	

SU = supplement-users, non-SU = non-supplement users, MCV = Mean Corpuscular Volume. Values are given as means ± SD or n (%) of the population in the different cut-off values. [a] One-way ANOVA, [b] Kruskal Wallis test.

Appendix Paper IV

Table S1. Sequences of qRT-PCR-Primers

human SUPT20H (forward)	AAC TTT TGC TTG AGA GCC AGC
human SUPT20H (reverse)	TTG CTG CCG ATT CAG AGA GG
human SIRT1 (forward)	CAA CTT GTA CGA CGA AGA C
human SIRT1 (reverse)	TCA TCA CCG AAC AGA AGG
human SIRT3 (forward)	CAG TCT GCC AAA GAC CCT TC
human SIRT3 (reverse)	AAA TCA ACC ACA TGC AGC AA
human SIRT4 (forward)	GCT GTG AGA GAA TGA AGA TGA GC
human SIRT4 (reverse)	CTT GGA AAG GGT GAT GAA GCG
human SIRT5 (forward)	AGT GGT GTT CCG ACC TTC AG
human SIRT5 (reverse)	CAT CGA TGT TCT GGG TGA TG

Table S2. Correlations of SIRT-activities with different parameters.

Sirtuin	Correlation with	p-value
SIRT1	coffee	0,238
	flavonoids	0,352
	polyphenols	0,324
	Vitamine B_{12} in serum	0,411
	Active vitamine B_{12}	0,433
	Glucose	0,416
	Insulin	0,305
	Calories	0,603
	W/kg body weight	0,979
SIRT3	coffee	0,190
	flavonoids	0,196
	polyphenols	0,146
	Vitamine B_{12}	0,349
	Active vitamine B_{12}	0,140
	Glucose	0,377
	Insulin	0,090
	Calories	0,897
	W/kg body weight	0,627
SIRT5	coffee	0,388
	flavonoids	0,067
	polyphenols	0,382
	Vitamine B_{12}	0,214
	Active vitamine B_{12}	0,075
	Glucose	0,274
	Insulin	0,111
	Calories	0,704
	W/kg body weight	0,623

Statistical analysis were conducted with Spearman correlation test.

Appendix Paper V

Table S1 Spearman correlation coefficients (*r*) and *p* values of biochemical parameters pre and post exercise.

Parameters	*r*	*p*
Oxidative stress/ NO metabolism		
MDA	0.360	**0.002**
Nitrate	0.708	**< 0.001**
Nitrite	0.444	**< 0.001**
Kidney function		
Creatinine	0.167	0.157
Amino acids		
Ala	0.802	**< 0.001**
Thr	0.797	**< 0.001**
Gly	0.861	**< 0.001**
Val	0.889	**< 0.001**
Ser	0.202	0.116
Sar	0.813	**< 0.001**
Leu+Ile	0.785	**< 0.001**
GAA	0.528	**< 0.001**
Asp+Asn	0.750	**< 0.001**
Pro	0.912	**< 0.001**
Met	0.682	**< 0.001**
Glu+Gln	0.732	**< 0.001**
Orn+Cit	0.818	**< 0.001**
Phe	0.811	**< 0.001**
Tyr	0.881	**< 0.001**
Lys	0.865	**< 0.001**
Arg	0.756	**< 0.001**
hArg	0.835	**< 0.001**
Trp	0.657	**< 0.001**

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Appendix Curriculum vitae

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