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„Physical Activity, Exercise and the Microbiome:
A Systematic Review“

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ABSTRACT

Zusammenfassung

Der Darm und Darmprobleme waren für eine lange Zeit ein Tabuthema. Die Forschung hat erst kürzlich begonnen die Bedeutung der Billionen von im Darm lebenden Mikroben und ihre Wichtigkeit für die menschliche Gesundheit zu verstehen. Das Darmmikrobiom unterscheidet sich zwischen Individuen und wird unter anderem von Umwelt- und Verhaltensfaktoren wie der Ernährungsweise und durch die Einnahme von Medikamenten (Antibiotika) beeinflusst. Im Allgemeinen ist Körperliche Aktivität für seine gesundheitsfördernden Wirkungen bekannt. Es gibt Hinweise, dass Bewegung die Mikroken Vielfalt und Zusammensetzung verändern kann. Das Ziel dieser systematischen Übersichtsarbeit ist es, die Auswirkung von akuten und chronischen Trainingsprogrammen auf die Darmmikroken zu untersuchen.

Methode

Die systematische Übersichtsarbeit wurde mittels der Richtlinien des PRISMA Statements durchgeführt. Die Literaturrecherche wurde am 25. Juni 2020 über die Datenbanken Scopus, Web of Science und PubMed durchgeführt. Schließlich erfüllten 18 Studien die Einschlusskriterien und wurden in die Auswertung mitaufgenommen.

Ergebnisse

In den ausgewählten Studien konnten folgende drei Hauptgruppen identifiziert werden: Personengruppe mit „gesunder Lebensstil“, Personengruppe mit „ungesunder Lebensstil“ (sitzend, fettleibig) und Patienten. Generell waren die Stichproben und Interventionen geprägt von hoher Heterogenität. Ergebnisse in der Gruppe für gesunden Lebensstil zeigte, dass die Alpha-Diversität vom Trainingsvolumen abzuhängen scheint. Hingegen bei Probanden mit ungesundem Lebensstil wurde ein Zusammenhang zwischen Beta-Diversität mit dem BMI gefunden. Aufgrund der verschiedenen Krankheitsbildern, wurde keine Auswertung in der Patientengruppe vorgenommen. Die folgenden vier Darmbakterien scheinen durch Training beeinflusst zu werden: *Bacteroidetes*, *Coprococcus*, *Lachnospiraceae* und *Veillonella*.

Fazit

Diese Arbeit zeigt, dass Bewegung das Darmmikrobiom beeinflussen kann. Unklar bleibt, welcher Trainingsmodus und welche Intensität am besten geeignet ist, um die Darmvielfalt zu modulieren. Intensivere Forschung am Menschen ist erforderlich, um die Auswirkungen von akutem und chronischem Training auf die Darmgesundheit zu verstehen.

Background

For a long time, the gut was considered rather taboo. Only recently research started to understand the importance of the trillions of microbes living in the intestine for human health. The gut microbiome differs between individuals and is influenced by environmental and behavioural factors such as diet and drug (antibiotics) intake. Physical activity is generally known for its health-promoting effects, but evidence exists that exercise can alter gut diversity and taxonomy. The aim of this systematic review was to examine the effect of acute and chronic exercise regimes on the gut microbiome.

Methods

The systematic review was conducted and reported based on the PRISMA statement for systematic reviews and meta-analyses. A literature search was performed across the databases Scopus, Web of Science and PubMed on 25th of June 2020. Finally, 18 studies met the inclusion criteria and were included in the systematic review.

Results

Across these studies three major groups could be identified: “healthy lifestyle” group, “unhealthy lifestyle” group (sedentary, obese) and patients. Within groups, study characteristics, sample characteristics and interventions were of large heterogeneity. In athletes, a subgroup of the healthy lifestyle group, alpha diversity seems to depend on training volume. In overweight, obese and or sedentary subjects, beta-diversity was connected to BMI. Due to the diverse illnesses observed in the patient group, no specific pattern could be identified, but gut taxonomy changes were reported in all studies. Four bacteria were found to be associated with exercising: *Bacteroidetes*, *Coprococcus*, *Lachnospiraceae*, and *Veillonella*.

Conclusions

This review showed that exercise can affect the gut microbiome, but it stays unclear which exercise mode and intensity would be most suitable to modulate gut diversity, gut taxonomy or specific species of bacteria. Further research in humans are needed to understand the effects of acute and chronic exercise on gut health.

Keywords: Exercise, Physical activity, Microbiome, Gut Health, Systematic Review

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Ethics and Dissemination

This systematic review does not collect any deeply personal, sensitive or confidential information and used publicly accessible documents as evidence. Therefore, no ethic approval was required.

This thesis has been conducted within the joint programme 'European Master of Science in Health and Physical Activity' following the specific rules of the University of Vienna as well as those of the coordinating institution, the University of Rome 'Foro Italico', and the other partners (Norwegian School of Sport Sciences, University of Southern Denmark, German Sport University Cologne) as outlined in the multi- and bilateral agreements between the universities

1 INTRODUCTION

Nearly 2500 years ago the ancient Greek physician Hippocrates quoted: „All disease begins in the gut“. Even though this concept is known for many decades, research is in a perimammary state. On the one hand, the gut and gut diseases were considered rather taboo (Bischoff, 2011). On the other hand, microbes were defined as pathogens (Dubos, 1995). Until the late 20th century, the concept that microorganism were beneficial for human's health was not proven (Metchnikoff, 1908). Recently, research started to explore the importance of gut health and the microbes living in the gastrointestinal tract. The biggest milestone was the development of specific scientific methods helpful to decipher the gut microbiome (Dubos, 1995), which will be explained later in this chapter. The first beneficial effect was found after consuming “probiotics” (Roberfroid et al., 2010). Since then, different scientific fields such as environmental science, medicine and industrial microbiology have begun to examine the microbiome within their respective discipline (O'Toole & Shiels, 2020). Now, the term “gut health” is more frequently used in literature (Cummings et al., 2004). In fact, a dysbiosis of intestinal microbiota is associated with a variety of pathologies as for example constipation (Zhu et al., 2014), obesity (Turnbaugh et al., 2009), diabetes (Larsen et al., 2010), colon cancer (Sobhani et al., 2011), coronary artery diseases (Emoto et al., 2016), inflammatory bowel disease (Tamboli et al., 2004) and depression (Cenit et al., 2017).

1.1 The Human Gut Microbiome

The human gut is a complex eco-system (Brestoff & Artis, 2013) and the gut microbiome is the sum of the microbial genes in the gut (Mailing et al., 2019). The terms microbiota and microbiome are often used as synonyms for each other but there is a slight difference between them. Various definitions of the microbiome exist. For this thesis, the definition of the Human Microbiome Project (HMP) has been used: “The Human Microbiome is the collection of all the microorganisms living in association with the human body. These communities consist of a variety of microorganisms including eukaryotes, archaea, bacteria and viruses” (Berg et al., 2020). The term “microbiota” is commonly described as living organisms of an ecosystem or a particular area” (Berg et al., 2020). Across studies, authors are using both terms “microbiota” and “microbiome”. The difference is that the term “microbiota” describes the microbes themselves, whereas “microbiome” refers to the microorganisms, their genes and sequence or collective coding capacity (O'Toole & Shiels, 2020).

The gut inhabits trillions of microbes, which play an essential role in human health. Between 500 to 1000 distinct microorganism have been identified (Gilbert et al., 2018). The gut contains

bacteria, fungi (mycobiome), eukaryotic virus (virome), prokaryotic viruses (phageome) and protozoa (Huttenhower et al., 2012). The large intestine hosts five phyla representing around 160 species (Rajilić-Stojanović & de Vos, 2014). The composition of the gut microbiome is different between individuals but the most representative phyla are: Firmicutes (60%–65%), Bacteroidetes (20%–25%) and Proteobacteria (5%–10%) (Huttenhower et al., 2012). It is assumed that, the human gut microbiome composition is considered to be relatively stable within a person across the lifespan (Faith et al., 2013), whereby approximately 70 % of the gut microbiota are steady over time but can rapidly change through diet and lifestyle (Faith et al., 2013). Although, research explores more and more about the gut microbiome, there is no accepted standard definition for a healthy gut microbiome (Faith et al., 2013).

Factors influencing the microbiome

The relationship between gut microbiome and host metabolism is very complex. Before birth, babies are growing in a sterile environment, free of microbes. Through birth, newborns get the first contact with microbes. In the first month up until 24 months of age, children create an adult-like microbiome. Colonization is depending on the mode of birth delivery, breast feeding and at what age they were weaned. (Bergström et al., 2014; Dominguez-Bello et al., 2010). Through life span, environmental stimuli and behavioral practices can modulate the composition and functionality of the gut microbiota (Allen et al., 2018). Research has shown that diet (David et al., 2014) and obesity status (Cani, 2013) can contribute to the metabolic and immune-regulating capacity of the gut microbiome (Allen et al., 2018). Also, the complex structure of the microbiome is modulated by host genetics (Bonder et al., 2016), age (Lozupone et al., 2012), medication (Maier et al., 2018), antibiotics, geographical region (Deschasaux et al., 2018) as well as psychosocial factors (O'Toole & Shiels, 2020). The figure of Hughes (2020) shows an overview of the most important influence factor of the human gut microbiome (**Figure 1**).

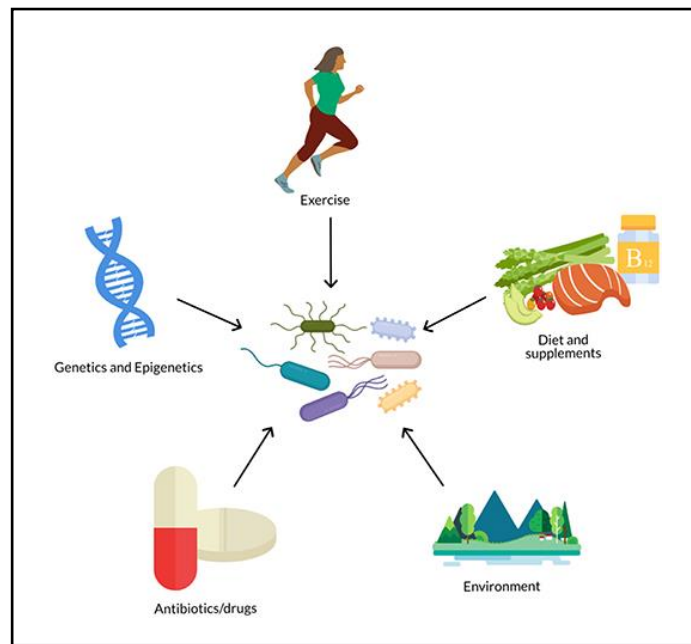


Figure 1 The gut microbiome is influenced by exercise, diet and supplements, environment, antibiotics/drugs and genetics (Figure from Hughes (2020)).

The effect of antibiotics is profound and rapid. Antibiotics lead to a loss of diversity and shift in community in the gut microbiome. In summary, antibiotic disturbance may lead to an alternative stable state but the full consequences of these disturbances are not fully known (Dethlefsen & Relman, 2011). A reduced species diversity of the intestinal microbiota is also associated with aging (Woodmansey et al., 2004).

Gut microbiome and diseases

Gut microbial profiles have been found to differ between healthy and unhealthy individuals (Cronin et al., 2017). Obesity is one of the major public health problems and is influenced by various factors, such as diet, parental obesity and decreased physical activity, especially in childhood (Isganaitis & Levitsky, 2008). The gut microbiota is an important factor that influences energy disposal and storage in overweight and obese (Ley et al., 2006). Recent research shows that chronic low-grade inflammation is associated with insulin resistance and the risk of acquiring diabetes (Shoelson et al., 2006). An altered gut microbiota composition could be a potential cause for developing diabetes (Nicholson et al., 2012). Another phenomenon, the leaky gut syndrome can be explained by a local inflammation in the gut and influencing its functions (Fasano, 2012). In people suffering from myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), gut microbiota is altered as compared to healthy controls (Frémont et al., 2013). On the contrary, athlete's microbiota has been found to have specific microbial

composition. Evaluated abundance has been found for *Veillonellaceae*, *Bacteroides*, *Prevotella*, *Methanobrevibacter* and *Akkermansia* (Clarke et al., 2014; Petersen et al., 2017). In summary, the gut microbiome plays an important role in regulating energy metabolism, oxidative stress, hydration status and systematic inflammatory response (Mach & Fuster-Botella, 2017). Therefore, optimizing and controlling those processes are important to maintain health and may be even beneficial in enhancing the performance of athletes (Clarke et al., 2014).

Gut diversity and gut taxonomy

The gut inhibits hundreds of diverse bacterial species and are grouped together by the microbial taxonomy, which is a classification in a hierarchical system. Outcomes and effects on the microbiome can be interpreted via changes in microbial diversity or microbial taxonomy. *Alpha diversity* is used as a measure to describe the diversity within a subject, whereas *beta diversity* describes the between-subject diversity (Anderson & Willis, 2003). As previously mentioned, Bacteroidetes and Firmicutes are the most abundant phyla in the human gut, representing 90% of the gut microbiota (Rinninella et al., 2019).

16rRNA sequencing and shotgun metagenomics

Microbes living in human and animals body sites are hard to replicate in growth conditions in laboratories. Some time ago, those microbes were considered unculturable, but this has changed over the years (Browne et al., 2016; Claesson et al., 2012). The reason for the increase in microbiome studies is that modern tools in molecular biology were made available for many disciplines. High through-put sequencing has revolutionized the depth and precision of complex microbial communities (Claesson et al., 2012). The gut harbors the largest load of bacterial loads and a diverse bacterial community (Browne et al., 2016). Therefore, it is not surprising that the majority of published microbiome studies focus on the gut microbiome. The driving factor in microbiome technology was to be able to routinely and affordably catalogue the composition of the microbiome at a given location (O'Toole & Shiels, 2020). Now, various ways to study the microbiome exists. Newest insight in research explored different areas of the microbiome, for example in what the bacterial community might do (total coding capacity), in the range of metabolites it produces (the metabolome), and in the total complement of proteins (the metaproteome) (O'Toole & Shiels, 2020). The most frequently used methods are 16S rRNA sequencing and shotgun metagenomics. In order to establish which microbes are present, the sequencing of ribosomal RNA (rRNA) gene amplicons is a cheap and quick method. Shotgun metagenomics is performed to move beyond 16S rRNA and to

better understand the role of the gut microbiome (Mailing et al., 2019). It involves the discovery of the encoded functions of these microbes. For this aim, true metagenomics is required (Fraher et al., 2012). A challenge these days is the standardization of protocols. Across studies, differences in underlying datasets, DNA extraction, and sample preparation for DNA sequencing exist (Costea et al., 2017). **Figure 2** gives an overview of the commonly used methods to access the gut microbiome.

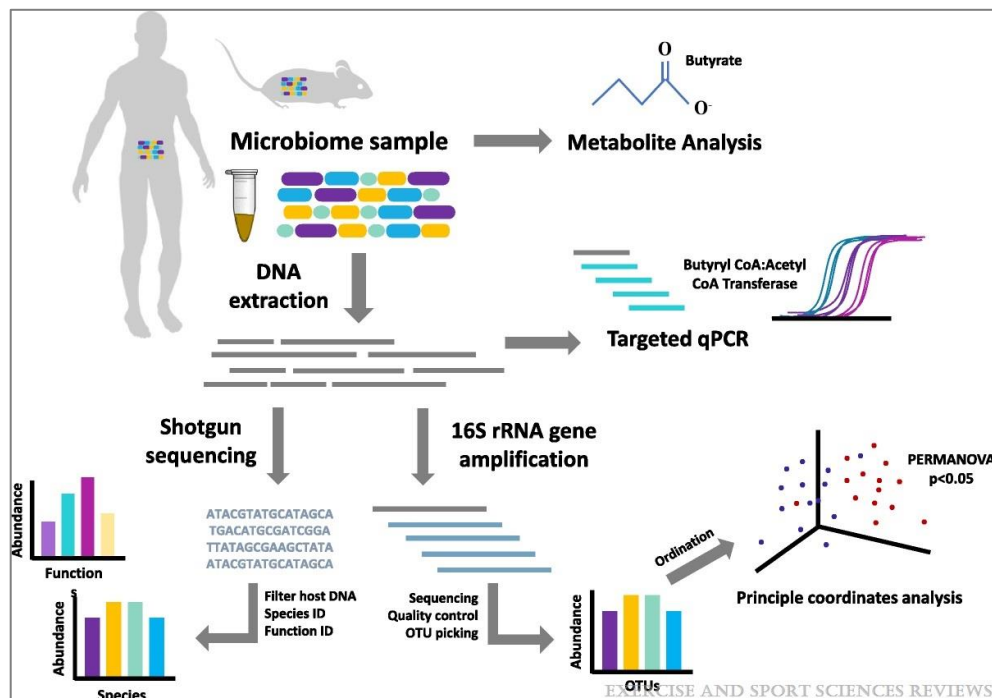


Figure 2 Overview of commonly used methods to assess the gut microbiome (Mailing et al. (2019))

1.2 Physical activity, Exercise and Impact on health

Physical activity has been shown to have protective effects against many diseases. Physical activity is powerful modifier in metabolic traits and is a key component for primary and secondary prevention in lifestyle-related diseases (Durstine et al., 2013). The World Health Organization defines physical activity as “any bodily movement produced by skeletal muscles that requires energy expenditure – including activities undertaken while working, playing, carrying out household chores, travelling, and engaging in recreational pursuits.” Physical activity and exercising are not synonyms for one another. Exercise is a subcategory of physical activity and is planned, repetitive and structured. The goal of exercising is to maintain or improve one or more components of physical fitness (WHO, 2010).

Exercise interventions can be categorized in “acute exercise” and “chronic exercise”. Within this thesis, one single bout of exercise was defined as “acute exercise” whereas an intervention over a period of time was defined as “chronic exercise”.

Numerous mechanisms have been found to affect human metabolism through physical activity. Those were: direct effects on targeted organs and skeletal muscles (Richter & Hargreaves, 2013) and indirect effects through fine-tuning energy metabolism (Beaulieu et al., 2018). As a consequence, exercise training can improve obesity status, increase insulin-sensitivity, reduce inflammation (Woods et al., 2009) and improve cardiorespiratory fitness (VO₂max) (Hammer et al., 1989). Also, in patients, exercise is known for its anti-inflammatory and multi-organ metabolic effects (Petersen & Pedersen, 2005). Especially in patients with type two diabetes mellitus (Schwingshackl et al., 2013) and non-fatty liver diseases (Hallsworth et al., 2015) exercise is used as first line treatment. Also, in cancer patients, exercise is suggested to improve outcome and quality of life (McTiernan, 2008). For example, regular running improves physical and mental fitness, including, bone, muscle, immune system and cardiovascular functions (Warburton et al., 2006). Exercise is known for modulating general metabolism (Zhao et al., 2018), but it can be assumed that also metabolic changes induced by metabolites of the gut microbiome contribute to the health-promoting effect of physical activity.

1.3 The Influence of Exercise on Gut Microbiota

The Human Microbiome Project (HMP) was initiated by the National Institute of Health in 2007. The purpose of the project is to characterize the contribution of human gut microbiota to health and diseases (Turnbaugh et al., 2007). Various studies have found a potential relationship between human gut microbiota and non-communicable diseases (NCD), such as cardiovascular diseases (Wang et al., 2011), cancer (Ahn et al., 2013) and diabetes (Larsen et al., 2010).

Available evidence on the influence of physical activity on the gut microbiota exists mainly in animal models, but studies in humans are catching up (Batacan et al., 2017). Physical activity is now considered as one of the main environmental factors that influence the gut microbiota, independent of age, weight, body composition and food intake. As evident from long-term studies it is shown that exercising can modulate the gut microbiota over a longer time (Pedersini et al., 2020). Physical activity is a cheap and useful tool to enhance health and prevent diseases and also helps as disease treatment (Kang et al., 2014). Up until now, the effect of exercise and physical activity on the microbiome remains elusive and is a popular topic of investigation.

Studies using Animal Models

Studying the effects of exercise on the microbiome using animal models has been deepened our understanding about the association between exercise and the gut microbiome. Several studies confirmed that exercise training can significantly modulate the gut microbiota in mice (Allen et al., 2015; Choi et al., 2013; Kang et al., 2014). Thereby, exercise leads to a favorable change in gut composition (Choi et al., 2013), and the abundance of certain bacterial strains (Lambert et al., 2015; Welly et al., 2016). Mechanistically, physical activity leads to increased butyrate production associated with positive health outcomes (Matsumoto et al., 2008). Interestingly, the alterations in microbial community structure are different between voluntary or forced exercise in mice (Allen et al., 2015). Alterations are also connected to enhanced performance (Scheiman et al., 2019). Thereby, endurance training can help to modulate the composition and diversity of the gut microbiota (Denou et al., 2016; Evans et al., 2014). Wheel-running exercises can reverse unhealthy states (Choi et al., 2013; Evans et al., 2014; Lambert et al., 2015) and lead to an increase in phylum Firmicutes and a decrease in phyla *Tenericutes* and *Bacteroidetes* (Choi et al., 2013). Another study showed that exercise in mice lead to an increased abundance of *Firmicutes* species and a decrease in abundance of *Bacteroidetes* (Lambert et al., 2015). However, studies are inconsistent as an increase in *Bacteroidetes* and a decrease in *Firmicutes* has been detected by Evans et al. (2014).

Cross-Sectional Studies in Humans

A number of cross-sectional human studies proved the association between physical activity and composition of the intestinal microbiota (Barton et al., 2018; Bressa et al., 2017; Clarke et al., 2014; Estaki et al., 2016). The first study investigating the gut microbiome in athletes examined the gut microbiota of rugby player and found a greater diversity in the composition in athletes compared to sedentary adults (Clarke et al., 2014). Also, cardiorespiratory fitness was associated with gut microbiota composition (Ahn et al., 2013; Clarke et al., 2014; Estaki et al., 2016; Yang et al., 2017). At taxonomy level, microbiota of normal-weight active woman differed from sedentary women (Bressa et al., 2017). Microbial diversity is greater in professional athletes than in sedentary controls (Barton et al., 2018). Adults who were exercising regularly during their childhood and onwards, demonstrate a greater potential fostering a healthy microbiome than those who exercised only in adulthood (Mika & Fleshner, 2016). It is unknown if improvements in cardiorespiratory fitness in adults can shift the gut microbiome to a more healthy one (Mika & Fleshner, 2016). The gut microbiota in elderly is less diverse compared to young subjects. Recent studies found an association between frailty index and the gut composition (Claesson et al., 2012)) and diversity (Jackson et al., 2016). Furthermore, the gut microbiota seems to be less

diverse in hypertensive patients (Li et al., 2017; Yang et al., 2015). In professional athletes, alpha diversity of the gut microbiome is increased presumably by the increased level of exercise (Barton et al., 2018; Clarke et al., 2014). Clarke et al. (2014) and Barton et al. (2018) found a significant high abundance in bacterial genus *Akkermansia* within athletes. Petersen et al. (2017) found out that professional cyclists have a higher abundance of *Prevotella*, a fast-growing bacterium involved in energy and carbohydrate metabolism when increasing the training volume. Cross-sectional studies proved that exercise can influence the gut microbiome but no understanding of microbial dynamics over time in response to exercise and physical activity is possible (O'Sullivan et al., 2015). Yet, a full understanding of the factors that can responsible modulate human gut microbiota is lacking (Relman, 2015). Until now, the mechanism of how exercise prevents diseases and improve health is poorly understood (Zhao et al., 2018). It seems to be evident from cross sectional studies that exercise can influence the gut microbiome but cause-effect relationships and mechanistic insights cannot be confirmed by those studies.

1.4 Research Question

Many studies researching the gut microbiome and exercise are of cross-sectional design. However, different lifestyle habits including the wide range of dietary regimes and physical habits shape the composition and function of the microbiome (Cronin et al., 2017). A review of Mohr et al. (2020) presented the actual knowledge of the microbiome and its association with nutrition and exercising in athletes. Another review of Mailing et al. (2019) also gave an overview of exercising and the mechanism behind the impact of exercise on the microbiome. Tzemah Shahar et al. (2020) published a systematic review of the attributes of physical activity and gut microbiome in adults. Only one systematic reviewed the influence of exercise on the microbiome, but only adults and did not include any other groups (Ortiz-Alvarez et al., 2020). Up until now, no systematic review on the effect of exercise interventions on the human gut microbiome across various populations has been published.

Therefore, the aim of the study is to explore if exercise has an effect on the human gut microbiome. The following sub questions will be investigated:

1. Does exercise influence gut diversity and gut taxonomy?
2. Is there a difference between acute and chronic effects of exercise onto the gut microbiome?
3. Are there specific bacteria, which can be associated to have an effect on the gut microbiome?

2 METHODS

In this chapter the methodological procedure of the systematic review will be explained. This includes detailed description of the literature research including search strategy, used databased and the initial search. Further, the selection criteria, especially inclusion and exclusion criteria, will be explained. Limitations to the search will be presented. Last, the process of data extraction is shown.

2.1 Protocol and Registration

The systematic review was reported based on the PRISMA statement for systematic reviews and meta-analysis (Moher et al., 2009). This systematic review was not prospectively registered at the International Prospective Register of Systematic Reviews (PROSPERO).

2.2 Eligibility Criteria

Longitudinal studies and clinical trials reporting the effect of physical activity and exercise on the human microbiome were included. The effect of lifestyle including physical activity and diet on the microbiome has been investigated mainly in cross-sectional study design during the last years. Newest research focused more and more on longitudinal studies. The effect of diet on the microbiome has been explored by plenty of researchers. Also, intervention studies focusing on weight-loss by regulating the gut microbiota have been published. Only studies which described a full exercise or physical activity program were included. Studies which just examined physical activity level through questionnaires were excluded. In total 8 exclusion and 5 inclusion criteria were defined. The concrete inclusion and exclusion criteria were:

Exclusion criteria

(i) any type observational studies (Cross-sectional, cohort studies or case-control studies)
(ii) reviews (iii) journal articles, letters, study protocols (iv) studies in other language than English or German (v) animal studies (vi) no exercise interventions (vii) Outcome: only physical activity parameters and (viii) irrelevant topic (f.e. cognition).

Inclusion criteria

The inclusion criteria were: (i) intervention studies (randomized and non-randomized control trials, clinical interventions, etc.) (ii) studies including any type of physical activity or exercise interventions (iii) studies which analyzed gut composition as outcome (iv) human studies (v) studies in German or English. No restriction was set with respect to study population (sample characteristic (age, gender, ethnicity) or sample size or setting). Further, there was no restriction concerning publication date. An overview of the inclusion and exclusion criteria can be found in **Table 1**.

Table 1 Overview of Inclusion and Exclusion criteria

Exclusion	1. Any type observational studies
	2. Reviews
	3. Journal articles, letters, study protocols
	4. Studies in other language than English or German
	5. Animal studies
	6. Other than exercise interventions
	7. Outcome: physical activity parameters
	8. Irrelevant topic (f.e. cognition).
Inclusion	1. Intervention studies (randomized and non-randomized control trials, clinical interventions, etc.)
	2. Studies including any type of physical activity or exercise interventions
	3. Studies which analyzed gut composition as outcome
	4. Human studies
	5. Studies in German or English

2.3 Information Sources

A computerized literature search was conducted across the databases Scopus, Web of Science and PubMed. Three databases were chosen to ensure all relevant papers are included, since it is possible that one database omits relevant research. Elsevier's Scopus is the largest abstract and citation database of peer-reviewed literature. Clarivate Analytics Web of Science, previously known as Web of Knowledge, provides subscription-based access to multiple databases that provide comprehensive citation data for many different

disciplines. PubMed including MEDLINE, is a database of references and abstracts on life sciences and biomedical topics. Also, two other databases, ScieLO and ScienceDirect, were initially searched but did not provide different results than the former three platforms. Therefore, the three databases were chosen due to the broad spectrum of different subjects and articles (see **Table 2**).

Table 2 Overview of databases

	Database	Provider	Time span
1	PubMed +MEDLINE	National Center for Biotechnology Information	all years
2	Scopus	Elsevier	all years
3	Web of Science	Clarivate Analytics	all years

2.4 Search

In March 2020, systematic reviews similar to the topic of the thesis were screened and relevant articles were screened for keywords. The purpose of this review is to give an overview of the effect of exercising and physical activity on the human microbiome. Four main categories (1-4) were selected. Each category was defined by one main keyword including various search terms. The search terms for each platform differed from each other to some extent due to different results from a trial search in May 2020. The keyword of category one was **Exercise** and included the search terms: exercise, physical activity, athlete, sedentary behavior and training. **Microbiome** was the keyword of category two including the following search terms: microbiota, microbial composition, gut health, microbiome and gastrointestinal microbiome. Keyword of category three was **Human**. The last category was **review** in order to be able to automatically exclude those from the hits. Different databases use different search types. **Table 3** gives an overview of the categories, keywords, search terms and search types for each database.

Table 3 Overview of keywords, search terms and search types

Category	Keyword	Search terms	Search type		
			Scopus	Pubmed	Web of Science
1	Exercise	<i>Exercise</i>	TITLE-ABS-KEY	All Fields, MeSH Terms	TOPIC
		<i>"physical activity" / physical activity/physical-activity</i>	TITLE-ABS-KEY	All Fields, MeSH Terms	TOPIC
		<i>Athlete*</i>	TITLE-ABS-KEY		TOPIC
		<i>"sedentary behaviour" /sedentary</i>	TITLE-ABS-KEY	All Fields, MeSH Terms	TOPIC
		<i>Training</i>			TOPIC
2	Microbiome	<i>Microbiota</i>	TITLE-ABS-KEY		TOPIC
		<i>"microbial composition"</i>	TITLE-ABS-KEY		
		<i>"gut health"</i>	TITLE-ABS-KEY	All Fields, MeSH Terms	TOPIC
		<i>Microbiome</i>	TITLE-ABS-KEY		TOPIC
		<i>Gastrointestinal microbiome</i>		All Fields, MeSH Terms	
3	Human	<i>Intestinal microbiome</i>			TOPIC
		<i>Human*/Human/Humans</i>	TITLE-ABS-KEY	Filter	TOPIC
4	Review	<i>Review</i>	TITLE-ABS-KEY	Publication Type	Document Types

On June 25th 2020, the literature search was conducted. All literature published up until this date was included. The search strings for the different databases differed from each other to some extent. Between search terms the operators AND, OR and NOT were used. Search types depended on the databases and previously described in **Table 3**.

In Scopus the following search term were used: physical activity, sedentary behavior, athlete, exercise, microbiota, microbial composition, gut health and human. Search terms were narrowed by search fields: Article title, abstracts and keywords to find fitting literature. No filter was set. **Table 4** gives an overview of the used search terms and the outcome of the search.

The exact search string for SCOPUS was:

(TITLE-ABS-KEY ("physical activity" OR "sedentary behavior" OR athlete OR exercise) AND TITLE-ABS-KEY (microbiota OR "microbial composition" OR "gut health") AND TITLE-ABS-KEY (human*) AND NOT TITLE-ABS-KEY (review))*

Table 4 Search strategy used for Scopus, and number of articles found

Search criterion 1	Search criterion 2	Search criterion 3	Search criterion 4	Items found (Combining criteria 1 "AND" 2 "AND" 3 "NOT" 4)
"physical activity" OR "sedentary behavior" OR athlete* OR exercise	Microbiota OR "microbial composition" OR "gut health"	Human*	review	344
All the searches were combined using the operator "OR" so that duplicated articles were excluded from the final number of articles found. Search terms were narrowed by the search fields: Article title, abstract and keywords. The final search term was:				
Total items found	(TITLE-ABS-KEY ("physical activity" OR "sedentary behavior" OR athlete* OR exercise) AND TITLE- ABS-KEY (microbiota OR "microbial composition" OR "gut health") AND TITLE-ABS- KEY (human*) AND NOT TITLE-ABS- KEY (review))			344

The search recruited articles published until June 25th 2020; no lower date limit was set.

Search terms used for PubMed were: physical activity, sedentary behavior, microbiome, gastrointestinal microbiome and review. For all search terms “all fields” and MeSH terms were applied. **Table 5** gives an overview of the used search terms and the outcome of the search.

The final string in PubMed was:

((((((((((("exercise"[MeSH Terms] OR "exercise"[All Fields]) OR "exercises"[All Fields]) OR "exercise therapy"[MeSH Terms]) OR ("exercise"[All Fields] AND "therapy"[All Fields])) OR "exercise therapy"[All Fields]) OR "exercise s"[All Fields]) OR "exercised"[All Fields]) OR "exerciser"[All Fields]) OR "exercisers"[All Fields]) OR "exercising"[All Fields]) OR (((("exercise"[MeSH Terms] OR "exercise"[All Fields]) OR ("physical"[All Fields] AND "activity"[All Fields])) OR "physical activity"[All Fields])) OR "sedentary behavior"[All Fields]) AND (((((((("microbiome s"[All Fields] OR "microbiomic"[All Fields]) OR "microbiomics"[All Fields]) OR "microbiota"[MeSH Terms]) OR "microbiota"[All Fields]) OR "microbiome"[All Fields]) OR "microbiomes"[All Fields]) OR (("gastrointestinal microbiome"[MeSH Terms] OR ("gastrointestinal"[All Fields] AND "microbiome"[All Fields])) OR "gastrointestinal microbiome"[All Fields])))) NOT (("review"[Publication Type] OR "review literature as topic"[MeSH Terms]) OR "review"[All Fields])

Table 5 Search strategy used for PubMed, and number of articles found.

Search criterion 1	Search criterion 2	Search criterion 3	Items found (Combining criteria 1 "AND" 2 "AND" 3 "NOT" 4)
Exercise OR physical activity OR "sedentary behavior"	Microbiome OR gastrointestinal microbiome	review	271
All the searches were combined using the operator "OR" so that duplicated articles were excluded from the final number of articles found. Search terms were narrowed by the search fields: Article title, abstract and keywords. The final search term was: (exercise OR physical activity OR "sedentary behavior") AND (microbiome OR gastrointestinal microbiome) NOT (review)Filters: Humans ((((((((((("exercise"[MeSH Terms] OR "exercise"[All Fields]) OR "exercises"[All Fields]) OR "exercise therapy"[MeSH Terms]) OR ("exercise"[All Fields] AND "therapy"[All Fields])) OR "exercise therapy"[All Fields]) OR "exercise s"[All Fields]) OR "exercised"[All Fields]) OR "exerciser"[All Fields]) OR "exercisers"[All Fields]) OR "exercising"[All Fields]) OR (((("exercise"[MeSH Terms] OR "exercise"[All Fields]) OR ("physical"[All Fields] AND "activity"[All Fields])) OR "physical activity"[All Fields])) OR "sedentary behavior"[All Fields]) AND ((((((("microbiome s"[All Fields] OR "microbiomic"[All Fields]) OR "microbiomics"[All Fields]) OR "microbiota"[MeSH Terms]) OR "microbiota"[All Fields]) OR "microbiome"[All Fields]) OR "microbiomes"[All Fields]) OR ((("gastrointestinal microbiome"[MeSH Terms] OR ("gastrointestinal"[All Fields] AND "microbiome"[All Fields])) OR "gastrointestinal microbiome"[All Fields])))) NOT ((("review"[Publication Type] OR "review literature as topic"[MeSH Terms]) OR "review"[All Fields])			
Total items found			271

The search recruited articles published until June 25th 2020; no lower date limit was set.

Filter: Humans

Translation:

exercise: "exercise"[MeSH Terms] OR "exercise"[All Fields] OR "exercises"[All Fields] OR "exercise therapy"[MeSH Terms] OR ("exercise"[All Fields] AND "therapy"[All Fields]) OR "exercise therapy"[All Fields] OR "exercise's"[All Fields] OR "exercised"[All Fields] OR "exerciser"[All Fields] OR "exercisers"[All Fields] OR "exercising"[All Fields]

physical activity: "exercise"[MeSH Terms] OR "exercise"[All Fields] OR ("physical"[All Fields] AND

microbiome: "microbiome's"[All Fields] OR "microbiomic"[All Fields] OR "microbiomics"[All Fields] OR

gastrointestinal microbiome: "gastrointestinal microbiome"[MeSH Terms] OR ("gastrointestinal"[All Fields] AND "microbiome"[All Fields]) OR "gastrointestinal microbiome"[All Fields]

review: "review"[Publication Type] .or. "review literature as topic"[MeSH Terms] .or. "review"[All Fields]

In Web of Science the following search term were used: exercise, training, physical activity, sedentary, athlete, microbiome, microbiota, gut health, intestinal microbiome, human and review. Search terms were narrowed by search fields: Article title, abstracts and keywords to find fitting literature. No filter was set. **Table 6** gives an overview of the used search terms and the outcome of the search.

The search string for Web of Science was:

exercise OR training OR physical-activity OR sedentary OR athlete AND microbiome OR microbiota OR „gut health" OR intestinal microbiome AND Human NOT Review.

All topics were searched. Only the document type was set to “review”. No restrictions were applied for date of publication, gender, age, subject or language.

Table 6 Search strategy used and number of articles found in Web of Science

Search criterion 1	Search criterion 2	Search criterion 3	Search criterion 4	Items found (Combining criteria 1 “AND” 2 “AND” 3 “NOT” 4)
exercise OR training OR physical-activity OR sedentary OR athlete	microbiome OR microbiota OR "Gut health" OR intestinal microbiome	Human	Review	284
All searches were combined using the operator “OR” so that duplicated articles were excluded from the final number of articles found. The final search term was:				
TOPIC(exercise OR training OR physical-activity OR sedentary OR athlete) AND TOPIC: (microbiome OR microbiota OR "Gut health" OR intestinal microbiome) AND TOPIC: (human) NOT DOCUMENT TYPES: (Review)				284
Timespan: All years. Indexes: SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC.				

The search recruited articles published until June 25th 2020; no lower date limit was set.

Hand search

Other articles were included by hand search based on the screening of the reference lists of the selected articles. After, they counted into the selected articles and further analyzed.

Limitations

No limitation occurred during the search.

2.5 Study Selection

The screening process was administered with the help of the reference management software “EndNote”. First, result the literature search from each database was imported into EndNote. After, all literature duplicates within each database and then across databases were removed. After removing duplicates, literature published in other languages than English and German were sorted out. The next step was screening for predefined inclusion and exclusion criteria by a two-step procedure. First, literature was screened by title and abstract to assess conformity with predefined inclusion criteria and exclusion criteria (**Table 1**). The remaining articles were selected to the full-text analysis. Each study was read in depth and either included or excluded. Reason for exclusion were given and summarized in an Excel sheet (see **APPENDIX**). The Prisma Flowchart was used to give an overview of the process.

2.6 Data Collection Process & Data Items

Only the master thesis student extracted the information from the papers. The following information was selected from each included study:

General study characteristics

- Author name and the bibliographic references
- Country
- Study design
- Study setting
- Enrolled and completed study samples
- Duration of intervention and follow-up

Sample characteristics

- Sample number
- Sex
- Sample category
- Age
- Baseline subject characteristics
- Control for antibiotic and diet

Intervention characteristics (training protocols)

- Type of exercise
- Exercise duration
- Exercise frequency
- Exercise intensity
- Control group

Sample collection and analysis methods

- Sample
- Fecal sample time point
- Sample transport
- DNA Extraction
- Gut microbiota analysis techniques
- Sequencing target
- Sequencing platform
- DNA analysis platform
- Reference sequence database

Outcome

- Gut diversity changes (alpha and beta diversity)
- Gut taxonomy changes (at domain, phylum, class, order, family, genus and species level)
- Physiological changes (BMI, body fat and VO_{2max})

2.7 Risk of Bias

Risk of Bias was assessed using the PEDro scale by searching the included studies in the Physiotherapy Evidence Database (de Morton, 2009). This tool is reliable, consists of 11 items and is able to detect potential bias with good reliability (Maher et al., 2003). Scores can range from 2 – 11. A score of ≤ 3 is defines as studies of low methodological quality, 4–6 those of moderate quality, and ≥ 7 those of high methodological quality (de Morton, 2009)

3 RESULTS

The next chapter will present the results of the literature search of the systematic review. Study selection including the PRISMA Flow Diagram and characteristics of the studies will be explained. In detail, study characteristics, sample characteristics, training protocols and study methods will be listed for each study. Main outcomes will be explained in detail. Primary outcomes of how exercise can influence the gut microbiome will be presented. Results were analyzed for alpha and beta-diversity and taxonomy of the microbiome. Secondary outcomes on physiological changes after intervention will be represented. Finally, further risks of bias within studies will be shown.

3.1 Study Selection

In total, 899 articles were found across the three databases Scopus (n=344), PubMed (n=271) and Web of Science (n=284). Another four articles were included by hand search. Hand search was performed by screening reference lists of relevant articles. First, duplicates were searched within databases and then across the other databases. In total, 240 duplicates were excluded. After reading the title and abstract of the remaining 663 articles, 591 articles did not meet inclusion criteria. The remaining 72 articles were selected to read in full-text. From the chosen articles, another 54 were excluded with several reasons (see **Appendix** for full inclusion and exclusion analysis). In summary, eleven different exclusion reasons were assessed. Articles were excluded with the following reasons: cross sectional study design (n=18), reviews (n=6), overview articles (n=7), studies with only outcome: physical parameter VO_{2max} (n=3), intervention studies focusing on diet (n=4) or weight-loss (n=4), supplementation with probiotics (n=2), study protocol of future interventions studies (n=4), studies with animal models (n=3), full-text not in English or German (n=1) and other reasons (n=2). In total, 18 studies met inclusion criteria. **Figure 3** on the next page shows the PRISMA flowchart of the study selection process.

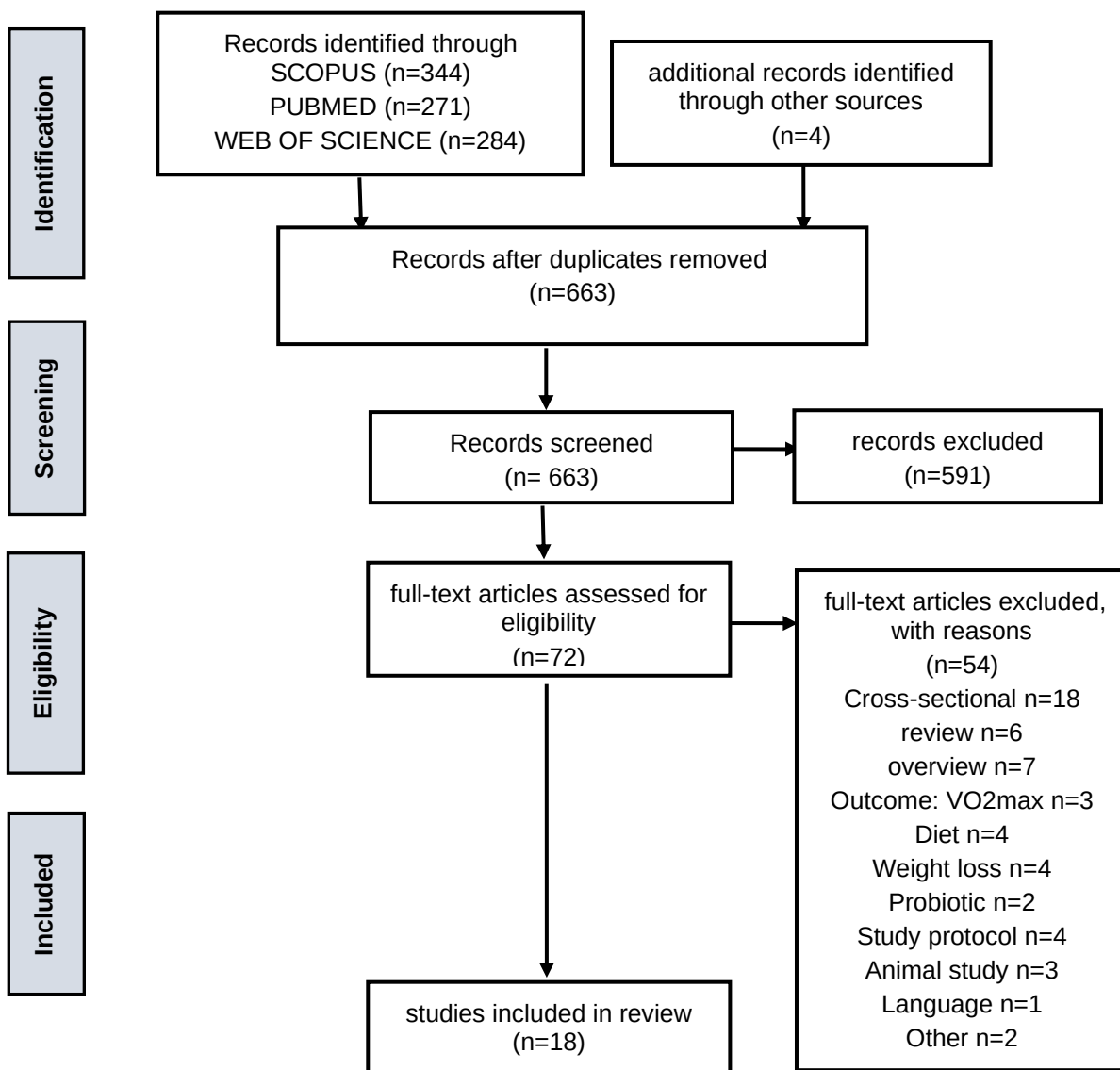


Figure 3 PRISMA flowchart illustrating the process for the selection of the included articles for the systematic review. PRISMA- Preferred Reporting Items for Systematic Reviews and Meta-Analyses

3.2 Study Characteristics

The 18 included studies were published between 2009 and 2020. Subjects were recruited from USA, Ireland, China, Japan, Ireland, Italy, Spain, Denmark and Finland. Studies enrolled between 1 to 130 subjects. Across the studies three population types were involved: “patients”, “participants with a healthy lifestyle”, “participants with an unhealthy lifestyle”. The settings of the exercise intervention studies including any kind of patients were hospitals, university laboratories or university gymnasiums. When samples with a healthy lifestyle were involved, studies were conducted at training or competition locations. In the groups with an unhealthy lifestyle, three studies were performed in a “free-living” setting (Morita et al., 2019; Paulsen et al., 2017; Santacruz et al., 2009) and one study involved a fitness studio (Morita et al., 2019). The included studies were either experimental or observational studies. Studies had different study designs. Four studies (Allen et al., 2018; Morita et al., 2019; Shukla et al., 2015) were non-randomized controlled trails and four studies were randomized controlled trails (Barton et al., 2018; Kern et al., 2020; Motiani et al., 2020; Paulsen et al., 2017; Santacruz et al., 2009). Crossover design was observed in two studies (Cronin et al., 2019; Taniguchi et al., 2018).

Three studies (Hampton-Marcell et al., 2020; Munukka et al., 2018; Zhao et al., 2018) were cohort studies. The study of Scheiman et al. (2019) was a case-control study for one experiment and a cohort study for the second experiment. Other study designs across the 18 studies were: Case study (Grosicki et al., 2019), clinical trial (Liu et al., 2020), prospective, repeated measures within subject report (Keohane et al., 2019) and open label trials (Pasini et al., 2019). Duration of the intervention ranged from one day to six months. Four studies followed up participants (Allen et al., 2018; Grosicki et al., 2019; Keohane et al., 2019; Morita et al., 2019; Motiani et al., 2020; Zhao et al., 2018)). Follow-ups ranged from 48 hours (acute effect of exercise) to three months post-intervention (chronic effect of exercise). Detailed information on acute and chronic effects of exercise on the microbiome will be explained later in the result part. **Table 7** summarizes the characteristics of the included studies.

Table 7 Study characteristics of included studies (n=18)

Year	Author	Country	Design	Setting	N Enrolled	N Completed	Duration of intervention	Follow-up
2018	Allen et al.	USA	Non-randomized controlled trial	University laboratory	32	32	6 weeks	6 weeks
2019	Cronin et al.	Ireland	Single-Centre, randomized, partial cross-over trial	University gymnasium	17	13	8 weeks	
2018	Cronin et al.	Ireland	Randomized-controlled trial , parallel group design	University gymnasium	90	74	8 weeks	
2019	Grosicki et al.	USA	Case study	Training period	1	1	21 weeks	10 days
2020	Hampton- Marcell et. al	USA	Cohort study	Training period	13	13	6 weeks	
2019	Keohane et al.	Cross countries	Prospective, repeated- measures, within- subject report	Competition	4	4	33 days 22 h	3 month
2020	Kern et al.	Denmark	Randomized controlled trial	Free-living	130	88	6 months	
2020	Liu et al.	China	Clinical trial	University laboratory	20	20	12 weeks	
2019	Morita et al.	Japan	Non-randomized comparative trial, clinical trial	Sports Center & free-living	32	29	12 weeks	
2020	Motiani et al.	Finland	Randomized controlled trial	Hospital	26	18	2 weeks	
2018	Munukka et al.	Finland	Cohort study	University laboratory	20	17	6 weeks	
2019	Pasini et al.	Italy	Controlled open-label trial	Hospital	30	30	6 months	
2017	Paulsen et al.	USA	Pilot study of a two- site, randomized controlled trial	Free-living	12	12	3 months	
2009	Santacruz et al.	Spain	Non-randomized controlled trial	Free-living	36	36	10 weeks	
2019	Scheimann et al.	USA	Case-control study	Competition	25	25	10 days	
2019	Scheimann et al.	USA	Cohort study	Training period	87	87	1 day	
2015	Shukla et al.	USA	Non-randomized controlled trial	University laboratory	20	20	1 day	48 h
2018	Taniguchi et al.	Japan	Randomized crossover trial	University laboratory	33	31	10 weeks	
2018	Zhao et al.	China	Cohort study	Competition	20	20	1 day	

3.3 Sample Characteristics

The total sample size of the included studies was 578 (202 women, 224 men and 152 sex unknown) shown in **Figure 4**. Studies were of diverse characteristics, which made it impossible to put data of the studies together (see data in **APPENDIX**). For a better understanding and comparison later in this review, samples were sorted into following three groups: Subject with healthy lifestyle (n=181), subject with an unhealthy lifestyle (n=286) and patients with various diagnosis (n=111) (see **Figure 5**).

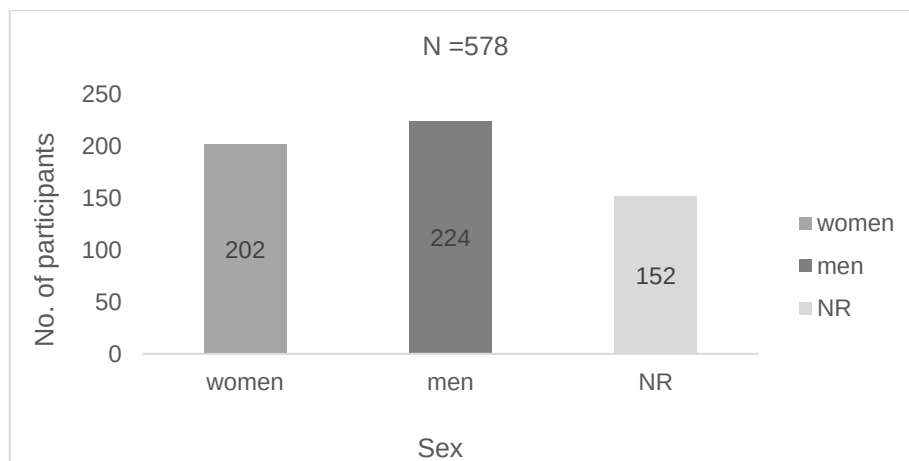


Figure 4 Total number of participants across the included studies (n=578) and differences in sex: women (n=202), men (n=224) and unknown (n=152).

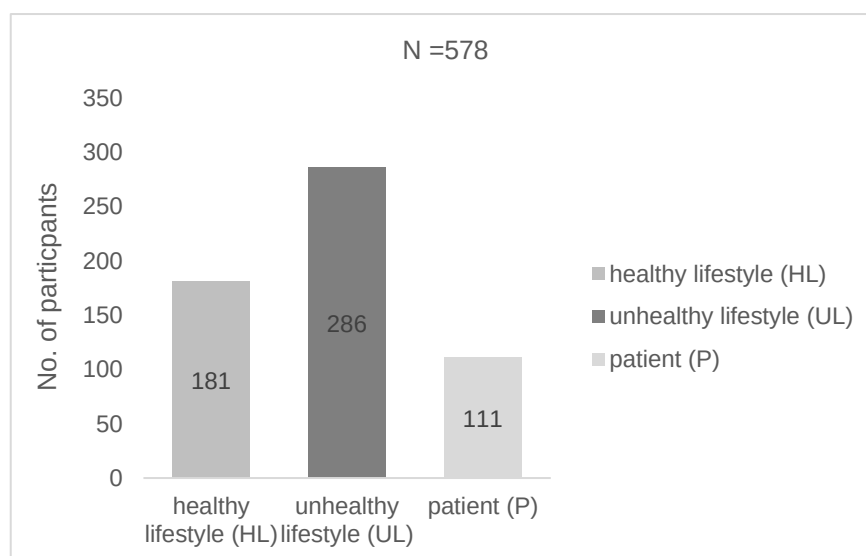


Figure 5 Different characteristics across included studies: healthy lifestyle (n=181), unhealthy lifestyle (n=286), patient with a diagnosis (Type 2 Diabetes, IBD, cancer or ME/CFS) n=111) of total sample size (n=578)

Each subgroup comprised 6 studies. The “healthy lifestyle” (HL) group included athletes and healthy controls. Overweight, obese and sedentary study samples were combined in the “unhealthy lifestyle” (UL) group. Subjects with a clinical diagnosis were summarized under patients (P).

Healthy lifestyle group

Six studies involved athletes and healthy participants (Grosicki et al., 2019; Hampton-Marcell et al., 2020; Keohane et al., 2019; Scheiman et al., 2019; Taniguchi et al., 2018; Zhao et al., 2018). Studies involved between 1 to 87 participants with age ranging von 18 to 76. years. Three studies only involved men (Grosicki et al., 2019; Keohane et al., 2019; Taniguchi et al., 2018), two studies men and women (Hampton-Marcell et al., 2020; Zhao et al., 2018) and one studies did not report the sex (Scheiman et al., 2019). Subjects in the healthy lifestyle group were mainly young and endurance-trained athletes. Those were half-marathon runners (Scheiman et al., 2019), marathon-runners (Scheiman et al., 2019), ultra-marathon runners (Grosicki et al., 2019; Scheiman et al., 2019), rowers (Keohane et al., 2019; Scheiman et al., 2019) or swimmers (Hampton-Marcell et al., 2020). Only one study involved healthy and elderly subjects (Taniguchi et al., 2018). VO_{2max} in athletes (Keohane et al., 2019) was higher than in healthy, elderly subjects (Taniguchi et al., 2018) Two studies did not control for diet or antibiotic usage (Grosicki et al., 2019; Hampton-Marcell et al., 2020) (see **Table 8**).

Table 8 Overview of the study characteristics of the HL group.

Study/Year	N	Sex	Category	Age (mean±SD)	(BMI (kg/m ²); Fat Mass (%), VO_{2max} :mL/min/kg)	Control for antibiotic intake	Control for diet
Grosicki et al. 2019	1	male	Healthy lifestyle	32 years	BMI: 22.1 Body fat %: 14.8, VO_{2max} : 66.7	No	No
Hampton-Marcell et. al 2020	13	8 female, 5 male	Healthy lifestyle	18-24 years	fat mass 22.0 ±- 6.9 %	No	No
Keohane et al. 2019	4	male	Healthy lifestyle	26.5±1.3	BMI: 24.4±1.4, Body Fat%: 17.7±7.9, VO_{2max} : 48.16±2.8	Yes	Yes
Scheimann et al. 2019	25	NR	Healthy lifestyle	mean age 27	NR	Yes	Yes
Scheimann et al. 2019	87	NR	Healthy lifestyle	mean 29 years	NR	Yes	Yes
Taniguchi et al. 2018	31	males	Healthy lifestyle	62-76 years	E: BMI:22.9±2.5, Body Fat %: 21.0±5.0, VO_{2peak} : 24.5±4.1, C: 22.9, Body Fat %:20.6±5, VO_{2peak} : 26.0±5.3	Yes	Yes
Zhao et al. 2018	20	16 males/4 females	Healthy lifestyle	31.3±6.1	BMI 22.6±2.1	Yes	Yes

NR = Not reported; BMI =Body Mass Index

Unhealthy lifestyle group

The group of unhealthy lifestyles included six studies (Allen et al., 2018; Barton et al., 2018; Kern et al., 2020; Morita et al., 2019; Munukka et al., 2018; Santacruz et al., 2009). Studies included between 18 and 88 subjects with age ranged from 13 to 75 years. Two study only involved women (Morita et al., 2019; Munukka et al., 2018), the other studies involved men and women. Participants were predominantly overweight or obese, expect one study where subjects had a normal weight, but were sedentary (Morita et al., 2019). Across the groups, all studies showed a low VO₂max score. One study did not collect cardiorespiratory fitness of the individuals (Santacruz et al., 2009). All studies controlled for diet. Two of the studies did not control for antibiotic usage (Kern et al., 2020; Morita et al., 2019). **Table 9** gives an overview of the study characteristics of the UL group.

Table 9 Overview of the study characteristics of the UL group

Study/Year	N	Sex	Category	Age (mean±SD)	(BMI (kg/m ²); Fat Mass (%), VO ₂ max:mL/min/kg	Control for antibiotic intake	Control for diet
Allen et al. 2018	32	Lean: 9 female/ 9 male; Obese :11 female/3 males	Unhealthy lifestyle	Lean: 25 ± 6.52 (lean), Obese : 31.14±8.57	Lean: BMI:22.21±2.76, Body Fat %: 26.04 ±6.12, rel. VO2max: 38.99±4.74 Obese : BMI: 35.71±5.11,Body Fat % 38.42±4.98, rel. VO2max: 28.76±6.01	yes	yes
Cronin et al. 2018	74	E: 14 females/11 males, EP : 12 females, 10 males), rest= control	Unhealthy lifestyle	E: 35 (28, 38), EP : 32 (28,35)	E: BMI: ≈ 27.9, Body Fat %: ≈32.8, VO2max measured(no data), EP : BMI: ≈ 27.5, Body Fat %: ≈34.7, VO2max measured (no data)	yes	yes
Kern et al. 2020	88	49 % females	Unhealthy lifestyle	36 (30;41)	BMI: 29.7 (27.9; 31.4), Body Fat %: 38 (33;43), Vo2max: 30 (25.7; 33.1)	no	yes
Morita et al. 2019	29	females	Unhealthy lifestyle	70 (66-75)	BMI: 21.4 (18.8-23.1.), Body fat ≈ 29%, 6MWT for cardiorespiratory fitness assessed	no	yes
Munukka et al. 2018	17	females	Unhealthy lifestyle	36.8 ± 3.9	BMI: 31.8 ±4.4, Body Fat%: 43.6±5.4, VO2 max 29.5 ±5.24	yes	yes
Santacruz et al. 2009	36	18 females/18 males	Unhealthy lifestyle	14.5(13-15)	BMI: 32.8(29.4-35.2)	yes	yes

Patients

Six studies were included into the patient's group (Cronin et al., 2019; Liu et al., 2020; Motiani et al., 2020; Pasini et al., 2019; Paulsen et al., 2017; Shukla et al., 2015). Sample size ranged from 12 to 30 participants with an age from 25 to 72.5 years. Two studies involved men and women (Cronin et al., 2019; Motiani et al., 2020), one study only examined women (Paulsen et al., 2017), one study only examined men (Pasini et al., 2019) and two studies did not report the sex (Liu et al., 2020; Shukla et al., 2015). Participants were patients with diagnosis of Inflammatory Bowel Disease (IBD) (Cronin et al., 2019), subjects with prediabetes (Liu et al., 2020), sedentary, insulin-resistant subjects (Motiani et al., 2020), patients with diagnosis of Type 2 Diabetes (T2D) (Pasini et al., 2019), breast cancer survivors (Paulsen et al., 2017) and patients with a diagnosis of Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) (Shukla et al., 2015). **Table 10** gives an overview of the study characteristics of the patients group.

Table 10 Overview of the study characteristics of the patient group

Study/Year	N	Sex	Category	Age (mean±SD)	(BMI (kg/m ²); Fat Mass (%), VO _{2max} :mL/min/kg	Control for antibiotic intake	Control for diet
Cronin et al. 2019	13	4 females/ 9 males	Patient	25±6.5	BMI: 28.9 k/m ² ± 3.8, Body Fat %≈35 & receiving disease maintenance therapy	Yes	No
Liu et al. 2020	20	NR	Patient	R: 43.29 ±3.27, NR: 36.00± 4.55	R: BMI: 28.78±1.08, Body Fat %: 36.13 ±1.33, VO _{2max} : 25.27±1.30 mL/kg/min, NR: BMI: 29.82±1.75, Body Fat%: 35.78±1.75, Vo _{2max} : 26.83±1.38	Yes	Yes
Motiani et al. 2020	26	10 females/ 16 males	Patient	45-55	VO _{2peak} : <40mL*kg ⁻¹ *min ⁻¹	No	No
Pasini et al. 2019	30	males	Patient	70±2.3 year	BMI: 29.89±3.7, Body Fat%: 27.67±5.15, VO _{2max} : 16.87±2.74	Yes	Yes
Paulsen et al. 2017	12	females	Patient	55±-13	BMI 30.4±-5.1, VO _{2max} : 20.2±3.7	Yes	Yes
Shukla et al. 2015	20	NR	Patient	P: 48.6 ± 10.5 C: 46.5±13.0	P: BMI:23.9±4.3, VO _{2peak} : 28.6±9 , C: 24.6±3.3, VO _{2peak} : 28.2±9.6	Yes	No

3.4 Training Protocols

The next part will provide an overview of the training protocols and exercise effects of studies within the three groups: healthy lifestyle, unhealthy lifestyle and patients.

Healthy lifestyle (HL) group

Across the studies of the healthy lifestyle group, three studies investigated acute exercise effects (Scheiman et al., 2019; Zhao et al., 2018) and three studies chronic exercise effects (Allen et al., 2018; Grosicki et al., 2019; Hampton-Marcell et al., 2020; Keohane et al., 2019; Taniguchi et al., 2018). Only endurance-based disciplines were represented. Exercise interventions were of various designs. Keohane et al. (2019) followed rowers for a continuous, transoceanic race over a period of 33 days with a follow up after three months. Scheiman et al. (2019) observed marathon runners pre- and post-race over a period of 10 days and controlled them against sedentary controls. Also, the study investigated the effect of a single bout of exercise but no further description was given. Zhao et al. (2018) investigated the acute effect of running a half-marathon. Grosicki et al. (2019) observed an ultra-marathon runner for 21 weeks of training; a 100-mile race and post-race 10 days follow up. Hampton-Marcell et al. (2020) investigated the reduction of training volume in swimmers over a period of six weeks. Training volume started at peak season with 32.4 ± 4.8 km per week and was reduced to 11.3 ± 8.1 km per week at the end of week six. The study of Taniguchi et al. (2018) undertook ergometer sessions, three times per week over a period of five weeks controlled by a control group. After five weeks the groups changed to the other group design (cross-over). Intensity was gradually increased and the duration of each session ranged from 30 minutes to 90 minutes. A summary of the training protocol of healthy lifestyle groups can be found in **Table 11**.

Table 11 Physical Intervention characteristics of HL group

Author/Year	Exercise Effect	Type of exercise	Duration	Training description	Control group
Keohane et al. 2019	acute	continuous, unsupported, rowing race	33 days, 3 mth follow up	4998.66 km transoceanic rowing race in 33 days 22h, average distance 151.8km/day, crew split in 2 pairs (each rowing for 2 hrs/2hrs rest)	
Scheimann et al. 2019	acute	Experiment 1: Marathon	10 days	Running 42.195 km	sedentary control
	acute	Experiment 2: 1 bout of exercise	1 day	athletic specific exercise (not described)	
Zhao et al. 2018	acute	Half-marathon	1 day	Running 21,1 km	
Grosicki et al. 2019	chronic	Training and race period	21 wk	• 6 wks pre 21 weeks training ($\approx$ 115 km/ week running + little strength training) • 21 wk training block ($\approx$124 km running + 120 min strength + stretching) • 4-5 wks pre-race: 8 day training camp (177 km in total) • week 4: 50k trail race & 100 k run • WSER: 163 km mountain footrace • 10 days post-race no activity	
Hampton-Marcell et. al 2020	chronic	Phase 1: Peak training Phase 2: Start of taper Phase 3: End of taper	6 week	• Phase 1: 32.4$\pm$4.8 km/wk • Phase 2: 19.6$\pm$8.2 km/wk • Phase 3: 11.3$\pm$ 8.1 m/wk	
Taniguchi et al. 2018	chronic	exercise program	2 x 5 wk	3x/wk cycle ergometer sessions gradually increased 60% of preexercise VO ₂ peak during week 1, 70% during weeks 2 and 3, and 75% during weeks 4 and 5, 30min at week 1, 2 and 45 min week 3,4,5	vica versa control

Unhealthy lifestyle group

Across the six included studies, all investigated chronic effects of exercising (Allen et al., 2018; Cronin et al., 2018; Kern et al., 2020; Morita et al., 2019; Munukka et al., 2018; Santacruz et al., 2009). Across the studies, various types of intervention were used (see **Table 12**). Allen et al. (2018) carried out a supervised aerobic exercise training, three times per week for 30 to 60 minutes over a period of six weeks. Intensity was increased gradually. Cronin et al. (2018) examined three groups: exercise only, exercise plus protein supplementation and protein only group (control group). Participants of the first two groups undertook a mixed aerobic and resistance exercise training, three times per weeks over a period of eight weeks. Intensity was only increased in the resistance exercise part. Kern et al. (2020) compared four groups over a period of six months for five days a week. The groups received the following interventions: active commuting by bike, leisure-time exercise of moderate intensity, leisure-time exercise of vigorous intensity and a control group. Morita et al. (2019) split the participants in two groups. One group received resistance exercise once a week of 45 minutes. The other group was told to increase brisk walking with intensity of over 3 metabolic equivalents (METS) daily. Both interventions lasted for 12 weeks. Munukka et al.

(2018) carried out an endurance intervention in groups of two to four subjects, three times per week over six weeks. Santacruz et al. (2009) received one hour of moderate to vigorous intensity physical activity for 3 to 5 days a week over 10 weeks. Intensity was depending on the physical activity level and participant's energy intake was reduced by 10 - 40 %.

Table 12 Physical Intervention characteristics of UL group

Author/Year	Exercise Effect	Type of exercise	Duration	Frequency	Intensity	Control group
Allen et al. 2018	chronic	supervised aerobic exercise	6 wk	3x week, 30-60 min/session	increased HRR from 60% (wk1) to 75% HRR (w6)	
Cronin et al. 2018	chronic	Exercise only group (E): mixed aerobic and resistance exercise training program Exercise + whey protein (EP): (30g protein supplement + aerobic and resistance exercise training program)	8 wk	3x/week	aerobic training=moderate intensity based on couch-5km training, Resistance=70% of 1 RM, 3 sets / 8 Rep, increased 15-20%	only whey protein group (P) (30g protein supplement)
Kern et al. 2020	chronic	active commuting by bike (BIKE), leisure-time exercise of moderate (MOD or VIG), weekly EE of 1600kcal for women, 2100kcal for men)	6 month	5x/wk	MOD: 50% of VO ₂ peak reserve) or vigorous intensity (VIG, 70% of Vo ₂ peak-reserve	CON group
Morita et al. 2019	chronic	trunk muscle (TM) group (resistance training) ; AE (aerobic-exercise group)	12 wk	TM: 1x/ week a 45 min, AE: daily 60 min	brisk walking at an intensity of ≥ 3 metabolic equivalents	
Munukka et al. 2018	chronic	Endurance intervention and carried out in groups of 2–4 subjects)	6 wk	3x/wk	wk 1– 2 (3 x 40 min steady-state cycling of low intensity) ; Wk 3-4 (3x 50 min every second time interval training (3x 10 min interval, the rest steady speed) , Wk 5-6 3x 60 min interval training (4x10 min)	control period (6weeks pre exercise period)
Santacruz et al. 2009	chronic	PA	10 wk	1 hr/ day MHPA 3-5 days a week	depending on Level (15-23 kcal/kg body weight/wk) + energy restricted diet (10-40% reduction)	

Patients

Across the six studies including patients, one study examined acute effects of exercising (Motiani et al., 2020; Shukla et al., 2015) and five investigated chronic effects of exercising (Cronin et al., 2019; Liu et al., 2020; Pasini et al., 2019; Paulsen et al., 2017). Across the studies, various types of intervention were carried out (see **Table 13**). Shukla et al. (2015) undertook a maximal exercise challenge on an electronically braked cycle ergometer until total exhaustion in patients and healthy controls. In the remaining studies, frequency of exercises was mostly three times per week. Motiani et al. (2020) compared sprint interval training and moderate

intensity continuous training three times per week over 2 weeks. The SIT group intensity increased every 30 seconds until exhaustion with four minutes of recovery. Cronin et al. (2019) performed a mixed aerobic and resistance exercise program with increased intensity in the resistance exercise part. Also, Pasini et al. (2019) carried out a mixed exercise intervention, including aerobic, resistance and flexibility exercise for six months. Repetition of resistance exercises increased during intervention. Further, Liu et al. (2020) conducted a high-Intensity training, including aerobic and resistance exercises. One study handed out written information on the benefits of physical activity in breast cancer patients and motivated them to increase physical activity up to 150 minutes per week (Paulsen et al., 2017).

Table 13 Physical Intervention characteristics of the patient group

Author/Year	Exercise Effect	Type of exercise	Duration	Frequency	Intensity	Control group
Shukla et al. 2015	acute	maximal exercise challenge	1 days	electronically braked cycle ergometer	(5 W every 20 seconds (15 W/min) until total exhaustion)	healthy controls
Motiani et al. 2020	chronic	Sprint interval Training (SIT) & Moderate intensity continuous training (MICT)	2 wk	3 sessions/ week	SIT: each 30 sec of all out cycling, 4 min recovery, 4-6 exercise bouts, MICT: group 40- 60 min session at 60 %VO ₂ peak cycling	
Cronin et al. 2019	chronic	mixed aerobic and resistance exercise training program	8 wk	3x/week	aerobic training=moderate intensity based on couch-5km training, Resistance=70% of 1 RM, 3 sets / 8 Rep, increased 15-20%	control group for 8 wk and then cross over to exercise group
Liu et al. 2020	chronic	High-Intensity Exercise Training one-to-one manner, Combined aerobic and resistance training, after: seperated into responders /non responders depending on insulin resistance	12 wk	3x/wk a 70 min	10min warm up, HI Treadmill intervals station (10min, 85-95%VO ₂ max), HI resistance and chalistenics exercise station (10min,) and HI stationary bike intervals station(10min, 90-95%VO ₂ max), 10-15 min cool down, 3-4 min recovery between stations,	sedentary control
Paulsen et al. 2017	chronic	physical activity behaviour change intervention	3 month	up to 150 min PA /wk	All received written material regarding benefits of PA for breast cancer, one group (n=5) received PA behaviour change intervention to increase PA to 150min/week	
Pasini et al. 2019	chronic	endurance, resistance and flexibility training	6 month	3x/wk a 90 min/session	endurance cycling ergometer with increased Hr/session from 15 min to 35 min, Resistance = calistenics and repetition exercises whole body 3-8 rep until 12-15 rep with weights), Flexibility=static stretching	

3.5 Study Methods

Microbiome Analysis

Stool samples for gut microbiota profiling were used by all included studies. Samples and methodology characteristics of the included studies are shown in **Table 14**. The fecal sample collection varied from study to study. All of the studies collected fecal samples at baseline and after intervention. Additionally, some studies collected samples mid-intervention (Grosicki et al., 2019; Hampton-Marcell et al., 2020; Munukka et al., 2018; Taniguchi et al., 2018). One study accumulates stool samples continuous over a period of time, from 7 days pre-race to 7 days post-race (Scheiman et al., 2019). Four studies collected fecal samples as follow-up measurement (Allen et al., 2018; Grosicki et al., 2019; Keohane et al., 2019; Shukla et al., 2015). Sample transport varied from stool collection directly in laboratory or home self-collected samples. If fecal samples were home-collected, then samples were immediately put into the fridge. If samples were collected outside of homes or laboratories, samples were put into a DNA stabilizer until further analyzed. If reported, then DNA extraction was performed by different stool kits (Power Soil DNA kit, QIAmp stool minikit, Ubiome Explorer kit, OMNIgene kit, DNA and RNA Purification Kit and GTX stool kit). Across the included studies, three mainly used gut microbiota analysis techniques were: 16 rRNA gene amplification (n= 9), Metagenomic whole genome shotgun sequencing (n=5) and real-time PCR (n=1). 16S ribosomal RNA (rRNA) gene sequencing targeting the V4 or V3-V4, was used. Sequencing platforms across all studies were Illumina Miseq, Illumina Hiseq and Illumuni Nextseq. DNA analysis platform and reference databases varied from study to study. The outcomes of the study were alpha-diversity, beta diversity and gut microbiome alterations.

3.6 Risk of Bias

PEDro scores were only found for 5 out of 18 studies. Four studies scored 4/10 (Allen et al., 2018; Cronin et al., 2019; Kern et al., 2020; Motiani et al., 2020) and one study scored 5/10 (Taniguchi et al., 2018). Therefore, those studies were considered of moderate quality (*data not shown*).

Table 14 Sample collection and analysis methods

Author/ Year	Sample	Fecal sample timepoint	Sample transport	DNA Extraction	Gut microbiota analysis techniques	Sequencing Target	Sequencing platform	DNA Analysis Platform	Reference Sequence database
Allen et al. 2018	stool	Baseline (E0); after 6 weeks of exercising (E6) and after a 6 week washout period (W6)	directly to laboratory within 30 min	PowerSoil DNA Isolation Kit	16S rRNA gene sequencing	V4	Illumina Miseq	QIIME	RDp database
Cronin et al. 2019	stool	fecal sample 4 days prior and after exercise intervention	fresh within 6 hrs	QIAmp DNA stool minikit	Metagenomic whole genome shotgun		Illumina NextSeq	HUMAN2	MetaCyc database
Cronin et al. 2018	stool	fecal sample 4 days prior and after exercise intervention	fresh or cooled for not more than 12 hrs	QIAmp DNA stool minikit	Metagenomic whole genome shotgun		Illumina Hiseq	HUMAN2	
Grosicki et al. 2019	stool	4 times (baseline, week 19, week 21, week 23)	transport at ambient temperature	Ubiome Explorer kit	16S rRNA gene sequencing	V4	Illumina NextSeq		
Hampton-Marcell et al. 2020	stool	3 times (end of week 2, week 4 and week 6)	immediatly freezing	PowerSoil DNA Isolation Kit	16S rRNA gene sequencing	V4	Illumina Miseq	QIIME	Greengenes database
Keohane et al. 2019	stool	4 times (pre-race, mid-way point, immediatly before race finish and 3 mths post race)	stabilize until end of race	OMNigene kit	Metagenomic whole genome shotgun sequencing		Illumina NextSeq	HUMAN2	ChocoPHIAN database
Kern et al. 2020	stool	baseline, at 3mth and 6mth	immediatly freezing		16S rRNA gene sequencing	V4	Illumina Hiseq		
Liu et al. 2020	stool	before (0) and after 12 week exercise intervention2 responders and 2 non-responders: fecal microbial transplantaton	immediatly freezing or cooled and transported on ice to laboratory		Metagenomic whole genome shotgun sequencing		Illumina Hiseq		
Morita et al. 2019	stool	at baseline and post exercise (12 weeks)	DNA stabilizer, refrigerated and laboratory within 7 days		16S rRNA terminal restriction fragment length polymorphism analyses (T-RFLP)				Human Fecal Microbiota. T-RFLP profiling
Motiani et al. 2020	stool	before and after exercise intervention	immediatly freezing	DNA & RNA Purification Kit	16S rRNA gene sequencing	V3-V4	Illumina Miseq	QIIME	Greengenes database
Munukka et al. 2018	stool	pre intervention, mid intervention, post intervention	immediatly freezing	GTX stool kit	16S rRNA gene sequencing	V4	Illumina Miseq		
Pasini et al. 2019	stool	at baseline (o) ad six month (t6)			physical, growth and biochemical methods (grow organism in culture)				
Paulsen et al. 2016	stool	at baseline and after 3 mths	immediatly freezing		16S rRNA gene sequencing	V4	Illumina Miseq	QIIME	
Santacruz et al. 2009	stool	pre/post intervention			quantitative real-time PCR				
Scheiman et al. 2019	stool	collected the 7 days before until 7days after marathon	refrigerated, transported on ice and then freezing	PowerSoil DNA Isolation Kit	16S ribosomal DNA (rDNA) sequencing,				
Scheiman et al. 2019	stool	stool samples before and after exercising			shotgun metagenomic sequencing				
Shukla et al. 2015	stool	at baseline (0) and 48 hrs and 72 hrs after maximal exercise challenge	immediatly freezing	QIAmp DNA stool minikit	16S rRNA gene sequencing analysis				
Taniguchi et al. 2018	stool	samples baseline (0), end of exercise and end of period	bacterial growth culture and refrigerated	DNA 200 kit	16S rRNA gene sequencing	V3-V4	Illumina Miseq	QIIME	Greengenes database
Zhao et al. 2018	stool	morning before (BEF) the run, and morning after(AFT) the run			16S rRNA gene sequencing	V3-V4	Illumina Hiseq	QIIME	

3.7 Main Findings

3.7.1 Primary Outcome

The gut microbiome of patients could be influenced by diseases. Therefore, it has been decided together with the supervisor to analyze only results of healthy and unhealthy lifestyle groups. Primary outcomes of exercise on the gut diversity and gut taxonomy will be shown in the following chapter. Further, bacteria reported in two or more studies will be represented.

Healthy lifestyle (HL) group

Findings of gut diversity changes

In the group of healthy subjects, only two studies found a change in alpha diversity (Grosicki et al., 2019; Keohane et al., 2019) and no study did report an increase in beta diversity. Results were further separated into acute and chronic effect of exercise:

Acute effect of exercising on gut diversity

Keohane et al. (2019) reported an increase in alpha diversity in healthy athletes (n=3) up until end of 33-days rowing race and a decrease after three months follow up and found out that changes in training volume were significantly associated with shifts in microbial community structure. Running a half-marathon did not lead a significant difference in alpha diversity after finishing (Zhao et al., 2018)

Chronic effect of exercising on the gut diversity

Grosicki et al. (2019) explored that alpha diversity decreased, following 19 week of highly specific race preparation. Two hours post-race, changes in the gut microbiome were observed and alpha diversity (Shannon Diversity Index) increased. Hampton-Marcell et al. (2020) found out that alpha diversity was positively correlated by yardage per weeks. Beta diversity stayed the same across the observation. Additionally, a decrease in training volume over a short period influenced microbial diversity and community structure. Taniguchi et al. (2018) found no differences in alpha diversity between exercise and control period. Beta diversity was almost identical between exercisers and non-exercisers.

Table 15 Alpha and Beta diversity outcome in healthy lifestyle subjects

Author/ Year	Indices of α -Diversity	Gut Microbiota Diversity	α - Indices of β - Diversity	Gut Microbiota β -Diversity
Grosicki et al. 2019	Shannon Index	decreased following 19 week race preparation, 2hrs post race: alpha diversity increased	x	x
Hampton-Marcell et al 2020	Shannon Index	Alpha diversity was positively correlated by yardage per weeks	Bray-Curtis dissimilarity	Beta diversity between the 3 phases on average 43%
Keohane et al. 2019	Shannon Index	Increase in alpha diversity in healthy athletes (n=3) up until end of race and decrease after 3 mth follow up	x	x
Taniguchi et al. 2018	Shannon Index	No differences between exercise/control period	weighted and unweighted UniFrac metrics	almost identical between exercisers and non-exercisers time period
Zhao et al. 2018	NR	No significant difference after finishing the half-marathon	x	x
Scheimann et al. 2019	x	x	x	x

x= not measured, NR= not reported

Findings of gut taxonomy changes

Microbial Taxonomy changes in healthy lifestyle group were reported in all six studies. Different microbes increased or decreased depending on the mode of intervention. A change in microbial taxonomy of acute exercising was reported in three studies (Grosicki et al., 2019; Scheiman et al., 2019). The remaining studies (n=3) reported a change of microbial taxonomy due to chronic exercise effects.

The effect of acute exercise on microbial taxonomy

After a 33-day continuous, rowing race, Keohane et al. (2019) reported a decrease in *Bacteroides finegoldii* and an increase in *Dorea Longicatena*, *Roseburia hominis* and in unclassified members of *Subdoligranulum* post-race. After three months follow up any of the adaptations in gut microbiome community structure and metaproteomics persisted. Scheiman et al. (2019) reported that bacterial genus *Veillonella* was the most differently abundant between pre and post marathon and was more prevalent in runners than non-runners although it was not statistically significant. In contrast, running a half-marathon and testing the acute effect of it showed different results. Zhao et al. (2018) detected 6 OTUs after the run (15 before) which two are unknown: *Lentisphaerae* and *Acidobacteria* (phylum level). Also 20 bacterial clades were enriched post-run, while 7 taxa were more abundant in the before group. *Pseudobutyrvibrio*, *Coprococcus_2*, *Collinsella* and *Mitsoukella* (genus level) increased after running. *Coprococcus_2* was the most increased taxa. As a result, half-Marathon can strike changes in gut environment and changes in gut microbiome composition.

The effect of chronic exercise on microbial taxonomy

Grosicki et al. (2019) found out that Firmicutes/Bacteroidetes ratio was stable pre -race and nearly tripled two hours post-race due to 69 % reduction in Bacteroidetes. Proteobacteria increased more than fivefold post-race. Also, an increase in *Haemophilus* (29-fold), *Veillonella* (+14,229%) and *Streptococcus* (+438%) and a decrease in *Alloprevotella* (-79%) and *Subdoligranulum* (-50%) were examined. Reducing the exercise frequency in collegiate swimmers lead to a decrease of *Firmicutes* (Hampton-Marcell et al., 2020). During peak training period, Bacteroidetes/Firmicutes ratio was 2:1 and after reduction in training the ratio decreases to 1:1. The most abundant families were *Bacteroidaceae* (39.5 %), *Lachnospiraceae* (16.6 %) and *Ruminococcaceae* (14 %). Reduction in two genera: *Coprococcus* (10.7 %) and *Faecalibacterium* (5.7 %). A comparison of the microbiome of the same swimmers in 2016 and 2017 lead to the result, that 82 % of the core microbiome were stable over time. Aerobic training on an ergometers of a longer period lead to no significant changes in relative abundance of taxa (Taniguchi et al., 2018). Additionally, relative abundance of the *Oscillospira* genus significantly increased in during exercise period and the relative abundance of *Clostridium difficile* (species level) significantly reduced in the elderly.

Table 16 Taxonomy in healthy subjects and athletes

Kingdom	Domain	Phylum	Class	Order	Family	Genus	Species
Bacteria	Bacteria	↓ Bacteroidetes (4)	Bacteroidia	Bacteroidales	Bacteroidaceae	Alistipes	
					Prevotellaceae	↓ Alloprevotella (4) Bacteroides	↓ Bacteroides finegoldii (6)
		↓ Firmicutes (5)	Clostridia	Clostridiales	Clostridiaceae	Clostridium	↓ Clostridium difficile (17)
						↓ Subdoligranulum (4)	↑ unclassified members of Subdoligranulum (6)
						↑ Coprococcus (18)	
						↓ Coprococcus (5)	
					Ruminococcaceae	Roseburia	↑ Roseburia hominis (6)
						Ruminococcus	
						↑ Oscillospira (17)	
						↓ Faecalibacterium (5)	
					Lactobacillaceae	Dorea	↑ Dorea Longicatena (6)
						Lachnospira	↑ Pseudobutyrvibrio (18)
						↑ Streptococcus (4)	
						↑ Mitsuokella (18)	
					Veillonellaceae	↑ Veillonella (4, 15)	
		↑ Proteobacteria (4)	Gamma proteobacteria	Pasteurellales	Pasteurellaceae	↑ Haemophilus (4)	
		Lentisphaerae				↑ Collinsella (18)	

The table shows specific bacterias, which were reported to increase (green) or decrease (orange). The six studies were reported: Grosicki et al. 2019 (4); Hampton-Marcell et. al 2020 (5); Keohane et al. 2019 (6); Scheimann et al. 2019 (15); Taniguchi et al. 2018 (17); Zhao et al. 2018 (18).

Unhealthy lifestyle group

Findings of gut diversity changes

In the group of unhealthy subjects, two studies found an increase in alpha diversity (Grosicki et al., 2019; Keohane et al., 2019) and three studies did report an change in beta diversity (Grosicki et al., 2019; Keohane et al., 2019). Further, all studies explored chronic exercise effects. Therefore, to gain a deeper understanding, results were separated into chronic effects of endurance-based and mixed exercise interventions on gut diversity.

Chronic effect of endurance-based training on gut diversity

Allen et al. (2018) reported that six weeks of endurance-based exercise training had no effect on alpha diversity, neither pre nor post intervention. Beta diversity changed and resulted in no difference between lean and obese groups, also after washout. Munukka et al. (2018) found no differences in alpha diversity. In contrast, beta diversity increased in response to training.

Chronic effect of endurance and resistance training on gut diversity

Kern et al. (2020) reported that alpha diversity significantly increased at three months in VIG group and decreased in heterogeneity following vigorous intensity. Beta diversity changed in all exercise groups (BIKE; MOD; VIG) compared to CON. On the contrary, Cronin et al. (2018) found out that short-and medium-term combined training does not increase beta diversity. Post intervention bacterial diversity was greater in “Exercise plus protein” group.

Table 17 Alpha and beta diversity outcome in unhealthy lifestyle subjects

Author/ Year	Indices of α - Diversity	Gut Microbiota Diversity	α -	Indices of β - Diversity	Gut Microbiota β -Diversity
Allen et al. 2018	Chao1	No differences pre or post intervention		weighted and unweighted UniFrac metrics	before: beta diversity was different between lean/obese after: no differences between groups, also after washout
Cronin et al. 2018	Shannon Index	trend of median increase in bacterial diversity for E and EP, after intervention: diversity was greater in EP than E		PCoA	Short-and medium-term combined training does not increase beta diversity ,
Kern et al. 2020	Shannon Index	potential increase in alpha diversity in MOD group, but not bike group, Shannon's diversity index: significant increase at 3 mth in VIG group		Bray-Curtis dissimilarity, weighted UniFrac metrics	Beta diversity changed in all exercise groups compared to CON
Munukka et al. 2018	NR	after exercise: no difference in alpha diversity		Jaccard index	Beta Diversity increased in response to training
Morita et al. 2019	x	x		x	x
Santacruz et al. 2009	x	x		x	x

x= not measured, NR= not reported

Findings of gut taxonomy changes

All studies (n=6) report a change in microbial taxonomy. Chronic effects of endurance-based training was reported by three studies (Allen et al., 2018; Munukka et al., 2018; Santacruz et al., 2009) and chronic exercise effects in mixed aerobic and resistance training were reported by three studies (Cronin et al., 2019; Kern et al., 2020; Morita et al., 2019).

Chronic exercise effects of endurance-based interventions on changes in microbial taxonomy

Allen et al. (2018) reported changes in three bacterial genera (*Collinsella* spp., *Faecalibacterium* spp., *Lachnospira* spp.). Also, the study reported an increase in butyrate-regulating bacterial taxa (*Roseburia* spp., *Lachnospira* spp., *Chlostridiales* spp., *Faecalibacterium* spp., *f lachnospiraceae*). Return to sedentary behavior lead to reversion in gut microbiome composition (BMI dependent). Munukka et al. (2018) found out that training induced modest shifts in gut microbiota both at taxonomical and functional level. After control period no changes on gut microbiota were investigated. After exercise genus *Streptococcus* and *Proteobacteria* decreased and increase in *Verrucomicrobia*, *Verrucomicrobiaceae* and *Bifidobacteriaceae*, genus: *Dorea*, *Anaerofilum* and *Akkermansia*

increased but *Porphyromonadaceae*, *Odoribacter* *Desulfovibrionaceae* and *Enterobacteriaceae* decreased. Santacruz et al. (2009) increased counts of *B. fragilis* group and *Lactobacillus* group and decreased *C. coccoides* group, *Bididobacterium longum* and *Bididobacterium adolescentis* among whole samples after a physical activity intervention. After intervention, groups were divided into two groups: low weight-loss group (<2kg) and high weight-loss group (<4kg). No significant differences in the low weight group were found but this was different in the high weight loss group. High counts of total bacteria *B.fragilis*, and *Lactobacillus* group could be found, whereas *C.coccoides* and *B. longum* decreased after intervention.

Chronic exercise effects of mixed exercise interventions on changes in microbial taxonomy

The diversity of Archaea found by Cronin et al. (2018) was a significant altered post-intervention in the protein- group (control group), but not in the exercise or exercise-protein group. Biking, moderate or vigorous intensity exercise did not lead to changes in microbial taxonomy in (Kern et al., 2020). Morita et al. (2019) found an increase in relative abundance of Bacteroidetes in aerobic group (AE), but not in the trunk-muscle group (TM). Clostridium subcluster XIVa was increased in TM group and decreased in AE group. Other microbiota groups remained unchanged in both group post interventions. An increased of relative abundance of intestinal Bacteroides was marked in subjects which increased the time in brisk walking for more than 20 minutes.

Table 18 Overview of unhealthy lifestyle group on microbial taxa

Kingdom	Domain	Phylum	Class	Order	Family	Genus	Species
Bacteria	Bacteria	↑ Bacteroidetes (9)	Bacteroidia	Bacteroidales	Bacteroidaceae	Alistipes	
					↓ Porphyromonadaceae (11)	↓ Odoribacter (11)	
					Prevotellaceae	Bacteroides	↑ Bacteroides fragilis (14)
		Firmicutes	Clostridia	↑ Clostridiales spp.(1)	Clostridiaceae	Clostridium	↓ C. coccides (14)
							Clostridium subcluster XIVa ↑ (9)
						Roseburia	Clostridium subcluster XIVa ↓ (9)
							↑ Roseburia spp.(1)
					Ruminococcaceae	Ruminococcus	
						Faecalibacterium	↑ Faecalibacterium spp. (1)
						↑ Dorea (11)	
						Lachnospira	↑ Lachnospira spp. (1)
						↑ Anaerofillum (11)	
						↑ Lactobacillus (14)	
			Bacilli	Lactobacillales	Lactobacillaceae		
					Streptococcaceae	Streptococcus ↓ (11)	
		↑ Verrucomicrobia (11)	Gammaproteobacteria	Pasteurellales	↑ Verrucomicrobiaceae (11.)	↑ Akkermansia (11)	
					Pasteurellaceae		
				Enterobacterales	↓ Enterobacteriaceae (11)		
			Deltaproteobacteria	Desulfovibrionales	↓ Desulfovibrionaceae (11)		
		Actinobacteria			↑ Bifidobacteriaceae (11)	Bifidobacterium	↓ Bifidobacterium adolescentis (14)
							↓ Bifidobacterium longum (14)
		Lentisphaerae					↑ Collinsella spp (1)
	↓ Archaea (3)						
	↓ Virus (3)						

The table shows specific bacterias, which were reported to increase (green) or decrease (orange). The six studies were reported: Allen at al. 2018 (1); Cronin et al. 2018 (3); Kern et al. 2020 (7); Morita et al. 2019 (9), Munukka et al. 2018 (11); Santacruz et al. 2009 (14).

Changes in microbes across all groups

Across all studies (n=18) microbes were searched which were reported to have changed in two or more studies. Changes were found in *Bacteroidetes* (phylum taxa), *Coprococcus* (genus level), *Lachnospiraceae* (family taxa) and *Veillonella* (genus taxa). *Coprococcus*, *Lachnospiraceae* and *Veillonella* belong to the phyla “Firmicutes”. Similarities across the studies, which reported a change, was searched, but studies were too diverse in intervention types and characteristics to come to a final conclusion (Data not shown, see table **APPENDIX**)

➤ *Bacteroidetes*

Three studies reported an increase in *Bacteroidetes* (Morita et al., 2019; Motiani et al., 2020; Shukla et al., 2015) and one study a decrease in *Bacteroidetes* (Grosicki et al., 2019). Of the three studies, one study reported an increase in the unhealthy lifestyle group (Motiani et al., 2020) and two studies an increase in the patient group (Morita et al., 2019; Shukla et al., 2015). The decrease was found in the healthy lifestyle group (Grosicki et al., 2019).

➤ *Coprococcus*

Two studies reported a change in *Coprococcus*, from which one study found an increase (Zhao et al., 2018) and one study a decrease (Hampton-Marcell et al., 2020) of the microbe following intervention. Zhao et al. (2018) reported an increase in the healthy lifestyle group whereas Hampton-Marcell et al. (2020) reported a decrease in the healthy lifestyle group.

➤ *Lachnospiraceae*

Two studies found an increase in *Lachnospiraceae*, from which one was in the unhealthy lifestyle group (Allen et al., 2018) and one in patients (Liu et al., 2020).

➤ *Veillonella*

Three studies reported an increase in *Veillonella* post-intervention, two studies in the healthy lifestyle group (Grosicki et al., 2019; Scheiman et al., 2019) and one study in patients (Motiani et al., 2020).

3.7.2 Secondary Outcome

Physiological changes and improvements were reported in most studies. In the healthy lifestyle group, Grosicki et al. (2019) reported that VO₂ max and lactate threshold of the ultra-marathon runner were comparable to highly trained distance runners. In healthy elderly, VO₂peak significantly increased in the exercise period (Taniguchi et al., 2018). In contrast, Hampton-Marcell et al. (2020) examined that a reduction in training volume (32.6±4.8 km/week to 11.3±8.1 km/week) did not cause any significant changes in weight, fat mass or fat free mass. In the unhealthy lifestyle group, studies reported an improvement in body composition (Allen et al., 2018; Cronin et al., 2018; Kern et al., 2020) and an improvement in cardiorespiratory fitness (Allen et al., 2018; Cronin et al., 2018; Kern et al., 2020; Morita et al., 2019; Munukka et al., 2018). Interventions led to an improvement in physiological parameters across patients. In the exercise group compared total body fat decreased by 2.1 % and total lean tissue mass increased by 1.59 % (Cronin et al., 2019). Two studies reported improvements in body weight, fat mass and visceral fat (Liu et al., 2020; Motiani et al., 2020). Motiani et al. (2020) only found an increase in VO₂ peak after SIT training but not after MICT training whereas whole body fat decreased in both groups. Pasini et al. (2019) reported a decrease in weight, BMI, fat mass and waist circumference and an increase in lean mass and VO₂ max increased.

4 DISCUSSION

4.1 Summary of Evidence

The aim of the current systematic review was to examine the effect of exercise on the gut microbiome. Firstly, a distinction between changes in gut diversity and gut taxonomy was made. Secondly, exercise interventions were differed by acute and chronic effects. Thirdly, specific bacteria, which show an influence by training, were searched. The search identified 18 studies, which showed a large heterogeneity. Therefore, the included studies were grouped by three sample characteristics: healthy lifestyle group, unhealthy lifestyle group and patients. The results were only shown for the first two groups. In general, the findings suggested that exercise and physical activity interventions of various characteristics, have the potential to positively change the microbial diversity and microbial taxonomy. This was also found in previous reviews (Mitchell et al., 2019; Monda et al., 2017; Ortiz-Alvarez et al., 2020; Tzemah Shahar et al., 2020). Details, of findings within the groups will be discussed in detail.

Healthy lifestyle group

The healthy lifestyle group included mainly athletes in a setting of training or competition and one study included healthy elderly. Two studies showed that alpha diversity was increased after acute exercise (marathon and ultramarathon) (Grosicki et al., 2019; Keohane et al., 2019). This was not found in amateur runners finishing a half-marathon (Zhao et al., 2018). Neither acute nor chronic exercise was shown to have an effect on beta diversity in the healthy lifestyle group. These findings are consistent with findings of Mörkl et al. (2017), who reported that alpha diversity was highest in athletes, compared to Anorexia patients, normal weight and obese subjects. Further, the study also reported that beta diversity was not different between athletes and the other groups. On the contrary, beta diversity changes were reported in patients with psoriasis compared to healthy controls, whereas alpha diversity did not (Sikora et al., 2020). Microbial taxonomy changes were reported in all included studies. Both acute and chronic exercise intervention showed an effect on the gut taxonomy and changes were found in the bacteria domain at phyla, genus and species level. This was consistent with finding that in active individual's gut microbiota, which is known to inhibit a higher abundance of health promoting bacterial species (Mohr et al., 2020). An increase in genus *Veillonella* was reported in two studies (Grosicki et al., 2019; Scheiman et al., 2019). The bacteria of the genus *Veillonella* is known to grow well anaerobically on lactate (Kolenbrander, 2006). Fernández-Sanjurjo et al. (2020) criticised Scheiman et al. (2019) conclusion that *Veillonella* is a “performance-enhancing microbe that functions via the utilization of lactate”. In their

study in mice they did not find any association between *Veillonella atypica* and training status. It stays unclear if *Veillonella* can enhance performance in athletes. Further, two studies found a change in the Firmicutes/Bacteroidetes (F/B) ratio. In detail an increase in ratio was found post-race (Grosicki et al., 2019) and a decrease after reduction in training volume (Hampton-Marcell et al., 2020). One could argue that the two phyla *Firmicutes* and *Bacteroidetes* represent 90% of the gut microbiota (Arumugam et al., 2011). An altered F/B ratio has been associated various comorbidities such as cardiovascular diseases (Yoshida et al., 2018), obesity (Crovesy et al., 2020) and insulin resistance (Moreno-Indias et al., 2016), which considers the F/B ratio as an important marker of gut microbiota state. An interesting finding was that the relative abundance of *Clostridium difficile* was significantly reduced in elderly (Taniguchi et al., 2018). *Clostridium difficile* is responsible for infections worldwide, causes diarrhea due to production of toxins and is a major problem in hospitalized elderly (Kelly & LaMont, 2008; Lessa et al., 2015). Up until now, no other study was found that showed a similar effect. For the group of athletes, it would be important to know how performance could be influenced by changes in alpha diversity and to find out if *Veillonella* play an important role when for example running a marathon. This could lead to a better understanding how to boost performance through modulating the gut.

Unhealthy lifestyle group

The unhealthy lifestyle group included overweight or obese and/or sedentary individuals. Findings show that alpha and beta diversity could be influenced by chronic exercise. As already mentioned, Mörkl et al. (2017) showed that alpha diversity in overweight and obese participants was found to be lower compared to athletes. Allen et al. (2018) reported that alpha diversity did not differ between lean and obese before or after exercise intervention. This was proven by Lin et al. (2015), who found out that alpha diversity is independent of BMI, but beta-diversity is. Our findings also suggest that alpha diversity shows to be influenced by combined endurance and resistance training whereas beta diversity may only be influenced by endurance-based intervention. Denou et al. (2016) found out that High-Intensity Interval training (HIIT) influences alpha diversity in in diet-induce obese mice. Until now, no human studies have proved that yet. Summarized, it is suggested that beta-diversity is dependent on the obesity status whereas alpha-diversity is independent of BMI. More research is needed, to find out which type of exercise leads into positive changes in gut diversity. On the opposite, from our findings nothing is known about the effect of acute exercise on the gut diversity and gut taxonomy in individuals with an unhealthy lifestyle. Findings reported in the healthy lifestyle group indicate, that also acute exercise affects the

gut diversity. In this systematic review, gut taxonomy changes were reported in 5 out of 6 studies after chronic exercise interventions. Results show that endurance-based interventions influence various bacteria at family, genus and species level. Across studies different microbes were reported, which makes a comparison impossible. In fact, a study found out that in the gut microbiome in obese patients is different across the world (Medina et al., 2019). Sheng et al. (2018) suggested that an enrichment in *Akkermania muciniphila* could provide a possible therapy in the treatment of obesity. In this review, only one study reported an increase in genus *Akkermansia* (Munukka et al., 2018). Beside of dietary implementation to treat obesity, physical activity is known for its cost-effective treatment and is used for primary and secondary prevention. An important area of interest should be to include microbiome findings into physical activity interventions. It is important to know of how much exercise is needed to build up and maintain a healthy gut, independently of diet.

Patients

Results of patient were not reported, as each included study reported exercise intervention in different patient groups. The gut microbiome is influenced by various diseases. Therefore, the comparison across different diseases is not meaningful. A review of Sikora et al. (2020) showed that there is a significant association between alterations in the gut composition and psoriasis but also studies were of high heterogeneity. For the treatment of diseases, it would be important to find out of how exercise can help to build up a diverse and functional gut microbiome after medication intake. Findings of the healthy and unhealthy lifestyle group show that exercise has the potential to change gut diversity and gut taxonomy. Exercise intervention should be implemented in the treatment of patients (Motiani et al., 2020)

Changes in specific bacteria

Across all included studies (n=18), we searched for bacteria which were reported in two studies or more. Those four microbes were found: *Bacteroidetes*, *Coproccoccus*, *Lachnospiraceae*, and *Veillonella*. *Bacteroidetes* are the one of the most species in our gut (Rinninella et al., 2019), which explains that the majority of studies found a change. Especially the group of patients benefit from an increase in *Bacteroidetes*. A decrease was only found in athletes. As already mentioned, the Firmicutes/*Bacteroides* ratio is considered to give a better understanding of gut microbiota state. The F/B ratio considered an important marker for gut health (Crovesy et al., 2020) and should be preferred against the interpretation of *Bacteroidetes* alone. Changes in *Coproccoccus* had only been reported in athletes. A study of Murtaza et al. (2019) investigated the effect of dietary pattern with intensified training of elite race walkers. *Coproccoccus* was increased in athletes consuming a periodised Carbohydrate diet. It stays unclear if these microbes are affected by diet or training. *Lachnospiraceae* showed a change in unhealthy people and in patients. A study of Yu et al. (2018) examined the association of gut microbiota and reduced exercise capacity in elderly patients with hypertension. They found an enrichment in *Lachnospiraceae* in the group with a reduced exercise capacity. Further research is needed to find out if *Lachnospiraceae* can be considered a marker to detect potential pathogenesis. *Veillonella* shows to be affected by endurance-based intervention and was a change which was found in athletes and in patient. Earlier in this chapter we discussed the changes of *Veillonella* in athletes. Motiani et al. (2020) detected a significant higher abundance of *Veillonella* after moderate-intensity training in patient with endotoxemia. A recent study of Stokholm et al. (2018) reported that *Veillonella* was involved in the asthma emergence in childhood. Zhu et al. (2014) found out that *Veillonella* increased in elderly compared to adults. In summary, further research is needed to find out which function *Veillonella* has in different group of people.

Physiological changes

All studies reported a change in BMI, Body fat and cardiorespiratory fitness. As athletes are well-training, this group is not comparable to the other groups. As expected, exercise and physical activity intervention positively change body composition and cardiorespiratory fitness.

4.2 Strength and Limitations

Animal studies have found out that exercise can modulate the gut microbiome (O'Toole & Shiels, 2020). Previous reviews argued that mainly cross-sectional studies showing a link between physical activity and the gut microbiome were published. In the last 10 years, more and more experimental studies were published. A strength of this systematic review was the high number of included studies. This proves, that research in this topic is booming. Also, studies could be narrowed down by defining three groups. Another strength of this review was that studies used the same gut microbiota analysis techniques. 16 rRNA gene sequencing is a cheap and quick method, but shotgun metagenomics offers deeper insights into the taxonomy and function of microbes. Also, almost all studies used Shannon Index to report alpha diversity, whereas different Indexes were reported to beta diversity. Gut diversity is one tool that is able to characterize the microbiome. Precaution should be taken when only analysing gut diversity. Alpha diversity can still be similar, even though microbial shifts may experience a complete shift (Scepanovic et al., 2019).

This systematic was marked by many limitations. Even though the number of studies which were of experimental design, was high, the studies show a large heterogeneity in almost all areas. Studies had various study designs with various inclusion and exclusion criteria. Most studies controlled for diet and antibiotic intake but still some did not report it. From the introduction it should be remembered that gut microbiota is affected by the diet and antibiotic intake ((David et al., 2014; Dethlefsen & Relman, 2011). It is crucial that diet is controlled and not only assessed by various questionnaires. Within groups, samples were of different age ranges. It is well-known that an altered gut microbiome is correlated with age. Some studies did not report the sex. From literature it is known, that women have a significantly different gut microbiome composition overall and a lower abundance of Bacteroidetes compared with men (Domianni et al., 2015). Intervention had different types of training, durations, frequencies and intensities. In endurance-based intervention mainly running and cycling was investigated. There was no study, only focusing on resistance training. In the group of patients, no similarities between study characteristics including diagnosis or intervention were found. Also, some studies included a control group but the majority did not. Also, almost no study provided a follow-up of the participants. Except of the same used gut microbiota analysis technique, different DNA analysis platforms and reference databases were used. With an annotation error rate of around 10 %, the Ribosomal Database Project is considered to be the most accurate platform (Edgar, 2018). This database was only reported in one study (Allen et al., 2018). Each step in the microbiota profiling could be a potential risk of bias (Panek et al., 2018). At outcome level, results across groups were not comparable. Within studies, results gave an indication that exercise has the potential to influence gut diversity,

gut taxonomy and even some specific bacteria. There is a need of proven studies with the same setting to find a valid. Another limitation was that this review did not include the function of the different microbes as well as the role of metabolites.

Risk of bias was only found for five studies and those were of moderate quality. This is an important limitation of this systematic review. More studies with a high quality are needed. Also, data was only reviewed by the master thesis student. On top, the interpretation of the changes in the microbiome were diverse and require a deep knowledge in the field of molecular biology. Future research faces a lot of questions. A multidisciplinary approach of microbiologists, doctors and sport scientists is needed.

The next steps in the microbiome research could be the following:

- **Defining a healthy gut status**
- **Using Standard Protocols for Exercise Interventions**
- **Replicate studies with the same study and sample characteristics**

Nothing is known about how physical activity is influencing the gut in children. There is an urgent need as gut microbiota is known to be built up in early years and stays stable over the lifetime (Faith et al., 2013). It could be considered that exercise prescription is a cheaper and more effective way to enhance gut health compared to the intake of probiotic.

4.3 Conclusion

For a long time, the gut was not a topic of interest and nothing was known about the functions and potential of the microbes living in our gut. This changed as due technical processes made it possible and available. Now, microbiome, especially the gut microbiome is a popular topic of investment. Physical activity and exercising are known as a cost-effective method and used as primary and secondary intervention. A few years ago, studies started to explore the potential that exercise may modulate the gut microbiome. Animals models have already shown that. The included studies showed a large diversity in all areas. In summary, it is possible that alpha diversity is influenced by training volume and beta diversity is dependent on the BMI status. Gut taxonomy changes in all taxonomy levels have been found and four microbes have been associated to exercising. Evidence gives a hint that different exercise interventions would lead to different changes in our gut ecosystem. In conclusion, this review shows that the gut microbiome is different in healthy, unhealthy and diseased individuals and that training can affect different areas within the gut microbiome. Gut diversity and Gut taxonomy changes are only the beginnings of possible areas of how physical activity can improve gut health in humans. Further research is needed to understand and prove the mechanism of how exercise influences our gut microbiome.

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APPENDICES

APPENDIX 1: Inclusion and Exclusion process

Author	Year	Title	Include	Exclude	Cross sectional	Review	Overview	Outcome V02	Diet	Weight loss	Probiotic	Language	Study protocol	Initial	Other
			18	54	18	6	7	3	4	4	2	1	4	3	2
Allen et al.	2018	Exercise Alters Gut Microbiota Composition and Function in Lean and Obese Humans	x	•											
Bai & Bruner	2019	Composition of gut microbiota and its association with body mass index and lifestyle factors in a cohort of 7–18 years old children from the American Gut Project	x	•											
Barton et al.	2018	The microbiome of professional athletes differs from that of more sedentary subjects in composition and particularly at the functional metabolic level	x	•											
Bressa et al.	2017	Differences in gut microbiota profile between women with active lifestyle and sedentary women	x	•											
Carter et al.	2019	Gut microbiota diversity is associated with cardiorespiratory fitness in post-primary treatment breast cancer survivors	x	•											
Castellanos et al.	2020	Key bacteria in the gut microbiota network for the transition between sedentary and active lifestyle	x	•											
Castellanos et al.	2020	A Critical Mutualism – Competition Interplay Underlies the Loss of Microbial Diversity in Sedentary Lifestyle	x	•											
Castro-Mejia et al.	2020	Physical fitness in community-dwelling older adults is linked to dietary intake, gut microbiota, and metabolomic signatures	x					•							
Cheng et al.	2018	What Makes a 97-Year-Old Man Cycle 5,000 km a Year? Exercise and associated dietary extremes impact on gut microbial diversity	x												•
Clarke et al.	2014	Exercise has the guts; How physical activity may positively modulate gut microbiota in chronic and immune-based diseases	x	•											
Codella et al.	2018	Moderate-intensity aerobic and resistance exercise is safe and favorably influences body composition in patients with quiescent inflammatory Bowel Disease; a randomized controlled cross-over trial	x		•										
Cronin et al.	2019	A Prospective Metagenomic and Metabolomic Analysis of the Impact of Exercise and/or Whey Protein Supplementation on the Gut Microbiome of Sedentary Adults	x												
Cronin et al.	2018	Gut microbiota; implications for sports and exercise medicine	x	x		•									
D'Argenio & alvatore	2015	The role of the gut microbiome in the healthy adult status	x		•										
Durk et al.	2019	Gut microbiota composition is related to cardiorespiratory fitness in healthy young adults	x				•								
Estaki et al.	2016	Cardiorespiratory fitness as a predictor of intestinal microbial diversity and distinct metagenomic functions	x				•								
Frost et al.	2019	A structured weight loss program increases gut microbiota phylogenetic diversity and reduces levels of Collinsella in obese type 2 diabetics; A pilot study	x						•						
Grosicki et al.	2019	Rapid gut microbiome changes in a world-class ultramarathon runner	x												
Hampton-Marcell et al.	2020	Comparative Analysis of Gut Microbiota Following Changes in Training Volume Among Swimmers	x												
Hawley, J. A.	2020	Microbiota and muscle highway - two way traffic	x		•										
Hold, G. L.	2014	The gut microbiota, dietary extremes and exercise	x			•									
Huang, et al.	2019	Investigation of the Effects of Microbiota on Exercise Physiological Adaptation, Performance, and Energy Utilization Using a Gnotobiotic Animal Model	x											•	
Jang, et al.	2019	The combination of sport and sport-specific diet is associated with characteristics of gut microbiota; An observational study	x	•											
Keohane et al.	2019	Four men in a boat; Ultra-endurance exercise alters the gut microbiome	x												
Kern et al.	2020	Structured exercise alters the gut microbiota in humans with overweight and obesity-A randomized controlled trial	x												
Langsetmo et al.	2019	The Association between Objectively Measured Physical Activity and the Gut Microbiome among Older Community Dwelling Men	x	•											
Liang et al.	2019	Characteristics of the gut microbiota in professional martial arts athletes; A comparison between different competition levels	x	•											
Liu et al.	2014	Effect of aerobic exercise and low carbohydrate diet on pre-diabetic non-alcoholic fatty liver disease in postmenopausal women and middle aged men - The role of gut microbiota composition; Study protocol for the AELC randomized controlled trial	x										•		
Liu et al.	2020	Gut Microbiome Fermentation Determines the Efficacy of Exercise for Diabetes Prevention	x												
Louis et al.	2016	Characterization of the gut microbial community of obese patients following a weight-loss intervention using whole metagenome shotgun sequencing	x							•					
Marlicz & Loniewski	2015	The effect of exercise and diet on gut microbial diversity	x	x		•									
Moreno-Pérez et al.	2018	Effect of a Protein Supplement on the Gut Microbiota of Endurance Athletes; A Randomized, Controlled, Double-Blind Pilot Study	x					•							
Moreno et al.	2019	Lifestyle modulation of gut microbiota	x								•				
Morita et al.	2019	Aerobic Exercise Training with Brisk Walking Increases Intestinal Bacteroides in Healthy Elderly Women	x												
Mörkl et al.	2017	Gut microbiota and body composition in anorexia nervosa inpatients in comparison to athletes, overweight, obese, and normal weight controls	x	•											
Motiani et al.	2020	Exercise Training Modulates Gut Microbiota Profile and Improves Endotoxaemia	x												
Munukka et al.	2018	Six-Week Endurance Exercise Alters Gut Metagenome That Is not Reflected in Systemic Metabolism in Over-weight Women	x												
Murtaza et al.	2019	The effects of dietary pattern during intensified training on stool microbiota of elite race walkers	x					•							
Newton et al.	2019	Does exercise impact gut microbiota composition in men receiving androgen deprivation therapy for prostate cancer? A single-blinded, two-armed, randomised controlled trial	x										•		
O'Donovan et al.	2020	Distinct microbiome composition and metabolome exists across subgroups of elite Irish athletes	x	•											
Pane et al.	2018	Gut Microbiota, Probiotics, and Sport; From Clinical Evidence to Agonistic Performance	x			•									
Pasini et al.	2019	Effects of chronic exercise on gut microbiota and intestinal barrier in human with type 2 diabetes	x												
Paulsen et al.	2017	Gut microbiota composition associated with alterations in cardiorespiratory fitness and psychosocial outcomes among breast cancer survivors	x												
Pedersini et al.	2020	Gut microbiota and physical activity; Is there an evidence-based link?	x			•									
Petersen et al.	2017	Community characteristics of the gut microbiomes of competitive cyclists	x	•											
Potera, C.	2013	Running interference? Exercise and PCB-induced changes in the gut microbiome	x											•	
Rankin et al.	2017	Microbes in sport: The potential role of the gut microbiota in athlete health and performance	x			•									

APPENDIX 2: Study characteristics overview (n=18)

Study/Year	N	Sex	Age (mean±SD)	Category	Baseline Subjects Characteristic (BMI (kg/m ²); Fat Mass (%), VO _{2max} :mL/min/kg)	Assessment of parameters	Assessment of Cardiorespiratory Fitness	Control for antibiotics	Control for diet
Allen et al. 2018	32	Lean: 9 female/ 9 male; Obese : 11 female/3 males	Lean: 25 ± 6.52 (lean), Obese : 31.14±8.57	Unhealthy lifestyle	Lean: BMI: 22.21±2.76, Body Fat %: 26.04 ±6.12, rel. VO _{2max} : 38.99±4.74 Obese : BMI: 35.71±5.11, Body Fat % 38.42±4.98, rel. VO _{2max} : 28.76±6.01	Physical activity (GSLTQ; Godin-Leisure score) Body composition and Bone density (DEXA), blood samples	Maximal oxygen uptake test (Parco Medics True Max 2400, Sandy, UT) using the Bruce testing protocol	Yes	Yes
Cronin et al. 2018	74	E: 14 females/11 males, EP : 12 females, 10 males), rest= control	E: 35 (28, 38, EP : 32 (28,35)	Unhealthy lifestyle	E: BMI: ≈ 27.9, Body Fat %: ≈32.8, VO _{2max} measured (no data), EP : BMI: ≈ 27.5, Body Fat %: ≈34.7, VO _{2max} measured (no data)	Body composition (DEXA) • Blood composition • Urine composition • Resting heart rate • Sitting time (hours per week) • Motorized transport (hours per week)	Submaximal testing (Rockport one-mile walk test)	Yes	Yes
Cronin et al. 2019	13	4 females/ 9 males	25±6.5	Patient	BMI: 28.9 kg/m ² ± 3.8, Body Fat %≈35 & receiving disease maintenance therapy	• Body composition (DEXA) • disease activity scores (depending on disease) • Quality of life (SF36V2) • Anxiety and depression indices (HADS, STAI, BDI-II) • Serum pro-inflammatory cytokine measures	Submaximal testing (Rockport one-mile walk test)	Yes	No
Grosicki et al. 2019	1	male	32 years	Healthy lifestyle	BMI: 22.1 Body fat %: 14.8, VO _{2max} : 66.7	Body composition and Bone mineral content (DEXA), training volume (GPS watch)	Submaximal testing (4 Font, Woodway), Maximal VO _{2max} testing 8	No	No
Hampton-Marcell et. al 2020	13	8 female, 5 male	18-24 years	Healthy lifestyle	fat mass 22.0 ± 6.9 %	Body composition (Skinfold Thickness, Caliper) Training Volume (booklet)		No	No
Keohane et al. 2019	4	male	26.5±1.3	Healthy lifestyle	BMI: 24.4±1.4, Body Fat%: 17.7±7.9, VO _{2max} : 48.16±2.8	Body composition (DEXA)	VO ₂ max testing("7 x4min step" cardiorespiratory test protocol	Yes	Yes
Kem et al. 2020	88	49 % females	36 (30;41)	Unhealthy lifestyle	BMI: 29.7 (27.9; 31.4), Body Fat %: 38 (33;43), Vo _{2max} : 30 (25.7; 33.1)	Body composition (DEXA)	VO ₂ peak (indirect calimetry, graded exercise test)	No	Yes
Liu et al. 2020	20	NR	R: 43.29 ±3.27, NR : 36.00± 4.55	Patient	R: BMI: 28.78±1.08, Body Fat %: 36.13 ±1.33, VO _{2max} : 25.27±1.30 mL/kg/min, NR : BMI: 29.82±1.75, Body Fat%: 35.78±1.75, Vo _{2max} : 26.83±1.38	Max strength (1-RM Method), blood sample	VO ₂ max testing using Balke treadmill protocol	Yes	Yes
Morita et al. 2019	29	females	70 (66-75)	Unhealthy lifestyle	BMI: 21.4 (18.8-23.1), Body fat ≈ 29%, 6MWT for cardiorespiratory fitness assessed	• Body composition (BIA) • Muscle strength (Dynamometer), Physical performance tests (MSL, TUG, Single-Leg Standing, • Clinical laboratory analyses • Daily physical activity levels (number of steps via accelerometer) • Bowel habits (CAS-I)	Cardiorespiratory Fitness by 6 minutes walking test (6MWT)	No	Yes
Motiani et al. 2020	26	10 females/ 16 males	45-55	Patient	VO ₂ peak: <40mL*kg ⁻¹ *min ⁻¹	• Body composition (BIA) • Body fat (MRI) • Intestinal Glucose Uptake	VO ₂ Peak (Cycle Ergometer)	No	No
Munukka et al. 2018	17	females	36.8 ± 3.9	Unhealthy lifestyle	BMI: 31.8 ±4.4, Body Fat%: 43.6±5.4, VO ₂ max 29.5 ±5.24	• Body composition (DEXA) • Blood composition • Heart rate • Max. Muscle strength, (dynamometer), Muscle thickness (Ultrasound)	Incremental submaximal endurance exercise test (bicycle ergometer)	Yes	Yes
Pasini et al. 2019	30	males	70±2.3 year	Patient	BMI: 29.89±3.7, Body Fat%: 27.67±5.15, VO _{2max} : 16.87±2.74	• Body composition • Biochemical measurements		Yes	Yes
Paulsen et al. 2017	12	females	55±13	Patient	BMI 30.4±5.1, VO _{2max} : 20.2±3.7	Anxiety and depression levels (Hospital Anxiety and Depression Scale Question- naire). • Fatigue intensity (Fatigue Symptom Inventory Questionnaire) • Sleep dysfunction (Pittsburgh Sleep Quality Index).	Submaximal treadmill test	Yes	Yes
Santacruz et al. 2009	36	18 females/18 males	14.5(13-15)	Unhealthy lifestyle	BMI: 32.8(29.4-35.2)	• Body composition (Skinfold Thickness - Caliper)		Yes	Yes
Scheimann et al. 2019	25	NR	mean age 27	Healthy lifestyle	NR	Questionnaire to collect health, dietary and athletic background information (adapted from The American Gut)		Yes	Yes
Scheimann et al. 2019	87	NR	mean 29 years	Healthy lifestyle	NR	Questionnaire to collect health, dietary and athletic background information (adapted from The American Gut)		Yes	Yes
Shukla et al. 2015	20	NR	P: 48.6 ± 10.5 C:46.5±13.0	Patient	P: BMI:23.9±4.3, VO ₂ peak: 28.6±9. C: 24.6±3.3, VO ₂ peak: 28.2±9.6	• Body composition • Blood composition • Urine composition • Symptom assessment (4 Questioners) • Physical activity level (self-reported)	Maximal exercise test, protocol (Cook et al.)	Yes	No
Taniguchi et al. 2018	31	males	62-76 years	Healthy lifestyle	E: BMI:22.9±2.5, Body Fat %: 21.0±5.0, VO ₂ peak: 24.5±4.1. C: 22.9, Body Fat %:20.6±5, VO ₂ peak: 26.0±5.3	Body composition (magnetic resonance spectroscopy) • Blood composition • Resting blood pressure • Arterial stiffness (cardio-ankle vascular index CAVI)	Maximal-graded exercise test (VO ₂ peak)	Yes	Yes
Zhao et al. 2018	20	16 males/4 females	31.3±6.1	Healthy lifestyle	BMI 22.6±2.1	• Body composition (only BMI) • Training (in month)		Yes	Yes

APPENDIX 3: Gut taxonomy changes across all studies (n=18)

Kingdom	Domain	Phylum	Class	Order	Family	Genus	Species										
Bacteria	Bacteria	Bacteroidetes ↑ (9,10,16)	Bacteroidia	Bacteroidales	Bacteroidaceae	Alistipes	↓ Alistipe shahii(8) ↑ Alistipe shahii(8)										
		Bacteroidetes ↓ (4)				↓ Alloprevotella (4)											
		↓ Porphyromonadaceae (11)				↓ Odoribacter (11)											
		Prevotellaceae				Bacteroides	↑ Bacteroides fragilis (14) ↓ Bacteroides finegoldii (6) ↓ Bacteroides xylanisolvens (8)										
		↓ Firmicutes (5)	Clostridia	Clostridiales	Clostridiaceae	↑ Anaerofilum (11)											
		↑ Clostridiales spp.(1)		Blautia		↓ Blautia spp (10)											
						Clostridium		↓ C.coccides (14)									
								↓ Clostridium difficile (17)									
								Clostridium subcluster XIVa ↑ (9) & ↓ (9)									
								↓ Clostridium spp. (10)									
						↓ Subdoligranulum (4)	↑ unclassified members of Subdoligranulum (6)										
		↑ Lachnospiraceae (1 & 8.)		Coprococcus ↓(5) ↑ (18)													
				Roseburia		↑ Roseburia hominis (6)											
				Ruminococcaceae		↑ Roseburia spp.(1) ↓ Ruminococcus ganvus (8)											
				↑ Oscillospira (17)													
				↓ Faecalibacterium (5)		↑ Faecalibacterium spp. (1)											
				↑ Dorea (11)		↑ Dorea Longicatena (6)											
				↑ Lachnospira (10)		↑ Lachnospira spp. (1)											
						↑ Pseudobutyrvibrio (18)											
						Lactobacillus spp											
						Streptococcus ↓ (11)	↑ Streptococcus spp (1)	↑ Streptococcus spp (1)	↑ Streptococcus spp (1)	↑ Streptococcus spp (1)	↑ Streptococcus spp (1)						
						Streptococcus ↑ (4)	↑ Streptococcus spp (1)	↑ Streptococcus spp (1)	↑ Streptococcus spp (1)	↑ Streptococcus spp (1)	↑ Streptococcus spp (1)						
		↑ Veillonella (4, 10, 15)	↑ Veillonella (4, 10, 15)	↑ Veillonella (4, 10, 15)	↑ Veillonella (4, 10, 15)	↑ Veillonella (4, 10, 15)	↑ Veillonella (4, 10, 15)										
		↑ Mitsukella (18)	↑ Mitsukella (18)	↑ Mitsukella (18)	↑ Mitsukella (18)	↑ Mitsukella (18)	↑ Mitsukella (18)										
		↑ Verrucomicrobia (11)	Gammaproteobacteria	Pasteurellales	↑ Verrucomicrobiaceae (11.)	↑ Akkermansia (11)											
		↑ Proteobacteria (4)		Pasteurellaceae	↑ Haemophilus (4)												
				Enterobacteriales	↓ Enterobacteriaceae (11)												
		Deltaproteobacteria		Desulfotribionaceae (11)													
		Acidobacteria															
		Actinobacteria															

Unhealthy lifestyle		Healthy lifestyle		Patient	
Number	Author/year	Number	Author/year	Number	Author/year
1	Allen et al. 2018	4	Grosicki et al. 2019	2	Cronin et al. 2019
3	Cronin et al. 2018	5	Hampton-Marcell et. al 2020	8	Liu et al. 2020
7	Kem et al. 2020	6	Keohane et al. 2019	10	Motiani et al. 2020
9	Morita et al. 2019	15	Scheimann et al. 2019	12	Pasini et al. 2019
11	Munukka et al. 2018	17	Taniguchi et al. 2018	13	Paulsen et al. 2016
14	Santacruz et al. 2009	18	Zhao et al. 2018	16	Shukla et al. 2015

STATUTORY DECLARATION

I, Bianca Heschl, declare that I have authored this thesis independently, that I have not used other than the declared sources / resources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

1st October 2020

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Date

A rectangular box containing a handwritten signature in blue ink that reads "B. Heschl".

.....

(signature)