JANNINA VILJAKAINEN-DIOP

EATING HABITS, BODY MASS AND SALIVA MICROBIOTA IN FINNISH ADOLESCENTS

FACULTY OF MEDICINE
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DOCTORAL DISSERTATION

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Pour mon mari et notre fille

ABSTRACT

Healthy eating habits, such as the consumption of fruits and vegetables and eating regular meals, reduce the risk of being overweight and obese in childhood as well as other noncommunicable diseases. However, eating habits have been examined only to a limited extent in Finland, such that further and updated information on eating habits and body mass is needed among adolescents. Healthy eating habits maintain health. Moreover, dietary choices and food timing shape the gut microbiota, although less is known about their relevance to saliva microbiota.

This study aims to: 1) identify the eating habits of Finnish adolescents; 2) examine the associations between eating habits and body mass; and 3) examine the associations between eating habits and the saliva microbial diversity and composition.

The study dataset consisted of 9- to 14-year-old Finnish adolescents participating in the Finnish Health in Teens (Fin-HIT) study between 2011 and 2014. Study I and study II consisted of 10 569 participants from 496 schools in 44 municipalities in southern, middle, and northern Finland. Study III included 842 randomly selected participants from the Fin-HIT study. These participants answered a web-based questionnaire on an electronic tablet. The questionnaire assessed lifestyle factors, such as diet and eating-related health behaviours. Participants also provided unstimulated saliva samples and trained fieldworkers measured their height and weight in a standardised way in school. Measurements used to calculate body mass index (BMI). BMI was categorised as underweight, normal weight and overweight or obese.

The results from the current study identified three eating habit groups in adolescents: ‘healthy eaters’ (4 661; 44.1%), ‘unhealthy eaters’ (1 298; 12.3%) and ‘fruit and vegetable avoiders’ (4610; 43.6%). Healthy eaters most frequently ate a regular breakfast and other meals (lunch and dinner) and had parents with a high education level. Unhealthy eaters, however, most frequently ate breakfast and other meals irregularly and had parents with a low education level (study I). Avoiding fruits and vegetables was associated with a high risk of being underweight. Eating breakfast irregularly was associated with a high risk of being overweight and obese and a decreased risk of being underweight. Similarly, eating dinner irregularly was associated with a decreased risk of being underweight (study II). More diverse saliva microbiota composition was observed among regular breakfast eaters compared to irregular breakfast eaters. Similarly, a tendency for a higher diversity in the saliva microbiota was found among regular dinner eaters compared to irregular dinner eaters. More specifically, *Prevotella* was highly abundant among fruit and vegetable avoiders and irregular breakfast and dinner eaters (study III).

The results presented here on eating habits emphasise that more attention must be devoted to adolescents' eating habits, particularly to those who avoid fruits and vegetables and those who irregularly eat meals since they are at high risk for adverse health outcomes, such as being overweight and obese. Futhermore, the results here on eating habits and body mass will assist public health personnel in guiding adolescents regarding how to maintain a normal weight. The association between avoiding fruits and vegetables and eating irregular meals, respectively, and the abundance of *Prevotella* in saliva warrants further studies on *Prevotella* and eating habits.

TIIVISTELMÄ

Terveelliset ruokailutottumukset, kuten hedelmien ja kasvisten nauttiminen ja säännöllinen ateriointi, pienentävät ylipainon ja lihavuuden sekä muiden kroonisten tautien riskiä lapsuusiässä. Ruokailutottumuksia on Suomessa tutkittu suppeasti, jonka vuoksi päivitettyä tietoa nuorten ruokailutottumuksista ja painoindeksistä tarvitaan lisää. Terveelliset ruokailutottumukset tukevat terveyttä. Lisäksi ruokavalinnat ja aterioiden ajoitus muokkaavat suoliston mikrobiotaa, vaikka niiden merkityksestä syljen mikrobiostolle tiedetään vielä vähän.

Tämän tutkimuksen tavoitteina oli: 1) tunnistaa ruokailutottumusten piirteitä suomalaisilla nuorilla; 2) tutkia ruokailutottumusten ja painoindeksin yhteyttä; ja 3) tutkia ruokailutottumusten yhteyttä syljen mikrobiotan monimuotoisuuteen ja koostumukseen.

Tutkimusaineisto koostuu 9–14-vuotiaista suomalaisista nuorista, jotka osallistuivat Hyvinvointi Teini-iässä (Fin-HIT) –tutkimukseen vuosina 2011–2014. Ensimmäinen ja toinen osatutkimus koostui 10 569 osallistujasta, jotka osallistuivat 496 koulusta yhteensä 44 kunnassa Etelä-, Keski- ja Pohjois-Suomessa. Kolmas osatutkimus koostui 842 satunnaisesti valitusta tutkittavasta, jotka osallistuivat Fin-HIT-tutkimukseen. Osallistujat vastasivat internetissä kyselylomakkeeseen elektroniselta tabletilta. Kyselylomake arvioi elintapatekijöitä, kuten ravitsemusta ja ruokailuun liittyvää terveyskäyttäytymistä. Tutkittavat antoivat stimuloimattomat sylkinäytteet ja koulutetut kenttätyöntekijät mittasivat pituuden ja painon standardisoidulla tavalla koulussa. Mittoja käytettiin painoindeksin laskemiseen. Tutkittavat jaettiin painoindeksin perusteella alipainoon, normaalipainoon ja ylipainoon tai lihavuuteen.

Tässä tutkimuksessa löydettiin kolme ruokailutottumusryhmää nuorilla: terveellisesti syövät (4 661; 44,1 %), epäterveellisesti syövät (1 298; 12,3 %) ja hedelmien ja kasvisten välttelijät (4 610; 43,6 %). Terveellisesti syövät nuoret söivät säännöllisemmin aamupalan ja muita aterioita (lounas ja illallinen) ja heidän vanhempansa olivat korkeammin koulutettuja. Sen sijaan epäterveellisesti syövät nuoret söivät epäsäännöllisemmin aamupalan ja muita aterioita ja heidän vanhempansa olivat matalammin koulutettuja (osatutkimus I). Nuoret, jotka välttelivät kasviksia ja vihanneksia, olivat useammin alipainoisia. Nuoret, jotka söivät epäsäännöllisen aamupalan, olivat useammin ylipainoisia ja lihavia ja harvemmin alipainoisia. Samoin nuoret, jotka söivät epäsäännöllisen illallisen, olivat harvemmin alipainoisia (osatutkimus II). Syljen monimuotoisen (diversiteetin) mikrobiotan koostumus havaittiin runsaammaksi nuorilla, jotka söivät säännöllisen aamupalan, verrattuna niillä, jotka söivät epäsäännöllisen aamupalan. Samoin runsaampi syljen mikrobiotan monimuotoisuus löydettiin nuorilla, jotka söivät säännöllisen illallisen, verrattuna niillä, jotka söivät epäsäännöllisen

illallisen. Erityisesti, *Prevotella*-bakteeria oli runsaasti nuorilla, jotka välttelivät kasviksia ja vihanneksia, ja söivät epäsäännöllisesti aamupalan ja illallisen (osatutkimus III).

Ruokailutottumusten tulokset korostavat, että nuorten ruokailutottumuksiin tulisi kiinnittää enemmän huomiota, etenkin niihin nuoriin, jotka välttelevät hedelmiä ja kasviksia sekä syövät aterioita epäsäännöllisesti, koska he ovat alttiita terveydellisille haittavaikutuksille, kuten ylipainolle ja lihavuudelle. Lisäksi, ruokailutottumusten ja painoindeksin tulokset auttavat henkilöitä, jotka edistävät kansanterveyttä ja pystyvät opastamaan nuoria ylläpitämään normaalia painoa. Kasvisten ja hedelmien välttelemisen ja aterioiden epäsäännöllisen syömisen yhteys syljen *Prevotella*-bakteerin runsaaseen määrään antaa aihetta jatkotutkimuksille *Prevotella*:sta ja ruokailutottumuksista.

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This doctoral thesis is based on the following publications:

I De Oliveira Figueiredo RA, Viljakainen J, Viljakainen H, Roos E, Rounge TB*, Weiderpass E*. Identifying eating habits in Finnish children: a cross-sectional study. *BMC Public Health* 2019; 19:1-11.

II Viljakainen J, Figueiredo RAO, Viljakainen H, Roos E, Weiderpass E*, Rounge TB*. Eating habits and weight status in Finnish adolescents. *Public Health Nutrition* 2019; 22:2617-2624.

III Viljakainen J, Raju SC, Viljakainen H, Figueiredo RAO, Roos E, Weiderpass E*, Rounge TB*. Meal regularity plays a role in shaping the saliva microbiota. *Frontiers in Microbiology* 2020; 11:1-11.

Authors marked with an * indicate an equal contribution.

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ABBREVIATIONS

ANCOVA	Analysis of co-variance
BMI	Body mass index
CI	Confidence interval
FAO	Food and Agriculture Organisation of the United Nations
FDR	False discovery rate
FFQ	Food frequency questionnaire
Fin-HIT	Finnish Health in Teens
GI	Gastrointestinal
HBSC	Health behaviour in school-aged children
IOTF	International Obesity Task Force
ISO–BMI	Children's body mass index
KMO	Kaiser-Meyer-Olklin
OR	Odds ratio
OTUs	Operational taxonomic units
PC	Principal component
PERMANOVA	Permutational analysis of variance
rRNA	Ribosomal RNA
SSBs	Sugary-sweetened beverages
TS	TruSeq
WHO	World Health Organisation

1 INTRODUCTION

Healthy eating habits play a role both in health and disease (1). Healthy eating habits including a healthy diet (2) are associated with eating healthy foods, avoiding unhealthy foods and having regular meals such as breakfast, lunch and dinner (3). Healthy eating habits are typically established in adolescence, a time considered the transitional period to adulthood (4). This period is also important in terms of adopting healthy eating habits. Healthy eating habits involve, for instance, the high consumption of fruits and vegetables (5–7) and whole-grain foods (2, 8), while unhealthy eating habits consist of, among other things, the high consumption of sweets and sugary-sweetened beverages (SSBs) (9–10) and skipping breakfast (11–12). Currently, adolescents eat unhealthy foods such as SSBs and salty snacks (13), and eat fewer fruits and vegetables than recommended (13–14). The World Health Organisation (WHO) and the Food and Agriculture Organisation of the United Nations (FAO) currently suggest consuming at least 400 g of fruits and vegetables per day (15).

Several studies have found an association between the frequent consumption of unhealthy foods, including sweets and SSBs, and being overweight and obese (10). Unhealthy eating habits have also been associated with being underweight (16). Additionally, skipping breakfast has been strongly associated with being overweight and obese among adolescents (11–12, 17–21). Yet, the relationship between body mass and other meals, such as lunch (22–23) and dinner (24) or family dinner (25–27), remain less conclusive.

Healthy eating habits are also important for shaping the human microbiota composition (28). Moreover, diverse ecosystems are advantageous for human microbiota (29). Research indicates that healthy eating habits alter the gut microbiota (28, 30–32). For instance, a high abundance of *Prevotella* in the gut was found among children with a plant-based diet (32). However, inconsistency exists between healthy eating habits and saliva microbiota, since the saliva diversity and composition did not differ between vegans, ovo-lacto-vegetarians and omnivores (33). Yet, another study (34) reported that vegans had a high abundance of *Neisseria* and less *Prevotella* in their saliva. Lastly, little evidence exists on the timing of eating and saliva microbiota (35), and no previous research has examined the association between eating habits and saliva microbiota in adolescents.

Therefore, this thesis aims to identify the main eating habits of Finnish adolescents. Additionally, this thesis examines the associations between eating habits and body mass as well as the associations between eating habits and the saliva microbial diversity and composition.

2 REVIEW OF THE LITERATURE

2.1 Eating habits

2.1.1 Definition of eating habits

Healthy eating habits consist of eating healthy foods and avoiding unhealthy foods (3). Healthy eating habits are influenced by socio-environmental factors, including parental permissiveness and school support (36). Additionally, healthy eating habits contribute to both boosting health and limiting disease development, such as obesity, hypertension and stroke (1). Healthy eating includes the consumption of healthy foods (3) such as a high intake of fruits and vegetables (5–7) and whole-grain foods (2, 8), and a low intake of processed meat (37).

Unhealthy eating habits including the consumption of unhealthy food items such as SSBs and sweets are harmful to one's health (9–10). Moreover, skipping breakfast (11–12) and following an irregular meal pattern (24, 38) are also considered unhealthy eating habits and appear unbeneficial to health. Thus, improving the quality of healthy eating habits by decreasing the consumption of unhealthy foods and preferring regular meals are vital to one's well-being. In this study, eating habits consisted of the frequency of food items and breakfast and meals such as lunch and dinner.

2.1.2 Eating habits among adolescents

Adolescence is the transitional period before reaching adulthood when healthy eating habits are adopted (4). Therefore, the importance of adopting healthy eating habits is emphasised during adolescence. The current challenge is that adolescents eat more sweets together with SSBs and salty snacks such as potato crisps than recommended (13). In Finland, sugary snacks and SSBs are also popular among adolescents (14). Moreover, the low consumption of fruits and vegetables both at the international (13) and national levels (14) and skipping breakfast (19, 39) and other meals (24) appear common among adolescents. A worrying issue is that unhealthy eating habits including the irregular consumption of breakfast increases the risk of adverse health outcomes, such as being overweight and obese among adolescents (11–12, 17–21).

Although daily sweets consumption has decreased in all Nordic countries (40), there is still an urgent need to improve healthy eating habits including increasing the consumption of fruits and vegetables among Nordic adolescents. As such, the Nordic Nutrition Recommendations advocate avoiding the abundant consumption of sugar-rich foods and restricting SSBs and salt. Futhermore, they recommend favouring vegetables, fish and poultry instead of processed meat and red meat (41). WHO and FAO recommend consuming at least 400 g of fruits and vegetables per day (15). Similarly, the

Finnish Nutrition Guidelines emphasise increasing the consumption of fruits and vegetables by up to 500 g per day and following a regular meal pattern including breakfast, lunch and dinner along with two snacks (42).

Healthy eating habits also differ based on gender and age groups. In particular, girls appear to eat breakfast less than daily compared to boys (14, 19, 43). Gender differences were also reported according to the consumption of fruits and vegetables and SSBs. For instance, a low daily intake of fresh vegetables is more common in boys than in girls (14, 44). Boys also appear to drink more SSBs than girls (14). In addition to gender, irregular breakfast consumption has been linked to an older age (19, 43). Thus, awareness of gender and age differences in eating habits is warranted.

2.2 Body Mass

2.2.1 Definition of body mass

Body mass is derived from BMI [weight (kg)/ height squared (m^2)]. Different reference measurements exist, such as those from WHO (45), ISO–BMI (children's body mass index) (46) and the International Obesity Task Force (IOTF) (47), which are used to categorise body mass among adolescents. In this study, body mass is categorised as underweight, normal weight and overweight or obese referring to IOTF age- and gender-specific cut-off points (47). The IOTF cut-off curves are as follows: <18.5 kg/m^2 for underweight, 18.5 kg/m^2 to 25 kg/m^2 for normal weight, 25 kg/m^2 to 30 kg/m^2 for overweight and >30 kg/m^2 for obesity (47).

2.2.1.1 Measurements

Typically, height and weight measurements rely on either self-reports by adolescents or are measured by someone such as a fieldworker from the study. Self-reported weight is typically underestimated (48), whilst self-reported height is typically overestimated compared to when the values are measured by study personnel (49). Weight can be measured on an electronic scale while adolescents are wearing light indoor clothing (49). In addition, a portable stadiometer can be used to measure height (48). Both the measured weight and height are used to calculate BMI [weight (kg)/ height squared (m^2)]. The classification of BMI into categories differs between the reference values from ISO–BMI, WHO and IOTF. Additionally, the reference values vary between countries. Furthermore, ISO–BMI refers to the Finnish age- and gender-specific cut-off points, which correspond to adult BMI values in children and adolescents (46). Yet, the WHO reference values are based on growth charts for healthy children in the US (45). IOTF uses more recent data from six reference populations worldwide and is widely used in studies on children and adolescents (45). Gonzalez-Casanova et al. (50) also reported differences between the reference values used by WHO and IOTF. The WHO classification yielded the highest prevalence of being overweight and obese in both genders, whereas the IOTF classification reported the lowest prevalence of being overweight and obese among females (50). When choosing the reference values for BMI, it is important that those values are based on recent information vis-à-vis adolescents' body mass.

2.2.2 Prevalence of being underweight, overweight and obese

Being overweight and obese in childhood have increased globally (51–52), while very recent studies have reported a plateau in mean BMIs in more affluent countries, including Finland (52). The prevalence of childhood overweight and obesity increased by 47% among 2- to 19-year-olds worldwide between 1980 and 2013 (51). In turn, being underweight has declined in recent years (52). According to the Register of Primary Health Care Visits (Avohilmo), 24% of 2- to 16-year-old children were overweight and obese in Finland in 2014 and 2015 (46). In 2018, 29% of Finnish boys and 18% of Finnish girls aged 7- to 12-years old were overweight (53). Obesity was more common among 7- to 12-year-old Finnish boys (9%) than among girls (3%) in 2018 (53). Yet, data from the Fin-HIT study shows that 11.0% of 9- to 14-year-old Finnish adolescents were underweight, 12.6% were overweight and 2.6% were obese (54).

Other factors such as differences in the age range used in Finnish studies (46, 53–54) may partly explain the differences in the prevalences of being overweight and obese among Finnish children and adolescents. Thus, it is not only important to consider how body mass is measured, but the age range applied is also important when reporting body mass of adolescents.

The consequences of being overweight and obese in childhood include an increased risk of several comorbidities, such as type 2 diabetes, cancer and coronary heart disease in adulthood (55–56). Additionally, obese adolescents are a high risk of being obese in adulthood (57). Moreover, being underweight has been associated with a higher risk of cardiovascular disease than being normal weight (58). Being overweight and obese in childhood are a multifactorial disease and need to be prevented at the social and individual levels (59). A healthy lifestyle, including promoting physical activity (60–61) and following healthy eating habits, plays a major role in decreasing the risk of being overweight and obese in childhood (5, 59).

2.2.3 Eating habits and body mass among adolescents

Healthy eating habits have emerged as particularly interesting when explaining a part of the childhood obesity epidemic. A meta-analysis of healthy eating habits and body mass including the high intake of fruits, vegetables and whole-grain foods, showed a low risk of being overweight and obese among the highest category. In comparison, unhealthy eating habits consisting of the abundant consumption of processed meats, starchy foods and sweets revealed an increased risk of being overweight and obese in the highest category (2). Additionally, a dietary pattern consisting of among others the high intake of sweets (e.g., biscuits, cakes and chocolate), salty snacks (e.g., crisps and savoury snacks) and SSBs and the infrequent intake of high-fibre bread, vegetables and fresh fruits were inversely associated with being overweight among Australian boys (62). Moreover, studies on some unhealthy foods such as sweets and SSBs were associated with both being underweight (16) and overweight and obese (10).

To date, the strongest evidence on eating habits and body mass stems from an irregular breakfast pattern together with skipping breakfast, which associated with being overweight and obese globally (11–12, 17–21). In Finland, an irregular breakfast pattern has also been associated with a high BMI (24, 63).

Fewer studies have investigated the association between other meals such as lunch and dinner, and body mass. A previous Finnish study (23) found that those who consumed either a balanced school lunch (3–4 school days/week) containing a main dish, a salad and bread or an imbalanced school lunch (never or rarely) did not differ in terms of body mass (23). Yet, a Norwegian study showed that a free school lunch consisting of bread, cold cuts, milk, fruits and vegetables increased BMI among boys (22). Different results between the Norwegian study (22) and Finnish study (23) may stem from the free warm school lunch served to all children in Finland (64). A free warm school lunch is prepared according to national nutritional recommendations, while a purchased lunch can contain both unhealthy and healthy foods.

Most studies have examined the associations between family dinners—defined as eating dinner together with one's family—and body mass (25–27). Less is known about the relationship between dinner patterns and body mass (24). One study (27) reported that the frequency of family dinners (1–7 meals/week) did not associate with being underweight (27). However, Canadian adolescents who ate family dinners (three or more times a week) exhibited a lower risk of being overweight (25). Taveras et al. also found that family dinner frequency associated with a lower risk of being overweight at baseline (26). In general, eating a family meal (65) and adhering to a regular meal pattern (66) (including five meals) decreased the risk of being overweight and obese among adolescents.

2.3 Microbiota

2.3.1 Definition of microbiota

Human microbiota is the ecological community consisting of commensal, symbiotic and pathogenic microorganisms (67) that can include bacteria (68), viruses (69), fungi and archaea (70). This community lives in or on the human body, and is essential for human health and disease (71). Several diseases have been associated with human microbiota. In particular, periodontitis (72–73), obesity (74–76) and oral cancer (77) have been associated with saliva microbiota. Colorectal cancer (78–79) and depression (80), however, have been associated with the gut microbiota. Microbiota play a role in the modulation of the immune system (81). Additionally, microbiota participate in human biological processes, including a metabolic exchange that affects disease development (82). Environmental factors, such as eating habits (28, 30–31) and using antibiotics (83), have been associated with the composition of the human microbiota (Figure 1). Figure 1 presents the factors shaping the microbiota and their composition in various anatomical locations including the gut (28, 30) and the oral cavity (84–85). Additionally, the well-known human microbiome project has identified several microbial communities from the skin, nostrils, gastrointestinal tract and urogenital tract (86). However, the types of human microbiota differ based on the anatomic location (87).

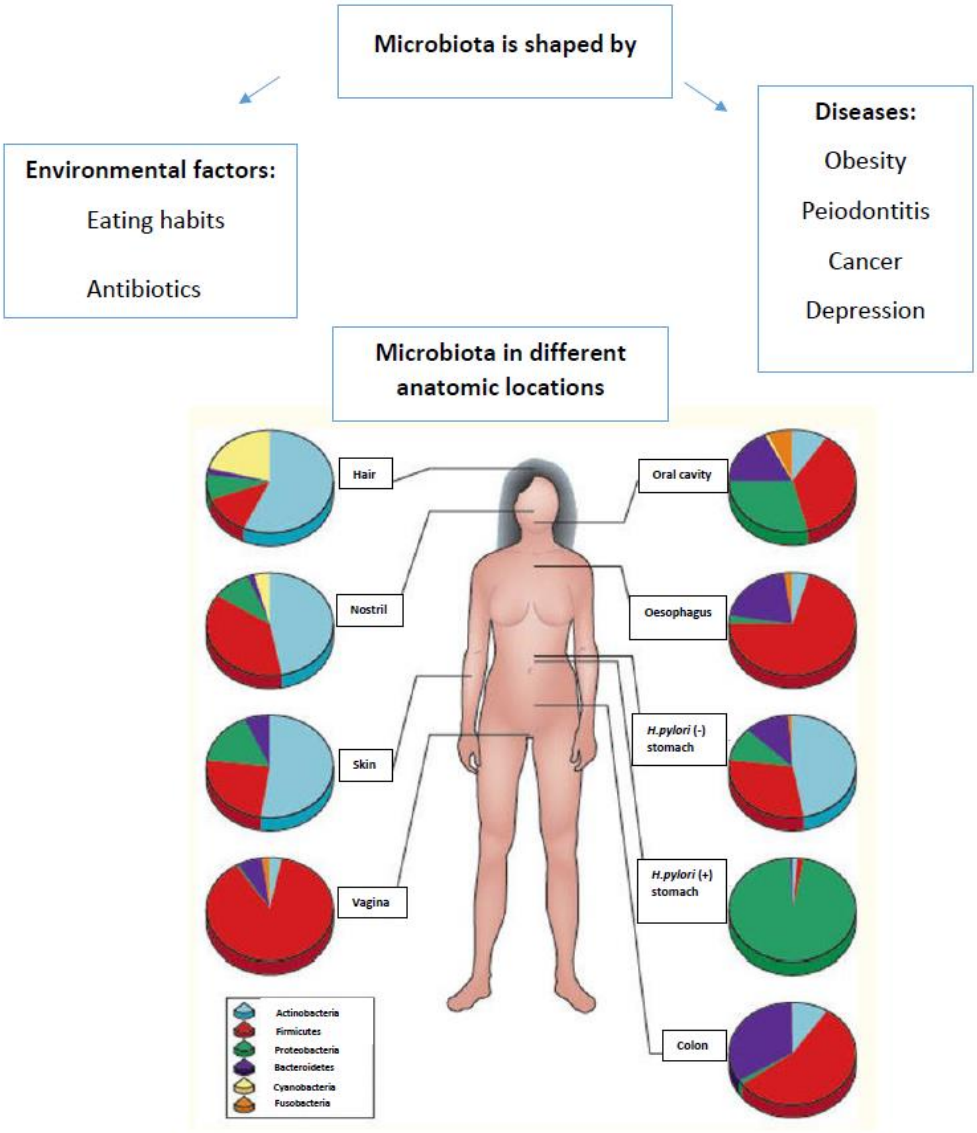

Figure 1. Factors that play a role in shaping the microbiota and the composition of dominant bacteria in various anatomical locations. Figure adapted from Cho and Blaser 2012.

2.3.2 Measurements

The 16S ribosomal RNA (rRNA) gene has been used to analyse microbial communities and identify bacteria (88). Moreover, the 16S rRNA gene can produce details regarding the phylogenetic composition of the microbiota (89). The 16S rRNA gene amplication sequencing data are compared to a reference database and presented as operational taxonomic units (OTUs), where bacteria are classified according to a sequence similarity and then counted (90–91). Furthermore, OTUs can be presented at several taxonomic levels: domain, kingdom, phylum, class, order, family, genus and species. The microbial community is also assessed based on alpha and beta diversities (92). Alpha diversity refers to the richness (the number of different OTUs). Both richness and evenness (93) can be assessed using, for instance, the Shannon and Simpson indices (93). In comparison, beta diversity refers to the composition dissimilarities and evaluates the between-sample differences using, for example, the Bray–Curtis dissimilarity index or UniFrac (94). Abundance data show differences in the number of microbes between groups (95).

2.3.3 Eating habits and gut microbiota

During the first year of an infant's life, the microbiota matures due to, among other things, the introduction of solid food to the infant's diet (96). Different nutrients such as a larger variety of carbohydrates are processed in the infant's gut microbiota (96). Children at the age of 3 obtain adult-like gut microbiota (97). In the adult gut, the dominant phyla consists of *Bacteroidetes* and *Firmicutes* (98). However, the gut microbiota differs between healthy individuals (99).

In the gut, diversity and microbial composition have been shown to differ between modern and more traditional societies (100–101). The observed differences in terms of diversity and composition in the gut are possibly explained by the effects of eating habits in these two types of societies. Specifically, a protein-rich diet characterises the modern society (97), while a fibre-rich diet predominates in traditional societies (101–102).

Furthermore, several studies have investigated the association between healthy eating habits and the gut microbiota (28, 30–32, 103–104). The quality of the diet appears to influence the gut microbiota among 30-year-old overweight and obese pregnant women. Specifically, the gut microbiota diversity was higher in the group with the highest dietary quality compared to the lowest dietary quality group (103). The same study reported that a high Shannon diversity index was associated with the daily consumption of whole grains and vegetables (103). In a multiethnic adult population, a high-quality diet was also associated with a high gut microbiota diversity and composition (104). Moreover, a high gut microbiota diversity was found among those multiethnic individuals who had a high total intake of vegetables, greens and beans and fruits (104). Additionally, a plant-based diet (e.g., Mediterranean) was associated with a high abundance of *Prevotella* in the gut of Egyptian children (32). In comparison, a Western diet involving the high intake of animal protein associated with *Bacteroides* in the gut of American children (32) and dysbiosis, indicating less microbiota diversity (105). Gut microbiota differences were also found when comparing Burkina Faso and Italian children (106). Children from a rural village in Burkina Faso followed a vegetarian diet and had a higher abundance of *Prevotella* compared to Italian children who consumed a Western diet and children from the capital city of Burkina Faso with a diet similar to the industrialised world (106).

Healthy eating habits and the relationship to gut microbiota have been examined to some extent, while research on the timing of eating (early and late lunch eaters) and gut microbiota remains scarce. To date, only one study (107) has investigated eating behaviour, including the timing of eating, and reported differences in both the composition and functions of gut microbiota.

2.3.4 Eating habits and saliva microbiota

The primary entry to the gastrointestinal (GI) system is the oral cavity (108), which contains diverse bacteria communities located, for instance, in the saliva and on the tongue (109). The oral cavity includes approximately 700 species (110). Common oral bacteria include *Streptococcus*, *Veillonella*, *Prevotella* and *Fusobacterium*, which are illustrated in Figure 2 (111–112). Similar oral bacteria also exist in the gut microbiota (113). Oral and saliva microbiota are altered by environmental factors such as healthy eating habits (33–34, 84–85, 114). However, few studies exist on healthy eating habits in relation to the oral (84–85) and saliva microbiota (35–37). In the oral microbiota, the frequency of fermentable carbohydrates were investigated in samples from 12-year-old adolescents (85). In that study (85), two diet groups were created and evaluated: 1) low-consumption diets of ≤1 time and 2) high-consumption diets of ≥2 times between meals. A higher alpha diversity was obtained from adolescents in the low-consumption carbohydrate group compared to adolescents in the high-consumption carbohydrate group. The bacterial abundance also differed between the low-carbohydrate consumption and high-carbohydrate consumption groups (85).

However, research on healthy eating habits and saliva microbiota remains inconsistent. No differences were found in the diversity and composition of saliva microbiota between vegan, omnivore and ovo-lacto-vegetarian adults (33). Yet, a high abundance of *Neisseria* and less *Prevotella* were found in saliva from vegan adults (34). Moreover, an association between saliva microbiota and a dietary treatment was observed among children (114). After a 60-day dietary treatment, African children following an Italian gluten-free diet experienced changes in their saliva microbiota (114). Eating habits include both the content of the food and the frequency of meals, but no studies exist on the frequency of different meals on the saliva microbiota. The closest measurement comes from eating time, suggesting that the alpha diversity of saliva microbiota is high among those who eat a late lunch (35).

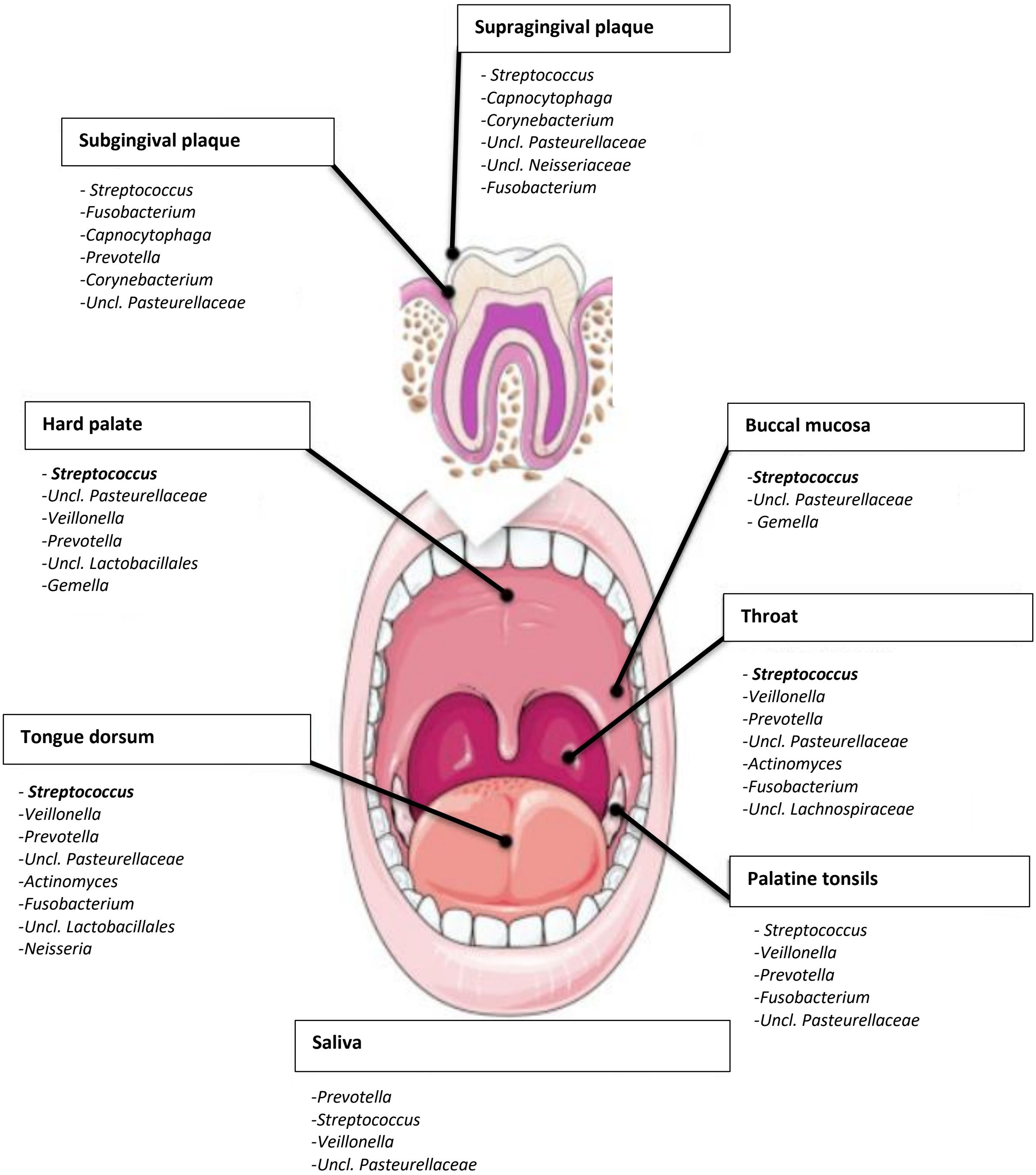

Figure 2. Composition of dominant bacteria in the oral microbiota. Figure adapted from Sampaio-Maia et al., 2016.

2.4 Gaps in the literature

The eating habits, including food consumption as well as breakfast and meal patterns, within this study have been examined only to a limited extent in Finland. Healthy eating habits are affected by other factors such as socio-cultural and environmental factors. Evaluating different eating habits across several countries is also challenging since the definitions of eating habits vary between studies. Many Finnish studies on eating habits have typically been conducted in one or two cities or areas of Finland (38, 115–116).

Healthy eating habits are obtained during the transitional period from adolescence to adulthood. However, most studies on eating habits have been examined among Finnish children and young or older adults (117–118), but less research has been carried out among adolescents (39, 119–121). This might explain why adolescents' eating habits remain poorly understood and why more updated research remains necessary in order to promote healthy eating habits among adolescents.

Studies on body mass are heterogeneous, since measuring BMI has relied both on self-reports and measurement by someone such as a fieldworker. Additionally, age groups among children and adolescents differ between BMI studies. Some studies rely on more recent data in calculating reference values that play a part in body mass. Being overweight and obese are often investigated, although less is known about being underweight. It is important to understand both being underweight and overweight as well as obese among adolescents. Furthermore, adolescents are still growing, which affects body mass and renders adolescence a crucial period in their lives.

Few studies have detected different eating habits that possibly play a role in the development of being underweight, overweight or obese among adolescents. More studies exist on the relationship between breakfast consumption and body mass than on the associations between eating lunch and dinner with body mass. Furthermore, comparing international and Finnish studies remains difficult since school lunch is not provided free-of-charge in most countries. Thus, Finland remains an exceptional country vis-à-vis school lunch.

The primary focus has remained on the association between healthy eating habits and the gut, although a limited number of studies exist on healthy eating habits and saliva microbiota. Findings on healthy eating habits in relation to saliva microbiota remain inconsistent, thus requiring further research to confirm the relationship between eating habits and saliva microbiota. No studies exist on eating habits and the relationship to the oral and saliva microbiota among adolescents. Additionally, previous saliva microbiota studies have featured small sample sizes and, therefore, more large-scale studies on the association between eating habits and saliva microbiota among adolescents are essential. Finally, studies on eating times and the microbiota are non-existent, although a growing need exists to examine different meal times and meal frequencies among adolescents.

3 AIMS OF THE STUDY

Overall, this doctoral dissertation aims to examine the associations between eating habits, body mass and saliva microbiota among Finnish adolescents.

To achieve this, the specific research questions addressed are as follows:

1. Can eating habits be identified among Finnish adolescents (study I)?

2. Do eating habits associate with body mass (study II)?

3. Are eating habits associated with the saliva microbial diversity and composition (study III)?

As such, the specific hypotheses are as follows:

1. Finnish adolescents might have healthy or unhealthy eating habits (study I).

2. Eating habits are associated with body mass (study II).

3. Unhealthy eating habits, including an irregular meal pattern, are associated with less bacterial diversity and have a dissimilar composition of saliva microbiota when compared to healthy eating habits (study III).

4 MATERIALS AND METHODS

4.1 Data and participants

The study data stem from the Fin-HIT cohort, which includes children aged 9- to 14-years old (henceforth, referred to as 'adolescents') and one of their parents. The Fin-HIT study aimed to assess long-term changes in body mass from adolescence to adulthood. Figueiredo et al. (54) described in detail the cohort profile and the preliminary findings.

Baseline recruitment included a pilot study in 2011 and a main study which took place in 2013 and 2014. The pilot study was carried out in participants' homes and the main study took place in schools. In the pilot study, invitations were sent via mail to 11 000 randomly selected households, which resulted in a response rate of just 14%. Therefore, the recruitment strategy was amended. Adolescents and their parents were contacted through 496 schools that agreed to participate. Fieldworkers briefly explained the Fin-HIT study to the adolescents. Thereafter, fieldworkers delivered invitations to 27 000 adolescents and their parents via schools. In the main study, the response rate climbed to 36%. Thus, the overall response rate at baseline was 30%, and in total 11 407 adolescents participated in the Fin-HIT baseline study (54). Here, I present the data and findings from 1 000 of these participants, who were randomly selected for the microbiota analyses.

Figure 3 provides a flow chart of baseline recruitment and participant selection in studies I–III and a more specific description of the selection of the adolescents. Study I and study II consisted of 10 569 participants aged 9- to 14-years old from the baseline Fin-HIT study cohort. Study III included 842 adolescents aged 11- to 14-years old from the baseline Fin-HIT study cohort.

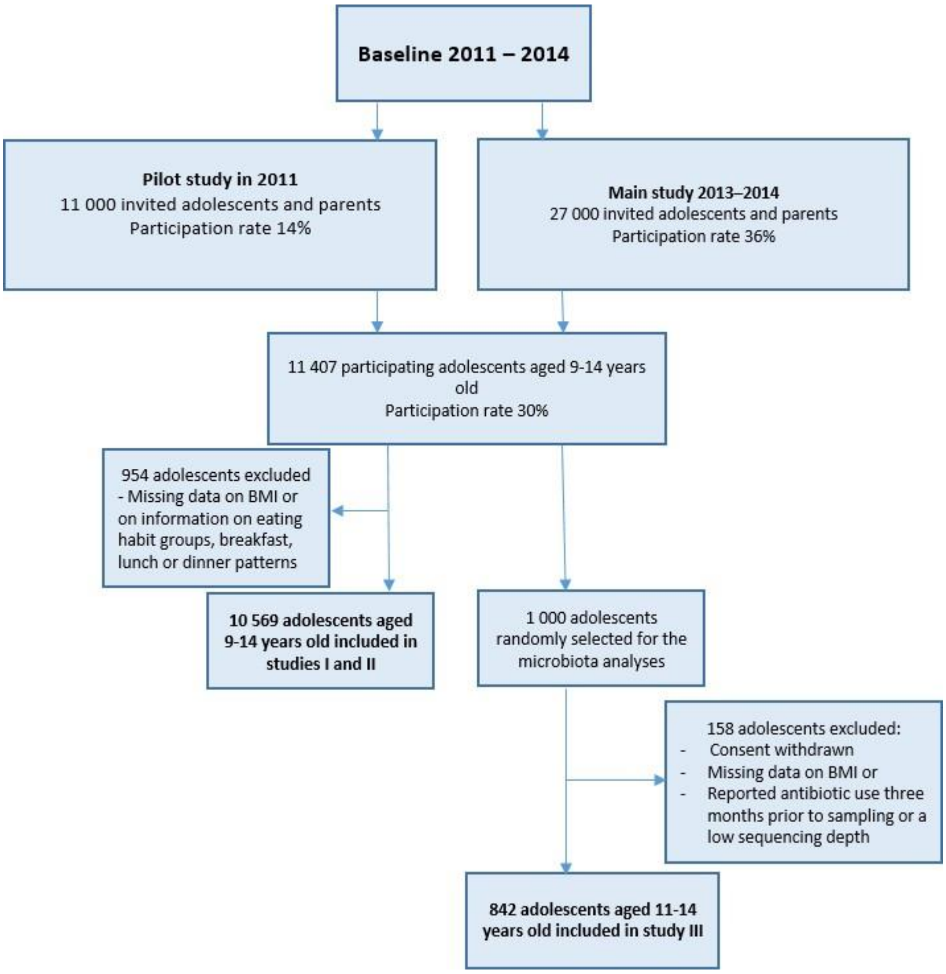

Figure 3. Flow chart of participants in studies I–III.

The Fin-HIT study was conducted in southern, middle and northern Finland between 2011 and 2014 (Figure 4). Through a home-based recruitment process, invitations were mailed to participants, who reported their height, weight and waist circumference via the home-based recruitment process. In the school-based recruitment process, a web-based baseline questionnaire completed on an electronic tablet was provided to participants in schools. The questionnaire assessed lifestyle factors, such as diet and eating-related health behaviours. Participants completed the questionnaires in Finnish and Swedish. Trained fieldworkers measured height and weight from participants and collected their saliva samples in schools.

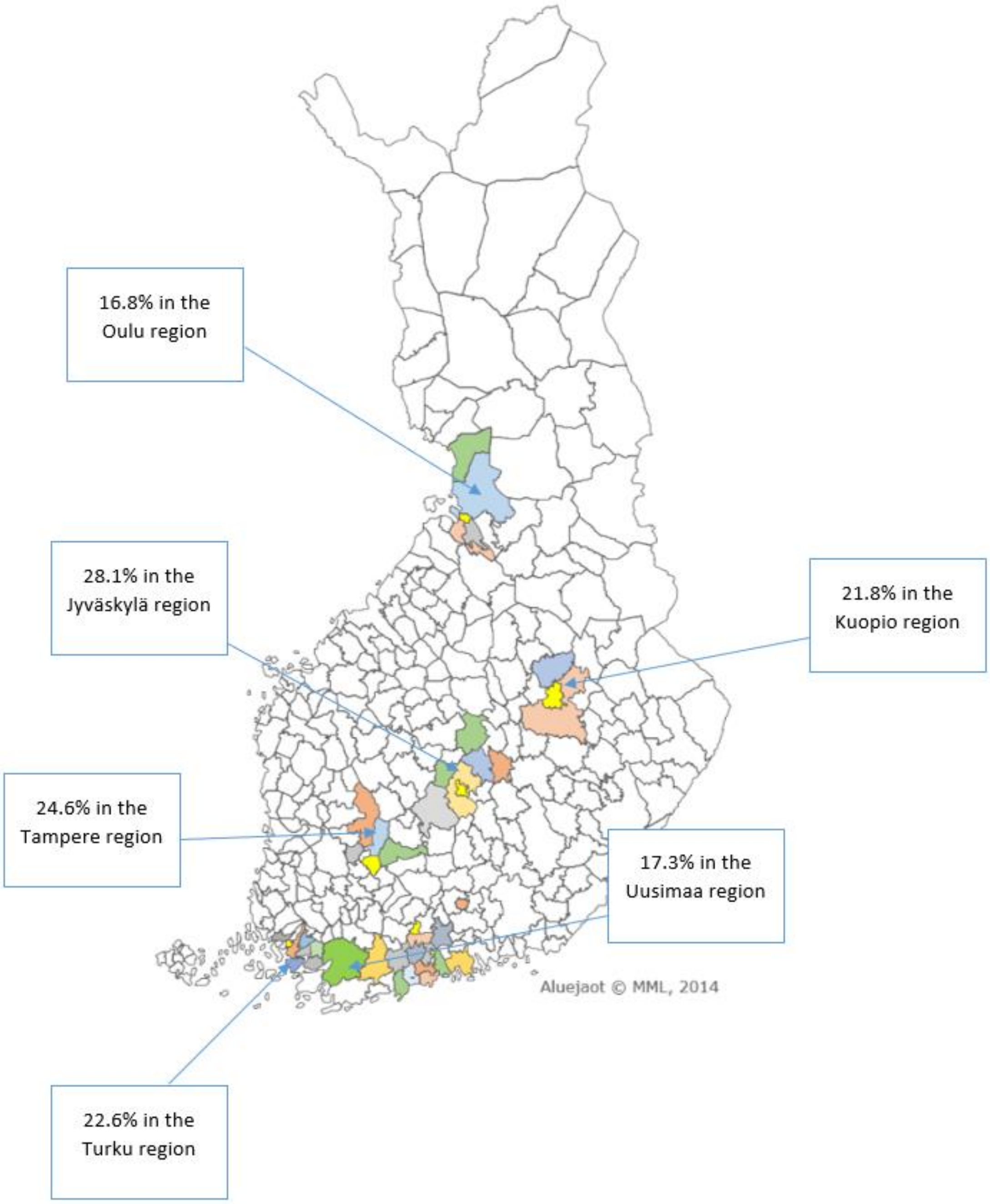

Figure 4. Map of Finland showing the study areas in southern, middle and northern Finland in detail, which included 496 schools in 44 municipalities. The percentages in the figure represent the proportion of adolescents in a specific region who took part in the Fin-HIT study (n = 8 632) (122). Adolescents included in the study were recruited in schools, since they had information on the specific region. The specific region includes all invited municipalities.

4.2 Measurements

4.2.1 Socio-demographic characteristics (studies I–III)

Parent's education level was recorded from the parent's questionnaire and grouped as a low education level (up to a technical high school diploma) and as a high education level (beyond a technical high school diploma; study I). Here, participants' gender, age (in years) and language spoken at home (Finnish, Swedish or other) were recorded from the consent forms or questionnaires and linked to the National Population Information System at the Population Register Centre to obtain accurate and updated information on participants (54) (studies I–III).

4.2.2 Food frequency questionnaire (FFQ; studies I–III)

A 16-item food frequency questionnaire (FFQ) detailing eating habits during the past month was used to create eating habit groups. The FFQ considered both school and non-school days. The 16 selected food items consisted of: water; chocolate and sweets; cooked vegetables; milk or sour milk; fresh juice; sugary soft drinks; pizza; hamburgers or hot dogs; biscuits or cookies; sweet pastries; ice cream; salty snacks; sugary juice drinks (SSBs); dark grain bread; fruits or berries; and fresh or grated vegetables and salad. These items were key indicators of healthy and unhealthy dietary habits among adolescents and recommended earlier by the Health Behaviour in School-Aged Children (HBSC) study protocol (123–124). Additionally, the HBSC study was carried out in several European countries (123–124). Participants reported the frequency of food consumption, whereby information on the frequency of food consumption was obtained through the following question: 'Thinking about the past month, how often did you eat and drink the following food and drinks?' The intake frequency of selected food items was recorded using the following scale: 1) not at all; 2) less than once a week; 3) once a week; 4) 2 to 4 times a week; 5) 5 to 6 times a week; 6) once a day; and 7) several times a day (studies I–III).

The frequency of eating breakfast, lunch and dinner was recorded using a single question: 'How often do you usually eat the following meals during a school week?' This question was used earlier in the HBSC study protocol (123–124). The frequency of these three meals was assessed on a 6-point scale: 1) rarely or never; 2) one day a week; 3) two days a week; 4) three days a week; 5) four days a week; and 6) five days a week. Breakfast, lunch and dinner were considered regular when those were consumed every school day and irregular if consumed less than every school day. In study I, breakfast consumption was analysed separately, whilst lunch and dinner were combined into a single meal pattern. In study II, all three meals—that is, breakfast, lunch and dinner—were examined separately in the analyses. In study III, breakfast and dinner were analysed separately. The questions related to eating habits included no information on portion size or energy intake, nor did they include information about weekend meals (studies I—III).

4.2.3 Body mass (studies II-III)

In the pilot study, height and weight were self-reported by participants and their parents. In the main study, trained fieldworkers measured participant's weight and height in a standardised way in schools (54). During the measurement period, participants wore light indoor clothing, with the weight of the indoor clothing evaluated using a list of 14 clothing items. A daily-calibrated digital scale (CAS model PB) was used to measure weight to the nearest 0.01 kg. A portable stadiometer (Seca Model 217) was used to measure height to the nearest 0.1 cm at the end of exhalation. Height was recorded twice and weight was measured once. The weight of the indoor clothing was substracted from the measured weight and used together with height to calculate BMI [weight (kg)/ height squared (m^2)]. BMI was categorised as underweight, normal weight, overweight and obese (BMI categories) based on age- and gender-specific IOTF cut-off points (47). The overweight and obese groups were combined given the small number of obese adolescents (2.6%). Approximately 13% of participants self-reported their body measurements due to, for instance, a school absence on the day of data collection (54) (studies II—III). However, a validation study from the Fin-HIT cohort (125) showed that home-measured BMI is also appropriate to use.

4.2.4 Saliva sample (study III)

In the pilot study, saliva samples were self-collected at participant's homes. Participants received instructions regarding how to provide a saliva sample. In the main study, participants provided saliva samples in school. Saliva samples were collected primarily between breakfast and lunch on school days according to the manufacturer's instructions. The unstimulated saliva samples were collected using the Oragene-DNA (OG-500) Self-Collection Kit (DNA Genotek Inc., Canada), which included a stabilising reagent. The unstimulated saliva samples and stimulated saliva samples showed no differences regarding the microbiota diversity. However, the bacterial composition differed between unstimulated and stimulated saliva samples at the species level, but not at the genera level (126). After collecting the saliva samples, they were stored at room temperature. Intensive lysis, bead-beating and an automated Chemagic protocol (PerkinElmer, UK) were used to extract DNA (127), which Raju et al. (128—129) used for their microbiota analyses. An intensive lysis protocol, including both enzymatic and mechanical lysis, was used since it takes into account all bacteria regardless of robustness to release DNA, enabling a high-diversity microbiota profile. More specifically, the intensive lysis protocol consisted of two phases. In the first phase, three components—50-µl lysozyme (10 mg/ml, Sigma-Aldrich), 6-µl mutanolysin (25 KU/ml, Sigma-Aldrich) and 3-µl lysostaphin (4000 U/ml, Sigma-Aldrich)—were included in a cell suspension, which consisted of 500-µl aliquot. Afterwards, samples were incubated for 1 h at 37 °C. In the second phase, the lysate was mixed with 600 mg of 0.1-mm-diameter zirconia/silica beads (BioSpec, Bartlesville, OK, USA). After lysis, Mini-BeadBeater-96 (BioSpec, Bartlesville, OK, USA) mechanically disrupted the microbial cells at 2100 rpm for 1 min (127). Finally, Chemagic MSM1 nucleic acid extraction robot (PerkinElmer) was performed in isolation and the total genomic DNA was purified from the lysates (study III).

4.2.5 Amplification and sequencing (study III)

The 16S rRNA gene is a culture-independent method developed for identifying bacteria (88). The 16S rRNA gene enjoys frequent use since it is common for all bacteria, but also works in identifying species-specific variation. Amplification and sequencing of this gene can provide throughput at a low cost. 16S provides information on the phylogenetic composition, but is not directly capable of providing information on the functional capacity of a community (89). The 16S amplification and sequencing protocol were utilised and adapted from Raju et al. (129) and used previously (128). In microbial analyses, amplicon sequencing, defined as sequencing a specific marker gene such as the 16S rRNA gene, is amplified using PCR. The V3–V4 gene regions were amplified using the two 16S primers—S-D-Bact-0341-b-S-17 (5′-CCTACGGGNGGCWGCAG-3′) and S-D-Bact-0785-a-A-21 (5′-GACTACHVGGGTATCTAATCC-3) (130)—using in the TruSeq (TS)-tailed one-step amplification protocol (129). The Agilent 2100 Bioanalyser was obtained to quantify the amplification performance and yield. The Illumina HiSeq1500 platform (Illumina, Inc., San Diego, CA, USA) was applied for sequencing 271 x 2 bp paired-end reads to provide a sufficient read-pair sample overlap and a high-quality assembly (129). In all samples, there were a total of 133 442 780 assembled read pairs. The average per sample was 1 584 831 read pairs and the median was 118 641 read pairs. The maximum was 1 316 321, while the minimum was 29 read pairs (study III).

4.2.6 Operational taxonomic units (OTUs; study III)

Operational taxonomic units (OTUs) were categorised based on sequence similarity and counted (90—91). The sequencing data were processed using MiSeq SOP and the mothur pipeline (Version V.1.35.1) (131). The sequences were filtered based on quality and the read pairs were assembled. The assembled reads were aligned to the SILVA 16S rRNA gene database (Version V119) and clustered into OTUs with a >98% similarity cut-off point. The SILVA 16S rRNA gene database was utilised since it is extensive, quality-checked and regularly updated (132). For the analyses, subsampling extended up to 500 to maximize the included samples. After rarefaction, the Shannon and Inverse Simpson's indices for the alpha diversity were calculated to evaluate the richness and the evenness of the saliva microbiota. Beta diversity was calculated using the Bray—Curtis dissimilarity index to test the composition of the saliva microbiota. Data were prefiltered to only OTUs with at least 20 read pairs in total for the abundance analyses (study III).

4.3 Statistical methods

4.3.1 Study I

Study I aimed to identify eating habits among 10 569 Finnish adolescents. The chi-square test evaluated associations between categorical variables. Water and chocolate and sweets from 16 food items were excluded. Water was excluded since it is non-nutrient containing. Chocolate and sweets as a variable was not available for all participants since the pilot study questionnaire lacked the ‘chocolate and sweets’ option. Subsequently, factor analysis and cluster analysis were performed to identify different eating habit groups using 10 food items. Then, this study excluded (**bolded**) food items that were highly correlated. The four excluded food items were: 1) **cooked vegetables** associated with fresh or grated vegetables and salad; 2) **sugary soft drinks** associated with SSBs; 3) **fresh juice** associated with fruits or berries; and 4) **milk or sour milk** associated with other healthy food items. Finally, ten food items were selected for the cluster analysis: pizza; hamburgers or hot dogs; biscuits or cookies; sweet pastries; ice cream; salty snacks: SSBs; dark grain bread; fruits or berries; and fresh or grated vegetables and salad. All ten food items were regrouped into three new categories based on the consumption of different food items: dark grain bread (rye bread), fruits or berries, fresh or grated vegetables and salad (maximum once a week, 2–6 times per week and at least once a day); sweet pastries, biscuits or cookies and SSBs (less than once a week, once a week and more than once a week); pizza and hamburgers or hot dogs and salty snacks (not at all, less than once a week and at least once a week).

Factor analysis was used to obtain factors indicating different food consumption patterns. In the factor analysis, five factors were extracted based on a principal components analysis and the varimax method performed using rotation. The Kaiser-Meyer-Olklin (KMO) and Bartlett’s sphericity test assessed the applicability of the factor analysis model. Values >0.70 and $p < 0.05$, respectively, were considered further. The K-means cluster analysis utilised these five factors. Robustness was tested and repeated in the cluster analysis five times, and then used to compare the analysis with previous results from the Kappa analyses. A p value of $p < 0.001$ and a Kappa index >0.70 were applied in all comparisons. This study excluded from the analyses 954 adolescents for whom no information on BMI, eating habit groups and breakfast patterns, lunch patterns or dinner patterns was available. The statistical significance level was set to 5%. This study used SPSS statistical software version 24.0 (IBM Corp., Armonk, NY, USA) for all statistical analyses.

4.3.2 Study II

The eating habits previously identified were utilised in study II. In study II, the association between eating habits and body mass was examined among 10 569 adolescents. Associations using categorical variables were tested through the chi-square test. The association between eating habit groups and breakfast and dinner patterns, respectively, and body mass (underweight, normal weight, overweight and obese) were assessed using multinomial logistic regression analyses, which provided the odds ratios (ORs) and 95% confidence intervals (CIs). In the multinomial logistic regression models, adjustments were participant's age, gender and language spoken at home. The reference category was normal weight, which was compared to the underweight and overweight and obese categories. Other comparisons included unhealthy eaters and fruit and vegetable avoiders against healthy eaters; an irregular breakfast pattern against a regular breakfast pattern; and an irregular dinner pattern against a regular dinner pattern. The likelihood ratio was used to evaluate models with and without interactions. This study excluded from the analyses 954 adolescents for whom no information on BMI, eating habit groups, breakfast patterns, lunch patterns or dinner pattern was available. The statistical significance level was set to 5%. SPSS version 24.0 (IBM Corp., Armonk, NY, USA) and SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA) were utilised for the statistical analyses.

4.3.3 Study III

Study III examined the associations between eating habits and the saliva microbial diversity and composition among 842 adolescents. In the saliva microbiota, both the alpha and beta diversities and the relative bacteria composition and their associations to eating habit groups and breakfast and dinner patterns were examined. The Shapirov–Wilk test checked the normality of the alpha diversity (Shannon and Inverse Simpson's indices). Differences in the alpha diversity between eating habit groups were tested using ANOVA without adjustments. The Levene's test was used to test the homogeneity of the variances. The alpha diversity was then tested against the breakfast and dinner patterns using the t-test. Additionally, the analysis of covariance (ANCOVA) assessed the alpha diversity between eating habit groups and breakfast and dinner patterns when adjusted for covariates. Both the alpha and beta diversities and bacterial abundance models using eating habit groups and breakfast and dinner patterns were adjusted for gender, age, language spoken at home, BMI categories and sequencing depth. In addition, this study evaluated interactions for eating habit groups and gender; breakfast patterns and gender; and dinner patterns and gender.

A permutational analysis of variance (PERMANOVA) with 999 permutations assessed the beta diversity (the Bray–Curtis index) against eating habit groups and breakfast patterns and dinner patterns. The homogeneity of the multivariate dispersion was checked between groups. The Adonis and Betadisper functions were utilised within the Phyloseq and Vegan R packages. In the analyses, variability in the homogeneity existed between eating habit groups and dinner pattern groups, whilst heterogeneity was observed between breakfast pattern groups. This study used random sampling with the regular breakfast eaters to obtain balanced groups given this heterogeneity. PERMANOVA was utilised because it is strong in a balanced design (133–134). For the analyses, this study included 142 regular breakfast eaters and 142 irregular breakfast eaters achieving a balanced number of adolescents. The analyses were rerun 30 times to test the confidence of the PERMANOVA analyses.

Differential abundances of OTUs between eating habits, breakfast and dinner patterns were calculated using the DESeq2 R package. This study used a negative binomial distribution in a general linear model and a false discovery rate (FDR) for the p value adjustment. This study used FDR = 0.05 for multiple test corrections. For the eating habit groups, a multiple comparison method was applied. The models for breakfast and dinner patterns in different abundances of OTUs were as follows: irregular breakfast vs. regular breakfast and irregular dinner vs. regular dinner patterns. This study excluded from the analyses 25 adolescents who withdrew their consent, 21 adolescents for whom BMI measurement information was missing, 21 adolescents who reported antibiotics three months prior to sampling and 91 samples with a low sequence depth (<500 read pairs). R version 3.2.4 and SPSS version 24.0 (IBM Corp., Armonk, NY, USA) were used for the statistical analyses.

4.4 Ethical issues

The Coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa (169/13/03/00/10) approved the current study protocol in 2010, and participation in the study was voluntary. Both adolescents and parents could withdraw from the study at any time. Both adolescents and their parents completed a written informed consent form.

5 RESULTS

Table 1 summarises the baseline characteristics of the adolescents in studies I and II. In study I, lunch and dinner were combined in the analysis of meal patterns. Almost one in five adolescents followed an irregular breakfast pattern and nearly one in four adolescents followed an irregular meal pattern and one in three ate breakfast, lunch and dinner irregularly. Yet, the majority of adolescents regularly ate breakfast (81.0%), lunch (87.6%) and dinner (83.6%) in study II. Most of the adolescents were normal weight (73.6%), whereas 11.1% were underweight and 15.3% were overweight and obese in study II. Overweight and obese adolescents were combined into a single group (overweight and obese group) in the subsequent analyses.

Table 1. Characteristics of adolescents (studies I–II).

Age, n (%)	
>11 years	1 415 (13.4)
11 years	6 709 (63.5)
<11 years	2 445 (23.1)
Gender, n (%)	
Boy	5 005 (47.4)
Girl	5 564 (52.6)
Language spoken at home, n (%)	
Finnish	9 850 (93.2)
Swedish	445 (4.2)
Other	274 (2.6)
Parental education[a,*] level, n (%)	
High	3 063 (55.0)
Low	2 509 (45.0)
Breakfast patterns, n (%)	
Missing values	628
Regular	8 563 (81.0)
Irregular	2 006 (19.0)
Lunch patterns[b], n (%)	
Missing values	636
Regular	9 261 (87.6)
Irregular	1 308 (12.4)
Dinner patterns[b], n (%)	
Missing values	633
Regular	8 840 (83.6)
Irregular	1 729 (16.4)
Breakfast, lunch and dinner, n (%)	
Regular	6908 (65.4)
Irregular	3661 (34.6)
Meal patterns (lunch and dinner)[a], n (%)	
Regular	8 001 (75.7)
Irregular	2 568 (24.3)
BMI categories[b], n (%)	
Missing values	725
Normal weight	7 784 (73.6)
Underweight	1 171 (11.1)
Overweight and obese	1 614 (15.3)

Variables included only in study I or study II are marked differently in the table.
[a]These variables were included in study I.
[b]These variables were included in study II.
[*]A low education level (up to technical high school) and a high education level (beyond technical high school).

5.1 Identifying eating habit groups (study I)

Factor analysis revealed five factors that explained 70.1% of the total variance. The five factors were (bolded): 1) **fast food** (including pizza; hamburgers or hot dogs); 2) **sweets** (including biscuits or cookies; sweet pastries; ice cream); 3) **salty snacks and SSBs**; 4) **dark grain bread**; and 5) **fruits and vegetables** (including fruits or berries; fresh or grated vegetables and salad).

Factor and K-means cluster analyses resulted in three eating habit groups. These were labelled as unhealthy eaters (n = 1 298; 12.3%), fruit and vegetable avoiders (n = 4 610; 43.6%) and healthy eaters (n = 4 661; 44.1%). Figure 5 displays the consumption of ten food items among the three eating habit groups.

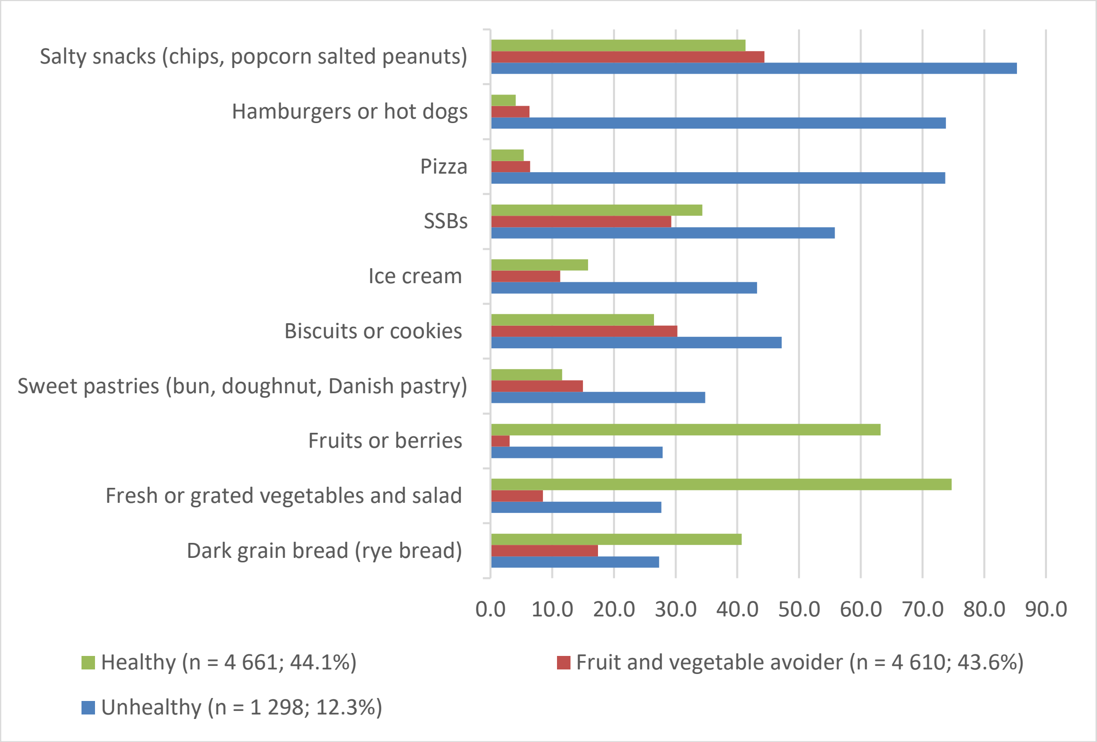

Figure 5. Consumption of ten food items among the three eating habit groups: unhealthy eaters (Unhealthy), fruit and vegetable avoiders (Fruit and vegetable avoider) and healthy eaters (Healthy) (studies I–II).

5.2 Description of the eating habit groups (study I)

Unhealthy eaters primarily ate sweet pastries, SSBs, hamburgers or hot dogs and salty snacks. Fruit and vegetable avoiders less frequently consumed both unhealthy and healthy foods, such as fresh or grated vegetables and salad as well as fruits or berries than others. Healthy eaters consumed more dark grain bread and fresh or grated vegetables and salad and fewer unhealthy food items compared to the other eating habit groups.

Figure 6 presents the distribution of breakfast and meal patterns, gender, age, language spoken at home and parental education level across the three eating habit groups. Unhealthy eaters were more often boys and more irregularly ate breakfast and followed an irregular meal pattern (lunch and dinner) and their parents were less educated compared to fruit and vegetable avoiders and healthy eaters (chi-square test: $p < 0.001$). In comparison, healthy eaters were more likely girls and more regularly ate breakfast and followed more a regular meal pattern, and their parents were more highly educated compared to other groups (chi-square test: $p < 0.001$).

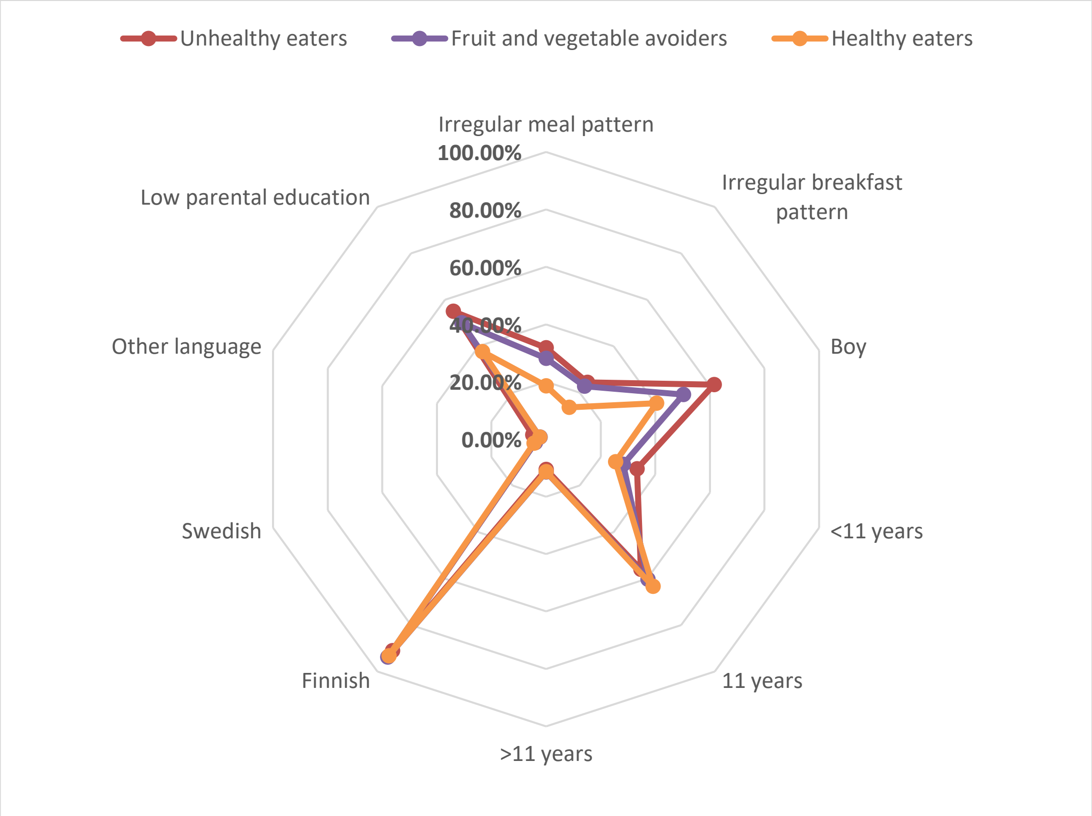

Figure 6. Breakfast and meal patterns, gender, age, language spoken at home and parental education among unhealthy eaters (Unhealthy), fruit and vegetable avoiders (Fruit and vegetable avoider) and healthy eaters (Healthy).

A chi-square test showed that girls more often irregularly ate breakfast than boys ($p < 0.001$). Adolescents older than 11 years old more irregularly ate breakfast and followed an irregular meal pattern compared to others (chi-square test: $p < 0.001$).

5.3 Eating habits and body mass (study II)

This study found that fruit and vegetable avoiders were more commonly underweight (46.6%) as well as overweight and obese (46.5%) than normal weight (42.6%). Underweight adolescents more regularly ate breakfast

(86.1%) and dinner (86.5%) than normal weight (81.8% and 83.8%, respectively) and overweight and obese adolescents (73.5% and 80.6%, respectively; chi-square test: p < 0.001).

Figure 7 shows that eating habit groups as well as breakfast and dinner patterns associated with body mass in individual comparisons examining underweight versus normal weight and overweight and obesity versus normal weight. For these analyses, normal weight was used as the reference category. Fruit and vegetable avoiders were associated with a higher risk of being underweight (multinomial logistic regression: OR = 1.28; 95% CI 1.12–1.46) compared to healthy eaters. Moreover, irregular breakfast (multinomial logistic regression: OR = 0.70; 95% CI 0.59–0.84) and irregular dinner eaters (multinomial logistic regression: OR = 0.83; 95% CI 0.69–0.99) were associated with a decreased risk of being underweight. Irregular breakfast eaters had a higher risk of being overweight and obese (multinomial logistic regression; OR = 1.56; 95% CI 1.37–1.77) than regular breakfast eaters.

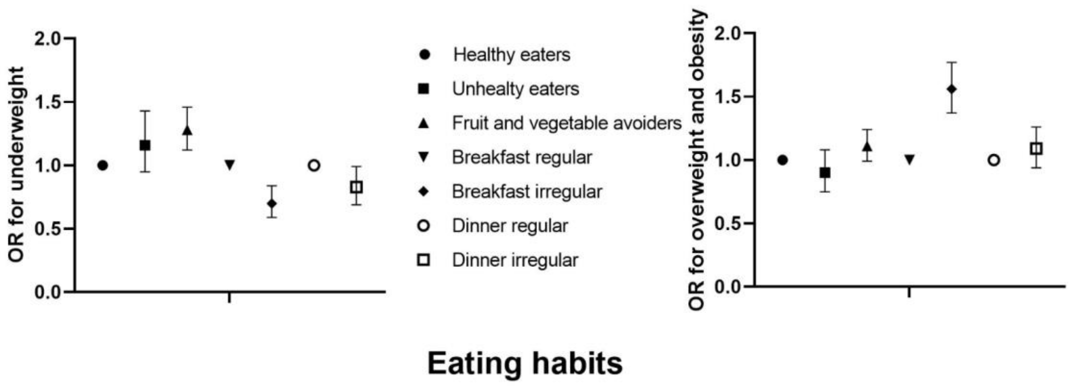

Figure 7. Eating habit groups and breakfast and dinner patterns among underweight (n = 1 171; 11.1%) and overweight and obese (n = 1 614; 15.3%) adolescents compared to normal weight adolescents. Odds ratios (ORs) for eating habit groups and breakfast and dinner patterns adjusted for adolescent's age, gender and language spoken at home. Results from a multinomial logistic regression.

Table 2 presents the eating habit groups according to the BMI categories analysed separately by dinner patterns given that an interaction was found between eating habit groups and dinner patterns (p = 0.049). In the regular dinner pattern group, fruit and vegetable avoiders were associated with a higher risk of being both underweight (multinomial logistic regression: OR = 1.27; 95% CI: 1.10–1.46) and overweight and obese (multinomial logistic regression: OR = 1.18; 95% CI 1.04–1.34) than healthy eaters. In the irregular dinner pattern group, however, unhealthy eaters were associated with a lower risk of being overweight and obese (multinomial logistic regression: OR = 0.58; 95% CI 0.39–0.87) than healthy eaters. This study found no interaction between eating habit groups and gender (p = 0.108) and between eating habit groups and breakfast patterns (p = 0.094).

Table 2. Odds ratios (ORs) and confidence intervals (CIs) for eating habits based on BMI categories stratified by dinner patterns[b] among adolescents.

	BMI categories[a]					
	Underweight*			Overweight and obese*		
	OR	(95% CI)	*p* value	OR	(95% CI)	*p* value
	Regular dinner pattern group[a]					
Eating habit groups						
Healthy eaters	1.00			1.00		
Unhealthy eaters	1.15	(0.92–1.45)	0.217	0.99	(0.81–1.21)	0.930
Fruit and vegetable avoiders	1.27	(1.10–1.46)	0.001	1.18	(1.04–1.34)	0.010
	Irregular dinner pattern group[a]					
Eating habit groups						
Healthy eaters	1.00			1.00		
Unhealthy eaters	1.30	(0.76–2.22)	0.336	0.58	(0.39–0.87)	0.009
Fruit and vegetable avoiders	1.41	(0.95–2.08)	0.085	0.78	(0.60–1.03)	0.083

[a]Adjusted for adolescent's age, gender and language spoken at home.
[b]Likelihood ratio test to assess models with and without an interaction between eating habit groups and dinner patterns (p = 0.049).
*Normal weight served as the reference category. Results presented from a multinomial logistic regression.

5.4 Eating habits and saliva microbiota (study III)

In study III, the majority of the adolescents were fruit and vegetable avoiders (42.9%) and healthy eaters (45.5%), and they regularly ate breakfast (83.1%) and dinner (82.4%), similar to findings from study II.

5.4.1 Alpha diversity

This study tested the association between eating habit groups and breakfast and dinner patterns against the alpha diversity. Alpha diversity was shown using the Shannon diversity index (2.26 ± 0.29) and the Inverse Simpson's index (6.12 ± 1.94). All of the diversity and abundance models were fully adjusted for gender, age, language spoken at home, BMI categories and sequencing depth. No significant associations between the alpha diversity were identified against eating habit groups using the Shannon diversity index (ANCOVA: p = 0.766) and the Inverse Simpon's index (ANCOVA: p = 0.941). However, a higher alpha diversity was found among regular breakfast eaters when compared to irregular breakfast eaters using the Inverse Simpon's index with mean (standard error of means) 6.27 (0.17) vs. 5.80 (0.02), ANCOVA: p = 0.010. The Shannon diversity index detected a borderline significance between regular and irregular breakfast eaters (2.27 (0.03) vs. 2.22 (0.03), ANCOVA: p = 0.056). Additionally, the Shannon diversity index detected a borderline significance in dinner patterns (2.27 (0.03) vs. 2.22 (0.03), ANCOVA: p = 0.054). Yet, the Inverse Simpson's index identified no differences between regular and irregular dinner eaters (6.23 (0.17) vs. 6.04 (0.22), ANCOVA: p = 0.275).

This study identified no interaction between gender and eating habit groups (Shannon diversity index, p = 0.181; Inverse Simpon's index, p = 0.212), between gender and breakfast patterns (Shannon diversity index, p = 0.974; Inverse Simpon's index, p = 0.910), and between gender and dinner patterns (Shannon diversity index, p = 0.850; Inverse Simpon's index, p = 0.959).

5.4.2 Beta diversity

This study evaluated the beta diversity using the the Bray–Curtis dissimilarity index. Here, no significant differences were detected between eating habit groups (PERMANOVA: p = 0.131) and dinner patterns (PERMANOVA: p = 0.076) in the beta diversity. However, this study detected a small but significant difference between regular breakfast and irregular breakfast eaters in the unbalanced design (PERMANOVA: p = 0.001; Figure 8). These results were confirmed through the balanced design.

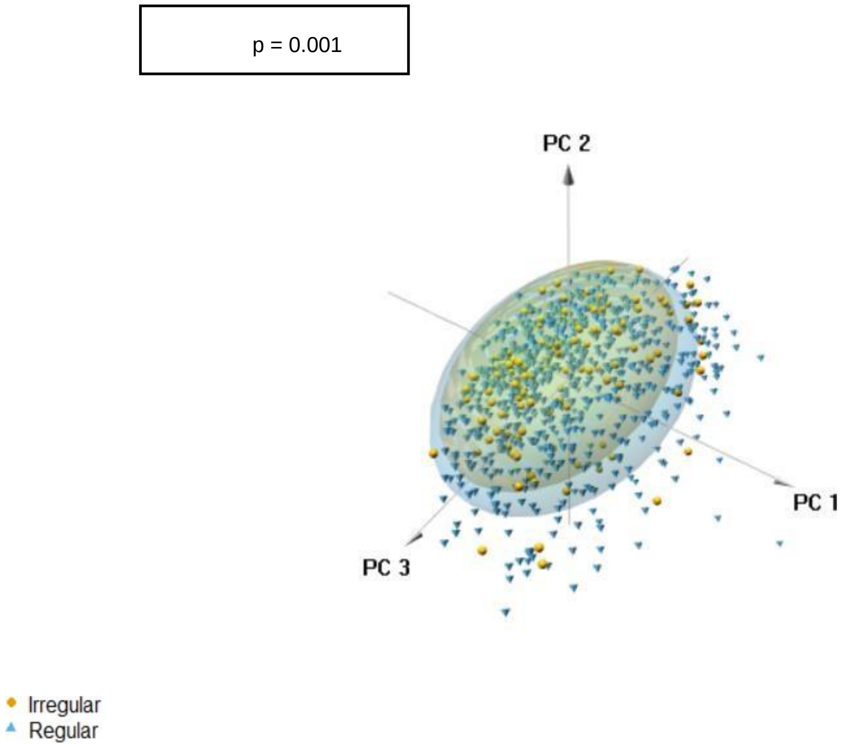

Figure 8. Bray–Curtis dissimilarity index among regular and irregular breakfast eaters analysed using PERMANOVA. Principal component (PC) plots explain the variance in breakfast patterns.

5.4.3 Bacterial abundance

Table 3 summarises the differentially abundant bacteria in OTUs between eating habit groups and breakfast and dinner patterns in the saliva microbiota. Six OTUs were highly abundant among fruit and vegetable avoiders. One unclassified bacterium belonging to the *Neisseriaceae* family and another unclassified bacterium from the *Prevotellaceae* family were highly abundant in the fruit and vegetable avoiders' group. Two OTUs were differentially abundant between unhealthy eaters and healthy eaters. Unhealthy eaters had a reduction in one unclassified bacterium belonging to the *Lachnospiraceae* family. Interestingly, *Megasphaera* increased among unhealthy eaters. However, fruit and vegetable avoiders and unhealthy eaters did not differ in terms of the abundance of various bacteria.

Next, this study compared irregular breakfast eaters to regular breakfast eaters. Six OTUs were differentially abundant when comparing irregular and regular breakfast eaters. *Prevotella* and two OTUs belonging to *Veillonella* were more abundant among irregular breakfast eaters compared to regular breakfast eaters. In turn, *Shingomonas* was less abundant among irregular breakfast eaters than among regular breakfast eaters. Finally, this study compared irregular dinner eaters to regular dinner eaters. Two OTUs were highly abundant and nine OTUs were less abundant among irregular dinner eaters compared to regular dinner eaters. *Prevotella* was also more abundant among irregular dinner eaters than among regular dinner eaters. However, *Prevotella*, which was found among irregular dinner eaters, belonged to a different OTU (OTU000063) than *Prevotella*, which was found among fruit and vegetable avoiders and irregular breakfast eaters (OTU000023). Additionally, *Leptotrichia* was more abundant among irregular dinner eaters than among regular dinner eaters. Yet, *Neisseria* and *Haemophilus* were less abundant among irregular dinner eaters than among regular dinner eaters.

Table 3. Bacterial abundance of OTUs between eating habit groups and breakfast and dinner patterns among adolescents.

Group	OTU	Nearest taxa	Base Mean OTU	LoG2Fold Change	*p*	Adjusted *p*
		Fruit and vegetable avoiders vs. Healthy eaters				
Eating habit groups[a]	Otu171	Unclassified bacterium from the *Neisseriaceae* family	3.20	2.44	2.74E-05	0.006
	Otu003	*Prevotella*	2370.05	0.38	7.28E-05	0.006
	Otu023	*Prevotella*	315.48	0.47	8.59E-05	0.006
	Otu106	Unclassified bacterium from the candidate division SR1 phylum	11.72	0.71	0.001	0.036
	Otu213	Unclassified bacterium from the *Prevotellaceae* family	1.20	1.43	0.001	0.036
	Otu113	*Dialister*	8.52	0.54	0.001	0.042
		Unhealthy vs. Healthy eaters				
	Otu587	Unclassified bacterium from the *Lachnospiraceae* family	0.05	-23.00	1.75E-06	0.003
	Otu280	*Megasphaera*	0.90	2.20	4.75E-06	0.004
		Fruit and vegetable avoiders vs. Unhealthy eaters				
			non-significant			

	Irregular vs. Regular patterns					
Breakfast patterns[a]	Otu023	*Prevotella*	315.48	0.64	2.24E-05	0.008
	Otu027	Unclassified bacterium from the candidate division TM7 phylum	153.31	0.80	0.0002	0.027
	Otu128	*Sphingomonas*	7.47	-2.00	0.0002	0.027
	Otu002	*Veillonella*	4320.25	0.55	0.0004	0.03
	Otu178	*Veillonella*	2.10	0.63	0.0004	0.03
	Otu035	*Derxia*	101.92	-0.53	0.001	0.048
	Irregular vs. Regular patterns					
Dinner patterns[a]	Otu063	*Prevotella*	39.4	1.02	1.80E-06	0.0003
	Otu078	*Porphyromonas*	23.55	-0.92	4.57E-05	0.003
	Otu088	*Campylobacter*	18.86	-0.82	3.57E-05	0.003
	Otu073	*Capnocytophaga*	28.69	-0.63	0.0003	0.013
	Otu039	*Haemophilus*	76.32	-1.15	0.001	0.019
	Otu052	*Actinobacillus*	55.06	-0.70	0.001	0.028
	Otu016	*Porphyromonas*	522.59	-0.47	0.002	0.046
	Otu050	*Johnsonella*	58.49	-0.39	0.002	0.046
	Otu157	*Neisseria*	3.11	-1.30	0.002	0.046
	Otu204	*Leptotrichia*	1.56	1.84	0.002	0.046
	Otu006	*Neisseria*	1903.29	-0.50	0.003	0.048

[a]Adjusted for gender, age, language spoken at home, body mass index (BMI) categories and sequencing depth. False discovery rate (FDR) adjustment for p value. FDR of 0.05 was used. Results presented from a general linear model. Log2fold change is a measure between the intial and final values of the groups.

6 DISCUSSION

Figure 9 summarises all of the main findings regarding the relationships between eating habits, body mass and saliva microbiota in studies I–III. In study I, three eating habit groups were identified: healthy eaters, unhealthy eaters and fruit and vegetable avoiders. The major eating habit groups were associated with breakfast and meal patterns including lunch and dinner, and socio-demographic characteristics such as gender and parental education level. In study II, adolescents who avoided fruits and vegetables were associated with being underweight. Those who ate breakfast irregularly were associated with being overweight and obese and inversely associated with being underweight. Similarly, adolescents who irregularly ate dinner were less likely to be underweight. In study III, a higher diversity was found among adolescents with regular breakfast and dinner patterns compared to adolescents who ate meals irregularly. However, only breakfast patterns differed in terms of composition. Different saliva bacteria were abundant when comparing eating habit groups and comparing breakfast and dinner pattern groups. *Prevotella* was highly abundant among adolescents who avoided fruits and vegetables and who skipped meals such as breakfast and dinner.

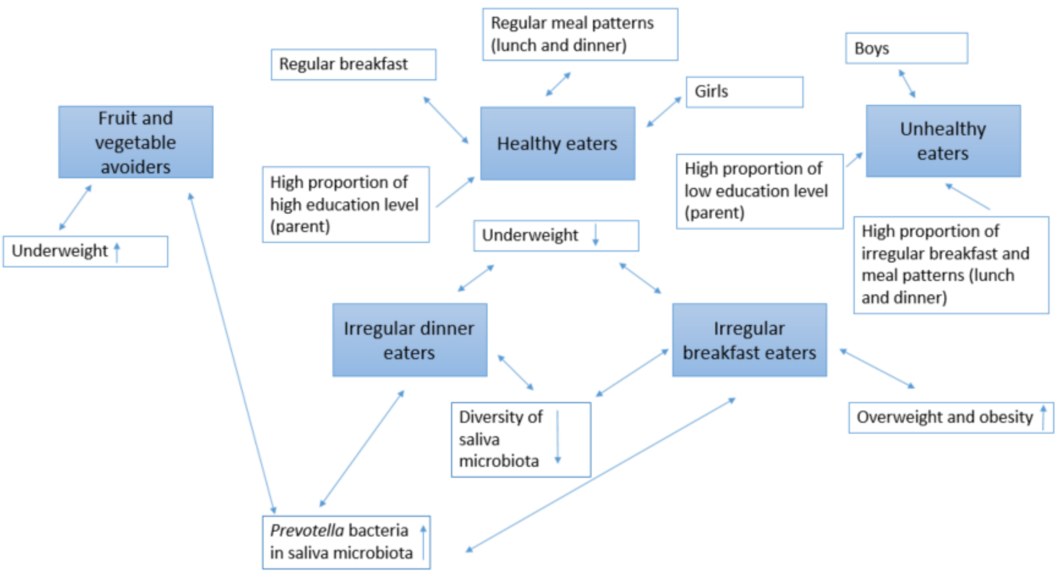

Figure 9. Summary of the main findings from studies I–III among adolescents.

6.1 Main findings and comparisons with previous studies

6.1.1 Identifying eating habits (study I)

Three different eating habit groups were identified in study I. The results of this study show that about 44% of Finnish adolescents avoid fruits and vegetables. Norwegian adolescents were clustered into a quite unhealthy group (n = 270; 38.5%) consisting of the infrequent consumption of fruits and vegetables and the low intake of snacks and SSBs (16), similar to the fruit and vegetable avoiders in this study. In Europe, adolescents consumed fewer fruits and vegetables than recommended and favoured meat and meat products, fats and sweets (13). Likewise in Finland, adolescents consumed very few fresh vegetables and fruits and frequently consumed SSBs and snacks (14). Additionally, young Finnish men less frequently ate fruits, berries and vegetables daily and preferred SSBs, sweets and fast food such as pizza and hamburgers (117), similar to the unhealthy eaters who were primarily boys in study I. Similar to healthy eaters in this study, a healthy group, including individuals with an adequate intake of fruits and vegetables and a low consumption of snacks and SSBs, was previously identified among Norwegian adolescents (16). This suggests that eating habits among adolescents might be comparable across the Nordic countries.

In Norway, adolescents who regularly ate breakfast more often had parents with a high education level (135). Other studies have also linked a low socio-economic status to unhealthy eating habits, such as the low consumptions of fruits and vegetables, frequent consumption of fast food and sweets (136–137) and skipping meals (breakfast, lunch and dinner) (138) in accordance with the results presented here. Therefore, this study strengthens the link between a low socio-economic status and unhealthy eating habits. Parental education should be taken into account when promoting healthy eating habits among adolescents with poor eating habits including skipping meals.

Finally, this study identified gender differences between eating habits. Similarly to the results presented here, girls less often consumed breakfast daily compared to boys in Europe and Australia (14, 19, 43). Moreover, Finnish boys ate fewer fresh vegetables (14, 44) and drank more SSBs (14) compared to Finnish girls. The results here emphasise the gender role in eating habits. Thus, more should be done to diminish gender differences in eating habits, particularly among boys with unhealthy eating habits including meal skipping.

Moreover, research on food consumpion utilises several tools such as dietary recalls (24 hours) (139), food records (140) and food frequency questionnaires (FFQs) (141). Therefore, findings on eating habits among adolescents may vary between studies. The limitations of dietary recalls and food records lie within reporting and recall biases (142–143). Thus, food FFQs are also used (144). This study utilised a FFQ, the appropriate tool to use in epidemiological studies (145). For instance, the HBSC study's FFQ consisted of key indicators of adolescents' healthy and unhealthy dietary habits and was established in different European countries (123–124). Here, the short FFQ adapted from the HBSC study was suitable, since it represents a reliable method to assess food groups in different countries including Finland (146). However, it is possible that food consumption was overestimated when using a short FFQ (147).

The results here provide more knowledge on major eating habit groups and their associations with breakfast and meal patterns and socio-demographic characteristics among Finnish adolescents. This study showed that more than one in three adolescents skipped breakfast, lunch and dinner. Therefore, there is a growing need to improve eating habits, such as increasing the intake of fruits and vegetables and following a regular meal pattern among adolescents.

6.1.2 Eating habits and body mass (study II)

Among the three major eating habit groups, only fruit and vegetable avoiders associated with being underweight in study II. Previously, an unhealthy group containing those who consumed few fruits and vegetables and an abundance of snacks and SSBs was inversely associated with BMI (16). That agrees with the results presented here. Picky eating can explain the association between fruit and vegetable avoiders and being underweight since picky eating has been associated with being underweight and a lower BMI (148). Additionally, the FFQ was not designed to examine whether fruit and vegetable avoiders consumed small portion sizes and a limited amount of energy.

No association was found between the intake of unhealthy food items, such as junk food, SSBs and biscuits, and being overweight among Norwegian school-aged children (149), findings similar to those presented here. Additionally, no association was found between an unhealthy diet containing among others ice cream, French fries, potato chips and SSBs, and being overweight among British children (150). Unhealthy eaters may maintain a normal weight given the consumption of the total energy of foods at a sufficient level.

Other studies support the association between an irregular breakfast pattern with being overweight and obese found in this study (11–12, 17–21). Previously, an irregular breakfast pattern was linked to a high BMI in Finland (24, 63). It is possible that other unhealthy behavioural factors including a sedentary lifestyle also play a role being overweight and obese (63). The inverse associations between being underweight and irregularly eating breakfast and irregularly eating dinner identified in this study represent intriguing findings. Yet, skipping breakfast has been associated with being underweight (151). The mechanism for a decreased risk of being underweight associated with irregular meals remains unclear. Thus, this study provides additional information regarding how meal frequency plays a role in being underweight. It is possible that breakfast skippers avoid some unhealthy food items which may lead to weight gain. However, information on the content of foods and portion sizes among those irregular breakfast eaters remains lacking in this study. Additionally, BMI associates with fat mass and waist circumference (152), showing that other body mass measures can provide additional information on being underweight. Thus, further research is needed to examine the associations between being underweight and breakfast and dinner patterns among adolescents.

The prevalence of being overweight and obese among children and adolescents may vary even between Finnish studies given, for instance, the coverage of the Register of Primary Health Care Visits (Avohilmo) (46). More specifically, data from the Register of Primary Health Care Visits (Avohilmo) reached 65% coverage showing a mean prevalence for being overweight (22%) and for obese (6%) among 7- to 12-year-old children (46). The data from the Register of Primary Health Care Visits (Avohilmo) revealed higher proportions than those reported here. Thus, this study's strength lies in its large cohort that included more than 10 000 Finnish adolescents from southern, middle and northern Finland. Additionally, findings from earlier studies may differ given the separate analyses on being overweight and obesity. In this study, however, the overweight and obese categories were combined.

Finally, 63.5% of the adolescents were 11 years old and 23.1% of the adolescents were older than 11 in this study. WHO defines individuals aged 10- to 19-years old as adolescents (153). Hence, the results presented here on the relationship between eating habits and body mass apply to both children and adolescents, since the study population here included participants aged 9 to 14 years.

6.1.3 Eating habits and saliva microbiota (study III)

This is the first study conducted on the association between eating habits and saliva microbiota. Previous studies have focused on the association between eating habits and saliva microbiota among adults (33–34). Saliva microbiota did not differ between adults aged 18 and 55 years following omnivore, ovo-lacto vegetarian and vegan diets (33). The previous study differed from this study since extreme diets were not examined here. Therefore, it is challenging to provide a comparison to previous findings. Saliva, however, appears to remain resilient toward diet among 12- to 22-year-old individuals (154), which may partly explain the results here.

In this study, high richness was found among adolescents following a regular meal pattern. A high diversity represents an advantage for humans due to the increased resistance to invaders (29), including pathogens. In the saliva microbiota, the timing of eating influenced the daily rhythm of diversity (35): late lunch eaters had a higher diversity at two time points compared to early lunch eaters. However, sample collection was undertaken between breakfast and lunch in school, possibly influencing the results here. More specifically, the timing of the saliva sample collection was unknown and, therefore, standardisation of meal behaviours was impossible. Previously, a low diversity in the saliva microbiota was linked to being overweight and obese among adolescents from the same cohort (128). Thus, it is possible that both BMI and irregular meals impact the saliva diversity among study participants. The results from this study on the associations related to diversity and composition in saliva microbiota in relation to breakfast and dinner eating may offer a more in-depth understanding of how regular eating contributes to health and preventing, for instance, being overweight and obese.

Moreover, a high abundance of *Prevotella* bacteria was found among adolescents who avoided fruits and vegetables and who skipped breakfast and dinner. *Prevotella* is an anaerobic Gram-negative bacterium (155) and plays a role in breaking down proteins and peptides into amino acids (156). Diseases, such as periodontitis are associated with *Prevotella* (157). Additionally, a high abundance of *Prevotella* was found in the gut (32, 106) and a low abundance in the saliva (34) of adults following a plant-based or a vegan diet. One study suggested that *Prevotella* could be used as a biomarker of diet and lifestyle (158). It is difficult to evaluate how big a role diet and meal frequency play in shaping the saliva microbiota given current evidence. Hence, the association between avoiding fruits and vegetables combined with eating irregular meals and the abundance of *Prevotella* in saliva may promote further studies on *Prevotella* and eating habits.

Veillonella bacteria was highly abundant among adolescents who irregularly ate breakfast. *Veillonella* is an anaerobic Gram-negative bacterium (159) which uses carbohydrates such as glucose or amino acids for energy mobilisation and receives energy from short-chain organic acids such as lactate (159). A high abundance of *Veillonella* has been linked to gingivitis (160) and discovered among children with poor oral hygiene (161). For study III, no information was available on the oral health status of adolescents in the Fin-HIT study. However, it is possible that irregular breakfast eaters also have poor oral hygiene. Previously, a low intake of daily meals associated with insufficient tooth brushing (less than twice a day) (162). That said, skipping breakfast was associated with less than one annual dental care visit among 16- to 30-year-old participants from low-, middle- and high-income countries (162). It is possible that saliva microbiota changed due to diseases such as periodontitis (157) or poor oral hygiene (161), justifying the inclusion of oral health status in future studies.

Futhermore, irregular dinner eaters had a higher abundance of *Leptotrichia* bacteria than regular dinner eaters in study III. *Leptotrichia* is a member of the anaerobic Gram-negative bacteria (163) utilising glucose to produce lactic acid (164). Adolescents suffering from caries have been found to have *Leptotrichia* (165). In caries, bacteria create acid from metabolised sugars (166). Other bacteria, such as Gram-negative *Neisseria* (167) and *Megasphaere* bacteria (168) were associated with caries (169–170). Fruit and vegetable avoiders had a high abundance of one unclassified bacterium from the *Neisseriaceae* family, whereas unhealthy eaters had a high abundance of *Megasphaere* in this study. The abundant consumption of sugary foods, such as SSBs and sweets, plays a role in the development of caries (166). Adolescents appear to favour SSBs and sweets (13) and eat fewer fruits and vegetables (14). Thus, adolescents with unhealthy eating habits, such as avoiding fruits and vegetables and preferring sugary and salty snacks and skipping meals, may be at a high risk of caries. Moreover, the results from this study provide additional understanding regarding how eating habits play a role in the saliva microbiota and may further improve knowledge on preventing of different diseases. However, it is difficult to specify which healthy microbiota are most important since it varies between healthy individuals (99). Finally, differences in the abundance of OTUs between eating habit groups, breakfast and dinner patterns were found in the saliva microbiota in this study. However, this study utilised DEseq2 to identify differences in the relative abundance. It is possible that DEseq2 may identify false-positive hits, since an increase in one OTU results in a decrease in the remaining OTUs.

6.2 Strengths and limitations

One key strength of this study stems from its large and well-characterised cohort. This study identified three main eating habit groups. Additionally, the associations beween eating habits and being underweight as well as overweight and obese and the associations between eating habits and saliva microbiota were examined using FFQ data participants provided. The size of the cohort allowed to explore rare eating habit groups such as unhealthy eaters, adjust for potential confounders and modifiers and examine interactions between eating habit groups and dinner patterns. This study also offers a broader understanding of eating habit groups and how the regularity of meals impacts saliva microbiota diversity and composition. Deep 16S microbiota represents an important strength given the large number of participants included in the analyses. Another well-known method is metagenomics (171). However, metagenomics remains expensive and better for the gut (171) than for the saliva microbiota given the large number of human cells and fewer bacteria in saliva (172).

The limitations of this study include the age group's ability to accurately answer the questionnaire. The questionnaire distributed to adolescents was assessed at the beginning of this study to determine the cognitive maturity of participants (54). Thus, this study chose a short questionnaire using indicator items taking cognitive maturity into account. This short FFQ was also considered suitable for this age group during a previous study (173).

A further limitation is that the FFQ questionnaire remains unvalidated. Information on the portion size, energy intake, weekend day meals and the entire diet also remain lacking. Fruit and vegetable avoiders may have avoided some other food items that were not included in the FFQ. Despite this, previous studies have utilised a short FFQ among children (40, 174) and the FFQ in this study contained key indicators of healthy and unhealthy diets among adolescents recommended by the HBSC study protocol (123).

It is possible that a reporting bias exists in this study since earlier research showed that overweight and obese adolescents underreported their dietary intake (175). That same study found that underweight adolescents overreported their dietary intake (175). Moreover, participating adolescents were most likely healthy since sick participants were absent from school during data collection.

Further limitations include a low response rate (30%) and obtaining data on parental education for only 57% of adolescents. However, the socio-demographic characteristics agreed with those reported in other studies of Finnish children (176–177). Additionally, this cross-sectional study design did not allow to examine causal relationships between eating habits and body mass or between eating habits and the saliva microbiota. Furthermore, the timing of the saliva sampling was unknown. Thus, future research should both attempt to replicate and validate the results presented here.

Finally, the study limitations include missing information on the oral health status of adolescents, a possible confounder in this study. Nonetheless, Finnish children appear to enjoy sufficiently good oral health (178).

6.3 Implications for health promotion and public health

This doctoral thesis provides a deeper understanding of adolescents' eating behaviours, including healthy and unhealthy eating habits, and adds knowledge to the field of eating habits, body mass and saliva microbiota. Based on the results presented here, actions to promote healthy eating habits are needed, specifically, among those who avoid fruits and vegetables, prefer unhealthy food items and skip meals. The results here also show that eating habits differed between genders. Previous studies found that girls ate more fruits and vegetables than boys. Moreover, fatty and sugary foods are more popular among boys than among girls (179), supporting the results here. Additionally, this study found that parental education plays a role in poor eating habits. Other studies also showed that unhealthy eating habits, such as a high intake of fast foods and sweets (136–137) and irregular meals (138) were linked to parental socio-economic status in accordance with this study's results. Thus, public health professionals promoting healthy eating behaviours should include targeted messages to boys and those who skip meals. Public health professionals should also consider parental education when improving eating habits among adolescents with unhealthy eating habits including irregular meals. Additionally, future research should verify the results of this study related to eating habits and their associations regarding breakfast and meal patterns among adolescents in Finland.

In Finland, school children are offered health checks in the first, fifth and eight grades in school by doctors (180). Nurses, however, check Finnish school children every year in schools (180). Therefore, school could serve as one of the potential health promotion places, providing additional lessons related to healthy eating habits. Finnish adolescents reportedly to consume school snacks, including vegetables and bread, but also energy-dense and nutrient-poor foods including sweets and SSBs (14, 181). The availability of fresh vegetables in schools and at home can increase consumption among adolescents (181). Additionally, increasing knowledge regarding how healthy eating habits relate to different health outcomes is crucial.

Eating habits play a vital part in human health and disease (1). Thus, it is important to assess eating habits and their synergistic and cumulative effects on health (182). Most studies agree with the results presented here on the association between irregular breakfast eaters and being overweight and obese (11–12, 17–21). This study's results also provide additional knowledge, particularly on the association between avoiding fruits and vegetables and being underweight. More actions are needed to prevent childhood obesity and promote a normal weight. These results also provide more in-depth understanding of the association between eating habits and body mass. The results here may help health personnel guide adolescents at risk of being underweight or overweight and obese to maintain a normal weight by increasing their intake of fruits and vegetables, decreasing their intake of unhealthy foods and eating regular meals, such as breakfast, lunch and dinner.

This study's results on eating habits and their association with body mass may be explained by the influence of the home environment on adolescents' BMI. Thus, the home environment can play a significant role in promoting healthy behaviours among children and adolescents. A child's food intake and parental feeding behaviours (183) including parenting practices (184) have been linked to a child's BMI. Parents carry a huge responsibility in terms of the availability of foods at home. Therefore, parents should adopt practices that promote healthy behaviours at home.

This study's results on eating habits and the saliva microbiota emphasise the importance of regular meals and their impact on health. Among other roles, the microbiota contribute to the immune system (81) and human biological processes (82), all of which are vital to human health. Previously, an unhealthy Western diet appeared to associate with dysbiosis in the gut (32). An increase in the pathobiont microbiota in the saliva was also linked to changes in the host's metabolism (35). The results presented here on the high diversity of the saliva microbiota among regular breakfast and dinner eaters provides further insight into the possible mechanisms of eating habits and body mass. Moreover, additional research is needed to determine how eating habits and the saliva microbiota link to health outcomes. Finally, given this cross-sectional study and its results, future research should examine the causal relationship between eating habits and body mass, and between eating habits and the saliva microbiota among adolescents through a longitudinal design.

7 CONCLUSIONS

This doctoral dissertation examined associations between eating habits, body mass and saliva microbiota among Finnish adolescents.

The three main conclusions and implications are as follows:

1. Three major eating habits groups were identified among adolescents: healthy eaters, unhealthy eaters and fruit and vegetable avoiders. Healthy eaters more often ate a regular breakfast and other meals (lunch and dinner) and had parents with a high education level. In turn, unhealthy eaters most often irregularly ate breakfast and other meals and had parents with a low education level. Promoting healthy eating habits remains important, particularly among adolescents who avoid fruits and vegetables and skip meals.

2. Fruit and vegetable avoidance was associated with being underweight. Eating breakfast irregularly was associated with being overweight and obese and inversely associated with being underweight. Eating dinner irregularly was also inversely associated with being underweight. The results presented here can help public health professionals to guide adolescents on how to maintain a normal weight and reduce their risks of being both underweight and overweight and obese.

3. Regular breakfast eating was associated with higher diversity and a different composition of the saliva microbiota compared to irregularly eating breakfast. Additionally, saliva microbiota diversity differed between regular and irregular dinner eaters. Different levels of bacterial abundance were found across eating habit groups, comparing regular and irregular breakfast eaters and comparing regular and irregular dinner eaters. The association between avoiding fruits and vegetables and eating skipping meals, respectively, and the abundance of *Prevotella* in the saliva may promote future studies on *Prevotella* and eating habits.

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