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Saliva microbiota differs between children with low and high sedentary screen times

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ABSTRACT

This study examined whether the diversity, composition and functional capacity of the saliva microbiota differed between children with low and high sedentary screen times. We analyzed the saliva microbiota using 16S rRNA (V3–V4) sequencing from 193 children with low and 183 children with high TV/screen viewing times while sitting. Microbiota diversity was higher among children with low screen times compared to children with high screen times. Furthermore, microbiota composition differed between the screen time groups. In addition, we identified ten differentially abundant taxonomic groups, including *Veillonella*, *Prevotella* and *Streptococcus*, and five differentially present metabolic pathways between the screen time groups. Children with high screen times exhibited a higher capacity to synthesize the fatigue- and activity-related amino acids ornithine and arginine. To conclude, children with high sedentary screen (sitting) times exhibited a lower diversity and a different composition and functionality of the microbiota compared to children with low screen times.

1. Introduction

The diversity and composition of both the gut and saliva microbiota seem highly important in human health and disease [1–4]. The saliva microbiota has a similar richness in species to elsewhere in the gastrointestinal tract, and the bacterial taxa are similar to those in stomach fluids and the placenta [5]. Moreover, the gut microbiome is influenced by the oral microbiota given the continuity of the gastrointestinal tract [6]. In recent years, saliva has attracted widespread interest as a means of simple, repeatable and rapid testing, and because the composition of the saliva microbiota might reflect the general health status [4]. Saliva is participant friendly to collect, minimally affected by collection or DNA extraction protocols, and has a temporal stability such that changes in the saliva microbiota profile may provide insight into the onset and progression of disease [2]. Nevertheless, the relationship between the saliva microbiota and health-related factors remains less studied than the relationship between the gut microbiota and health. In addition to the composition, the metabolic functions of the saliva microbiota warrant further analysis [4].

The microbiota is influenced by host genetics, age and environmental factors, such as delivery mode, antibiotic use and diet [7–10]. Recent studies indicated that physical activity may also modify the microbiota by increasing the presence of beneficial bacteria and microbial diversity. However, such studies remain scarce and primarily consist of animal studies [11–13]. From a recent review, existing human studies feature small numbers of participants, are mostly cross-sectional and have investigated the gut microbiota [13]. We found one pilot study addressing the oral microbiota, which showed that compared with 'off-season' athletes, 'in-season' athletes exhibited higher levels of total microbial counts accompanied with lower levels of potentially

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Table 1
Participant characteristics by sedentary screen times ($n = 376$).

Characteristic	All $n = 376$ (100%)	Children with low screen times $n = 193$ (51%)	Children with high screen times $n = 183$ (49%)	p[a]
Age, in years, mean (SD)	11.7 (0.4)	11.7 (0.4)	11.8 (0.4)	0.202
Gender, n (%)				
Girl	184 (48.9)	109 (56.5)	75 (41.0)	0.003
Boy	192 (51.1)	84 (43.5)	108 (59.0)	
Language spoken at home, n (%)				
Finnish	319 (84.8)	164 (85.0)	155 (84.7)	0.060
Swedish	39 (10.4)	24 (12.4)	15 (8.2)	
Other	18 (4.8)	5 (2.6)	13 (7.1)	
BMI categories, n (%)				
Underweight	52 (13.8)	30 (15.5)	22 (12.0)	0.297
Normal weight	274 (72.9)	143 (74.1)	131 (71.6)	
Overweight	41 (10.9)	17 (8.8)	24 (13.1)	
Obese	9 (2.4)	3 (1.6)	6 (3.3)	
Eating habit groups, n (%)				
Unhealthy	47 (12.5)	12 (6.2)	35 (19.1)	<0.001
Vegetable avoider	154 (41.0)	63 (32.6)	91 (49.7)	
Healthy	175 (46.5)	118 (61.1)	57 (31.1)	
Leisure time physical activity Categories, n (%)				
Low (≤4 h/week)	88 (23.4)	38 (19.7)	50 (27.3)	0.178
Medium (5–9 h/week)	196 (52.1)	103 (53.4)	93 (50.8)	
High (≥10 h/week)	92 (24.5)	52 (26.9)	40 (21.9)	

Low sedentary screen time = TV viewing ≤ 1 h/day on weekend days.
High sedentary screen time = TV viewing ≥ 4 h/day or more on weekend days.
BMI = body mass index; SD = standard deviation
[a] Results from the Chi-square test, except for age, which is from ANOVA.

pathogenic bacteria [14]. Physical activity may increase microbial diversity through several mechanisms, including promoting an anti-inflammatory state [12,15].

If physical activity provokes beneficial changes in the microbiota, sedentary behavior may, in turn, promote harmful alterations. Sedentary behaviors refer to activities in a reclining, seated or lying position requiring very low energy expenditure and body movement. To date, only a few studies have examined the relationship between physical inactivity or sedentary behavior with the gut microbiota [16–18] and, to the best of our knowledge, none focused on the saliva microbiota. An inverse relationship was observed, however, between sedentary time and gut microbiota richness among 40 adult women, and the abundance of some health-promoting bacterial species was higher among active women compared to inactive women [16]. Sedentary behaviors did not associate with the gut microbiota richness among 82 college students, although the microbiota composition differed based on daily sedentary times [17]. Moreover, a very recent study reported a lower diversity and network complexity of the gut microbiota among 45 inactive compared to 64 active healthy adults [18]. These findings indicate that not only physical activity, but also a sedentary lifestyle may induce changes in the gut microbiota. Examining the possible relationship of both physical activity and inactivity with the microbiota may lead to further understanding how physical movement diminishes the risk of disease and improves health [19].

Host–environment–microbial interactions are important throughout life, but these interactions can carry crucial and long-lasting implications particularly during critical developmental periods during childhood [20]. However, to our knowledge, the relationship between sedentary behavior and the microbiota in children remains unclear. We previously showed that high sedentary screen times among children associated with being overweight and central adiposity, and predicted a higher body mass index (BMI) among adolescents [21,22]. Here, we aim to identify potential differences in saliva microbiota richness, composition and functional capacity between children with high and low sedentary screen times. This study provides novel evidence on the possible factors associated with children's microbiota, and may reveal mechanisms regarding how sedentary behavior relates to health.

2. Results

2.1. Participant characteristics

Our study included a total of 376 children with low or high screen times and data available on the microbiota and the appropriate covariates. Table 1 shows the participant characteristics in all children and by screen time level. Children with low and high screen times differed based on gender and eating habits. A larger proportion of girls (57%) reported low screen times, whereas a larger proportion of boys (59%) reported high screen times. Among children with low screen times, a higher proportion comprised of healthy eaters, while a smaller proportion comprised of unhealthy eaters and vegetable avoiders when compared to children with high screen times.

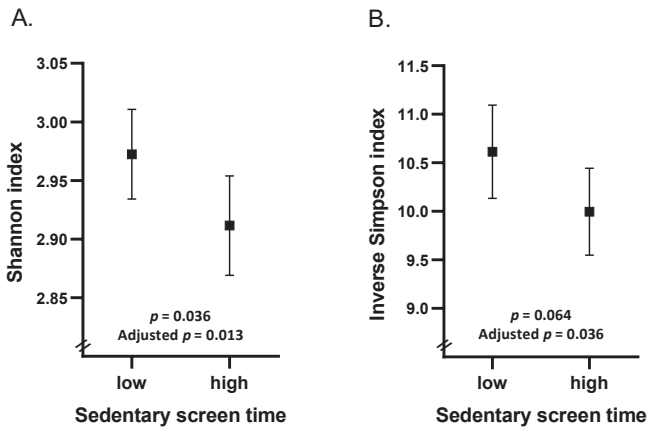

Fig. 1. The alpha diversity (means and 95% CIs) in the saliva microbiota among children with low ($n = 193$) and high ($n = 183$) sedentary screen times using A) the Shannon index and B) the Inverse Simpson index. Adjusted p is adjusted for age, gender, language, body mass index categories, physical activity and eating habits. Results reported from ANOVA and ANCOVA.

2.2. Sedentary screen time and the alpha diversity of saliva microbiota

We detected differences in the alpha diversity between children with low and high sedentary screen times using the Shannon and Inverse Simpson indexes (Fig. 1). Children with low screen times had a higher Shannon index compared to children with high screen times; the mean difference between groups was 0.06 (95% CI 0.00–0.12, $p = 0.036$, Partial Eta Squared = 0.012). After adjusting for age, gender, language and BMI categories, the difference was 0.07 (95% CI 0.01–0.12, $p = 0.027$, Partial Eta Squared = 0.013), and after adding physical activity and eating habits as covariates reached 0.08 (95% CI 0.02–0.14, $p = 0.013$, Partial Eta Squared = 0.017). The Inverse Simpson index did not differ between children with low and high screen times, with a mean difference of 0.62 (95% CI −0.04–1.27, $p = 0.064$, Partial Eta Squared = 0.009). After adjusting for age, gender, language and BMI categories, the mean difference was 0.67 (95% CI 0.00–1.35, $p = 0.051$, Partial Eta Squared = 0.010). We detected a significant difference after adding physical activity and eating habits as covariates, finding a mean difference of 0.76 (95% CI 0.05–1.47, $p = 0.036$, Partial Eta Squared = 0.012).

We conducted sensitivity analyses by repeating the crude alpha diversity analyses only among children reporting healthy eating habits. Our results were similar to those for the entire sample. Healthy eaters with low screen times ($n = 157$, mean 2.96, SD 0.27) exhibited a higher Shannon index compared to healthy eaters with high screen times ($n = 57$, mean 2.86, SD 0.30; mean difference 0.11, 95% CI 0.02–0.20, $p = 0.018$, Partial Eta Squared = 0.032), whereas the Inverse Simpson index did not differ between healthy eaters with low (mean 10.55, SD 3.62) and high screen times (mean 9.57, SD 2.81; mean difference 0.98, 95% CI −0.10–2.06, $p = 0.074$, Partial Eta Squared = 0.018).

2.3. Sedentary screen time and the beta diversity of the saliva microbiota

In addition, the composition (beta diversity) of the saliva microbiota was nearly significantly different between children with low and high screen times in our crude analysis ($p = 0.051$; Fig. 2). After adjusting for age, gender, language and BMI categories, the difference became more evident ($p = 0.040$), and remained significant after adding physical activity and eating habits as covariates ($p = 0.036$).

2.4. Sedentary screen time and saliva bacteria abundances

We observed 17 differently abundant OTUs when comparing children with low and high screen times in a crude model (Supplementary table 1). After adjusting the analyses for age, gender, language and BMI categories, 12 OTUs remained differentially abundant among children with low and high screen times (Supplementary table 2). Finally, after adding physical activity and eating habits as additional covariates, the differences between children with low and high screen times remained significant for 10 OTUs (Table 2).

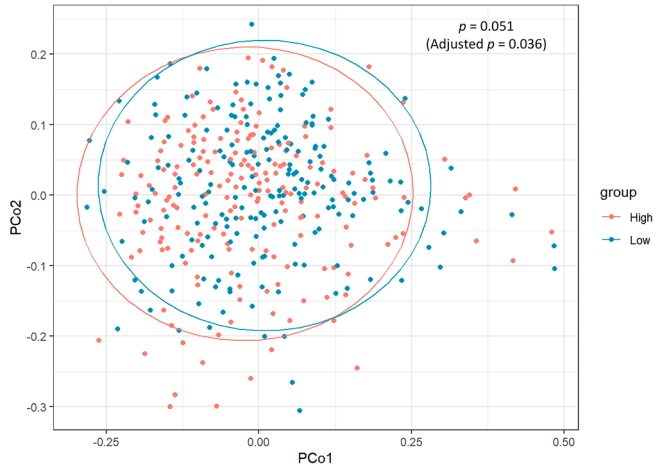

Fig. 2. Principal coordinate analysis (PCoA) based on the Bray–Curtis distances (beta diversity) for the saliva microbiota according to children's sedentary screen times: low ($n = 193$) or high ($n = 183$). Adjusted p is adjusted for age, gender, language, body mass index categories, physical activity and eating habits. Results reported from a PERMANOVA.

Table 2
Ten differentially abundant bacteria at the OTU level between children with low ($n = 193$) and high ($n = 183$) screen times after adjusting for age, gender, language, BMI categories, physical activity and eating habits.

OTU	Nearest taxa	Base mean	log2 fold change[a]	Adjusted p
Otu000001	Veillonella	9114.9	0.446	0.001
Otu000002	Veillonella	4561.2	0.565	0.034
Otu000005	Prevotella	2242.4	0.707	0.014
Otu000011	Streptococcus	1159.1	0.552	0.034
Otu000014	Streptococcus	932.0	0.527	0.040
Otu000031	Atopobium	174.8	0.567	0.014
Otu000045	Actinomyces	72.9	0.534	0.014
Otu000112	Streptococcus	10.5	0.907	0.003
Otu000150	Veillonella	4.0	0.788	0.040
Otu000170	Streptococcus	3.2	1.210	0.003

Base mean = mean of the normalized counts across all samples.
OTU = operational taxonomic unit.
[a] A positive value reflects a higher abundance among children reporting high screen times compared to those reporting low screen times.

2.5. Sedentary screen time and functional capacity of saliva microbiota

We created a metabolic profile using all OTUs; functional predictions identified five differentially present metaCyc pathways between children reporting low and high screen times (Fig. 3). Among these, three pathways comprised higher proportions of children with high screen times. The largest differences between the screen time groups were as follows: pathways for the superpathway of demethylmenaquinol-8 biosynthesis I, 2-carboxy-1,4-naphthoquinol biosynthesis, and superpathways phylloquinol biosynthesis, which in general relate to the syntheses of vitamin K2 (menaquinones) and vitamin K1 (phylloquinone). Children reporting low screen times exhibited lower proportions of biosynthesis of the amino acids L-ornithine I and L-arginine.

3. Discussion

We compared the diversity, composition and functional capacity of the saliva microbiota between 193 children with low and 183 children with high sedentary screen times. Our results showed that children with low sedentary screen times had a greater microbial diversity. In addition, we observed differences in the composition and functional capacity of the saliva microbiota between the sedentary screen time groups. Children reporting high sedentary screen times had microbiota with an advanced menaquinone biosynthesis, while children reporting low sedentary screen times had microbiota with less amino acid biosynthesis.

The use of screen-based devices has increased over the past two decades in children and adolescents [23]. This had led to higher sedentary times, which in turn associates with adiposity, an unhealthy diet and poor mental wellbeing among youth [24–26]. Some evidence, however, suggests that small amounts of daily screen use are not harmful and may carry some benefits to children's wellbeing [26]. We previously showed that high sedentary screen times associated with being overweight and central adiposity among the Fin-HIT children [21]. Our results here indicate that high screen times also associate with a lower diversity, an altered composition and a distinctive functional capacity of the saliva microbiota. To our knowledge, this study represents the first to examine the relationship between sedentary behavior and the saliva microbiota in children or in any population.

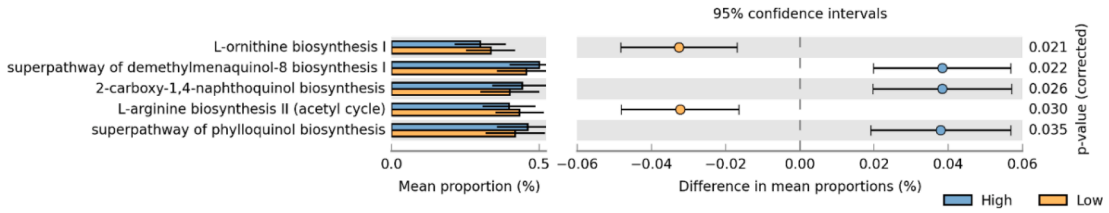

Fig. 3. Functionally predicted MetaCyc pathways in differing proportions between children reporting low and high screen times. The bar plot shows the mean proportions of differential MetaCyc pathways predicted using PICRUSt2. The difference in the proportions between groups are shown with the 95% confidence intervals. Only differences with $p < 0.05$ (Welch's *t*-test, FDR adjusted) are shown.

We showed that children's saliva microbiota was more diverse among children reporting low sedentary screen times compared to children reporting high screen times, even when taking into account several confounding factors, including BMI, physical activity and eating habits. Moreover, we detected a difference in the microbiota diversity between the screen time groups using both the Shannon and Inverse Simpson indexes, thereby strengthening the reliability of our findings. Diversity appears to serve as a generally good indicator of "healthy" microbiota [4,27]. The richer and more diverse the microbiota is throughout life, the better it withstands external threats [28]. Low bacterial richness correlates with adverse metabolic markers, such as adiposity, insulin resistance and overall inflammatory phenotypes [29].

We also showed that the beta diversity, that is, the microbial composition of saliva, differed between children reporting low and high screen times. These differences were small, but they may potentially carry health consequences in the long term. More precisely, we detected ten differentially abundant bacteria between low and high screen time groups. Children reporting high screen times had higher abundances of, for example, *Veillonella, Prevotella* and *Streptococcus*, all belonging to the core bacteria present in the saliva [30,31]. Moreover, their abundances were previously shown to differ between overweight and normal weight children [30]. In another previous analysis, Fin-HIT children with unhealthier eating habits or with an irregular dinner pattern exhibited a higher abundance of *Prevotella,* whereas children with an irregular breakfast pattern exhibited higher abundances of both *Prevotella* and *Veillonella* [32]. *Prevotella* and *Veillonella* associate with various infections in children [33,34]. Furthermore, the proportion of *Veillonella* in the saliva appears to increase and *Streptococcus* decreases with poor oral hygiene in children [35].

We observed that some metabolic pathways of the microbiome differed between children with low and high screen times. The pathways related to menaquinone biosynthesis were more frequently present in the saliva microbiome of children with high screen times. Local actions of menaquinones in the bacterial cytoplasmic membrane associate with electron transfer and antioxidant properties that protect cellular membranes [36]. The intestinal bacterial production of menaquinones may contribute to up to 50% of the total menaquinones intake [37]. Yet, their bioavailability and absorption remain unclear [37,38]. Menaquinones, similar to any other vitamin K form, are required for the hepatic production of Gla proteins, including various coagulation factors such as prothrombin, VI, IX and X and the well-defined matrix Gla protein and osteocalcin [38]. Interestingly, an adequate intake of menaquinones associates with improved bone health and a lower risk for cardiovascular disease [38,39]. In our study, this finding was reassuring, since it suggests that bacterial K2 synthesis is not jeopardized in otherwise sedentary children.

In addition, the saliva microbiota of children reporting low screen times produced lower levels of L-ornithine and L-arginine amino acids compared to children reporting high screen times. These amino acids interact, e.g. L-ornithine is a substrate of L-arginine. L-arginine plays a role in the maintenance of gut mucosal homeostasis and the gut barrier function [40,41]. Thus, our finding seems somewhat surprising. An impaired gut mucosal function has been associated with colitis and metabolism-associated diseases [40], yet several other factors play a role as well. A drastic drop in the endogenous synthesis of L-arginine results in ammonia detoxification, immune modulation, polyamine synthesis and the secretion of certain hormones [42–45] if not supplied from the diet.

We found no previous studies addressing the relationship between sedentary time and the saliva microbiota, although some studies examined the relationship between sedentary time and the gut microbiota among college students and adults [16–18]. One study reported no differences in gut microbiota species richness or evenness among 82 college students according to their physical activity or screen time levels. However, the microbiota composition differed by self-reported total sedentary time [17]. Moreover, the gut microbial taxa varied by physical activity level; *Paraprevotellaceae, Lachnospiraceae* and *Lachnospira* were more prevalent in college students reporting greater physical activity, and *Enterobacteriaceae* and *Enterobacteriales* appeared more enriched among college students reporting less physical activity [17]. In line with our results, another study reported an inverse relationship between sedentary parameters and the gut microbiota richness (number of species, and the Shannon and Inverse Simpson indices) among 40 women [16]. Moreover, *Odoribacter, Paraprevotella, Desulfovibrionaceae and Akkermansia*, correlated with sedentary time [16]. Interestingly, that study identified no difference between active and inactive groups (meeting and not meeting WHO physical activity recommendations) in the alpha or beta diversity, but found a higher abundance of health-promoting bacterial species among active women, including *Faecalibacterium prausnitzii, Roseburia hominis* and *Akkermansia muciniphila* [16]. Moreover, a rather recent study reported a lower diversity (phylogenic diversity, Chao, observed species and the Shannon index) and network complexity in the gut microbiota among 45 inactive compared to 64 active healthy adults [18]. However, the inactive group reported an unhealthier diet compared to the active group, possibly affecting the results. The inactive group reported more screen time than the active group, although the study did not directly examine the associations between sedentary time and microbiota.

High amounts of sedentary screen time can modify the microbiota through several possible mediating mechanisms. Physical activity–induced beneficial alterations in the microbiome may be related to alterations in the immune response and metabolism [12,46–48]. Furthermore, limited evidence from research indicates that low-grade inflammation and metabolic impairment serve as mediating mechanisms between sitting and an increased cardiovascular disease risk [49]. Thus, sedentary time may relate to the microbiota through inducing inflammation and metabolic impairment.

One limitation to our study includes the self-reported measurement of sedentary screen time. However, similar screen time questions were used previously in the WHO HBSC study, which showed fair to substantial test–retest reliability [50–52]. Self-report measurements of sedentary behavior may be somewhat biased due to either under- or over-reporting, but are relatively inexpensive and easy to administer, and, thus, remain more feasible. Self-report measures provide solid estimates of context-specific sedentary behavior in large-scale studies [53]. In addition, we only included children with extreme amounts of

sedentary screen times (low or high) in order to better compare children with different levels of screen times, and to minimize the possible bias related to self-report. This is the first study to examine the association between sedentary time and saliva microbiota, and therefore, we used the extreme amounts of sedentary screen time to examine whether we can detect any association. One limitation to our study is that there may be additional confounding factors, which we were not able to control for, in the relationship between sedentary time and saliva microbiota. These possible additional confounders include children's oral health and general health. However, we recently detected only minor differences in the saliva microbiota between children with and without caries in this same cohort [69]. Moreover, considering the young age of our participants recruited from the general population, most of them were healthy. Further studies are needed to show whether our findings can be replicated and to show their importance.

Despite these limitations, our study carries several strengths, including the large number of participants compared to studies examining the relationship between sedentary time and the gut microbiota. Furthermore, we could include many possible confounding factors in our analyses, including BMI, physical activity and eating habits. However, any residual confounding may persist even after adjustments because of possible measurement errors in the self-reported confounders. This study provides novel evidence on the possible relationship between children's sedentary screen times and the microbiota, suggesting that one mechanism behind the adverse health consequences of sedentary time may associate with the microbiota.

4. Conclusion

This study is the first to suggest that high amounts of sedentary behaviors, i.e. behaviors requiring very low energy expenditure and body movement, may be distinctly related to less diversity, different composition and distinct functional capacity in the children's saliva microbiota.

5. Materials and methods

5.1. Study design and participants

This cross-sectional study utilizes data from the Finnish Health in Teens study (Fin-HIT), a cohort study including more than 10 000 9- to 12-year-old children recruited from Finnish schools between 2011 and 2014. More detailed information on the Fin-HIT cohort appears elsewhere [54]. All study procedures adhered to the 1964 Helsinki Declaration and its later amendments or followed comparable ethical standards. The Coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa approved the study protocol (169/13/03/00/10), and we obtained written informed consent from all children who participated in the study and from one of their guardians. Microbiota profiles were obtained for 1000 randomly selected Fin-HIT participants [55]. In these analyses, we included children for whom information was available on the saliva microbiota, sedentary screen time and relevant confounders.

5.2. Measures

5.2.1. Sedentary screen time

We assessed children's sedentary screen time, that is, screen time while sitting, outside school hours using a web-based questionnaire. More precisely, we assessed time spent viewing TV programs and using a computer with questions adapted from the World Health Organization's (WHO) Health Behavior in School-Aged Children (HBSC) study [70]. The HBSC screen time questions have demonstrated a fair to substantial test–retest reliability depending on the criteria used [50–52]. We assessed screen time questions separately for school days and for weekends or days off, initially resulting in four sedentary screen time variables: viewing TV programs on school days, viewing TV programs on weekends, computer use on school days and computer use on weekends. We decided to only include viewing TV programs on weekends to represent screen time in the analyses because all four screen time variables correlated with each other ($r_s = 0.382–0.747$, $p < 0.001$ for all), and because viewing TV programs on weekends most strongly associated with BMI among the Fin-HIT children in our previous analysis [22]. In addition, TV viewing questions have shown best Intraclass Correlation Coefficients for test–retest reliability [50,51].

We assessed viewing TV programs through the following question: "How many hours a day during your free time do you normally watch TV, videos or DVDs? By TV, we mean programs that can be watched on TV as well as on a computer." Children answered the question by choosing between nine response options, ranging from (1) "I do not watch TV, videos or DVDs" to (9) "Around seven or more hours a day." Based on the responses for viewing TV programs on weekends, we categorized children into three groups with low (approximately the lowest 25%), medium (approximately the middle 50%) or high (approximately the highest 25%) screen times. The final categories consisted of the following: low as $\leq$ 1 h/weekend day (n = 201); medium as 2 to 3 h/weekend day (n = 410); and high as $\geq$ 4 h/weekend day (n = 191). In our analysis, we only included children with either low or high screen times because we specifically focused on comparing the saliva microbiota between children with the lowest and highest sedentary screen times. We did this because we believe that comparing the extreme amounts of screen times may minimize the possible bias related to self-reported behavior. In addition, this is the first study to examine the relationship between saliva microbiota and sedentary screen time, and therefore detecting any associations is important before proceeding to more detailed investigation.

5.2.2. Demographics

Parents reported their child's age, gender and language spoken at home, and we confirmed the child's birthday by linking data to the National Population Information System at the Population Register Center [54].

5.2.3. Body mass index (BMI)

Trained field workers measured children's height (cm) and weight (kg) at school, as described elsewhere [54]. We calculated BMI and categorized children as underweight, normal weight, overweight or obese according to age- and sex-specific cut-offs from the International Obesity Task Force (IOTF) classification [56].

5.2.4. Eating habits

We assessed eating habits through a 16-item food frequency questionnaire. Children used a seven-point scale ranging from 0 (not consumed) to 6 (consumed several times per day) to report how often they consumed each item during the past month. Based on a cluster and factor analysis, three types of eaters were previously identified in the Fin-HIT cohort: unhealthy eaters, fruit and vegetable avoiders and healthy eaters [57]. Unhealthy eaters represented the most frequent consumers of sweet pastries, biscuits or cookies, ice cream, sugary juice drinks, fast food (hamburgers or hot dogs) and salty snacks. Fruit and vegetable avoiders consumed the fewest fresh vegetables, fruits and berries, whereas healthy eaters consumed more dark bread, fresh vegetables, fruits and berries compared to others.

5.2.5. Physical activity

We assessed the leisure time physical activity duration through the following question: "How many hours a week do you normally exercise or do sports during your free time? Include all of the exercise you do in a club or team and any exercise by yourself, with family or friends. Do not count any exercise at school or on the way to school." Children responded using ten response options ranging from (1) "An hour or less each week" to (10) "About ten hours a week". The physical activity

questions were previously validated against an accelerometer among 11 year olds; children who reported higher amounts of physical activity, had higher amounts accelerometer-derived physical activity. In addition, a moderate capability was found for categorizing children according to their activity levels [58]. We categorized the children based on their responses into high (≥10 h/week), medium (5–9 h/week) and low (≤4 h/week) physical activity groups. We categorized the high, medium and low groups based on the distribution of responses: approximately the highest 25%; approximately the middle 50%, and approximately the lowest 25%.

5.2.6. Saliva samples and microbiota analysis

We previously described the detailed procedures for the saliva collection and microbial analysis [55]. Children provided unstimulated saliva samples mostly between breakfast and lunch on a school day using the Oragene® DNA (OG-500) Self-Collection Kit (DNA Genotek Inc., Ottawa, Ontario, Canada), a method with a demonstrated high quality [59]. The saliva microbiota was characterized with 16S rDNA sequencing from DNA extracted [60] through a standardized protocol, which contained an intensive lysis step using a cocktail of lysozyme and the mechanical disruption of bacterial cells, employing bead-beating [61]. 16S rDNA is the most widely used biomarker gene for microbial diversity. We used primers to amplify the V3–V4 region of 16S rDNA [62], sequenced on the Illumina platform (HiSeq 1500) (Illumina Inc., San Diego, CA, USA). MiSeq SOP in the mothur pipeline (Version V.1.35.1) was used to process sequences into operational taxonomical units (OTUs). The SILVA 16S rRNA database (Version V119) allowed us to classify the high-quality sequence reads, which were clustered into OTUs at a cut-off value > 98% sequence similarity to approach species resolution. Our previous study demonstrated the feasibility and reproducibility of this methodology; large-scale profiling of the microbiota can be consistently produced by 16S amplicon assays [55]. Only samples with > 10 000 OTU counts were analyzed. Diversity in the alpha and beta indices (Shannon and Inverse Simpson, and Bray–Curtis, respectively) were generated to describe diversities within and between samples, and we identified the relative abundance of species at several taxonomic levels. Shannon index is a metric that accounts for abundance and represents species evenness in the sample, whereas Inverse Simpson index measures community diversity, i.e. similarity between a pair of community samples, as well as the relative abundance on each species. For group comparisons, a sample size of 125 was sufficiently adequate to detect a 5% difference in the Shannon diversity index reflecting both the abundance and evenness of the species in groups and to ideally identify the top differentially abundant bacterial species [63]. The datasets used in this study can be found in the European Genome-phenome Archive (EGA) database (accession number EGAS00001003039).

5.3. Statistical analyses

We collected saliva samples from all children who participated in the Fin-HIT study, and the microbiota were analyzed from a randomly selected subsample (n = 972). OTU was standardized by subsampling with a threshold of 2000, excluding samples below this threshold (n = 83). We excluded those with a low sequencing depth < 10000 (n = 29). We also excluded children with missing data on TV viewing on weekends (n = 16) and on BMI (n = 19). In addition, we excluded recent antibiotic users (prescribed an antibiotic during the previous 3 months; n = 44) since antibiotic use during the previous three months associated with the saliva microbiota among Fin-HIT children [64]. Finally, we excluded children with a medium amount of TV viewing on weekends (n = 410), and only included children with low and high TV viewing on weekends or days off, that is, children with low and high screen times (n = 392).

We describe screen time by providing the number and percentage of children reporting low and high TV viewing. We visually examined the normal distribution of the variables using histograms. We compared participant characteristics between screen time groups, that is, children reporting low or high screen times, using the Chi-square test or ANOVA adjusted for the Brown-Forsythe when appropriate. We tested the difference in the alpha diversity indices between the screen time groups using ANOVA, and using ANCOVA when adjusting for confounders. We used Partial Eta Squared to calculate an effect size. We conducted sensitivity analyses by repeating the alpha diversity analyses only among children with healthy eating habits, because eating habits associated with the saliva microbiota [32] and with the screen time groups in our data. We conducted all analyses using IBM's SPSS statistical software program, version 25 (IBM Corp., Armonk, NY, USA).

We compared the beta diversity between low and high screen time groups using the permutational analysis of variance (PERMANOVA) and principal coordinate analysis (PCoA) based on Bray–Curtis distances to visualize the beta diversity between groups. Moreover, we employed general linear models (GLMs) with a negative binomial distribution to compare the bacteria abundances between the screen time groups as OTUs, considering the phylum and genus levels. All rare OTUs, that is, those with low counts (<20), were excluded. We corrected p values using the false discovery rate. PERMANOVAs were performed using Vegan (version 2.5–4) and phyloseq (version 1.25.2), and the GLM analysis relied on DESeq2 (Version 1.26.0) [65] in R (version 3.4.3). We considered adjusted $p < 0.05$ as statistically significant.

The functional profiling of the saliva microbiota was predicted using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2; v2.0.0-b.2) [66]. The MetaCyc database was used as the pathway reference. Differentially present pathways between low and high screen time groups were analyzed with the Welch t-test using STAMP (Version 2.1.3) [67]. We present the differentially present pathways using the False Discovery Rate (FDR) adjusted $p < 0.05$.

5.4. Covariates

We adjusted the analyses for the language spoken at home (Finnish, Swedish or other) since Swedish-speaking Finns and Finnish-speaking Finns differ by genetic background. Moreover, children who speak other languages are immigrants with a different genetic background compared to both Swedish- and Finnish-speaking Finns [68]. Therefore, language spoken at home served as an indicator of genetic background. We adjusted for gender (girl or boy) and BMI categories (underweight, normal weight, overweight or obese), because we previously found differences in the saliva microbiota between girls and boys, and between BMI categories among the Fin-HIT children [30]. Finally, we conducted additional analyses in which we added eating habits and physical activity as covariates. The justification for this lies in that diet and physical activity appear to modify the gut microbiota and possibly the saliva microbiota [11–14,16,17][32].

6. Declarations

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CRediT authorship contribution statement

Elina Engberg: Conceptualization, Formal analysis, Funding acquisition, Visualization, Writing - original draft. **Sajan C. Raju:** Data curation, Formal analysis, Methodology, Visualization, Writing - review & editing. **Rejane A.O. Figueiredo:** Conceptualization, Data curation, Formal analysis, Visualization, Writing - review & editing. **Elisabete Weiderpass:** Funding acquisition, Resources, Writing - review & editing. **Trine B. Rounge:** Conceptualization, Methodology, Writing - review & editing. **Heli Viljakainen:** Conceptualization, Funding acquisition, Project administration, Resources, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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