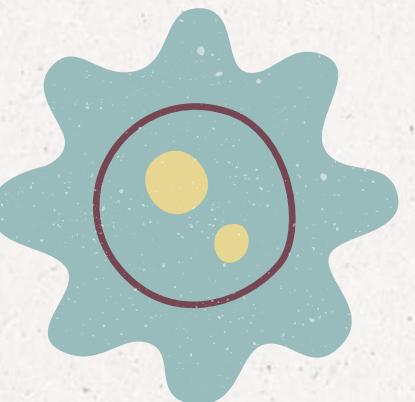


ANALYSIS OF GUT MICROBIOME VARIATIONS IN RELATION TO REFINED SUGARS CONSUMPTION

GAETANO CHIRIACO
GIANMARCO RUSSO



PROJECT OVERVIEW

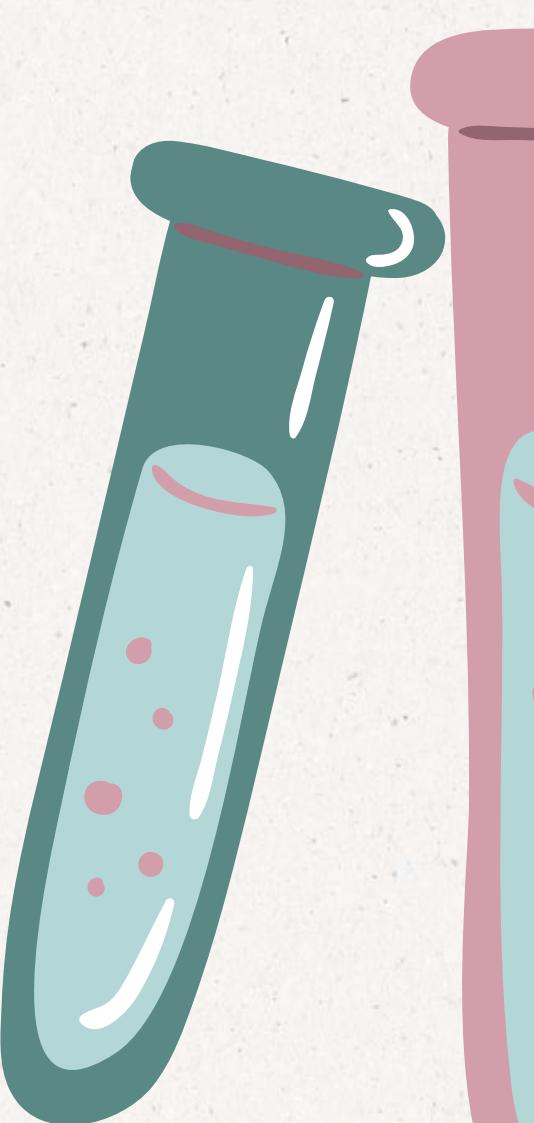


The aim is to determine how **dietary changes**, specifically **refined sugar** consumption, affect the human gut microbiota.

Research* has proved that this changes in nutrition have an effect on the gut microbiota.

The objective is the understanding of the effect of refined sugar consumption on a subsample of subjects in the american gut microbiome project.

*Sara C Di Rienzi , Robert A Britton, Adaptation of the Gut Microbiota to Modern Dietary Sugars and Sweeteners, Advances in Nutrition, Volume 11, Issue 3, May 2020, Pages 616–629 <https://doi.org/10.1093/advances/nmz118>



REFINED SUGAR AND HEALTH RISKS

Consuming large amounts of refined sugar has consistently been linked to **obesity** and excess belly fat, a risk factor for conditions like **diabetes** and **heart disease***.

Foods enriched with **HFCS** may cause you to become resistant to leptin, a hormone that signals your body when to eat and when to stop**.

*Vreman RA, Goodell AJ, Rodriguez LA, Porco TC, Lustig RH, Kahn JG. Health and economic benefits of reducing sugar intake in the USA, including effects via non-alcoholic fatty liver disease: a microsimulation model. *BMJ Open*. 2017 Aug 3;7(8):e013543. doi: 10.1136/bmjopen-2016-013543. PMID: 28775179; PMCID: PMC5577881.

**Vasselli JR, Scarpace PJ, Harris RB, Banks WA. Dietary components in the development of leptin resistance. *Adv Nutr*. 2013 Mar 1;4(2):164-75. doi: 10.3945/an.112.003152. PMID: 23493533; PMCID: PMC3649097.

WHAT ABOUT MICROBIOTA?



Adaptation of the Gut Microbiota to Modern Dietary Sugars and Sweeteners

Sara C Di Rienzi¹  , Robert A Britton¹

Gut microbiota composition in relation to intake of added sugar, sugar-sweetened beverages and artificially sweetened beverages in the Malmö Offspring Study

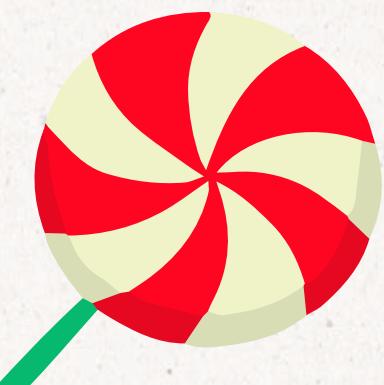
High Intake of Sugar and the Balance between Pro- and Anti-Inflammatory Gut Bacteria

[Reetta Satokari](#)

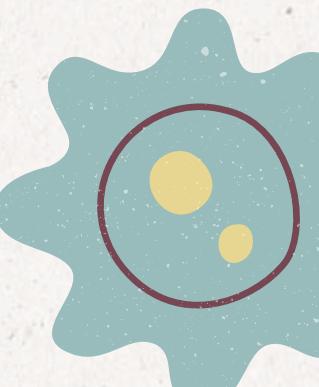
[Gray³](#), [Gunter G C Kuhnle⁴](#),
[...](#) 

Sugars and Gastrointestinal Health

Djésia Arnone¹, Caroline Chabot², Anne-Charlotte Heba³, Tunay Kökten³, Bénédicte Caron⁴, Franck Hansmannel³, Natacha Dreumont³, Ashwin N Ananthakrishnan⁵, Didier Quilliot⁶, Laurent Peyrin-Biroulet⁷



WHAT ABOUT MICROBIOTA?

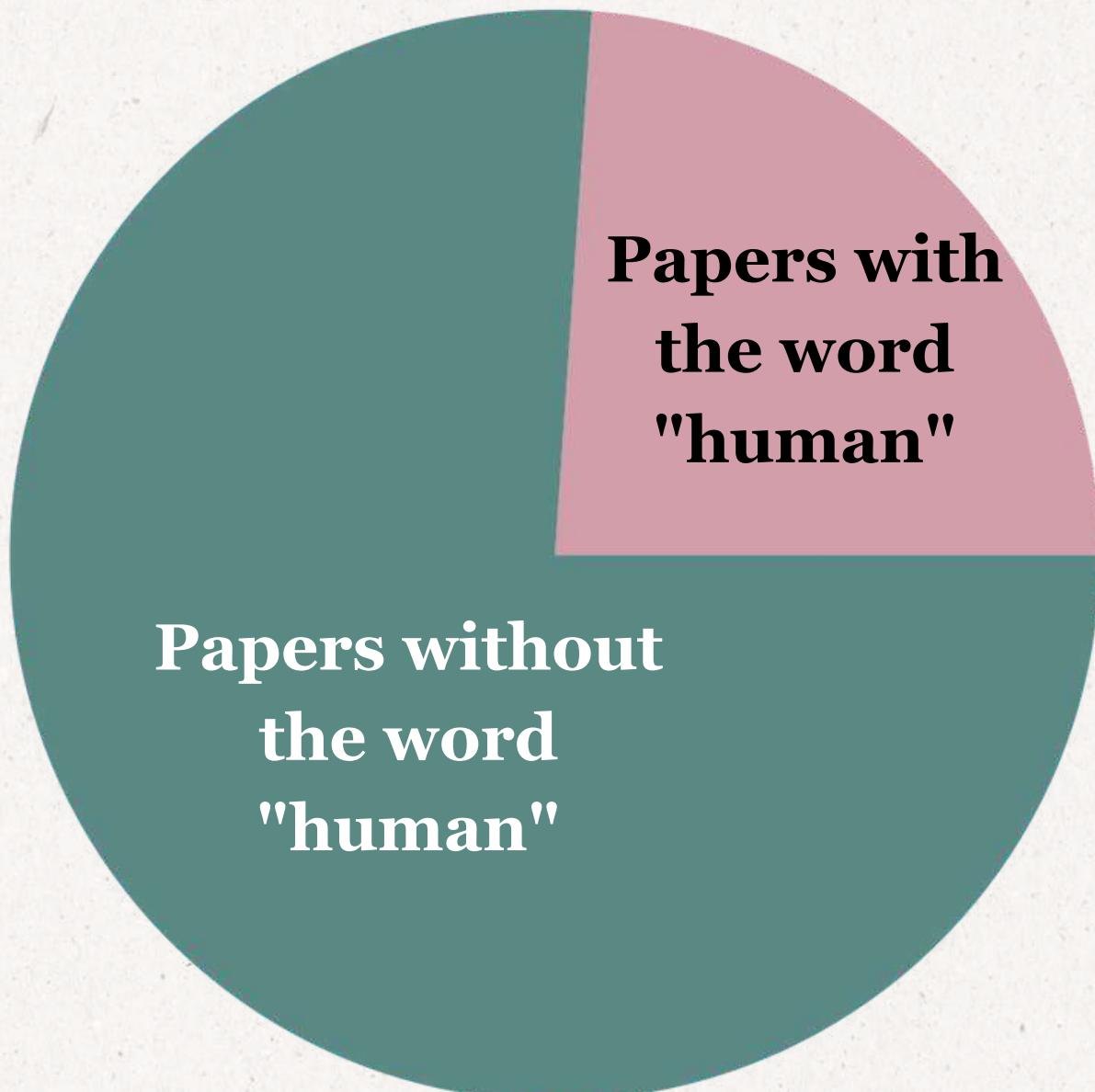


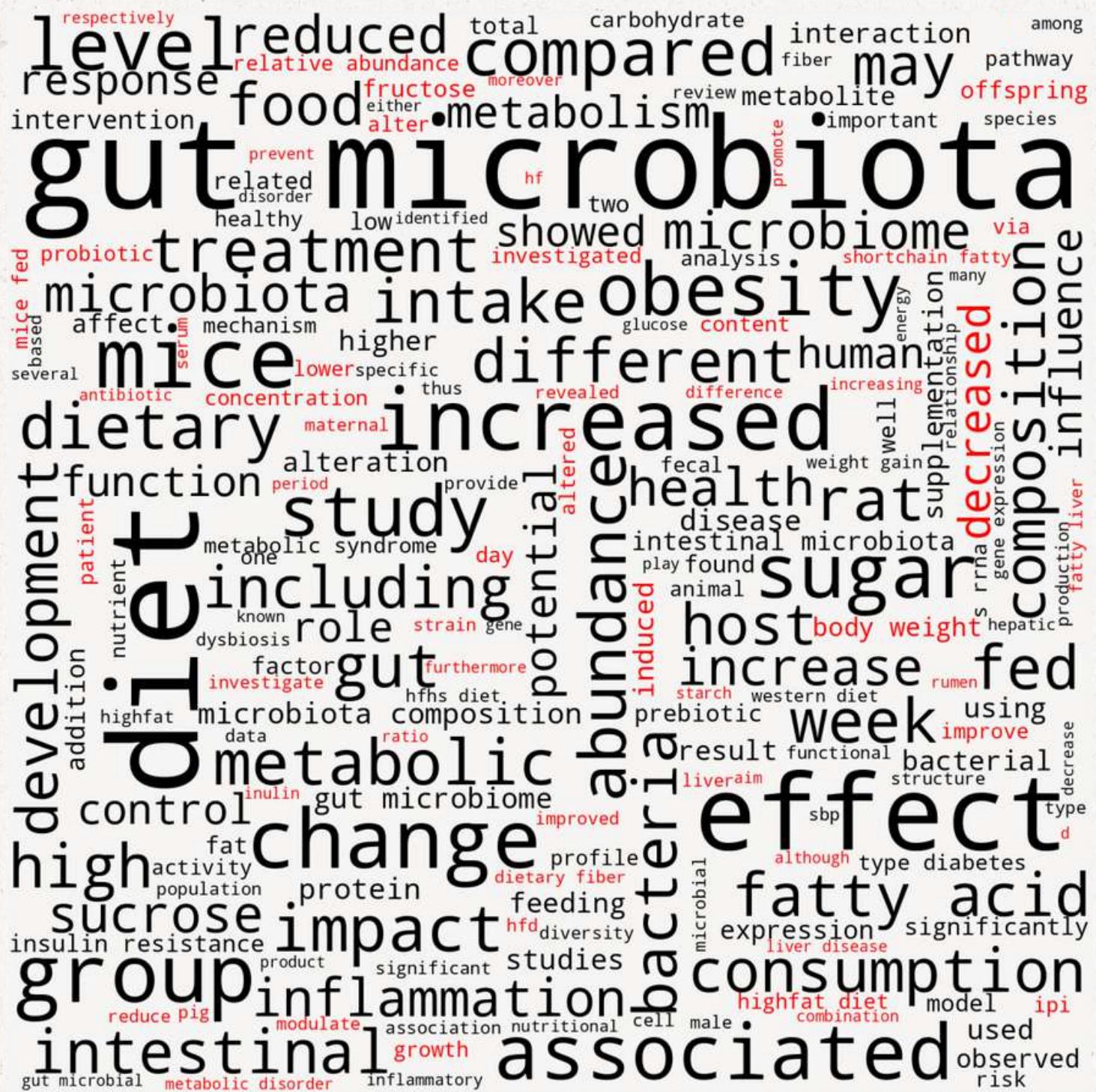
Found **675 articles** on PubMed using this query:

((refined AND sugar) OR (sugar AND diet) OR (sucrose AND diet)) AND microbiota

Most of the papers found don't talk about
human microbiota.

Adding "**human**" to the query reduces
the number of papers found to **161**





Wordcloud on all 675 papers



Wordcloud only on papers that contain the word "human"



TOPIC MODELLING USING LDA

LDA used to partition papers in groups and analyze the most characterizing words per topic.

Number of groups:

- 2
- 4

<https://grusso98.github.io/gut-microbiota-diet/>

Data:

- all 675 papers
- 161 on human microbiota



TOPIC MODELLING USING LDA

Papers with the highest probability to belong to the cluster that we found:

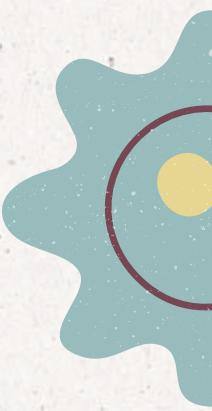
Data: All papers N. of topics: 2

Topic 1: Health Risks

1. Eucommiae cortex polysaccharides mitigate **obesogenic diet-induced cognitive and social dysfunction** via modulation of gut microbiota and tryptophan metabolism
2. **Consumption of a Western-Style Diet** Modulates the Response of the Murine Gut Microbiome to Ciprofloxacin
3. Amino Sugars Reshape Interactions between **Streptococcus mutans** and **Streptococcus gordonii**

Topic 2: Experiments

1. Dietary Phytase- and Lactic Acid-Treated Cereals Caused Greater Taxonomic Adaptations than Functional Adaptations in the Cecal Metagenome of **Growing Pigs**
2. Ganoderic acid A from Ganoderma lucidum ameliorates lipid metabolism and alters gut microbiota composition in **hyperlipidemic mice** fed a high-fat diet.
3. Effects of Silage Diet on Meat Quality through Shaping Gut Microbiota in **Finishing Pigs**





TOPIC MODELLING USING LDA

Papers with the highest probability to belong to the cluster that we found

Data: Human papers N. of topics: 2

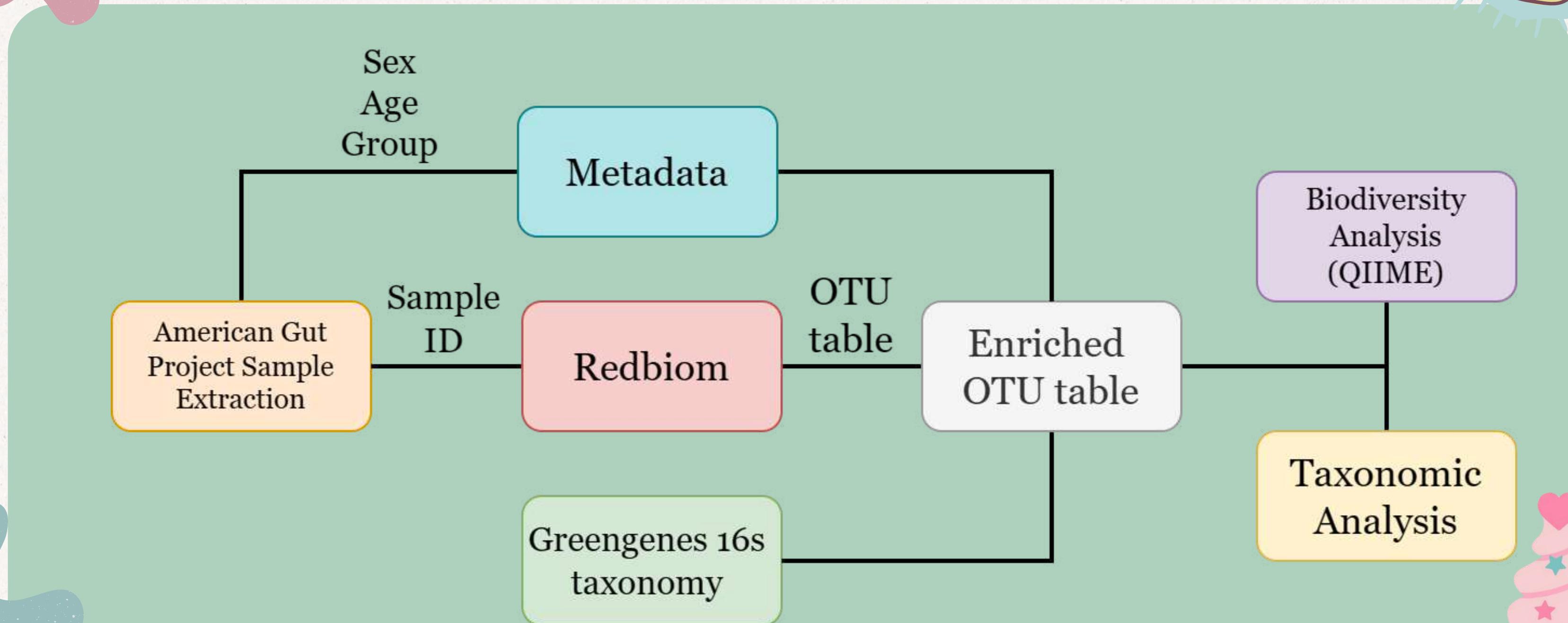
Topic 1: Diet Risks

1. Amino Sugars Reshape Interactions between *Streptococcus mutans* and *Streptococcus gordonii*.
2. Therapeutic potential of bioactive phytoconstituents found in fruits in the **treatment of non-alcoholic fatty liver disease**: A comprehensive review
3. The **Impact of Western Diet and Nutrients** on the Microbiota and Immune Response at Mucosal Interfaces

Topic 2: Experiments

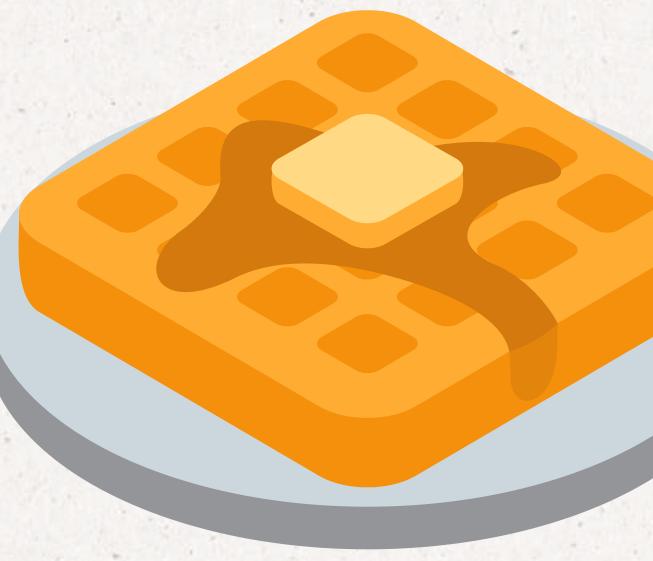
1. Dietary pomegranate extract and inulin affect gut microbiome differentially in **mice** fed an obesogenic diet
2. Impact of anthocyanin component and metabolite of Saskatoon berry on gut microbiome and relationship with fecal short chain fatty acids in diet-induced insulin resistant **mice**
3. Dietary Alaska pollock protein alters insulin sensitivity and gut microbiota composition in **rats**

WORKFLOW





SAMPLING STRATEGY



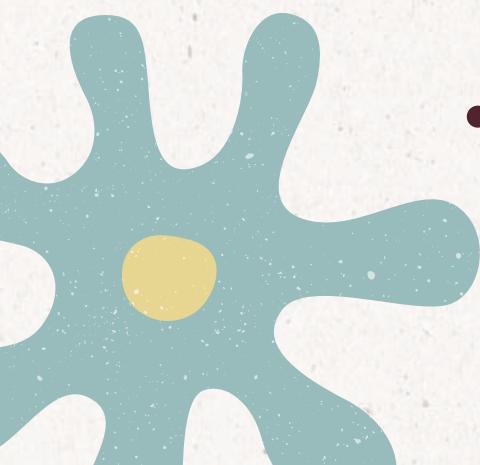
TREATMENT GROUP DEFINITION

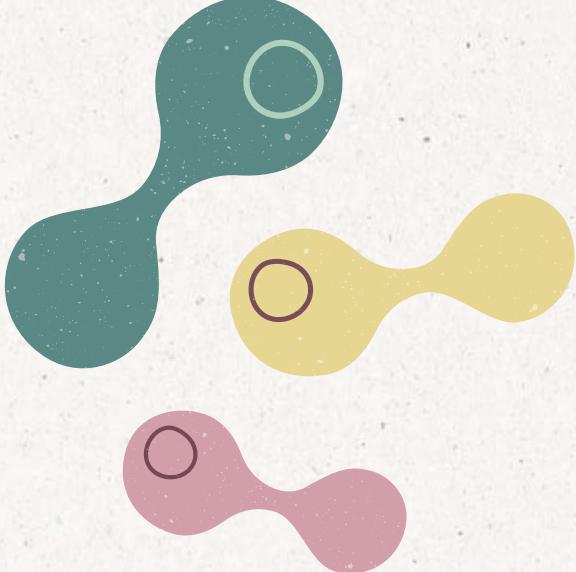
People included in the "exclude refined sugars" group are defined as follows:

- between **20-69 years old**
- those who declared to **exclude refined sugars**
- those who declared to **adopt particular diets** that automatically **exclude refined sugars** like:
 - **Primal diet**
 - **Paleo diet**
 - **Raw food diet**
 - **Weston Price diet**
- excluding people that eat any other kind of diet

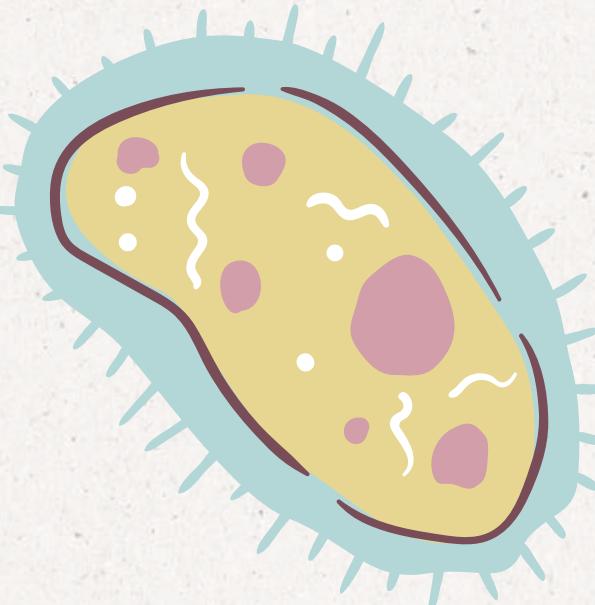


359 subjects in treatment group





SAMPLING STRATEGY



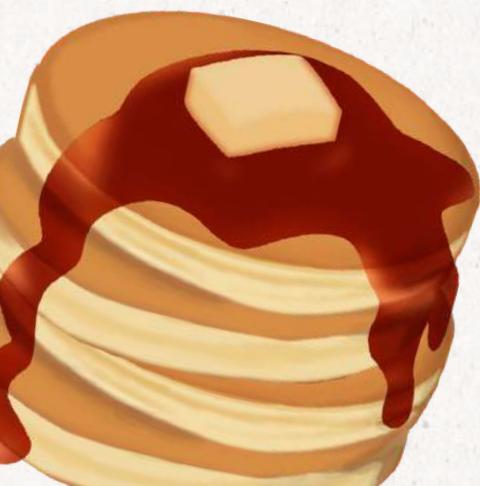
SPECIAL CONDITIONS FILTERING

Samples affected by particular conditions have been filtered to **exclude possible biases** in the analysis:

- *alcoholism*
- *autoimmune diseases*
- *cancer*
- *clostridioides difficile*
- *inflammatory bowel disease*
- *inflammatory bowel syndrome*
- *kidney disease*
- *liver disease*
- *anorexia*
- *bulimia*
- *small intestinal bacterial overgrowth*



~~359~~ 191 remaining in
Treatment group



METADATA

To be able to use the **QIIME tools** for diversity analysis, a **metadata file** has to be created. This table contains all the needed information of our samples.

sample-id	sex	age_cat	refined_sugars
10317000023139	female	60s	Yes
10317000027811	male	40s	Yes
10317000031513	female	30s	Yes
10317000033280	female	60s	Yes
10317000065684	female	20s	No

OTU 99 TAXONOMY

The complete **greengenes 16s taxonomy** has been downloaded* and later merged with our samples-id to add the taxonomy information.

id	bacteria
228054	k_bacteria; p_CyanoBacteria; c_Synechococco...
228075	k_bacteria; p_Firmicutes; c_Bacilli; ...
73627	k_bacteria; p_Firmicutes; c_Bacilli; ...
392021	k_bacteria; p_Proteobacteria, c_Alphaproteo...
3492492	k_bacteria; p_Firmicutes; c_Bacilli; ...

OTU TABLE

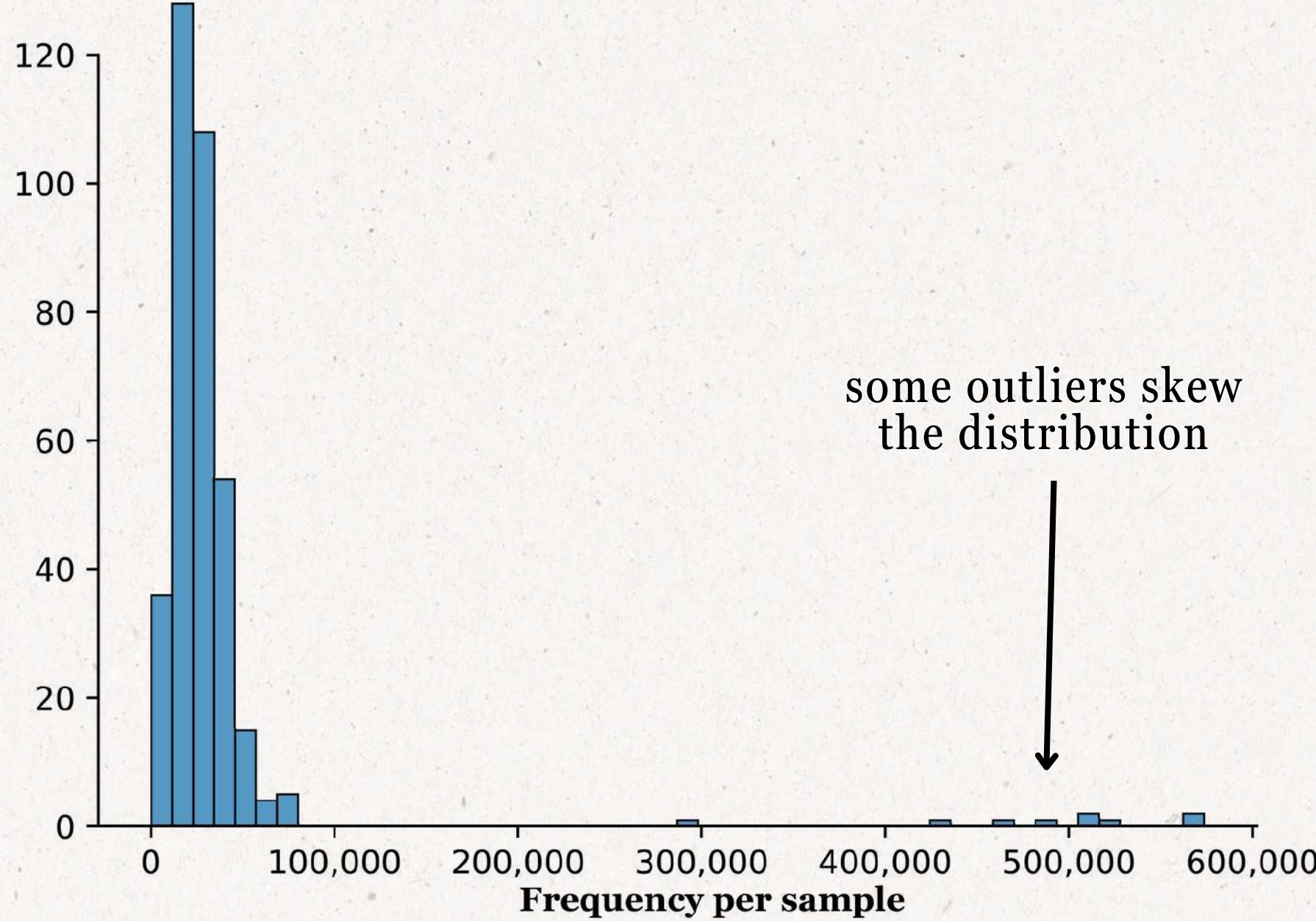
This table has been extracted from **RedBiom** using **sample-ids**. Each row is composed by the absolute frequency of every feature in our sample.

sample-id	208901	1117675	193418
10317000023139	0	1	134
10317000027811	1	0	789
10317000031513	0	0	102
10317000033280	27	0	87
10317000065684	2	0	30

*http://greengenes.microbio.me/greengenes_release/gg_13_8_otus/

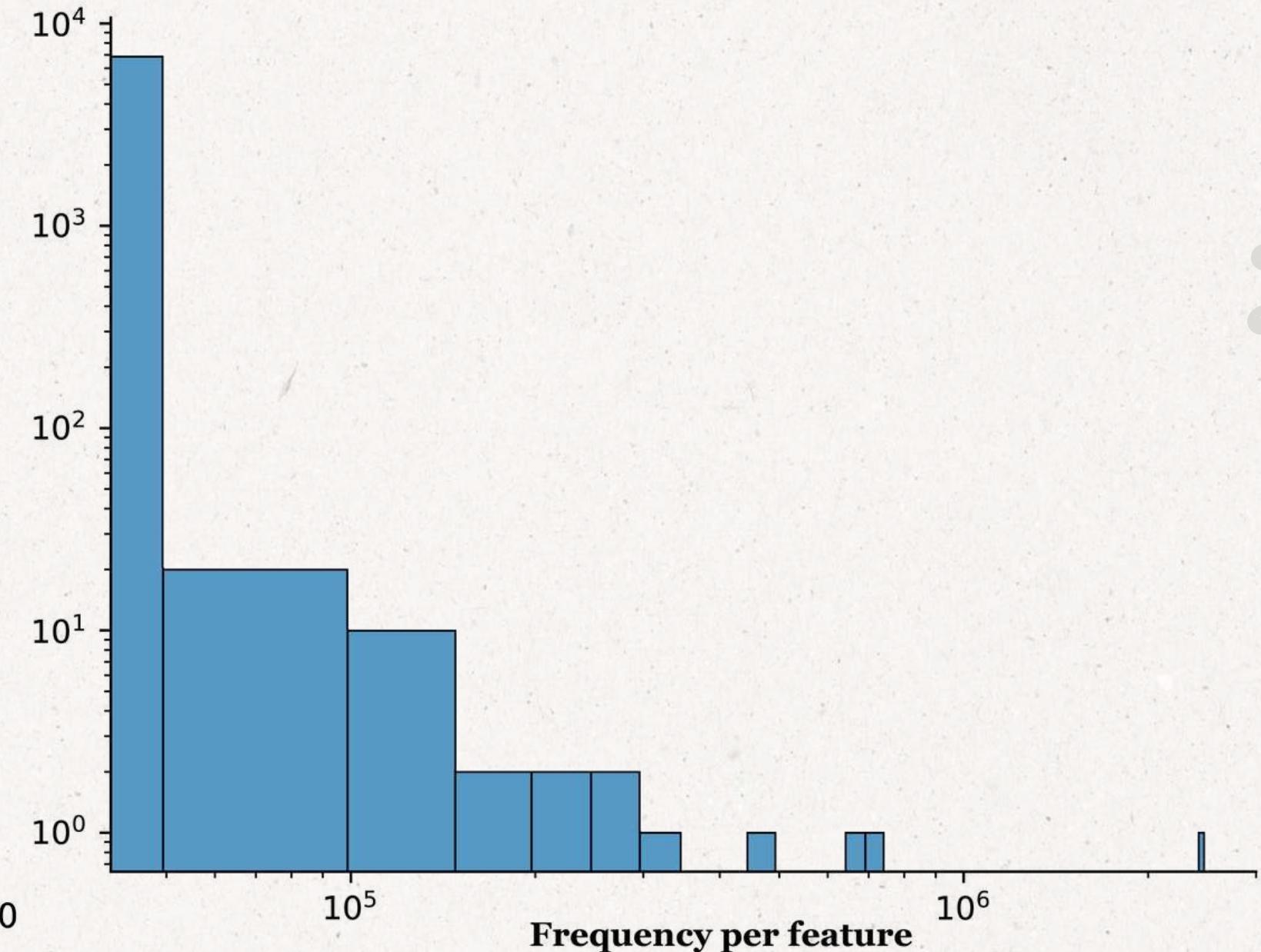
PRELIMINARY ANALYSIS

Number of samples



some outliers skew
the distribution

Number of features





ALPHA & BETA DIVERSITY



Next, we computed the unrooted and rooted tree using QIIME. The tree is useful to later obtain the scores of alpha and beta diversity.

Alpha diversity is measured as the **intra-group diversity**: defines how different the features are inside the same group

Shannon Entropy



p-value = 0.734 | test statistic=0,1145*

Pielou's Evennes



p-value=0.842 | test statistic=0,0397*

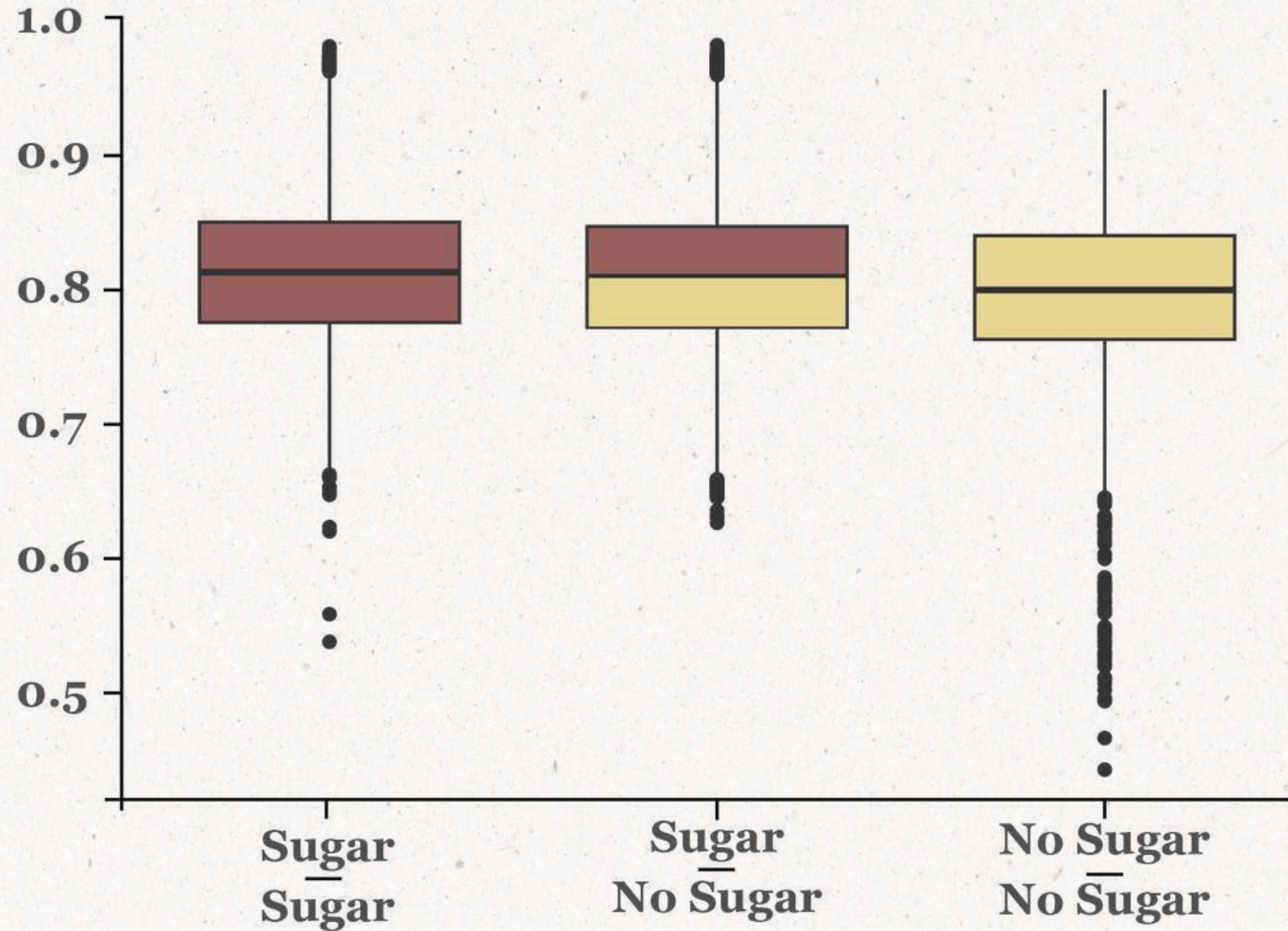
*A Kruskal-Wallis test is used to determine whether or not there is a statistically significant difference between the medians of independent groups



ALPHA & BETA DIVERSITY

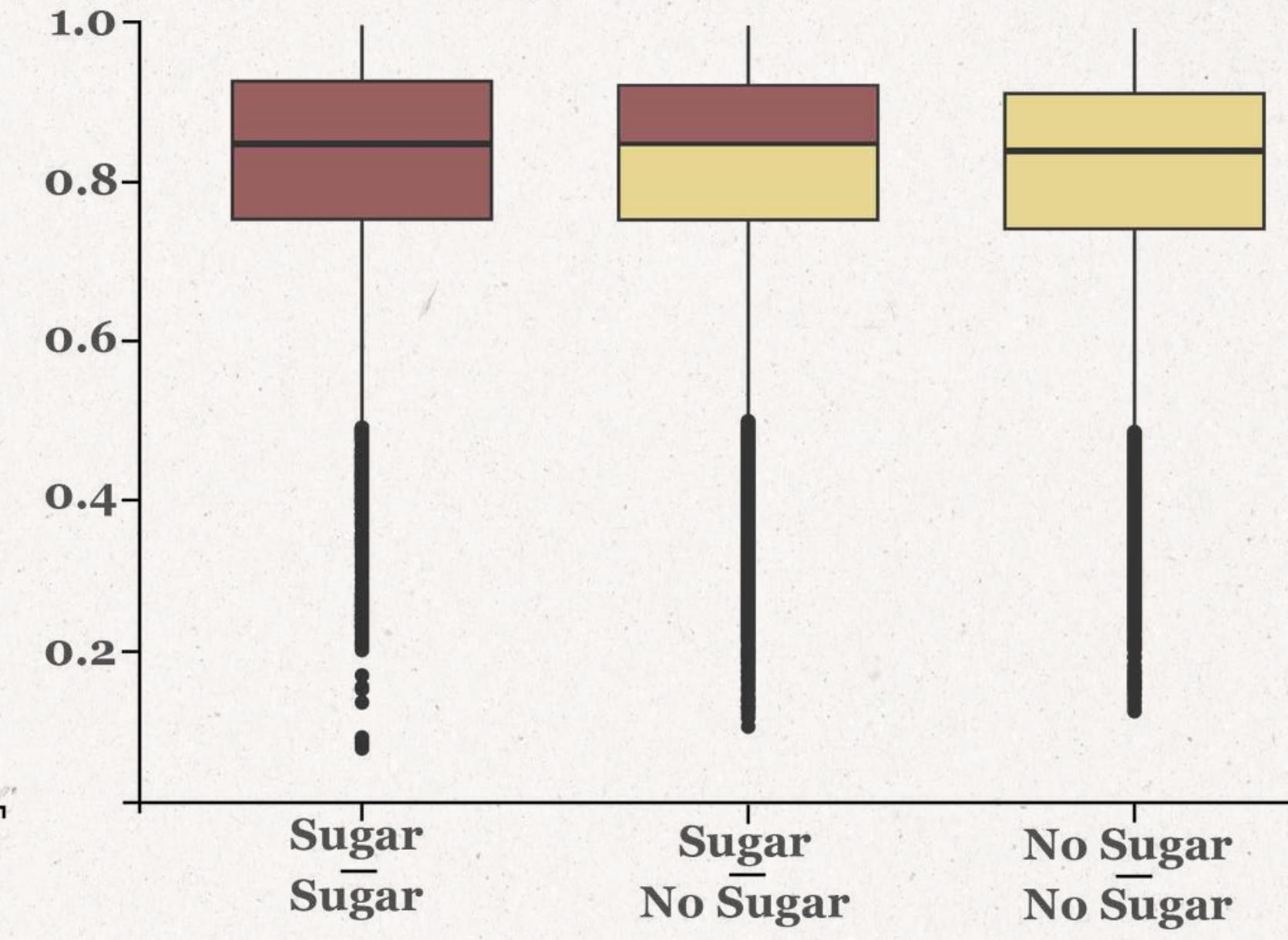
Beta diversity is measured as the **inter-group diversity**: defines how different the features are between the groups

Jaccard distance between samples



p-value=0.001 | test statistic=1,9005*

Bray Curtis distance between samples



p-value=0,003 | test statistic=2,8924*

*The null hypothesis tested by PERMANOVA is that, under the assumption of exchangeability of the sample units among the groups, H0: „the centroids of the groups, as defined in the space of the chosen resemblance measure, are equivalent for all groups.“

TAXONOMIC CLASSIFICATION ANALYSIS

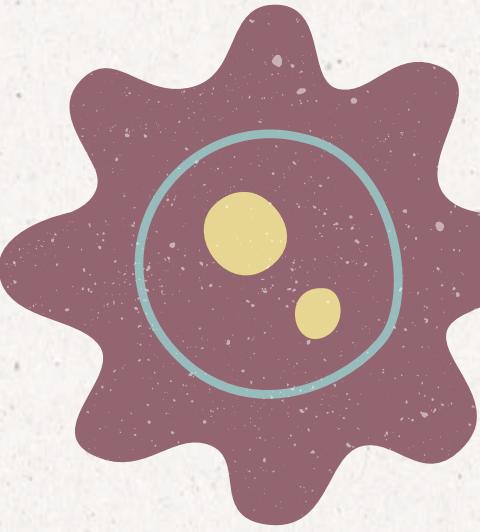
From the **Enriched OTU table**, we also studied the **taxonomic classification** assigned to each feature in our dataset.

For every **Feature ID**, we associated a taxonomy that express the membership of every feature to the associated **phylum**, **class**, **order**, etc.

In the next analysis, we tried to obtain better results by **dropping samples** with a number of features below a certain threshold. This type of filtering strategy didn't change the estimates that much, so we decided to keep all data points.

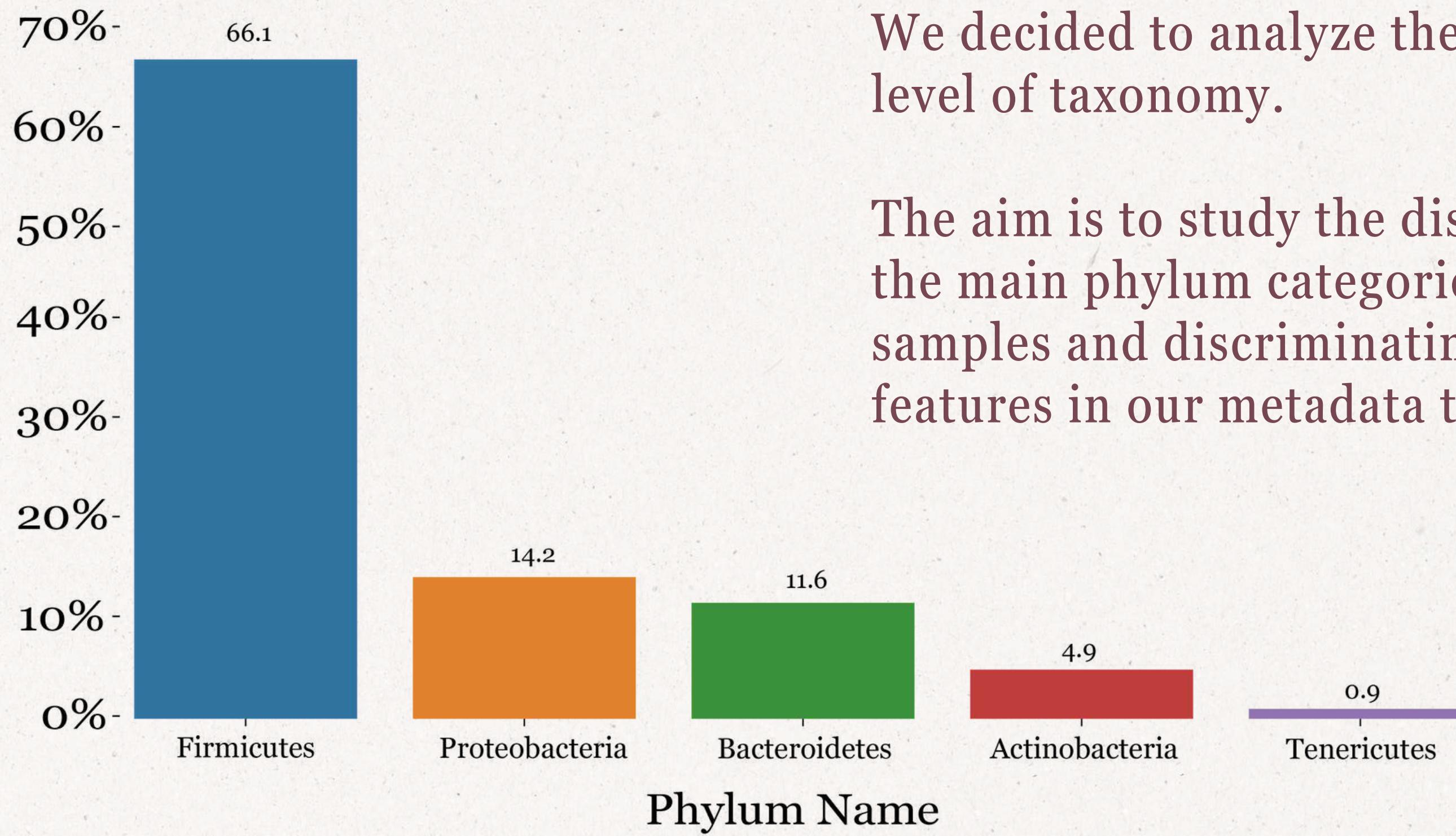
Proportion of taxonomy completeness per level

Kingdom	100%
Phylum	100%
Class	99.93%
Order	99.7%
Family	85%
Genus	49%
Species	11%



PHYLUM ANALYSIS

Phylum proportion



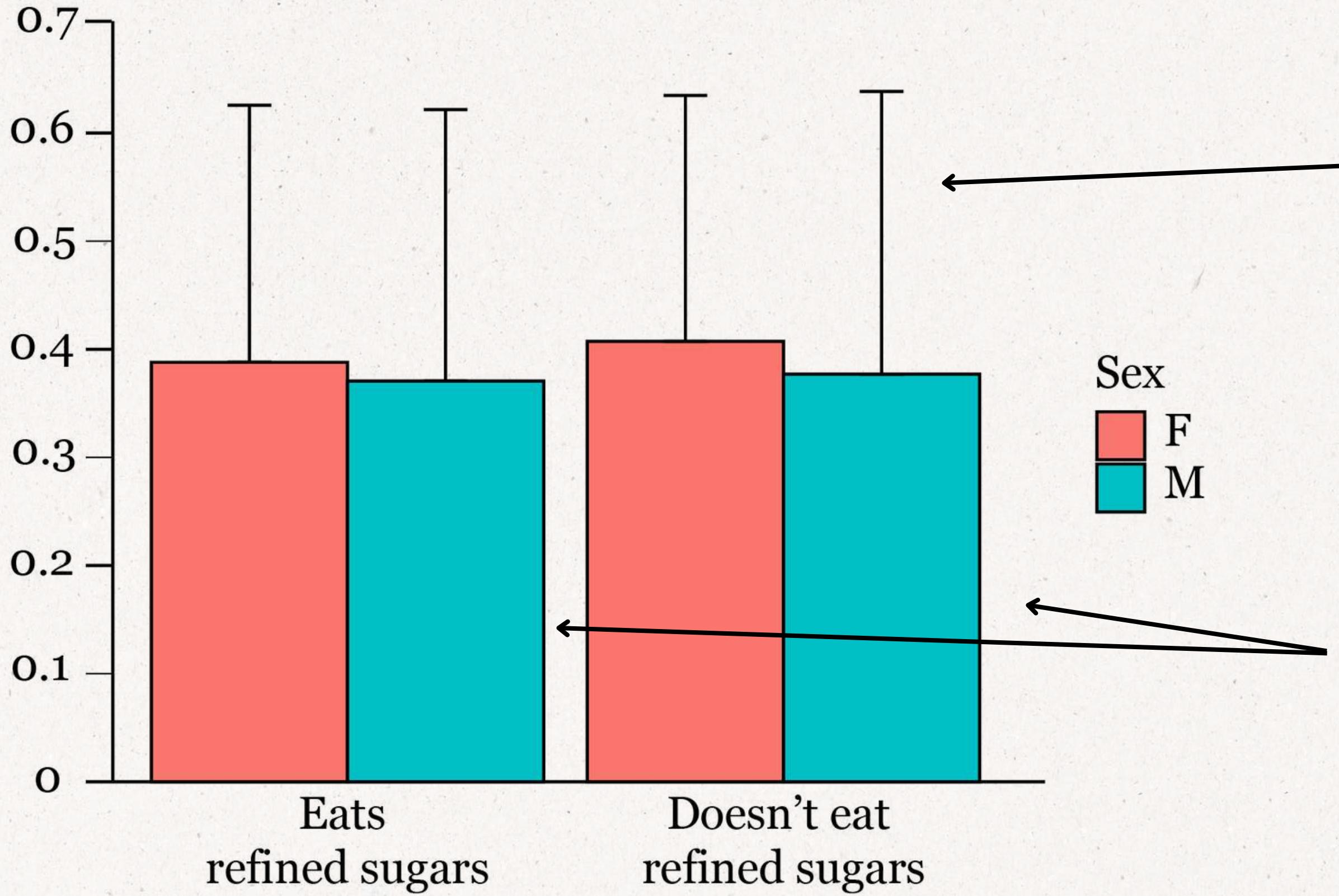
We decided to analyze the **phylum** level of taxonomy.

The aim is to study the distribution of the main phylum categories on all the samples and discriminating on the features in our metadata table.



FIRMICUTE

Average relative frequency of Firmicute per group



The **standard deviation** associated with the estimates is extremely high

The average proportion of Firmicute doesn't change much discriminating by **sex** and **sugar consumption**

FIRMICUTE

Average relative frequency of Firmicutes per group



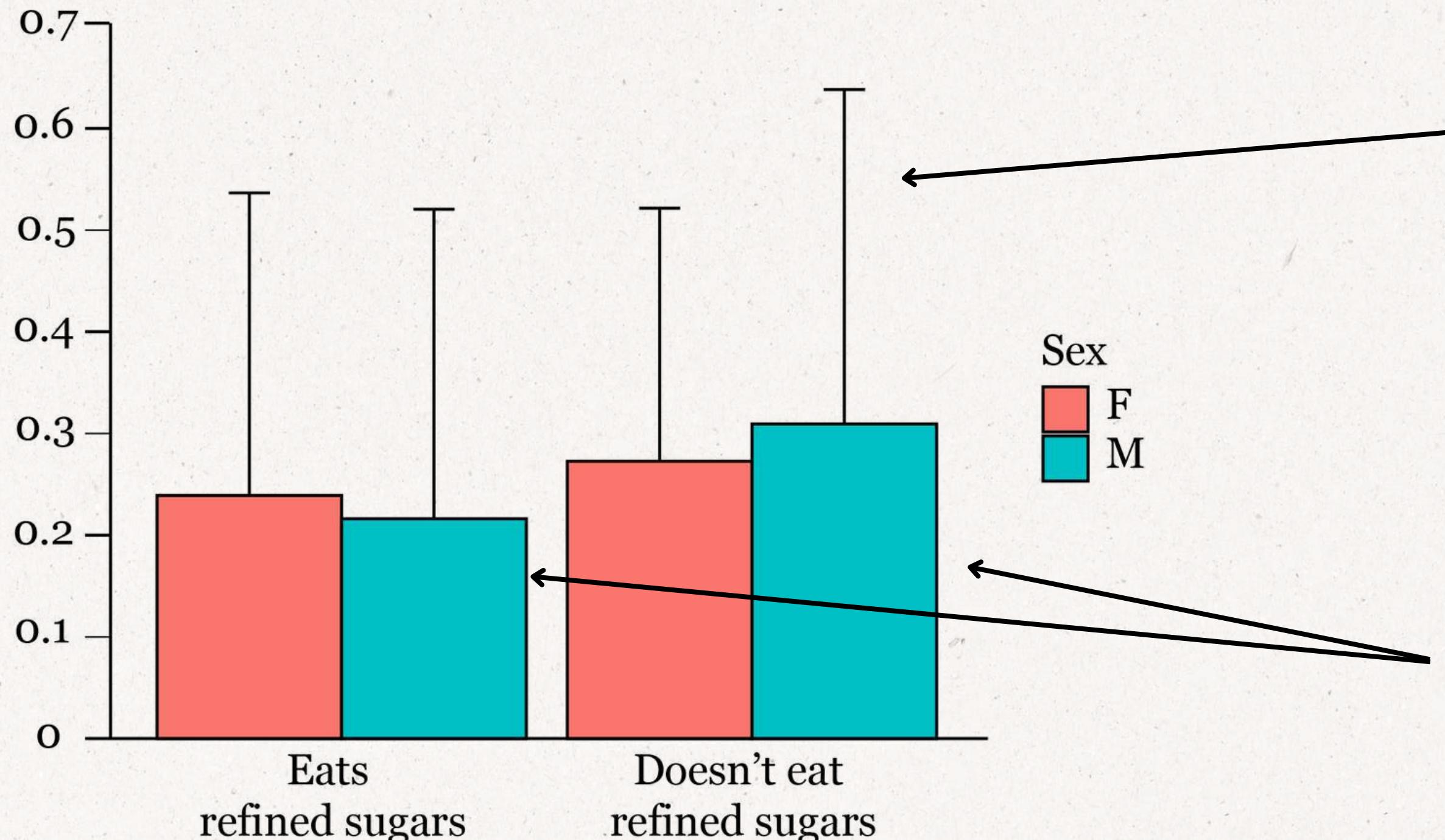
The average proportion
of Firmicutes is almost
equal between the two
samples but...

... it drops as **age**
increases*

*keep in mind that these
conclusions are not reliable
given the high std. dev.

PROTOBACTERIAS

Average relative frequency of Proteobacterias per group

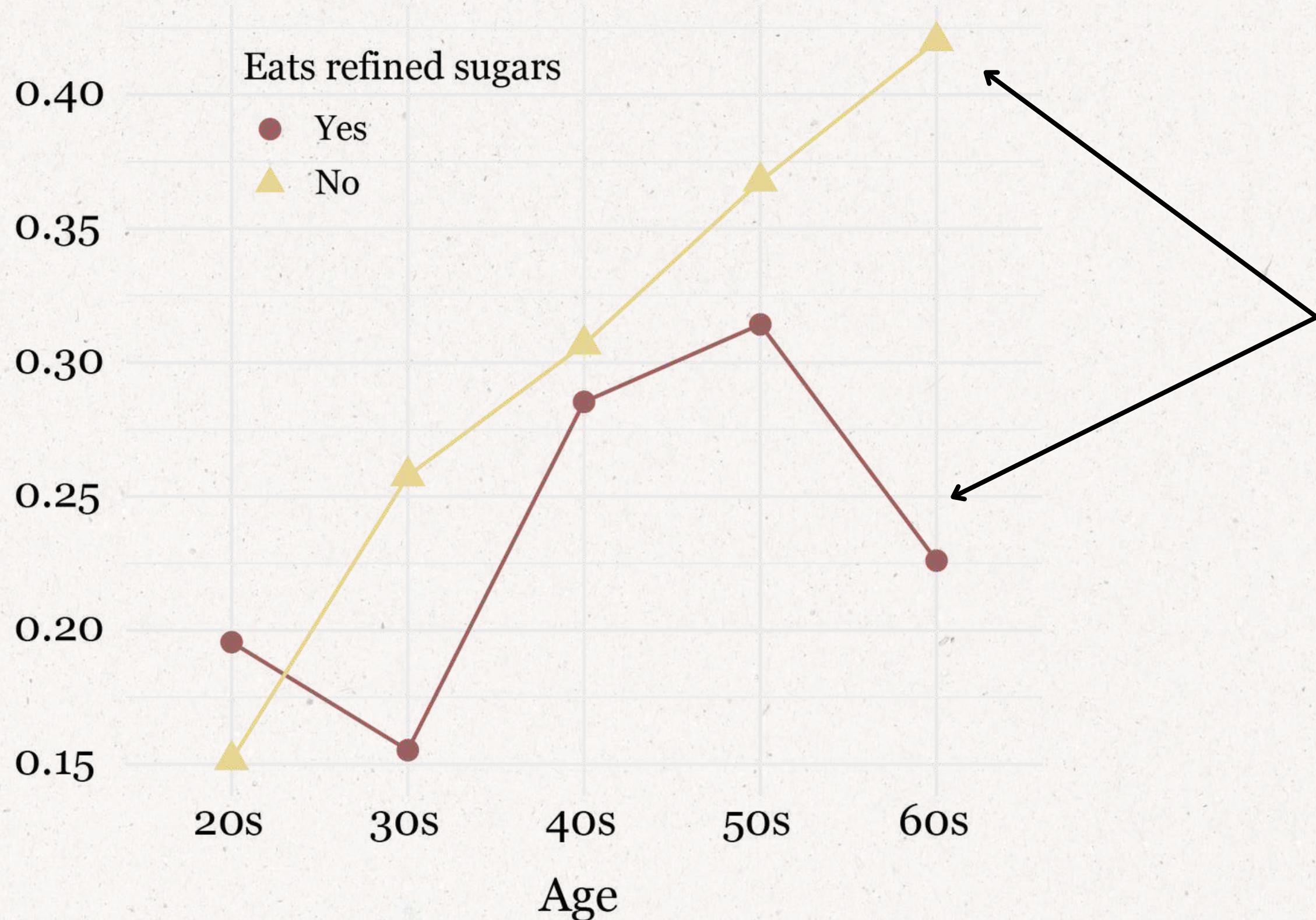


For some of these estimates the standard deviation is even higher than the average

The average proportion of Protobacterias is **45% higher in males** that don't eat refined sugars compared to those who do (14% higher in females)

PROTOBACTERIAS

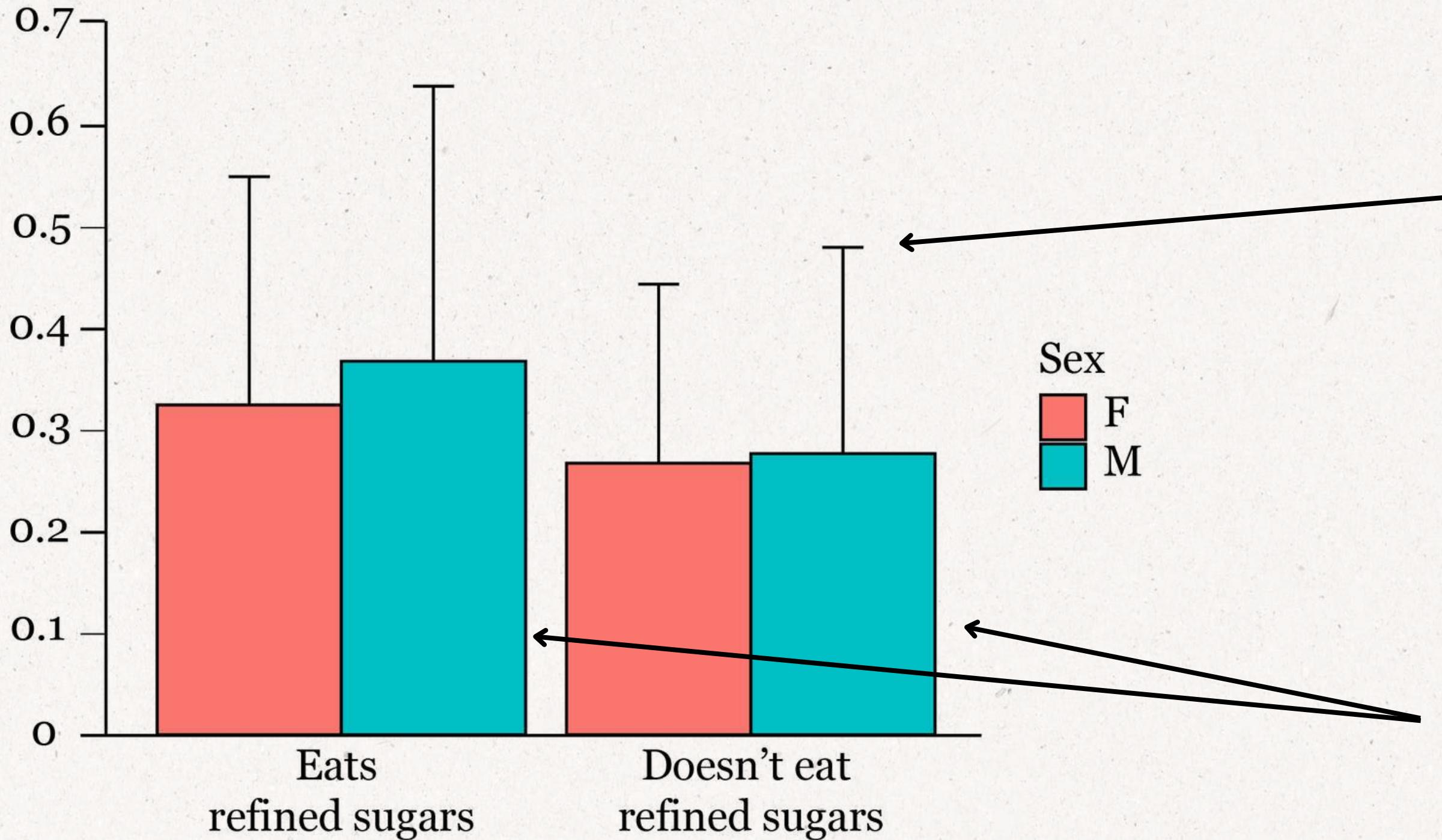
Average relative frequency of Proteobacteria per group



The average proportion of Protoprotobacterias increases with **age** in subjects that don't consume refined sugars, while it's much lower in subjects in the other group

BACTEROIDES

Average relative frequency of Bacteroides per group

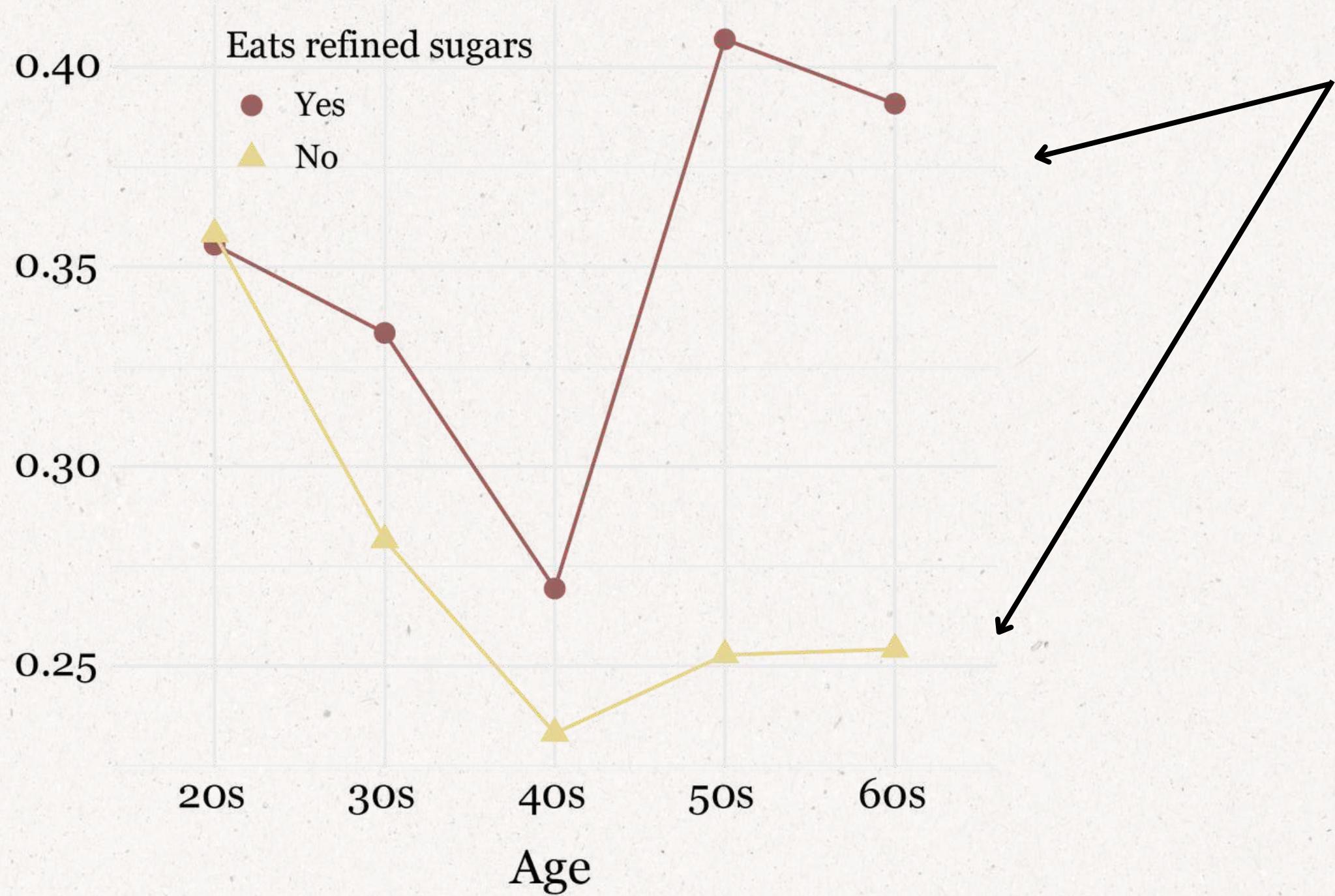


The uncertainty associated to the estimates is lower than the previous ones, but still very high

In contrast to what was seen previously, the average proportion of **Bacteroides** is higher in those who eat refined sugars

BACTEROIDES

Average relative frequency of Bacteroides per group



The average proportion of Bacteroides **decreases with age** in subjects that don't consume refined sugars, while we can observe an increase in subjects that eat refined sugars

NOTABLE DIFFERENCES

SUBJECTS THAT DON'T EAT REFINED SUGARS



SUBJECTS THAT EAT REFINED SUGARS



PAPERS ON THE SUBJECT

The Firmicutes/Bacteroidetes Ratio: A Relevant Marker of Gut Dysbiosis in Obese Patients?

[Fabien Magne](#),^{1,*} [Martin Gotteland](#),^{2,3,4,*} [Lea Gauthier](#),² [Alejandra Zazueta](#),¹ [Susana Pesoá](#),⁵ [Paola Navarrete](#),^{3,4} and [Ramadass Balamurugan](#)⁶

inflammatory and metabolic disorders in humans. Many of the current research efforts are focused on the identification of specific microbial signatures, more particularly for those associated with obesity, type 2 diabetes, and cardiovascular diseases. Some studies have described that the gut microbiota of obese animals and humans exhibits a higher Firmicutes/Bacteroidetes ratio compared with normal-weight individuals, proposing this ratio as an eventual biomarker. Accordingly, the Firmicutes/Bacteroidetes ratio is frequently cited in the scientific literature as a hallmark of obesity.

PAPERS ON THE SUBJECT

Gut microbiota composition in relation to intake of added sugar, sugar-sweetened beverages and artificially sweetened beverages in the Malmö Offspring Study

Stina Ramne ¹, Louise Brunkwall ², Ulrika Ericson ², Nicola Gray ³, Gunter G C Kuhnle ⁴,
Peter M Nilsson ², Marju Orho-Melander ², Emily Sonestedt ²

Results: Various genera nominally associated with intake of added sugar, SSBs, and ASBs. Only the negative association between SSB intake and Lachnobacterium remained significant after multiple testing correction. A positive association between SSB intake and the Firmicutes:Bacteroidetes ratio was also observed.

PAPERS ON THE SUBJECT

The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age

D Mariat¹, O Firmesse, F Levenez, Vd Guimarães, H Sokol, J Doré, G Corthier, J-P Furet

Results: Here we report on the comparative assessment of human fecal microbiota from three age-groups: infants, adults and the elderly. We demonstrate that the human intestinal microbiota undergoes maturation from birth to adulthood and is further altered with ageing. The counts of major bacterial groups Clostridium leptum, Clostridium coccoides, Bacteroidetes, Bifidobacterium, Lactobacillus and Escherichia coli were assessed by quantitative PCR (qPCR). By comparing species diversity profiles, we observed age-related changes in the human fecal microbiota. The microbiota of infants was generally characterized by low levels of total bacteria. C. leptum and C. coccoides species were highly represented in the microbiota of infants, while elderly subjects exhibited high levels of E. coli and Bacteroidetes. We observed that the ratio of Firmicutes to Bacteroidetes evolves during different life stages. For infants, adults and elderly individuals we measured ratios of 0.4, 10.9 and 0.6, respectively.

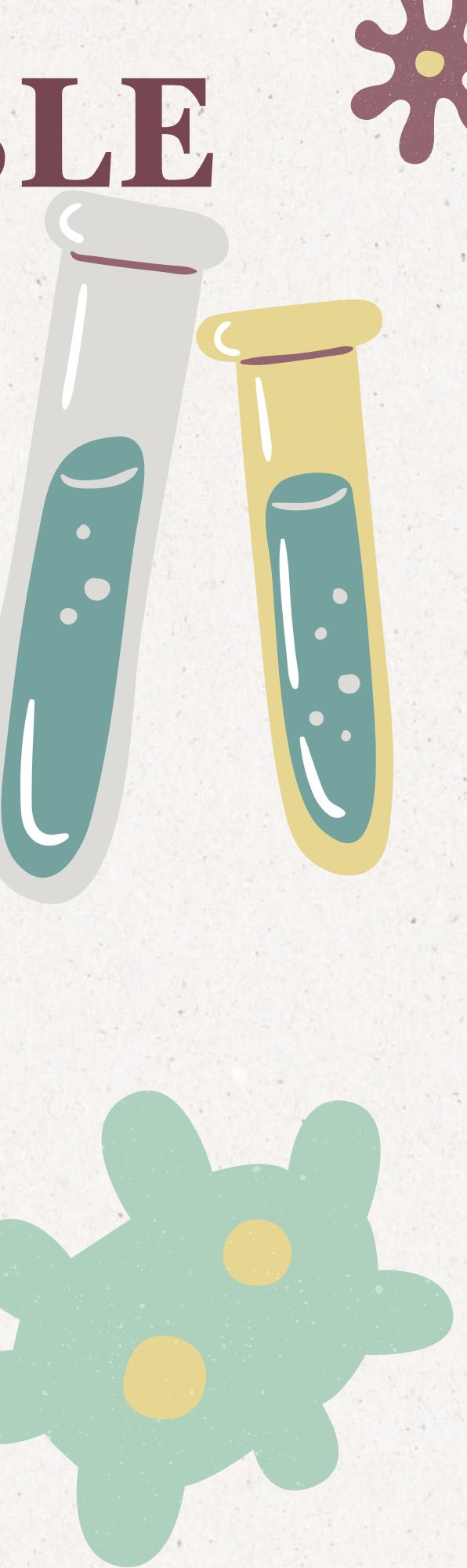
CONCLUSIONS AND POSSIBLE IMPROVEMENTS

In conclusion, this study aimed to investigate the **potential differences** between individuals who consume **refined sugars** and those who do not, but due to a **high variance** in the data, statistically significant results could not be obtained.

Despite the lack of significant findings, several noteworthy observations and trends were observed.

It is important to acknowledge that **dietary habits**, including sugar consumption, can **vary widely among individuals**, and this variability might have contributed to the high variance observed in the data.

Future improvements could be made by either obtaining a **less diverse and controlled sample** or by employing methods that account for and exclude the influence of other **correlated variables**.





THANKS FOR YOUR ATTENTION!

GITHUB PROJECT REPO:[HTTPS://GITHUB.COM/GRUSSO98/GUT-MICROBIOTA-DIET](https://github.com/grusso98/gut-microbiota-diet)

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DATA SCIENCE LAB IN
BIOSCIENCES | JUNE 2023