

The Fermented Soy Beverage Q-CAN® Plus Induces Beneficial Changes in the Oral and Intestinal Microbiome

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Research article

Keywords: Soy, Gut Microbiome, Oral Microbiome, Commensals, Obesity

Posted Date: May 11th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-26827/v1>

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Version of Record: A version of this preprint was published on March 4th, 2021. See the published version at <https://doi.org/10.1186/s40795-021-00408-4>.

Abstract

Background

Q-CAN® Plus is a pasteurized soy fermented product rich in isoflavones that has been used for over 30 years to aid in recovery from a wide range of conditions.

Objectives

To identify the changes in the oral and fecal microbiome in lean and obese subjects due to consumption of Q-CAN®, and to assess the expected consequences of these changes based on the published literature.

Methods

Prospective study of lean (10) and obese (9) subjects consuming Q-CAN® twice daily for 4 weeks with 8 weeks follow-up. Microbial DNA was extracted from saliva and stool samples, amplified against the V4 region of the 16S ribosomal RNA gene and data analyzed using QIIME 1.9.1 bioinformatics. 440 samples were collected in total, 424 of which were productive and yielded good quality data.

Results

STOOL. In the lean population Bifidobacteria and Blautia show a significant increase while taking Q-CAN®, and there was a trend for this in the obese population. ORAL. There were relatively fewer major changes in the oral microbiome with an increase in the family Veillonellaceae in the lean population while on Q-CAN®.

Conclusion

Q-CAN® consumption induced a number of significant changes in the fecal and oral microbiome. Most notably an increase in the stool microbiome of Bifidobacteria and Blautia, both of which are associated with positive health benefits, and in the saliva an increase in Veillonellaceae.

Introduction

Soybeans have long been recognized as sources of high-quality protein and beneficial lipids with several health benefits [1]. Unfermented soybeans, are very difficult to digest due to the high amount of protein enzyme inhibitors and indigestible sugar structures. The benefits of fermented soybeans have been recognized for many years and have recently been examined in an objective manner[2]. During

fermentation microbial enzymes digest the complex sugar molecules and the hard-to-digest proteins, significantly improving nutrient value. Consumption of fermented soybean foods is associated with many health benefits including reduced risks of type 2 diabetes (T2D) and blood pressure [3–5], improved fasting blood glucose and other metabolic syndrome symptoms [6], improved plasma triglyceride levels [7] and protection against the development of insulin resistance and Non Alcoholic Fatty Liver Disease (NAFLD) [8]. In addition, fermented soy may lower inflammatory markers [9], ameliorate colitis [10] and prevent the onset and progression of cardiovascular disorders and cancer [11] [2]. However, the mechanisms through which fermented soy may exert the above effects are unknown.

Q-CAN® is a fermented soybean beverage and has been used for over 30 years as a nutritional food supplement to aid in the recovery from a wide range of conditions and is also taken during health. The beneficial effects of Q-CAN® fermented soy may be attributed to the combination of the production of fiber, high levels of isoflavones such as Genistein and Daidzein, amino acids, trace elements, minerals, bioactive peptides and branched-chain fatty acids. Among the several putative ingredients of Q-CAN®, isoflavones were shown to exert several health benefits on the host via alterations in key bacteria that are associated with a beneficial effect [12]. In line with this, consumption of fermented soy milk was previously shown to increase healthy microbiota, such as *Bifidobacteria* and *Lactobacilli*, and to decrease the pathogenic ones, such as *Clostridia*, in healthy individuals [13, 14]. The possible restoration of the gut microbiome upon fermented soy consumption is of particular significance given that altered gut microbiome has been shown by many studies to contribute to the development and progression of cardiometabolic disorders, such as atherosclerosis, obesity, and T2D [15], NAFLD [16] and cancer [17, 18]. Q-CAN® may restore the gut and oral microbiome and through this mechanism exert its beneficial effects.

Here, we hypothesize that Q-CAN® exerts beneficial effects through reduction of pathogenic bacteria and increase of beneficial ones. Given that obesity is increasing dramatically [19] and is a risk factor for cardiometabolic disorders, NAFLD and cancer [20], we determined the effects of Q-CAN® on the microbiome of lean and obese subjects, and assessed the expected consequences of these changes based on the published literature.

Materials And Methods

Subjects

This study was approved by the Human Investigation Committee of Yale University. Subjects were recruited mostly from the campus of Yale University and had no history of abdominal surgeries (excluding cholecystectomy, appendectomy, hysterectomy, hernia repair), inflammatory bowel disease (e.g., ulcerative colitis, Crohn's disease), GI bleeding, radiation proctitis or other known poorly controlled medical conditions that could interfere with bowel function, acute or chronic diseases, allergy to soy, soy derivatives, milk protein, alcohol use disorder, anorexia nervosa, autoimmune disease, bulimia, celiac disease, chronic infections and illicit drug use. Other exclusion criteria were major changes in dietary

habits in the past 6 months, use of proton pump inhibitors, antibiotics, probiotics, laxatives (chronic use), anticholinergics, or systemic corticosteroid (within 3 months of enrollment) and medicines affected by modest dietary changes (including but not limited to, warfarin, immunosuppressives), pregnancy or history of pregnancy within the past 6 months or intent to get pregnant during study period, use of tobacco (cigarettes, smokeless tobacco, cigars, pipes) within past 30 days. Before executing this study, written informed consent was acquired from all subjects. Totally 19 subjects participated at the beginning. Subjects were advised to maintain their normal life style during the course of the study.

Q-CAN® composition

Q-CAN® contained 8% fermented soy powder in water with 290.12 mg of total soy isoflavones in 240 ml and 3.6% protein. A profile of over 300 herbicides, pesticides and fungicides was negative and no chemical residual solvents tested were above the limit of quantification. Lead, mercury, arsenic, and cadmium were all below the detectable limit (5 to 10 ppb). 24-month serial assessment (accelerated and shelf) of Q-CAN® determined that soy isoflavones did not decrease in potency and microbiology analysis revealed no contamination.

Study Design

20 subjects including 10 lean (3 males, 7 females, mean age 32 years, mean BMI 22 Kg/m²) and 10 obese individuals (7 males, 3 females, mean age 45 years, mean BMI 34 Kg/m²) were enrolled in this study. The participants performed in total 11 visits (Fig. 1A). The first 3 visits take place with one-week intervals and subjects provided stool and saliva without any intervention. After the 3rd visit they started the Q-CAN® consumption (237 ml) twice daily for 4 weeks until visit 7. Every week stool and saliva were collected. At the 7th visit they stopped the Q-CAN® consumption and were monitored for 8 weeks post Q-CAN®. They gave stool and saliva samples every 2 weeks in the post Q-CAN® period. The 8-week follow up was to identify how sustained the changes induced by Q-CAN® were after consumption ceased. 3 samples (2 saliva and 1 stool) were collected per visit. Saliva sample 1 was collected first thing in the morning when the participants awoke, and before brushing their teeth, eating or drinking. The stool sample was collected during the day and the saliva sample 2 was collected right after the collection of the stool sample.

Sample Collection And Microbiome Analysis

The saliva and stool samples were kept frozen at -80°C until their DNA isolation. For saliva samples collection the OMNI gene-oral (OM-501) tube was used and for stool the OMNI gene GUT (OMR-200) tube. About 1 g of stool was collected from each subject in each of the 11 visits. DNA was extracted from all the samples (both saliva and stool samples from all 11 visits) according to the manufacturer instructions of the OM-501 and OMR-200 kits (DNA Genotek). All purified DNA samples were quantified via nanodrop

and/or Qubit measurements. Acceptable Qubit value was > 20 ng/μl. Extracted microbial DNA from saliva and stool samples was amplified against the V4 region of the 16S ribosomal RNA gene. Raw DNA sequencing data was analyzed with the QIIME 1.9.1 bioinformatics pipeline. Samples producing > 5,000 reads were considered for analysis, and the cutoff abundance was 0.01 percent. Statistical validation was performed using the SAS software package to calculate Least Squares Means (LSM) and group difference of LSM. 440 samples were collected in total, 424 of which were productive and yielded good quality data.

The Shannon Diversity Index was calculated based on the following formula:

$$H = \sum_{i=1}^s - (P_i * \ln P_i)$$

where:

H = the Shannon diversity index

P_i = fraction of the entire population made up of species i

S = numbers of species encountered

∑ = sum from species 1 to species S

To calculate the index, the number of individuals of species found in our samples were divided by the total number of individuals of all species. This is the P_i. Afterwards, the P_i was multiplied with its natural log (P_i * ln P_i). At the end, the sum of all the - (P_i * ln P_i) products is the value H (Shannon Diversity Index).

Statistical Analysis

All data are presented as the mean ± SEM. *P* < 0.05 was the level of significance. Repeated measure analyses was done on outcomes at each level, taking into account the correlation on observations occurred among the same patient. Treatment stage, gender and age were entered as fixed effects. Unstructured covariance was used. Analyses was done on each BMI level. The relative abundance of bacteria that were present at 50% of participants was also presented at the level of phylum, family, genus and species as a heat maps with hierarchical clustering using GluCore.

Results

In this study saliva and stool were collected from healthy, lean or obese individuals before Q-CAN® consumption (Pre Q-CAN® group), during Q-CAN® consumption (On Q-CAN® group) and after the

cessation of Q-CAN® consumption (Post Q-CAN® group) (Fig. 1A). The Pre Q-CAN® group includes 3 visits with one-week interval between visits. The On Q-CAN® group includes 4 visits with one-week interval between visits. The Post Q-CAN® group includes 4 visits with two-week interval between visits. In each visit, stool and saliva were collected. Q-CAN® consumption had no effect on alpha diversity of stool or saliva bacteria species in both obese and lean participants (Fig. 1B-E). Consumption of fermented soy product significantly increased stool *Actinobacteria* phylum populations in lean subjects (Fig. 2A). This increase was not observed in obese subjects (Fig. 2B). The highest abundant phylum populations (*Firmicutes* and *Bacteroidetes*) and their ratio were not affected upon Q-CAN consumption or withdrawal in either lean or obese populations (Fig. 2C-E). On the other hand, in the low abundant populations, Q-CAN significantly increased *Fusobacteria* only in obese participants (Fig. 2F) that returned to pre-QCAN levels upon its withdrawal. The rest bacteria at the level of phylum were not changed in either lean or obese as it is shown in the heat maps of Supplementary Figure S1.

At the level of Family, QCAN consumption in the lean group was associated with decreased abundance of *S24-7*, *Enterobacteriaceae* (Fig. 3A) and *Gemellaceae* families (Fig. 3C). The decrease of the *S24-7* family was retained upon Q-CAN® withdrawal but not in *Enterobacteriaceae* and *Gemellaceae* families (Fig. 3A, C) that were significantly increased upon Q-CAN withdrawal. On the other hand, the *Bifidobacteriaceae* (Fig. 3A) and *EtOH8* families (Fig. 3C) were significantly increased upon Q-CAN® consumption. Their increase was not retained upon Q-CAN® withdrawal (Fig. 3A, C). Q-CAN® had opposite effect on the *Gemellaceae* family (Fig. 3D) in obese group compared to lean ones (Fig. 3A). Several other changes were also observed in non-identified bacteria in lean and obese upon QCAN consumption or withdrawal (Supplementary Fig. 2; heat map).

At the genus level, the most abundant bacteria genera were not affected by Q-CAN® consumption in either obese or lean groups. In the less abundant bacteria genera, Q-CAN® consumption increased the levels of *Blautia* (Fig. 4A) and *Bifidobacterium* (Fig. 4C) in lean subjects. This induction was reduced upon Q-CAN® withdrawal (Fig. 4A, C). In obese, only *Sutterella* (Fig. 4D) was increased significantly upon QCAN consumption. The changes in *Sutterella* in both lean and obese group were not maintained upon Q-CAN® withdrawal (Fig. 4C, D). In the very low abundant genera that were detectable in few lean or obese participants, leptotrichia was shown a trend of decrease upon QCAN consumption in the lean group (Fig. 4E) and lactobacillus was significantly decreased in the obese group (Fig. 4F). These genera were significantly increased in the Post-QCAN group compared to On-QCAN group (Fig. 4E, F). Several other changes were also observed in non-identified genera in lean and obese upon QCAN consumption or withdrawal (Supplementary Fig. 3; heat map).

At the level of species, fewer changes were observed and they were only in non-identified bacteria. Interestingly, at lean group the non-identified species belonging to *Blautia* and *Bifidobacterium* genera display the same alterations upon QCAN as the corresponding genera (Fig. 5; heap map). In particular, unidentified species of *Bifidobacterium*, *Blautia* and *Acidaminococcus* were increased in the lean group and unidentified species of *Parabacteroidetes* and *Comamonadacea* were increased in the obese group

upon QCAN consumption. Several other changes were also observed in non-identified genera in lean and obese upon QCAN consumption or withdrawal (Fig. 5; heap map).

In the saliva samples, fewer changes were observed at the phylum, family and genus level than in stool. Moreover, Q-CAN® consumption affected different oral microbes from the stool ones. At the phylum level there was no effect of Q-CAN® consumption in any of the bacteria in both lean and obese individuals (Supplementary Fig. 4; heat map). At the family level, *Veillonelaceae* (Fig. 6A) was significantly increased during fermented soy consumption only in lean population. These changes were maintained upon Q-CAN® withdrawal. On the other hand, *Leptotrichiaceae* family was not affected during Q-CAN® consumption but were increased in the post Q-CAN® when compared to the on Q-CAN® group, in the lean population (Fig. 6A). In the obese, with the exception of *Tisserelaceae* that was significantly increased during fermented soy consumption only in obese population, the *Peptrosreptococcaceae* and the *Commanonadaceae* families were not affected during Q-CAN® consumption but were increased in the post Q-CAN® when compared to the on Q-CAN® group, in the obese population (Fig. 6B). All the bacteria at family level that were identified in the different visits are presented also by heat map (Supplementary Fig. 5; heat map).

At the genus level, several genera were altered upon Q-CAN consumption in the lean whereas in the obese the most alterations were observed upon Q-CAN withdrawal. In particular, in the lean ones *Lachnospira*, *Bacteroides* and *Enterococcus* were increased upon Q-CAN consumption whereas *Lautropia* and *Mobiluncus* were decreased (Fig. 6C, E). *Plaudibacter* was increased only in post Q-CAN® when compared to the on Q-CAN® group in the same population (Fig. 6C). On the other hand, in the obese with the exception of *Anaerococcus* that was significantly increased during fermented soy consumption, the *Plaudibacter*, *Tennerella*, *Peptoniphillus*, *Lautropia*, *Bifidobacterium*, *Bacteroides*, *Enterococcus* and *Peptosptreptococcus* were not affected during Q-CAN® consumption but were increased in the post Q-CAN® when compared to the on Q-CAN® group, in the obese population (Fig. 6D, E). Several other changes were also observed in non-identified genera in lean and obese upon QCAN consumption or withdrawal (Supplementary Fig. 6; heat map).

At the level of species, fewer changes were observed and they were only in non-identified bacteria. These bacteria belong to Lachnospiraceae family, *Oribacterium* and *Moryella* genera in the lean and to Ruminococcaceae family, *Tennerella*, *Plaudibacter*, *Bacteroides* genera in the obese (Fig. 7). All the species that were identified in the different visits are presented also by heat map (Fig. 7; heat map). Each column represents a subject and each row a bacterial taxon.

All the above changes are summarized in Table 1 where we can see that Q-CAN® consumption increases several bacteria in lean (n = 8 in stool and n = 1 in saliva) and obese (n = 3 in stool, n = 2 in saliva) subjects whereas decreases few of them in lean (n = 2 in stool and n = 1 in saliva) and in obese (n = 2 in stool and n = 0 in saliva) subjects. Several bacteria are also altered upon QCAN withdrawal as compared upon QCAN consumption. In the lean 5 bacteria are increased in stool and 5 in saliva whereas 3 bacteria

are decreased in stool and 0 in saliva. In the obese 1 bacterium is increased in stool and 13 in saliva whereas 2 bacteria are decreased in stool and 0 in saliva.

Discussion And Conclusion

Fermented soy consumption has been shown to be protective against the development of several diseases including type 2 diabetes [6], NAFLD [8], alcoholic induced liver damage [21], colitis [10], cardiovascular disorders and cancer [11] [2]. However, the mechanisms through which fermented soy products exert the above beneficial effects are totally unknown. Here we found that fermented soy beverage Q-CAN® alters the microbiome in lean, and healthy obese individuals, with a number of the changes occurring in a direction expected to improve overall health. In detail, we found that there was no effect on alpha diversity in the microbiome in the stool or saliva. There was alpha diversity however: 1) While taking Q-CAN®, at phylum level, in the stool of lean individuals there was an increase in Actinobacteria, and in obese individuals there was an increase in Fusobacteria (Fig. 2); 2) While taking Q-CAN®, at the family level in the stool of lean individuals, there was an increase in Bifidobacteriaceae and EtOH8, and a decrease in S24-7 (Fig. 3).; and 3) While taking Q-CAN®, at a genus level in the stool of lean individuals, there was an increase in Blautia, Bifidobacterium and Staphylococcus (Fig. 4).

There is now a large amount of data associating changes in the microbiome with physiological changes with impact on health[22]. Microbiome diversity is considered to be positive and it was reassuring to see that Q-CAN® did not decrease microbiome diversity in the stool or saliva (Fig. 1B-E). The increase in Actinobacteria in lean individuals taking Q-CAN® (Fig. 2A), is of interest as Actinobacteria are one of the four major phyla of gut microbiota and has a crucial role in maintaining gut homeostasis. Actinobacteria are gram positive, multiple branching rods, non-motile, non-spore-forming anaerobic bacteria that include three main anaerobe families (Bifidobacteria, Propionibacteria and Corynebacteria)[23]. Within the Actinobacteria phylum the increase was found to be in the family Bifidobacteria (Fig. 3A). Bifidobacteria have beneficial effects in the maintenance of gut barrier thanks to their great production of short chain fatty acids (SCFA)[24]. They produce high concentration of acetate that can protect the host from enteropathogenic infections, such as entero-haemorrhagic *Escherichia coli* and *Shigella*[25, 26]. In addition to producing SCFA, Bifidobacteria provide many of the enzymes needed for biotransformation of nutrients in the diet[27]. The pathways involved include the fermentation of large polysaccharides, oligosaccharides, unabsorbed sugars and fibers, that release hydrogen, carbon dioxide and SCFAs, the degradation of proteins, the regulation of lipid metabolism through lipoprotein lipase (LPL), and the absorption and biosynthesis of vitamin K, iron, calcium and magnesium[28, 29]. Through the stimulation of intrahepatic lymphocytes, Bifidobacteria are also important in the maintenance of a tolerogenic immune environment[30, 31]. A decreased number of Bifidobacteria is associated to an enhancement of gut permeability that leads to the translocation of LPS into the serum[32]. This triggers the immune system activation and sustains chronic inflammatory conditions, such as insulin resistance, diabetes and liver diseases[33]. The administration of *Bifidobacterium pseudocatenulatum* CECT 7765 along with high fat diet in mice is able to down-regulate the inflammation by reducing the production of inflammatory cytokines and chemokines, especially IL-6 and MCP-1, which are usually increased in obesity and

metabolic disorders[34]. Overall Bifidobacteria are seen as improving gut barrier function and reduce the translocation of pro-inflammatory molecules such as lipopolysaccharide into the blood stream[32]. Bifidobacteria, together with Lactobacilli, represents the cornerstone of probiotic therapeutic approach. For example, the probiotic formulation VSL#3, a mixture of lyophilized four Lactobacilli and three Bifidobacteria strains has been demonstrated to be effective in several conditions including pouchitis, non-alcoholic steatohepatitis and in the prevention of antibiotic associated diarrhea[35–37]. Bifidobacteria treatment has also been demonstrated to improve symptoms of irritable bowel syndrome[38].

A significant increase in the phylum Actinobacteria and the family Bifidobacteria in the stool was not seen in obese individuals, however there was a trend in that direction (Fig. 2B, 3B). We believe this lack of positive association is due to the relatively small samples size of ten individuals in each group, and with larger samples sizes a significant increase will be seen. There was a statistically significant increase in the phylum Fusobacteria in the stool of obese individuals but this increase was not followed through at the family (Fusobacteriaceae) level and is of unclear significance. The species *Fusobacterium nucleatum* has been shown to be associated with colon cancer, although this association has not been universally reproduced[39, 40].

In lean individuals there was also an increase in the family of EtOH8 anaerobic bacteria, (Fig. 3C), but relatively little is known about the biological significance of this and it is difficult to speculate. The uncultured S24-7, a member of the Bacteroidetes family, was reduced in lean individuals while taking Q-CAN® (Fig. 3A). S24-7 are highly anaerobic bacteria that are localized to the gastrointestinal tracts of homeothermic animals and are increasingly being recognized as a numerically predominant member of the gut microbiota but due to the inability to culture them little is known about the nature of their interactions with the host[41].

At the genus level there was an increase in *Blautia* (family: Lachnospiraceae, order: Clostridiales, class: Clostridia, and phylum: Firmicutes) (Fig. 4A). Higher levels of *Blautia* have been associated with several positive health features including nutrient assimilation, immunological health, lower amount of visceral fat, reduced risk of graft versus host disease and [42–44], and administration of *Blautia* has been proposed as a treatment for cancer[45].

In the saliva of lean individuals there was an increase in family Veillonellaceae (phylum Firmicutes, with three genera *Veillonella*, *Acidaminococcus*, and *Megasphaera*) while on Q-CAN® (Fig. 6A). Members of the family Veillonellaceae are of particular interest for their probiotic effects but to date this has been investigated in animal husbandry with trials showing improvement in energy balance and inhibiting colonization by antibiotic resistance strains of bacteria[46, 47]. It will be interesting to see if such beneficial effects are also found in the future in humans.

When comparing the lean and obese populations it is clear that Q-CAN® consumption resulted in a greater number of changes in the lean than the obese (Phylum lean 1: obese 1, family lean 3: obese 0,

genus lean 3: obese 0). This may be due to the microbiota of obese individuals having less diversity and therefore less opportunity for Q-CAN® to interact with a range of microbes[48, 49].

In conclusion, Q-CAN® induced a number of changes in the stool and saliva microbiome. The changes which were most notable and for which we currently have the greatest information on physiological impact are the increase in stool microbiome of lean subjects of family Bifidobacteria which are known to have a wide variety of beneficial effects including producing SCFA, reducing intestinal permeability and improving immune function. The increase in Blautia is likewise proposed to have a number of beneficial effects including nutrient assimilation and cancer. This study has also generated a significant amount of data on bacteria of unclear biological functions and will be a reference source as more information on these bacteria is generated.

Declarations

Acknowledgments. This work was supported by a sponsored research agreement from BESO Biological Research Inc. The design and conduct of the study as well the data analysis and conclusions were conducted independently by the investigators.

Ethical Approval and Consent to participate: Approval was obtained from Yale HIC.

Consent for publication: All authors consent to publication.

Availability of supporting data: Supporting data is available on request.

Competing interests: There are no competing interests.

Funding: This project was funded by BESO Biological Research Inc.

Authors' contributions: 1. ED and AA designed research (project conception, development of overall research plan, and study oversight). 2. ED, RP, TRW, MRF, conducted research (hands-on conduct of the experiments and data collection). 3. ED, EK analyzed data and performed statistical analysis. 4. RP and EK wrote paper.

Acknowledgements: We acknowledge funding by BESO Biological Research Inc.

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Table

Due to technical limitations, table 1 is only available as a download in the supplemental files section.

Supplementary Figures

Supplementary Fig. 1: Intestinal microbiome analysis at the level of Phylum. A-B) Relative abundance of bacterial is visualized by heat map in both lean and obese. Each column represents a subject and each colored row a bacterial taxon. The intensity of the red color represents the highest abundance taxa and the intensity of the blue colour the lowest abundance taxa in lean and obese people. The results are the average of 3 visits in pre Q-CAN group, 4 visits in on Q-CAN group and 4 visits in post Q-CAN group for each participant. Obese (n = 9 participants), Lean (n = 10 participants).

Supplementary Fig. 2: Intestinal microbiome analysis at the level of Family. A-B) Relative abundance of bacterial is visualized by heat map in both lean and obese. Each column represents a subject and each colored row a bacterial taxon. The intensity of the red color represents the highest abundance taxa and the intensity of the blue colour the lowest abundance taxa in lean and obese people. The results are the average of 3 visits in pre Q-CAN group, 4 visits in on Q-CAN group and 4 visits in post Q-CAN group for each participant. Obese (n = 9 participants), Lean (n = 10 participants).

Supplementary Fig. 3: Intestinal microbiome analysis at the level of Genus. A-B) Relative abundance of bacterial genera is visualized by heat map in both lean and obese. Each column represents a subject and each colored row a bacterial taxon. The intensity of the red color represents the highest abundance taxa and the intensity of the blue color the lowest abundance taxa in lean and obese people. The results are the average of 3 visits in pre Q-CAN group, 4 visits in on Q-CAN group and 4 visits in post Q-CAN group for each participant. Obese (n = 9 participants), Lean (n = 10 participants).

Supplementary Fig. 4: Oral microbiome analysis at the level of Phylum. A-B) Relative abundance of bacterial is visualized by heat map in both lean and obese. Each column represents a subject and each colored row a bacterial taxon. The intensity of the red color represents the highest abundance taxa and the intensity of the blue color the lowest abundance taxa in lean and obese people. The results are the average of 3 visits in pre Q-CAN group, 4 visits in on Q-CAN group and 4 visits in post Q-CAN group for each participant. Obese (n = 10 participants), Lean (n = 10 participants).

Supplementary Fig. 5: Oral microbiome analysis at the level of Family. A-B) Relative abundance of bacterial is visualized by heat map in both lean and obese. Each column represents a subject and each colored row a bacterial taxon. The intensity of the red color represents the highest abundance taxa and the intensity of the blue color the lowest abundance taxa in lean and obese people. The results are the average of 3 visits in pre Q-CAN group, 4 visits in on Q-CAN group and 4 visits in post Q-CAN group for each participant. Obese (n = 10 participants), Lean (n = 10 participants).

Supplementary Fig. 6: Oral microbiome analysis at the level of Genus. A-B) Relative abundance of bacterial genera is visualized by heat map in both lean and obese. Each column represents a subject and each colored row a bacterial taxon. The intensity of the red color represents the highest abundance taxa and the intensity of the blue color the lowest abundance taxa in lean and obese people. The results are the average of 3 visits in pre Q-CAN group, 4 visits in on Q-CAN group and 4 visits in post Q-CAN group for each participant. Obese (n = 10 participants), Lean (n = 10 participants).

Figures

FIGURE 1

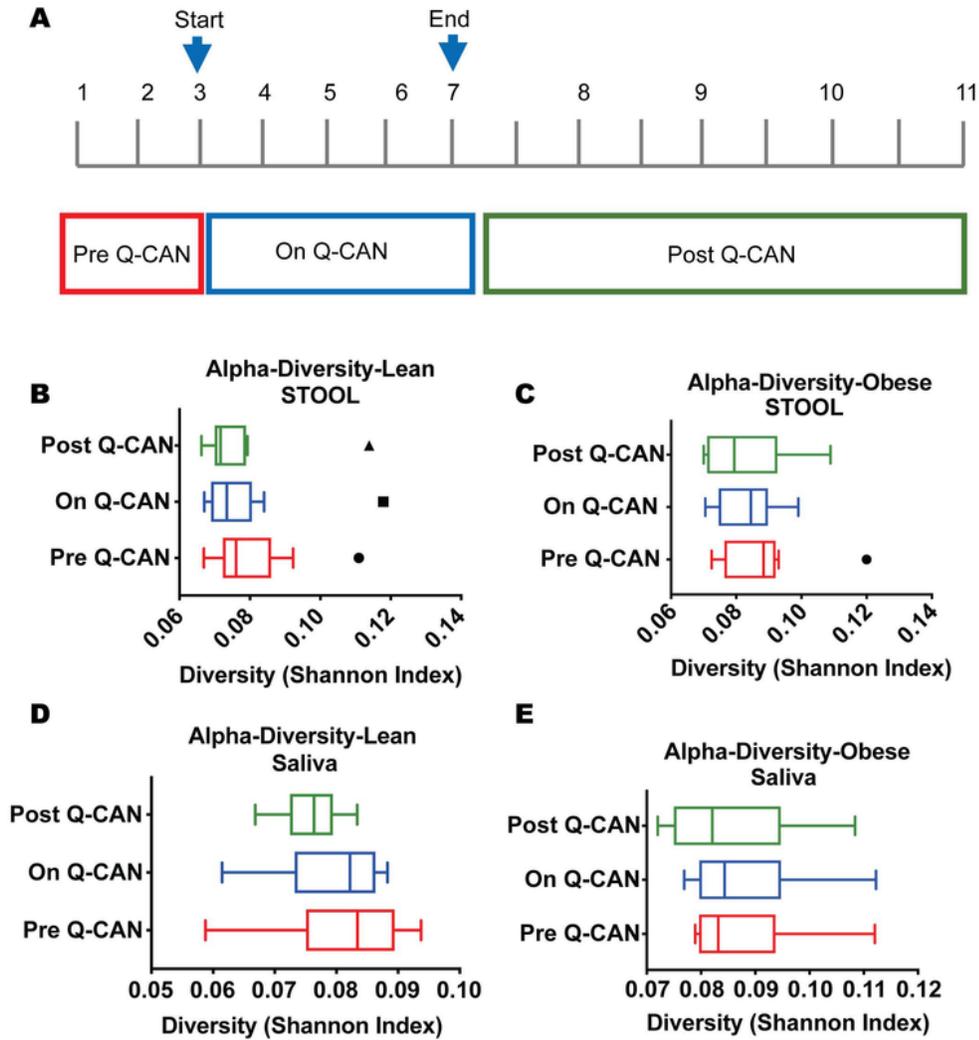


Figure 1

A) Depiction of the time-frame of Q-CAN consumption or withdrawal. B-D) Shannon Diversity Index of intestinal or oral microbiome is not altered upon Q-CAN consumption or withdrawal in lean or obese people. The results are the average of 3 visits in pre Q-CAN group, 4 visits in on Q-CAN group and 4 visits

in post Q-CAN group for each participant. Obese (n=9 participants), Lean (n=10 participants). The data are presented as Tukey box plots showing the median values.

FIGURE 2

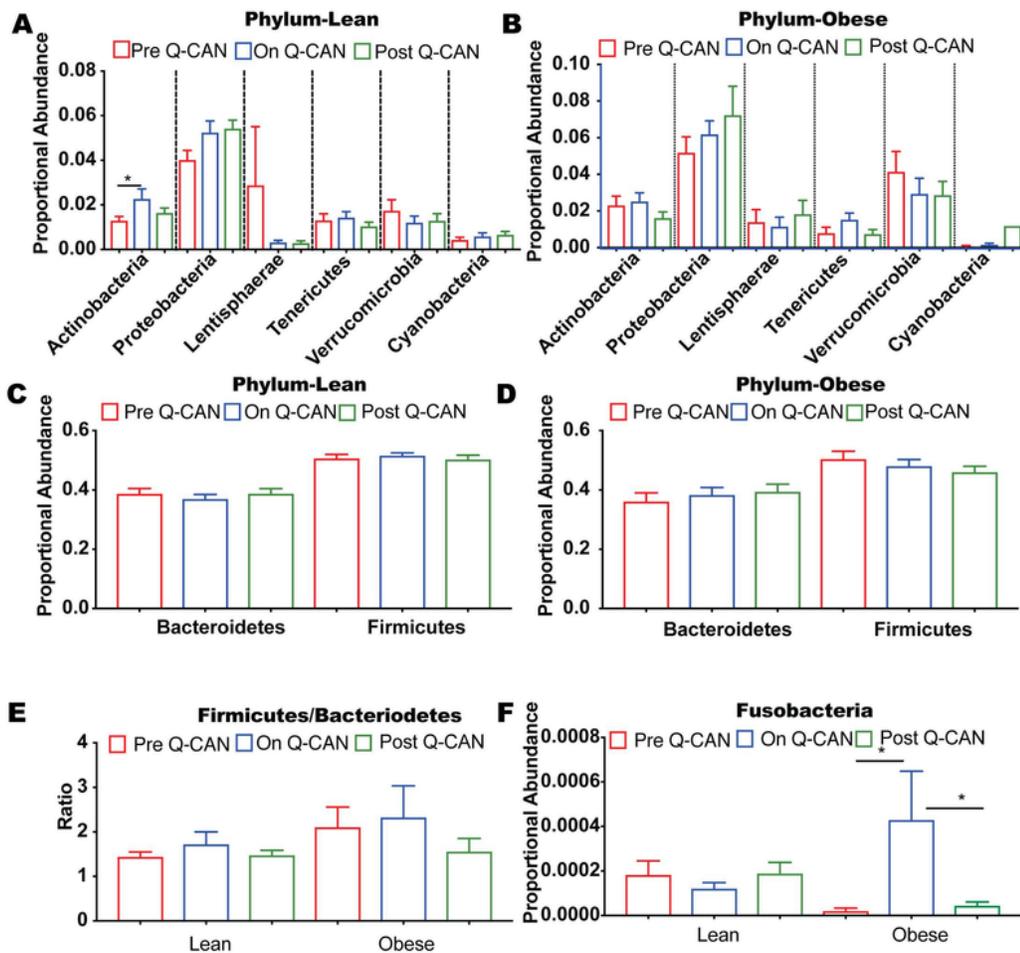


Figure 2

Intestinal microbiome analysis at the level of Phylum. A-F) Bacteria at Phylum level in both lean and obese shows that only Actinobacteria and Fusobacteria are altered upon Q-CAN consumption. E) The ratio of Firmicutes/Bacteroidetes has a trend for increase in obese compared to lean ones. The results are

the average of 3 visits in pre Q-CAN group, 4 visits in on Q-CAN group and 4 visits in post Q-CAN group for each participant. Obese (n=9 participants), Lean (n=10 participants). The data are presented as median with SEM, *p < 0.05.

FIGURE 3

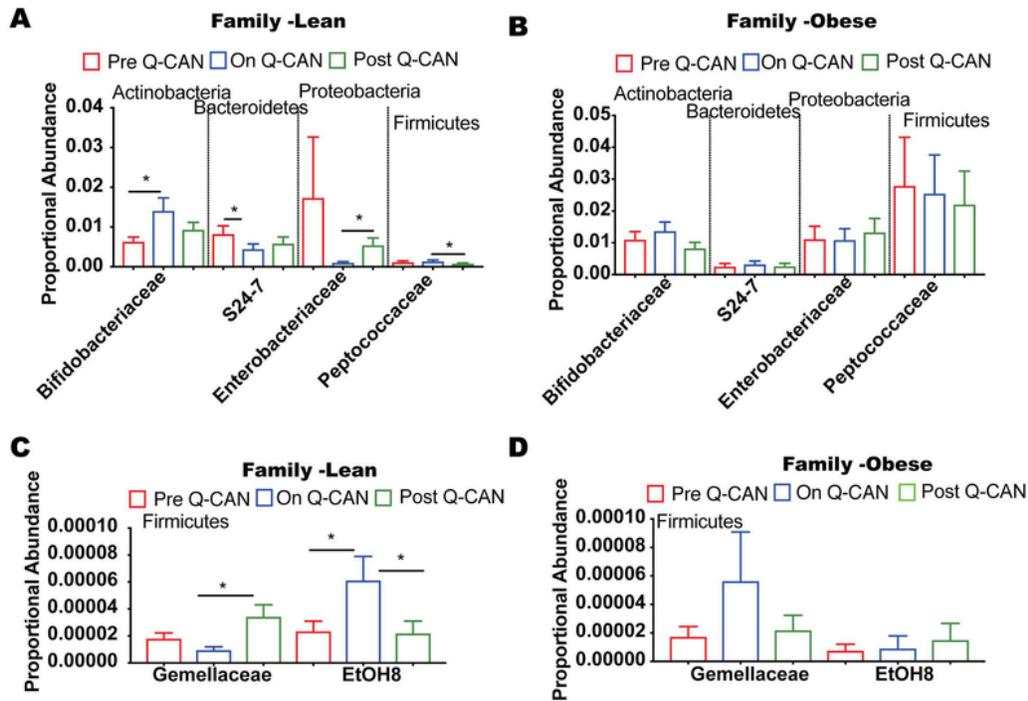


Figure 3

Intestinal microbiome analysis at the level of Family. A-D) Bacteria at Family level in both lean and obese shows that only Bifidobacteriaceae, S24-7 and EtOH8 are altered upon Q-CAN consumption. The results are

the average of 3 visits in pre Q-CAN group, 4 visits in on Q-CAN group and 4 visits in post Q-CAN group for each participant. Obese (n=9 participants), Lean (n=10 participants). The data are presented as median with SEM, *p < 0.05.

FIGURE 4

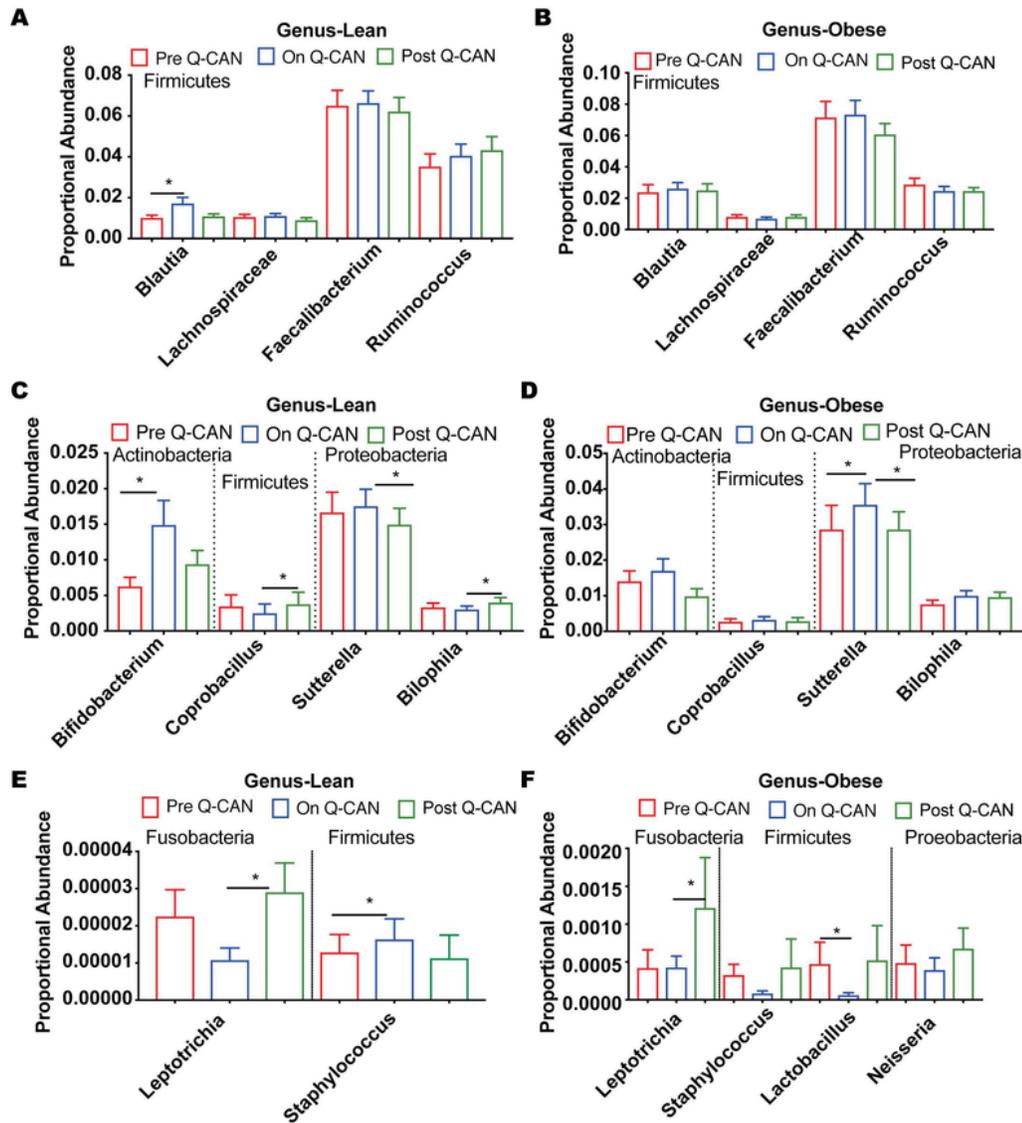


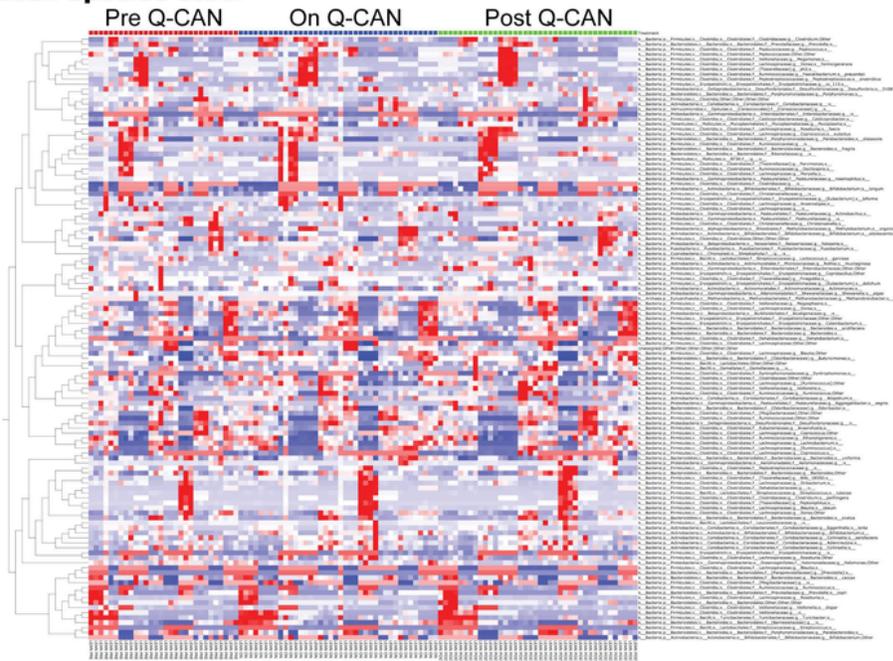
Figure 4

Intestinal microbiome analysis at the level of genus. A-F) Bacteria distribution at genus level in both lean and obese participants. The levels of *Blautia*, *Bifidobacterium* and *Staphylococcus* genera are altered

upon Q-CAN consumption in lean and the levels of *Sutterella* and *Lactobacillus* in obese. The results are the average of 3 visits in pre Q-CAN group, 4 visits in on Q-CAN group and 4 visits in post Q-CAN group for each participant. Obese (n=9 participants), Lean (n=10 participants). The data are presented as median with SEM, *p < 0.05.

FIGURE 5

A Lean-Species Stool



B Obese-Species Stool

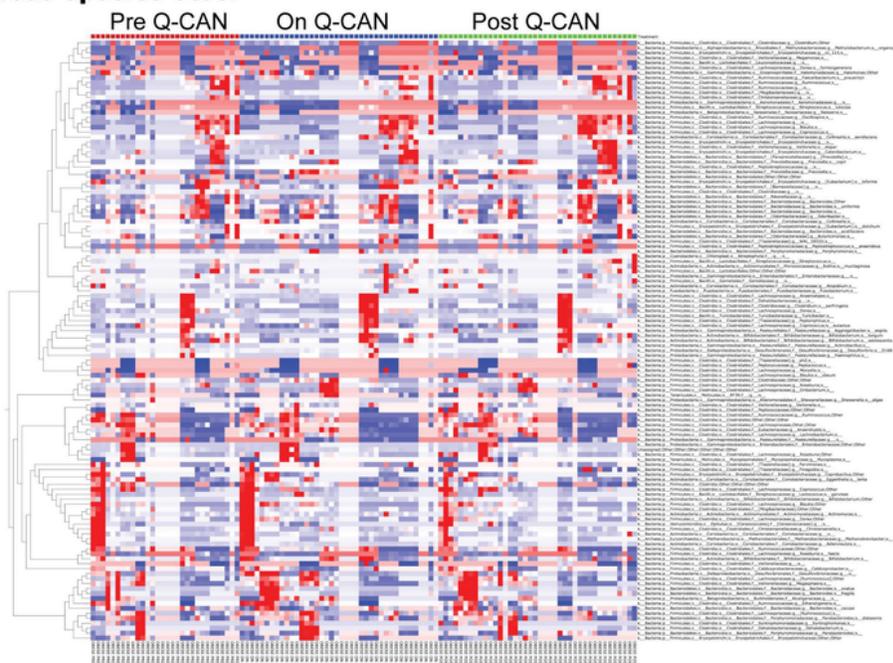


Figure 5

Intestinal microbiome analysis at the level of species. A-B) Relative abundance of bacterial species is visualized by heat map. Each column represents a subject and each colored row a bacterial taxon. The intensity of the red color represents the highest abundance taxa and the intensity of the blue colour the lowest abundance taxa in lean and obese people. The results are the average of 3 visits in pre Q-CAN group, 4 visits in on Q-CAN group and 4 visits in post Q-CAN group for each participant. Obese (n=9 participants), Lean (n=10 participants).

FIGURE 6

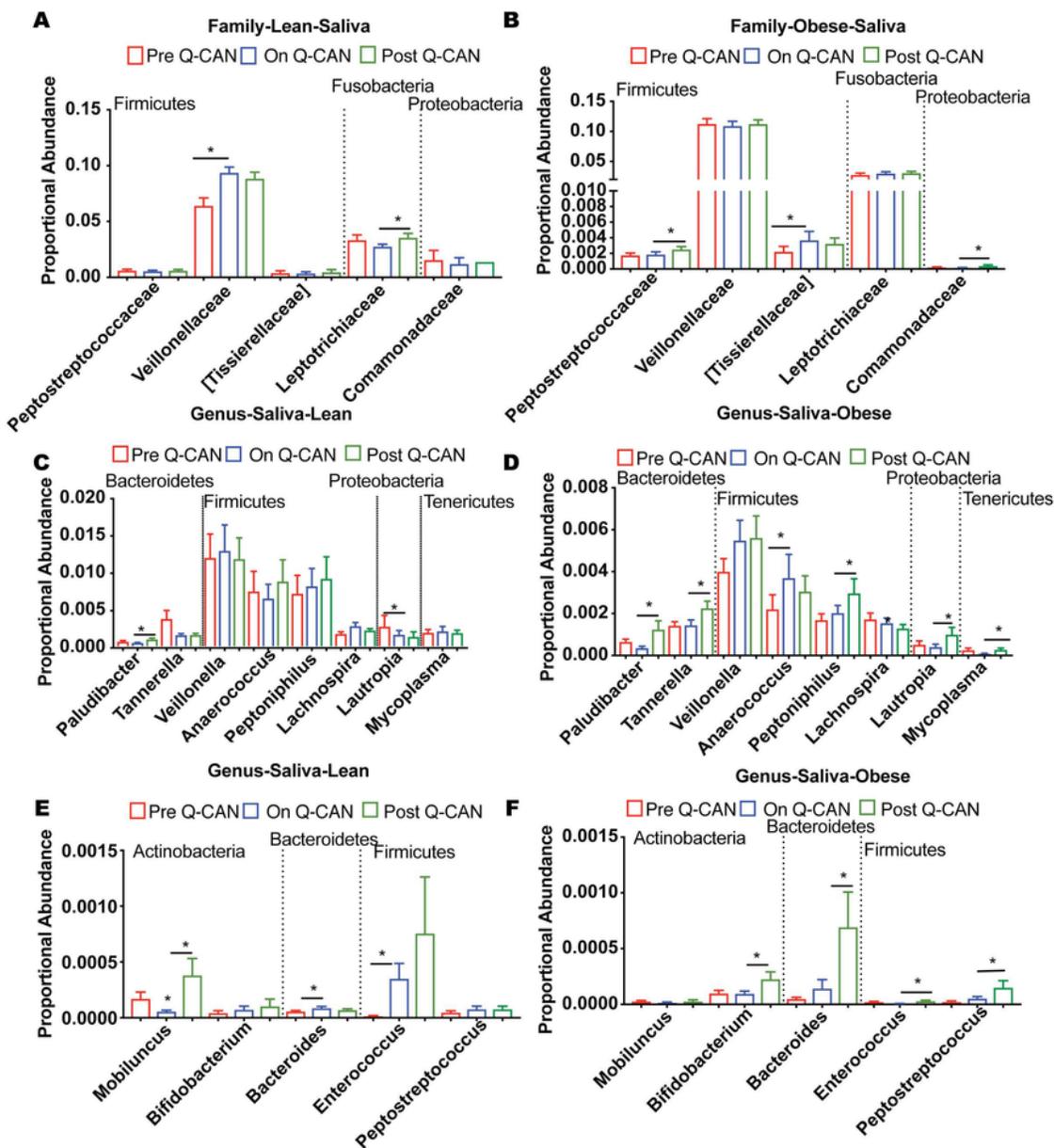
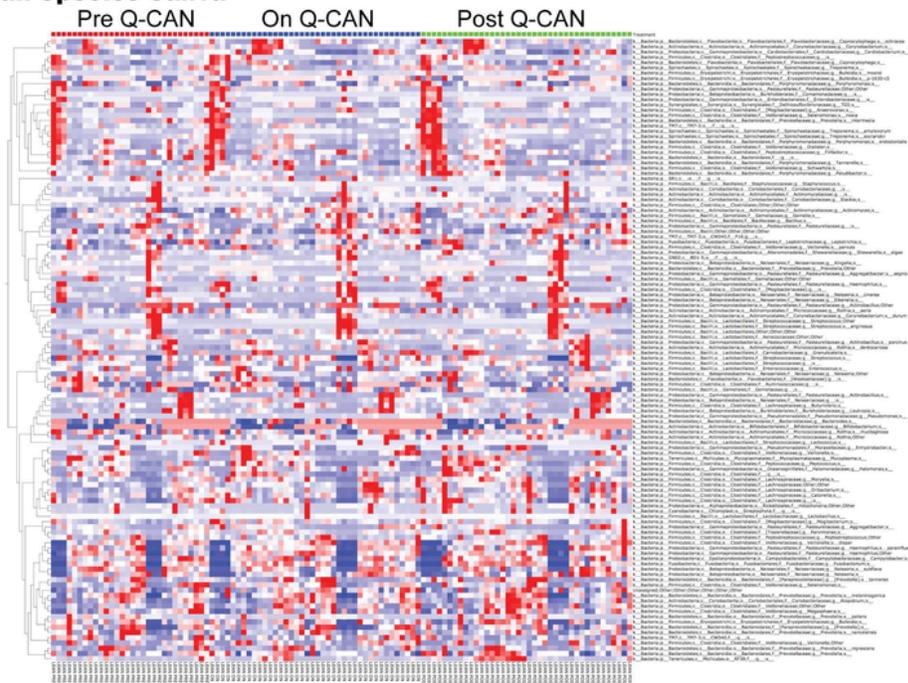


Figure 6

Oral microbiome analysis at the level of Family and Genus. A, B) Bacteria at Family level in both lean and obese shows increase of Veillonelleae in lean and Tissierallacea in obese upon Q-CAN consumption. C-F) Bacteria distribution at genus level in both lean and obese participants. The results are the average of 3 visits in pre Q-CAN group, 4 visits in on Q-CAN group and 4 visits in post Q-CAN group for each participant. Obese (n=10 participants), Lean (n=10 participants). The data are presented as median with SEM, *p < 0.05.

FIGURE 7

A Lean-Species Saliva



B Obese-Species Saliva

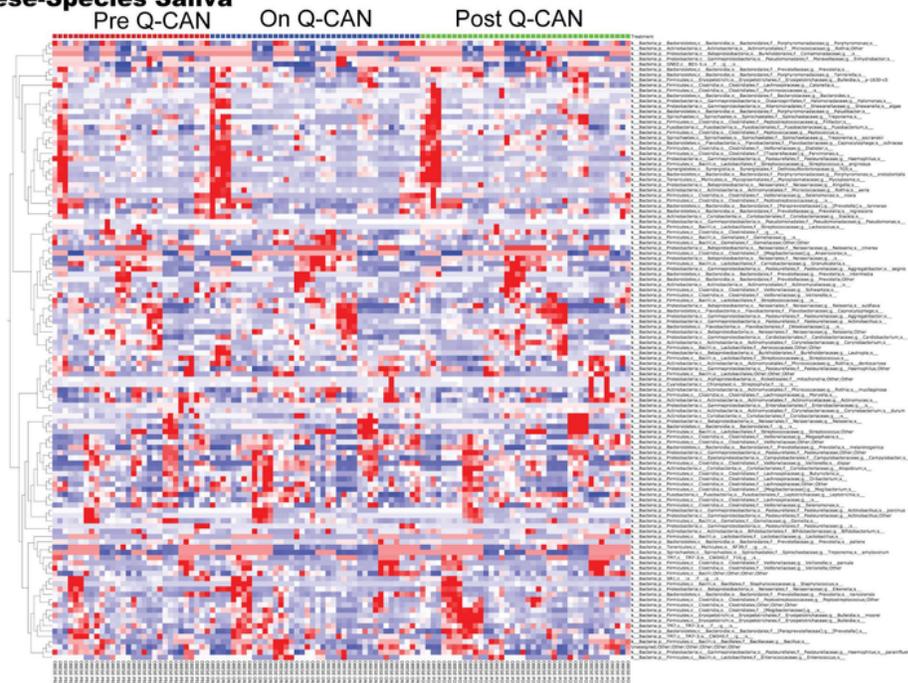


Figure 7

Oral microbiome analysis at the level of Species. A-B) Relative abundance of bacterial species is visualized by heat map. Each column represents a subject and each colored row a bacterial taxon. The intensity of the red color represents the highest abundance taxa and the intensity of the blue colour the lowest abundance taxa in lean and obese people. The results are the average of 3 visits in pre Q-CAN group, 4 visits in on Q-CAN group and 4 visits in post Q-CAN group for each participant. Obese (n=10 participants), Lean (n=10 participants).

Supplementary Files

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