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Communication

Short Communication: Obesity Intervention Resulting in Significant Changes in the Human Gut Viral Composition

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Abstract: Obesity is a health problem of global concern that negatively impacts quality of life. Various studies have implicated obesity in the disruption of the normal microbiome composition. The virome consists of a collection of all the viruses that inhabit a particular niche. However, the study of such viruses that compose the human gut microbiome in the context of obesity has been paid little attention. One interesting aspect of virome study is the description of phages that can specifically interact with the bacterial component of the microbiota and modulate the microbiome's dynamics. Previous work showed that the microbiota composition changes after obesity treatment and that these changes are country specific. In this work, we perform a quick gut viral composition of the gut microbiota of patients from Denmark, Italy, and Chile before and after obesity intervention using metagenomic sequences previously published and bioinformatics tools. Our results showed that obesity treatment results in significant changes in the human gut viral composition. These results conclude that the virome composition changes after obesity intervention by suggesting that changes can be related to the microbiota rearrangements reported in other works and may be involved in microbial dynamics after treatment.

Keywords: obesity; human gut microbiome; human gut virome; viral changes

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1. Introduction

At least 2.8 million people die each year because of obesity, which makes it akin to being considered an epidemic [1]. Obesity is a risk factor related to cardiovascular disease, diabetes, cancer, chronic diseases (osteoarthritis and liver and kidney diseases), depression, and sleep apnea [2]. The main treatments prescribed against overweight or obesity are physical activity and diet modification. However, bariatric surgery is majorly successful in patients with severe obesity by generating successful weight loss [3]. Common bariatric surgery procedures include a Roux-en-Y gastric bypass (RYGB), sleeve gastrectomy (SG), and laparoscopic adjustable gastric band (LAGB), although there is no clear difference in terms of the effectiveness of these procedures [4]. Vertical banded gastroplasty (VBG) was also a bariatric procedure used to treat obesity. However, it has been mainly replaced by RYGB intervention [5].

Gut microbiota has a direct function in host nutrient metabolism, drug metabolism, and xenobiotic metabolism, maintenance of the structural integrity of the gut mucosal barrier, immunomodulation, and protection against pathogens [6–8]. Moreover, the gut microbiota seems to be dominated by bacteria belonging to the Firmicutes and Bacteroidetes phyla, with Actinobacteria, Proteobacteria, and Verrucomicrobia being less represented [9]. Moreover, patients who are overweight and obese possess low fecal bacterial diversity, which is associated with high levels of adiposity and dyslipidemia, and may also include glucose homeostasis changes and mucosa inflammation [10].

Remarkably, bariatric surgery generates important changes in the composition of the gut microbiota, and it is even quite notorious for causing taxonomical and functional changes. This leads to an increase in taxonomical diversity of gut microbiota within the first three months after surgery, which continues to be maintained at a high level even one year later [11]. This high diversity of the gut microbiota is stable even nine years after RYGB surgery, where the long-term effects of both RYGB and VBG on the composition and functional capacity of the gut microbiota are prevalent [12]. These changes could be explained by an increased potential for oxygen tolerance and better potential to utilize macronutrients and micronutrients [11]. Other research compared the gut microbiota of obese and lean people from six different regions, showing that the obesity microbiota was country-specific. However, the country-specific microbiota composition in the obesity context contributes to similar metabolic functions [13]. In a study conducted on the stools of individuals with obesity from France, the United States, and Switzerland, comparing the microbiome composition before and after six months of SG and laparoscopic Roux-en-Y gastric bypass (LRYGB), some changes associated with the intervention type were identified: LRYGB produced a relatively higher abundance of aerotolerant bacteria, whereas anaerobes were only higher after SG, concluding that microbiota changes depend on the type of bariatric surgery. Nevertheless, both kinds of interventions produce an increase in *Akkermansia muciniphila* [14], whose higher abundance is associated with a healthier metabolic status [15].

Human stool samples contain more than 10^9 viruses per g of feces, which is approximately the number detected for bacteria, with DNA bacteriophages being the most predominant [16]. On the other hand, RNA viruses make up a small fraction of the gut, and most of them are described as plant-derived [17]. The human gut microbiome is highly diverse, with the virome being more heterogeneous than the bacterial microbiota [18]. A recent study analyzed fecal samples of healthy adults from different regions and ethnicities, showing urban residence association with multiple bacteriophages, such as Lactobacillus and Lactococcus phages [19]. In that sense, it has been shown that bacteriophages have an important role in the ecology of the human gut microbiome. Nayfach et al. (2021) assembled a Metagenomic Gut Virus catalog that contains 189,680 viral genomes from the data of human stool metagenomes. Interestingly, over 75% of the genomes are double-stranded DNA phages that can infect Bacteroidia and Clostridia members. However, there are a high number of unknown viral species in the existing databases. The authors proposed that some species-level viral operational taxonomic units (vOTUs) may infect some of the most prevalent species in the gut microbiome, including *Bacteroides uniformis*, *Faecalibacterium prausnitzii*, and *Agathobacter rectalis* [20]. However, in contrast with bacteria, the viral contribution to human gut microbiome dynamics is still poorly understood. In this work, we use shotgun DNA metagenomic sequences retrieved from previous works that characterize the microbiome changes after an obesity intervention to perform a quick description of the human gut viral composition. We compare the taxonomic abundance in each group to assess the differences in viral composition before and after treatment. Overall, we observed an increase in the alpha diversity index and significant differences in the viral composition after obesity treatment in comparison with individuals with obesity. Accordingly, these findings suggest that the gut viruses may play an important role in microbiota recovery after obesity intervention and open the possibility of designing viral therapies to support obesity treatments.

2. Materials and Methods

Shotgun metagenomics sequences from fecal DNA samples belonging to three independent studies were downloaded from the ENA-EMBL database (Supplementary Table S1) grouped by the project under the accession numbers PRJEB29060 [13], PRJEB8249 [12], and PRJEB12947 [11]. A total of 49 raw sequences obtained using Illumina HiSeq shotgun metagenomics were analyzed, including the 21 pre-treated and 28 post-obesity-treated subjects (measured after 6 months post-treatment), including 2 medical treatments based on exercise and diet (MT), 17 Roux-en-Y gastric bypasses (GBs), 2 sleeve gastrectomies (SGs), and 7 verticals banded gastroplasties (VBGs). To access the viral taxa and abundance, we analyzed the raw data sequences using the bioinformatics software FastViromeExplorer [21] and followed their guidelines for viral profiling from metagenomic data (https://fastviromeexplorer.readthedocs.io/en/latest/), (accessed on 15 March 2021). Briefly, paired-end raw data sequences were used simultaneously and analyzed using the NCBI RefSeq database (ncbi-virus-kallisto-index-k31.idx) provided by the author's tool. The output table obtained was exported to the R environment [22] for statistical analysis and figure representation. The R package vegan [23] was used for the diversity index estimation, and the microbiome package [24] was used for the virome core estimation [25] using a phyloseq object created from the merger of the FastViromeExplorer output using the phyloseq [26] and ape [27] packages under the R environment. The results obtained from the statistical analysis were represented using the ggplot2 library [28], and the significant differences were assessed using the unpaired Mann–Whitney test under R statistical language. Finally, to identify the differentially enriched biomarkers among the compared groups, we applied the LEfSe analytic method using the online interface Galaxy [29].

3. Results

3.1. Description of Raw Data Sequences Used

To investigate the effects of obesity treatments on the virome of individuals with obesity, a total of 49 raw data samples of shotgun metagenomic sequencing obtained from the stools were used. The sequences belonged to previous microbiome studies performed in the obesity context [11–13], including pre-treated (n = 21) and post-treated (n = 28) conditions, namely medical treatments based on exercise and diet (MT), Roux-en-Y gastric bypass (GB), sleeve gastrectomy (SG), and vertical banded gastroplasty (VBG) (Supplementary Table S1). All these studies reported significant changes in microbiome key microorganisms and functional pathways after an obesity intervention. However, no information about viral changes was reported. To ascertain the viral status in the described context, we used the metagenomic sequences and performed a viral characterization using the FastViromeExplorer bioinformatics tool [21].

3.2. Alterations of Gut Viral Diversity in Obesity and Post-Obesity Treatments

To describe the composition of the gut virome, we first analyzed the alpha diversity using the Shannon index in pre-treated and post-obesity-treated groups (Supplementary Table S2) based on the virome abundances obtained from the FastViromeExplorer analysis (Supplementary Table S3). We found a lower alpha diversity index of the gut virome in the pre-obesity group compared with the post-obesity group (Figure 1A), showing significant differences between the diversity of both groups.

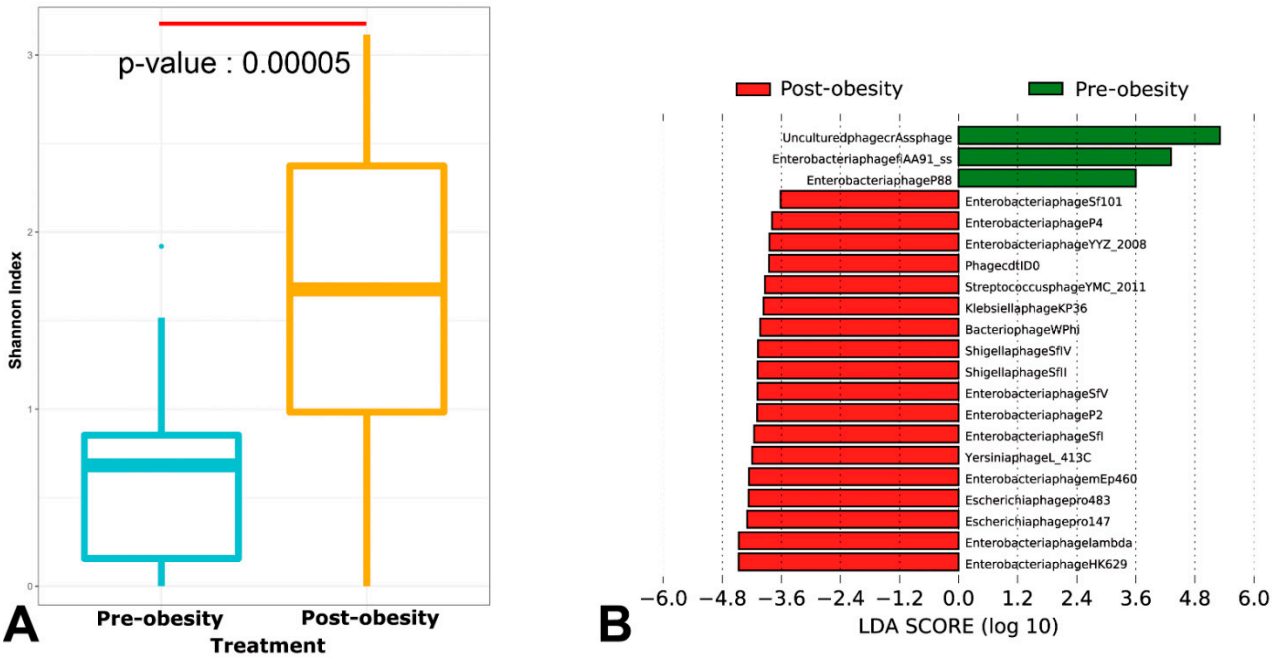

Figure 1. Alpha diversity metric measured by Shannon index and viral biomarkers identified in pre- and post-obesity groups. In (**A**), light blue boxplot indicates the index level of the pre-obesity group, and yellow represents the post-obesity treatment. The *p*-value indicates the statistical significance. In (**B**), the viral biomarkers presented in pre- (green) and post-obesity (red) intervention groups are shown.

3.3. Viral Biomarker Enrichment in Each Condition

In addition to diversity differences, we looked for the presence of viral biomarkers before and after obesity intervention using Lefse-LDA analysis [29]. We found a relatively low abundance in the pre-obesity group (Figure 1B), where only three viruses were found predominantly in this condition: *Uncultured phage crAssphage*, *Enterobacteria phage fiAA91_ss*, and *Enterobacteria phage P88*. Nevertheless, when we analyzed the post-obesity subjects, it was possible to see that there was a total of 18 viral biomarkers (Figure 1B). Some of these viruses correspond to *Enterobacteria phage HK629*, *Enterobacteria phage lambda*, *Escherichia phage pro147*, *Escherichia phage pro483*, and *Enterobacteria phage mEp460* among others.

3.4. Core Virome in Pre- and Post-Obesity Intervention Groups

To identify the core virome in individuals with obesity and post-obesity intervention, we used their relative abundances and performed an analysis to assess the prevalence of the main taxonomic groups in each condition [25]. To visualize the taxa with high prevalence across sample categories, we used a heatmap representation. In the pre-treatment condition, only four viruses were identified as members of the compositional core virome (Figure 2A), namely *Uncultured phage crAssphage*, *Enterobacteria phage phiX174 sensu lato*, *Parabacteroides phage YZ-2015b*, and *Enterobacteria phage lambda*. Conversely, using the same parameters to assess the core virome in individuals with obesity, the post-obesity group showed at least 13 viruses that shaped the core virome (Figure 2B), thus showing that, in this group, the virome has a higher core diversity of DNA viruses in comparison with the pre-obese samples.

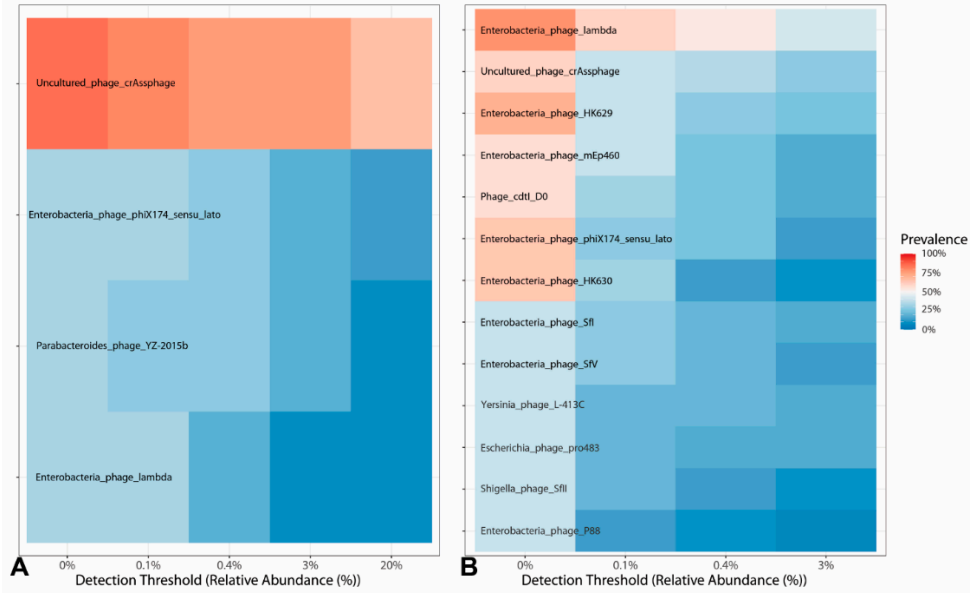

Figure 2. Heatmap of the core virome in pre- (**A**) and post-obesity (**B**) groups. Taxa prevalence is denoted red for higher values and blue for lower values.

4. Discussion

Obesity is a global health issue whose prevalence has been increasing rapidly. Severe differences in microbiota composition have been reported by several studies in obese people. Currently, the most common treatment for obesity is bariatric surgery. In this context, it has been described that the human gut microbiota composition and functionality perform a crucial role during obesity as well as in the recovery after medical intervention [30–34]. For example, Tremaroli et al. (2015) demonstrated that two types of bariatric surgery, namely Roux-en-Y gastric bypass and vertical banded gastroplasty, produced long-term alterations of the gut microbiome independently of BMI and that these alterations modulated host metabolism and fat mass deposition. However, the studies that have been carried out to understand how the microbiota is involved in the development and establishment of obesity have paid little attention to viruses.

The virome is starting to be recognized as a study object inside of microbiome research, where the identification of the viral community composition can be associated with adverse outcomes for the human host, whereas other states may be characteristic of health [35]. Some studies support evidence that prokaryotic viruses can have an impact on human health by affecting the structure and function of the bacteria community and its symbiotic interaction with humans [36,37]. Shotgun metagenomics provides a great quantity of data, which, however, are not always explored completely. Here, we used previously published sequences obtained from public databases from gut microbiome patients before and after undergoing gastric surgery to survey what occurred with the gut viral content. Our results show that during obesity, the alpha diversity index is lower compared with subjects that undergo obesity intervention, suggesting that the viral context turns out to be more diverse after treatment. Moreover, we identified some viral biomarkers in each condition, which can be associated with the bacterial changes reported in the works where the sequences used come from.

To date, bioinformatics databases may have ignored a large proportion of unknown phages in the metagenomic data, limiting the amount of information obtained from these kinds of studies. In a recent article, the authors managed to assemble a Metagenomic Gut Virus catalog, which comprised 189,680 viral genomes by using 11,810 published human stool metagenomes. They found that over 75% of genomes represented dsDNA phages, and they also identified 54,118 candidate viral species, of which, 92% were not found on previous databases studied [20]. These findings suggest that there is a low representation

of known viruses in public databases, which could generate biases during metagenomic studies, such as the one carried out in this work. Moreover, there is a great diversity of RNA viruses that are out of focus of shotgun DNA sequencing, pointing out the need to use metatranscriptomic analysis to survey the presence of RNA viruses in the gut microbiome. Given the above, optimized methods to characterize RNA-based viruses are needed to piece together the whole picture of the gut virome. In this sense, a recent study aimed to characterize gut virome alterations in both an individual with obesity and type 2 diabetes (T2DM) and an individual without it [38]. The authors found that obese patients with T2DM in particular, had a decreased gut viral richness and diversity when they were compared with lean control subjects. Moreover, they revealed a trans-kingdom correlation between phages (viruses) and bacteria in lean controls, but this correlation was weak in the case of obese patients [38]. The cited study points out that the virome may play an important role in the development of obesity and T2DM. Hence, understanding virome dynamics could eventually help to design microbiome-based phage therapies aimed at treating metabolic disorders, such as obesity and T2DM.

In this work, we identified biomarkers for individuals with obesity and post-obesity treatment. Comparing this information with the viral taxa identified in this research, we noticed differences between each condition. *Escherichia* phage, *Geobacillus* phage, and *Lactobacillus* phage had the relatively largest abundance in the obese group. On the other hand, we identified three viruses in the obesity condition: *Uncultured phage crAssphage*, *Enterobacteria phage fiAA91_ss,* and *Enterobacteria phage P88*. In contrast, we identified a higher abundance of *Escherichia phage pro483* and *Escherichia phage pro147* in post-obese patients. This difference could be the result of the direct contribution of the gut microbiota post-surgery and must be related to the differences observed in the core virome of pre- and post-obesity patients reported in this paper.

Although the nature of all viruses and their role in the health or dysbiosis states of the intestinal microbiome are not currently known, the evidence indicates that their role may be fundamental for bacterial dynamics, pointing to the need to perform deep gut virome studies to elucidate their ecological role in this niche. Given the increase in viral genomes that have been included in the International Committee on Taxonomy of Viruses (ICTV) [39–41], we can predict that the gut virome will be a hot topic within a short time.

A weakness of this study was that only 49 samples were retrieved from public databases to perform the quick bioinformatic comparison of the viral content in both conditions, namely obesity and post-obesity treatment. In the literature, some works use shotgun metagenomics to study the gut microbiome in that context. However, few of these publish their raw data sequences or the metadata associated with the raw data sequences, which is needed to understand and re-analyze the metagenomic sequences. Another weakness was the quick description performed using a bioinformatics tool based on viral recognition by the stored information in public databases. Altogether, these limitations could be complemented by carrying out a meta-study with a high sample number and bioinformatics tools that recognize and assemble viral genomes without the need to use database recognition. Moreover, new advances in functional metagenomics will be needed besides the development of new bioinformatics tools that integrate all the metadata that microbiome studies collect. Finally, these observations suggest that, at present, it is not possible to understand the whole complex virome dynamics and their implication on the human gut microbiome, but it is a topic that needs a great deal of attention.

5. Conclusions

Obesity is characterized by low viral taxonomic composition and diversity indexes compared with post-obesity treatments, signifying that gut viral content may play an important role in the development and establishment of obesity and its recovery after the intervention. Further studies are needed to determine the cause-effect role of the gut virome in the dysbiosis registered in the individual with obesity and how the virome may

be involved in microbiome dynamics. A better understanding could eventually help to propose viral-based therapies aimed at treating microbiome disorders.

Supplementary Materials: The following materials are available online at https://www.mdpi.com/article/10.3390/app112110039/s1, Table S1: Metadata for the raw data of metagenomic files used in this study, Table S2: Alpha diversity metrics measured by Shannon index obtained from virome abundance data, Table S3: viral abundance obtained from FastViromeExplorer analysis.

Author Contributions: D.S.-V. and N.D.C.-R. performed bioinformatics analysis and wrote the manuscript. P.N. and M.C. contributed to the writing and discussion and critically edited the manuscript. D.A.M. directed the study, conceived the work, performed the comparative analyses, and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The experimental raw data used in this study are available on ENA-EMBL under the accession numbers PRJEB29060 [13], PRJEB8249 [12], and PRJEB12947 [11]. The processed data are available in the Supplementary Material.

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