Published in final edited form as:

Diabetes Obes Metab. 2016 May ; 18(5): 519–527. doi:10.1111/dom.12645.

Prevention of antibiotic-associated metabolic syndrome in mice by intestinal alkaline phosphatase

K. P. Economopoulos¹, N. L. Ward², C. D. Phillips^{3,4}, A. Teshager¹, P. Patel¹, M. M. Mohamed¹, S. Hakimian¹, S. B. Cox³, R. Ahmed¹, O. Moaven¹, K. Kaliannan¹, S. N. Alam¹, J. F. Haller⁵, A. M. Goldstein⁶, A. K. Bhan⁷, M. S. Malo¹, and R. A. Hodin^{*,1}

¹Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA

²Departments of Molecular Biology and Botany, University of Wyoming, WY 82071, USA

³Research and Testing Laboratory, Lubbock, TX 79407

⁴Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409

⁵Lipid Metabolism Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA

⁶Department of Pediatric Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA

⁷Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA

Abstract

Aims—Early childhood exposure to antibiotics has been implicated in the pathogenesis of metabolic syndrome (MetS) later on in adulthood. Intestinal alkaline phosphatase (IAP) preserves the normal homeostasis of intestinal microbiota and restores the normal microbiota upon cessation of antibiotic treatment. We aim to examine whether co-administration of IAP with antibiotics early in life may have a preventive role against MetS in mice.

Materials and Methods—Fifty mice were allocated to four treatment groups after weaning. Mice were treated with azithromycin±IAP, or with no azithromycin±IAP, for three intermittent 7-day cycles. After the last treatment course, the mice were administered regular chow diet for five weeks and subsequently high-fat diet for five weeks. Animal body weight, food intake, water intake, serum lipids, glucose levels and liver lipids were compared. 16S rRNA gene pyrosequencing was used to determine differences in microbiome composition.

Corresponding author: Richard A. Hodin, MD, Department of Surgery, Massachusetts General Hospital, 15 Parkman Street, Boston, MA 02114; Telephone: (617) 724-2570; Fax: (617) 724-2574; rhodin@mgh.harvard.edu.

Declaration of interests:

The authors declare no conflict of interest.

Contribution statement:

K.P.E., M.S.M., R.A.H. designed research; K.P.E., A.T., P.P., M. M. M., S.H., S.B.C., C.D.P., R.A., O.M., K.K., S.N.A., J.F.H., conducted research and collected data; K.P.E., N.L.W., S.B.C., C.D.P., A.M.G., A.K.B., M.S.M., R.A.H. analyzed data; and K.P.E., C.D.P., N.L.W., and R.A.H. wrote the paper. All authors approved the final version of the manuscript.

Results—Azithromycin exposure early in life rendered mice susceptible to MetS in adulthood. Co-administration of IAP with azithromycin completely prevented this susceptibility by decreasing total body weight, serum lipids, glucose levels and liver lipids to the levels of control mice. These effects of IAP likely occur due to changes in the composition of specific bacterial taxa at the genus and species levels (e.g. members of *Anaeroplasma* and *Parabacteroides*).

Conclusions—Co-administration of IAP with azithromycin early in life prevents mice from susceptibility to the later development of MetS. This effect is associated with alterations in the composition of the gut microbiota. IAP may represent a novel treatment against MetS in humans.

Keywords

intestinal alkaline phosphatase; metabolic syndrome; mice; azithromycin

INTRODUCTION

The intestinal microbiota is a critical factor in the development of obesity and metabolic syndrome (MetS). Colonization of non-obese adult germ-free mice with microbiota of conventionally raised obese mice results in transfer of MetS-related features from the donor to the recipient [1]. There are several proposed mechanisms for the role of the gut microbiota in MetS which include an increase in energy harvest, the production of toxic bacterial metabolites, and an increased intestinal permeability leading to elevated levels of lipopolysaccharides (LPS) in the systemic circulation and consequently a low-grade systemic inflammatory state [2].

Epidemiological studies have shown that disruption of the gut microbiota by the administration of antibiotics during childhood is associated with an increased susceptibility to obesity later in life [3, 4]. Interestingly, growth-promoting effects have not been observed with antifungal or antiviral agents, indicating that the activity of antimicrobials is mainly due to alterations in the populations of the gut bacteria [5]. Furthermore, administration of antibiotics alters not only the gut microbiota composition but also the response of the host to specific microbial signals [6].

Many different antibiotics have been associated with obesity in childhood [7–9]. Macrolides are the leading class of broad-spectrum antibiotics prescribed in children, with azithromycin (AZT) being the most represented drug in this family [10]. In double-blind randomized placebo-controlled studies an obesogenic impact was noted using AZT in children with cystic fibrosis, [11–13] making AZT an important target for further investigation.

We have previously demonstrated that the brush-border enzyme intestinal alkaline phosphatase (IAP) preserves the normal homeostasis of intestinal microbiota [14]. Specifically, IAP knockout mice harbor far fewer commensal bacteria than their wild-type (WT) littermates and WT mice receiving oral antibiotics with IAP supplementation more rapidly restore the normal microbiota upon termination of antibiotic treatment compared with an un-supplemented control group [14]. Based on these observations, we hypothesized that co-administration of IAP with antibiotics early in life may protect against the future development of obesity and MetS.

MATERIALS AND METHODS

Experimental design

Detailed methods of this study regarding animal diets, mice and protocol details, chemicals, glucose tolerance test, serum lipid profile, liver histopathology, hepatic steatosis scoring system, serum assays, 16S rRNA gene pyrosequencing and processing, and quantification of liver total lipids and triglycerides can be found in the Supplementary material. We established a novel mouse model mimicking childhood antibiotic exposure in humans (Fig. 1). 50 male wild-type C57BL/6 (WT) mice were allocated to four treatment groups immediately after weaning (25 days). Mice (n=10–14 per group) were treated with AZT (50 mg/kg/day) with or without oral IAP (100 units/ml drinking water), or with no antibiotic with or without oral IAP, for three intermittent 7-day cycles (Fig. 1; treatment period). Both AZT and IAP were mixed together with the drinking water (pH = 8.2), which was provided to the mice *ad libitum*. The pH of the AZT-treated water and vehicle was similar. Control mice received an equal volume of “vehicle for IAP” (50 mM KCl, 10 mM Tris-HCl [pH 8.2], 1 mM MgCl₂, 0.1 mM ZnCl₂, and 50% glycerol) in the drinking water. Between the treatment cycles, the mice received LFD and autoclaved tap water for 7 days (Fig. 1; delay period). After the last treatment course, the mice were administered LFD for five weeks and subsequently HFD for five weeks (Fig. 1; HFD period). Animal body weight, food intake and water intake were monitored weekly. During the administration of antibiotics and IAP, the water tubes were replaced daily. Stool samples from all mice were collected: i) at the first day of the experiment, ii) after the last treatment cycle, iii) before the administration of HFD and iv) before sacrifice, and stored at –80°C for further analyses.

To investigate MetS, a GTT was performed in mice fasted for 6 hours. Serum lipids, LPS, IL-1 β and TNF- α quantification was also performed prior to termination of the experiment. After collecting blood, mice were sacrificed and fat pads wet weights were measured. Liver was immediately harvested and stored at –80°C for further analyses.

Statistics

Data were expressed as mean $\pm$ standard error (SEM). Analysis of variance (ANOVA) was used to test for group differences in all examined parameters (e.g. food intake, body weight, serum lipids, alpha diversity etc.) and an alpha value of 0.05 was employed as the significance threshold. Statistical testing for multivariate differences among groups were evaluated using a permutational multivariate ANOVA using the UniFrac distance matrices [15]. Bacterial lineages classified at the generic and OTU levels were screened for statistically significant differences in abundances among groups using count data and generalized linear modeling with a negative binomial distribution [16]. A false discovery rate of 5% was controlled with Benjamini-Hochberg *p*-value correction. All statistical analyses were conducted in SPSS (version 21 for Mac OS; SPSS, Inc., Chicago, IL) and R Software [17] using the vegan [15], lsdbsv [18], and DESeq2 [16] packages.

RESULTS

Oral administration of AZT in young mice makes them susceptible to MetS in adulthood

Given that AZT is the most commonly prescribed broad-spectrum antibiotic and has been associated with obesity in children [7–9], we postulated that administration of AZT soon after weaning would make WT mice more susceptible to high fat diet (HFD)-induced obesity as adults. Mice were treated for three intermittent 7-day cycles $\pm$ AZT $\pm$ IAP, followed by a 5-week period on a low fat diet (LFD) and then exposed to a HFD for 5 weeks (Fig. 1). The antibiotic- and non-antibiotic-treated mice had similar food and water intake (Fig. 2A and 2B). No difference in body weight between the experimental groups was evident during the low-fat diet delay period (Fig. 2C to E). Body weight was higher in mice receiving AZT + vehicle (AZT+V) compared with mice not receiving antibiotic (vehicle only group) during the HFD period (Fig. 2C to E). The AZT+V group also showed an overall increase in body fat compared to control, including both visceral and subcutaneous components (Fig. 2F and 2G).

We next investigated whether mice treated with AZT early on also displayed features of MetS after being on the HFD for 5 weeks. Serum total-, LDL- and non-HDL-cholesterol were not significantly increased in the AZT+V group compared to mice in the control group (V) (Fig. 3A to C). However, fasting blood glucose levels were significantly higher in the AZT+V group compared to mice in the V group after 5 weeks of HFD (Fig. 3D). Furthermore, the mice in the AZT+IAP group exhibited better glucose tolerance than mice in the AZT+V group (Fig. 3E and 3F).

Co-administration of IAP with AZT in young mice prevents HFD-induced MetS

We next assessed whether co-administration of IAP with AZT in young mice could prevent HFD-induced MetS. As expected, we observed higher body weight in mice treated with AZT alone (AZT+V group) compared to the mice treated with AZT and IAP (AZT+IAP group) (Fig. 2C to E). Serum total cholesterol (Fig. 3A), LDL cholesterol (Fig. 3B), and non-HDL cholesterol levels (Fig. 3C) were markedly increased in the AZT+V group compared to the AZT+IAP group. Fasting blood glucose levels were higher in AZT+V compared to AZT+IAP mice (Fig. 3D). The mice receiving IAP with AZT exhibited better glucose tolerance than mice receiving AZT alone, and this was mainly evident at the baseline levels and at the 90' time point of the glucose tolerance test (GTT) (Fig. 3E to F).

Co-administration of IAP with AZT prevents susceptibility to HFD-induced liver steatosis

We next examined the efficacy of co-administration of IAP with AZT in preventing HFD-induced liver steatosis. Histological analysis showed that mice treated with antibiotics had accumulated considerably higher amounts of hepatic fat, and these changes were not seen in the non-antibiotic-treated animals or in the animals that received IAP with the antibiotic (Fig. 4A to 4E). Mice treated with AZT+IAP had significantly lower levels of liver triglycerides (Fig. 4F) and liver total lipids (Fig. 4G) than those treated with AZT alone.

Antibiotic-related changes in microbiota composition

We initially assessed adequacy of sequencing effort to characterize individual microbiomes. Rarefaction plots of observed OTUs showed trends toward asymptotic approach for each sample (Supplementary Fig. 1A). In order to further gauge how sequencing effort provided accurate characterization of communities, we next statistically assessed the difference in the observed number of OTUs, and those estimated by the Chao1 index (which uses the frequency of singletons and doubletons to estimate true richness). Although the distribution of Chao1 values was larger than observed OTUs (Chao1 richness can never be smaller than observed OTUs), the distributions of these values were not significantly different from each other (Supplementary Fig. 1B) which indicated that sampling effort provide a good characterization of community membership and structure.

Given the impact of the AZT and IAP on the obesity/MetS phenotype, we performed a detailed analysis of the gut microbiota in each group. Richness, here defined by the Chao1 estimator of the total number of bacterial OTUs for each microbiome, of the AZT+V, AZT +IAP and V groups were similar (Fig. 5A). A similar relationship was observed for the Shannon diversity index, a measure of OTU diversity, which accounts for richness and evenness. In this model, there was no difference between the AZT-treated and control groups and between the AZT+IAP and the AZT+V group (Fig. 5B).

Multivariate analysis of phylogenetic distances of microbial communities (UniFrac distances) among groups using ADONIS showed significant differences between AZT-treated and V groups ($p=0.001$ unweighted; $p=0.001$ weighted). *Post hoc* pairwise ADONIS of AZT + V and AZT + IAP groups also confirmed significant compositional differences between these groups ($p=0.011$ unweighted; $p=0.012$ weighted). Principle coordinate analysis (PCoA) of unweighted and weighted UniFrac distances (Fig. 5C and 5D) were used to further interpret the relationships between treatment groups. PCoA of both unweighted and weighted UniFrac distances (Fig. 5C, Fig. 5D) showed segregation of the two AZT-treated groups from the control groups, and also segregation of the AZT+V from AZT+IAP groups.

We observed visually striking differences between AZT-treated mice (AZT+V and AZT +IAP groups) and the untreated control group (V) in the relative abundance of the 19 most dominant bacterial lineages classified at the genus or higher level (i.e. only confident genus-level OTU taxonomy is reported; Supplementary Fig. 2). In particular, AZT-treated mice appeared to lack the dominant *Bacteroides*, as well as the unclassified *S24.7* populations observed in the V mice, with a relative expansion in the number of sequences assigned to unclassified *Rikenellaceae*. Statistical testing supported differences between V and V+AZT groups in the abundance of these three genus-level groups, and also revealed a higher abundance of *Allobaculum* and unclassified *F16* in AZT+V relative to V (Supplementary Fig S3A). The AZT+V group was also depleted in a number of bacterial lineages including *Mucispirillum*, *Odoribacter*, *Prevotella*, *Sutterella*, *Coprococcus*, *Ruminococcus*, unclassified Bacteroidales, and unclassified YS2, relative to the V group.

Compositional differences between the AZT+V and AZT+IAP group

While it was expected that antibiotic treatment would change the gut microbiome (Supplementary Fig. 3A), we also observed striking differences with the co-administration of IAP. We observed significant differences in abundance of the genera *Parabacteroides* (depleted in AZT+IAP relative to AZT+V) and *Anaeroplasma* (displaying the opposite trend) (Supplementary Fig. 3B).

Examination of significant compositional differences between AZT+V and AZT+IAP at the Operational Taxonomic Unit (OTU) level revealed that the vast majority of discriminating OTUs belonged to the phylum Firmicutes (Supplementary Fig. 4A). The majority of these could not be classified to the genus level, but those Firmicutes were assigned to *Oscillospira*, *Ruminococcus*, and *Coprococcus*. In phyla other than Firmicutes, significant differences were observed for *Anaeroplasma* (Tenericutes) and *Parabacteroides* (Bacteroidetes). The value of characterization of differences at the OTU level, however, was underscored by the fact that comparison of statistical trends at the generic (Supplementary Fig. 3B) relative to the OTU level (Supplementary Fig. 4A) revealed a subset of genera that significantly differ, presumably because the many significantly different OTUs (identified in Supplementary Fig. 4A) did not contribute a large quantitative component to community composition for their respective genera.

To further explore the composition-related similarities and differences among groups, the relative abundance of the 20 most dominant genera was visualized by heat-map analysis (Supplementary Fig. S4B). The hierarchical clustering on the left side of the figure shows clear separation: (1) between the antibiotic-treated mice compared to the controls, and (2) between the antibiotic-treated mice that received IAP compared to those that did not. These results indicate that the microbiome of mice receiving the vehicle for IAP was, on average, more similar to that from mice receiving absolutely no treatment than to the microbiomes of AZT-treated mice.

DISCUSSION

The discovery of antibiotics in the early 20th century revolutionized medicine, industry, and farming, and their widespread use has clearly led to substantial health benefits for mankind. However, there is an increasing concern about overuse, as current data indicate that each child in the United States receives on average one course of antibiotics per year [19, 20]. This early exposure to antibiotics may have long-term consequences, mainly through alterations in the population and composition of the gut microbiota and alterations in the host immune system [21]. Specifically, selective disappearance of components of the microbiota has been associated with a variety of human diseases, including esophageal disease, inflammatory bowel disease, asthma, obesity, and MetS [22]. Obesity and MetS are among the most important global health problems in developed countries and it appears that the intestinal microbiota play a central role in their pathogenesis. Indeed, alterations in the infant human gut microbiome by antibiotics were recently proposed to have long-term consequences, predisposing adults to the development of obesity and MetS [23].

We have previously demonstrated that the brush-border enzyme intestinal alkaline phosphatase (IAP) preserves the normal homeostasis of intestinal microbiota [14]. IAP-KO mice display an overall decrease in the number of intestinal bacteria [14] and WT mice receiving oral IAP supplementation along with an antibiotic were able to rapidly restore the normal commensal microbiota, providing protection from subsequent infection with the pathogens *C. difficile* and *Salmonella* [14]. The bacteria-promoting effects of IAP may be due to its ability to dephosphorylate luminal ATP and other nucleotide triphosphates [24].

A critical role for IAP at the interface between the host and the microbiome is supported by studies in zebrafish by Bates et al., [25] who found that IAP expression was induced only when the fish were exposed to bacteria whereas the enzyme was absent under germ-free conditions. Jang et al. showed that endogenous IAP levels in rats decrease with age, indicating a possible role for 'loss of IAP' as a precipitating cause of MetS that is known to be more common in the elderly [26]. Based on the above observations, we hypothesized that co-administration of IAP with antibiotics early in life may have a preventive role against the future development of obesity and MetS.

In the present study, we established a novel mouse model to mimic childhood antibiotic exposure. We decided to focus on AZT for two reasons: 1) there is ample evidence from randomized trials suggesting an obesogenic effect of AZT in children with cystic fibrosis [11–13], and 2) AZT is the most commonly prescribed broad-spectrum antibiotic in children [10]. We were able to demonstrate that administration of AZT early in life renders mice more susceptible to obesity and MetS in adulthood. Furthermore, co-administration of oral IAP with AZT protected against the obesity and MetS sequelae.

To further delineate the mechanisms of beneficial effects of co-administration of IAP with AZT, we examined the composition of the gut microbiota. We observed no statistically supported differences in species richness or evenness between AZT-treated and untreated groups, or between AZT+IAP and AZT+V groups. This is not unexpected given the general nature of these ecological summary statistics. However, multiple analyses suggested treatment-based compositional differences. Analysis of Unifrac distances identified statistically supported differences between AZT-treated and untreated groups, as well as between AZT + V and AZT + IAP groups. PCoA-based exploration of unweighted and weighted Unifrac distances supported these compositional differences. Overall, these results suggest that co-administration of IAP with AZT influences the impact of the antibiotic on both bacterial presence and abundance.

The strongest signal for treatment-based differences was observed in the taxonomic analysis of relative abundance of specific genera. We will focus here on the differences associated with IAP supplementation, given the substantial evidence for an effect of this treatment on susceptibility to MetS [27], and the fact that the effects of antibiotic treatment alone on microbiome composition are well documented in other studies [21, 28, 29]. There were significant differences in the relative abundance of two genera between AZT-treated mice and mice that received the combination of AZT and IAP. Specifically, co-administration of IAP with AZT was associated with significant increases in the proportional representation of *Anaeroplasm*, while *Parabacteroides* were almost absent from the feces of IAP-treated

mice. These differences in genus-level abundances, together with more subtle differences in the abundance of other genera, contributed to separate clustering of the two groups in heat-map analysis. The abundance of species-level OTUs within *Parabacteroides* and *Anaeroplasma*, as well as *Oscillospira*, *Ruminococcus*, *Lactobacillus*, and *Helicobacter* differed between IAP-treated and untreated mice.

While some of these genera are intensively studied, others such as *Anaeroplasma* have very scant literature, making it difficult to draw useful conclusions. The abundance of *Parabacteroides* has been associated with HFD-induced obesity [30], as well as with other inflammatory disorders such as nonalcoholic steatohepatitis [31] and colonic tumors [32]. Conversely, *Parabacteroides* species have been found to be decreased in inflamed tissue or associated with remission in the context of inflammatory bowel diseases and irritable bowel syndrome [33, 34], suggesting that only certain *Parabacteroides* species/OTUs may be associated with reduced MetS risk in our study. Additionally, whole-genome phylogenetic analysis has shown that there is very little difference between the *Parabacteroides* and *Bacteroides* genera [35], which both belong to the phylum Bacteroidetes [36]. Studies in humans and mice have linked Bacteroidetes with weight loss [37, 38]. It is known that high fat intake causes more bile acids to be secreted [39] and that *Parabacteroides* is bile-resistant [40]. As such, the increased numbers of *Parabacteroides* in the AZT-treated animals compared with the mice receiving IAP with AZT may be partially explain the susceptibility to HFD-induced obesity.

There is very little literature on the genus *Anaeroplasma*, a relative of *Mycoplasma*, but it is interesting to note that obese mice had significantly less *Anaeroplasma* compared with lean mice [41]. We also found that two *Ruminococcus* OTUs were significantly increased in the mice treated with AZT and IAP compared with the mice treated with AZT alone. Conversely, two different *Ruminococcus* OTUs were also enriched in AZT alone compared to AZT+IAP. It was recently shown that formula-fed infants are more obese than their breast-fed counterparts and have a different gut microbiome that mainly includes higher levels of bacteria from the *Ruminococcus* genus and lower levels of bacteria from the *Lactobacillus* genus [42]. To conclude, there are some associations in the literature between diseases and conditions relevant to MetS and some of the genera we find to be enriched or depleted in IAP-treated microbiomes relative to untreated controls. However, reports of conflicting associations between these genera and various disease states suggest that comparison at the genus level may not be meaningful.

Because little is known about how this model of early life antibiotics works more evidence is needed to understand its limitations. Even though the obesogenic effects of azithromycin were evident in this study, we were not able to observe the same with other antibiotics [i.e. amoxicillin or a four antibiotic-regimen consisting of ampicillin (A), vancomycin (V), neomycin (N), and metronidazole (M); data not shown]. These results are in agreement with recently published studies in mice showing that pre-weaning amoxicillin treatment had no significant impact on either growth or body composition at adulthood even though transient changes in the composition of the fecal microbiota were initially observed [43]. It should be also noted that WT C57BL/6 mice repeatedly refrain from drinking *ad libitum* water mixed with the AVNM concoction at our facilities, as has been observed by other investigators

[44]. Further studies will be needed to determine whether our findings with AZT will also occur in the context of other antibiotic regimens. In addition, the use of intraperitoneal glucose tolerance test is a limitation of our study as the oral glucose tolerance test would be presumably a more optimum method to assess the role of the gut in glucose tolerance. Furthermore, given the results of the weight-gain assays we focused on the ability of IAP to mitigate AZT effects on microbiome composition rather than effects of IAP on the microbiome alone. Future work incorporating microbiome data from “IAP only” treatment group would be useful for further characterizing interaction effects between AZT and IAP. Finally, our study does not differentiate the direct effects of AZT from the microbiota-mediated changes on hepatic physiology.

It is well known that IAP exerts anti-inflammatory properties by directly inhibiting the pro-inflammatory effects of a variety of bacterially-derived mediators, such as uridine diphosphate, flagellin, CpG DNA, and LPS [45, 46]. As such, the beneficial effects of the co-administration of IAP with AZT could be related to these direct anti-inflammatory effects of IAP. However, we found that serum levels of TNF- α , IL-1 β and LPS were similar among the treatment groups during the HFD period (data not shown), so it is not clear whether the direct anti-inflammatory effects of IAP are an important factor in the context of preventing antibiotic-induced obesity/MetS. Another potential mechanism of the observed beneficial effects of IAP could be through the improvement of gut barrier function by regulating the expression of junctional proteins [46]. However, in our study intestinal permeability was similar among the treatment groups during the HFD period (data not shown). Further studies will be needed to determine whether gut barrier function is an important factor in antibiotic-induced obesity/MetS.

In summary, we have demonstrated that co-administration of IAP with AZT early in life prevents mice from susceptibility to the later development of HFD-induced obesity and MetS, and that this effect is associated with alterations in the composition of the gut microbiota. Although caution is needed in extrapolating findings in mouse models of inflammation to human diseases [47], our findings suggest that orally administered IAP could represent a novel preventive strategy against antibiotic-related obesity and MetS in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by grant from The Ellison Medical Foundation.

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Figure 1.

Schematic diagram summarizing the timeline of experimental procedures in this study. Fifty male wild-type C57BL/6 mice (IAP group: 12 mice, Vehicle group: 14 mice, AZT+IAP: 10 mice and AZT+Vehicle: 14 mice) were used. FC:Fecal collections, Seq:Microbiome sequencing, IAP:Intestinal Alkaline Phosphatase, LFD:Low-fat diet, HFD:High-fat diet.

Figure 2.

Food intake (A), water intake (B), body (C–E), visceral (F) and subcutaneous (G) fat weights among the experimental treatment groups (n=10–14 per experimental group; see legend of Figure 1). Panel C depicts the average body weight gain of the mice in each group during the HFD period (11–15 weeks). Body (D), visceral (F) and subcutaneous (G) fat weights were measured at the end of the study (at 15 weeks). IAP: Intestinal Alkaline Phosphatase, HFD: High-fat diet, Az: Azithromycin, Veh: Vehicle. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure 3.

Serum lipid profile (A–C) and serum glucose (D–F) among the experimental treatment groups (n=10–14 per experimental group; see legend of Figure 1) at the end of the study (at 15 weeks). IAP: Intestinal Alkaline Phosphatase, LDL: low density lipoprotein, HDL: high density lipoprotein, GTT: glucose tolerance test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure 4.

Histological specimens of liver tissue stained with oil-red-O in the IAP (A), Vehicle (B), Azithromycin+IAP (C) and Azithromycin+Vehicle (D) groups (n=10–14 per experimental group; see legend of Figure 1). Liver histopathologic scores (E), triglycerides (F) and total lipids (G) among the experimental treatment groups. Hepatic steatosis was graded based on the number and size of stained fat droplets: 0(none/minimal); 1+(mild); 2+(moderate); 3+(moderate/marked); 4+(marked). IAP: intestinal alkaline phosphatase.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure 5.

(A) and (B) Species richness (as estimated using the Chao1 estimator) (A) and Shannon diversity (B) within the microbiome data for each group (n= 5 mice per group). Operational taxonomic units (OTUs) are defined at 97% 16S rRNA gene sequence similarity. Mean values for each group are indicated by solid lines, and shading indicates standard error around estimates of the mean. Confidence intervals are shown for Chao1 estimates from individual mice (A). (C) and (D) Principle coordinates analysis of unweighted (C) and weighted (D) UniFrac distances for sequences obtained from treatment groups. Percentage values on axes indicate the amount of variability in the data explained by each of the first two principal coordinates. Contributions of individual taxa to the segregation of samples are indicated by labeled vectors. IAP: Intestinal Alkaline Phosphatase, AZT: Azithromycin.