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Rapid metformin effects on multiple intestinal functions in high-fat high-sucrose fed mice

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SUMMARY

Although the mechanism of action of the antidiabetic drug metformin is still a matter of discussions, it is well accepted that the gut plays an important role. To gain more insights into the mechanisms that occur in the different regions of the intestinal tract in response to metformin treatment, adult male mice were fed a high-fat-high sucrose (HFS) diet for 8 days. Metformin treatment by gavage (300 mg/day/kg body weight) during the HFS diet improved glucose tolerance. Metformin counteracted HFS diet-induced overexpression of a network of genes involved in the transport of glucose and fatty acids in the small intestine, and induced beneficial modification of secondary bile acid profile in the caecum, with reduction in DCA and LCA levels and increased abundance of UDCA and TUDCA, potentially leading to FXR inhibition. In parallel, metformin treatment was associated with specific changes in the microbiota composition in the lumen of the different regions of the gut, counteracting the effects of HFS diet on the abundances of some bacterial groups generally associated with metabolic disturbances (f-Lachnospiraceae, f-Petostreptococcaceae, g-*Clostridium*). In addition, metformin promotes a strong increase in the abundance of *Akkermansia muciniphila* in the intestinal lumen all along the gut. Therefore, the present work clearly emphasises the role of all the regions of the intestinal tract in the beneficial action of the antidiabetic drug metformin in a prediabetic mouse model.

Keywords

Type 2 diabetes, metformin, gut microbiota, bile acids

INTRODUCTION

The oral antidiabetic drug metformin (1,1-dimethylbiguanide hydrochloride) is the first line therapy of type 2 diabetes mellitus (T2DM). Despite its use for more than 60 years, the mechanisms contributing to its effects on blood glucose levels are still the subject of debates ^{1,2}. Clinical studies have demonstrated that metformin acts primarily by the reduction of exaggerated endogenous glucose production in diabetic patients, especially through a decrease of hepatic gluconeogenesis rate ³. Animal studies and *in vitro* experiments in hepatocytes revealed an inhibition of complex I of the mitochondrial electron transfer chain and activation of AMP-activated protein kinase (AMPK), leading to the repression of gluconeogenic gene expression ^{1,2}. Additional mechanisms, independent from AMPK, were also proposed, such as changes in cell redox state or inhibition of mitochondrial glycerol-3-phosphate dehydrogenase ^{1,2}. Several works have suggested that metformin may act primarily by targeting intestinal tract as it can accumulate and reach high concentrations in the small intestine ⁴. Furthermore, administration of metformin *per os* appeared to reduce blood glucose more efficiently than the intravenous route ⁵, and a gut-restricted formulation was shown to efficiently decrease glucose levels in T2DM patients ⁶. It was also shown that metformin is able to inhibit intestinal glucose absorption in the proximal small intestine ⁷, and to increase glucose uptake and utilization by the enterocytes ⁸. Additional modifications of intestinal functions have been reported, including increased production of the incretin hormone, glucagon-like peptide 1 (GLP1) ⁹, stimulation of Goblet cells specialized in mucin production ¹⁰ and bile acid pool modifications ¹¹. These effects can be related to a direct action of the drug on intestinal cells or to consequences of a modification of the gut microbiota. Indeed, it is currently well accepted that metformin is able to alter the overall structure and the functions of the gut microbiota ^{12,13} and it was shown that metformin can stimulate directly the growth of specific bacterial species, notably *Akkermansia muciniphila* ^{13,14}. However, the link between the modulation of microbiota and the metabolic actions of metformin is not yet fully established ¹⁵.

Therefore, there are still some uncertainties regarding the mechanisms of action of metformin in the intestinal tract, and it is also difficult to evaluate if the observed changes are direct effects of the drug or consequences of modifications of other parameters, including the overall metabolic status of the host upon treatment. In the present study, we investigated the changes in a number of biological parameters in

different regions of the intestinal tract after a short-term metformin treatment in high-fat high-sucrose (HFS) fed mice. In addition to its effects on the expression of key intestinal genes in different segments (duodenum, jejunum, ileum, colon), we evaluated the impact of the drug on the luminal microbiota composition of each gut segment and we performed a bile acid profiling in the caecum of the animals. Mice were fed HFS diet for 8 days and were treated with metformin by daily gavage. We found rapid multi-level effects of metformin, leading in one week to a restoration of most of the perturbations induced by HFS diet on gene expression, associated with modifications of the bile acid profile and of the luminal microbiota composition all along the gut.

RESULTS

Short metformin treatment prevented metabolic disturbances in HFS fed mice.

HFS feeding for a period of 8 days induced metabolic disturbances in adult C57BL/6J male mice, as evidenced by a significant rise in fasting glucose levels and a deterioration of glucose tolerance during ipGTT (Fig. 1). There was also an increase in body weight upon HFS feeding (mean gain of 1.7 ± 0.2 g in HFS vs 0.2 ± 0.1 in SD group). The administration of metformin was able to counteract these alterations, as reflected by the maintenance of normal body weight gain (mean gain of 0.1 ± 0.1 g) and the preservation of glucose tolerance, although the effect of metformin on fasting glucose levels did not reach statistical significance (Fig. 1).

Short metformin treatment restored the expression of important genes in intestinal regions.

The different intestinal segments express specific patterns of genes allowing a spatial organization of a number of functions along the intestinal tract, such as nutrient digestion and absorption, chylomicron production, bile acid uptake, gut hormone secretion, or immune response and microbe defense. Therefore, we analyzed for each intestinal segment the expression of a specific set of genes after 8 days of HFS feeding with or without metformin. Regarding the genes coding for the apical sugar transporters (Fig. 2), namely the sodium-glucose cotransporter SGLT1 (encoded by *Slc5a1*) and the fructose transporter GLUT5 (*Slc2a5*), we found that HFS diet increased GLUT5 and SGLT1 expression in the duodenum, the main region of sugar absorption, while metformin

counteracted these effects and significantly reduced GLUT5 expression in all segments of the intestine. We also observed that metformin was able to reduce Glut2 expression in the duodenum (data not shown).

Jejunum and ileum are the favored sites for lipid absorption. As shown in Figure 3, HFS diet feeding significantly increased the gene expression of the main actors of apical fatty acid uptake (FAT/CD36 and FATP4), intracellular fatty acid transport (FA2PB2) and chylomicron synthesis (MTTP) in these two regions, while metformin restored their expression to basal levels (Fig. 3). In contrast, the ileal expression of the apical sodium-dependent bile acid transporter (ASBT, coded by *Slc10a2*) and of the organic solute transporter-alpha (OST α , coded by *SLC51B*, which exports bile acid across the enterocyte basolateral membrane), was not significantly affected by HFS diet or by metformin (Fig. 3). The mRNA level of the cholesterol transporter NPC1L1 was strongly decreased upon HFS diet in the jejunum and not modified by metformin treatment (Fig.3).

Results regarding gut hormones and peptides are shown in Figure 4. We analyzed the expression of the glucagon gene (*Gcg*), encoding GLP-1, in different regions of the gut. HFS diet was associated with an increase in *Gcg* expression in duodenum and jejunum and metformin treatment tended to restore its expression to basal level in duodenum and to slightly decrease it in the colon (Fig.4). In the other regions, we did not find significant effect of metformin on *Gcg* gene expression. In contrast, the mRNA levels of gastric inhibitory polypeptide (GIP) and cholecystokinin (CCK) in duodenum, of neuropeptide Y (NPY) in jejunum and of fibroblast growth factor 15 (FGF15) in ileum, which were all increased upon HFS feeding, were restored or down-regulated in the presence of metformin (Fig. 4). Finally, the expression of markers of inflammation (IL1 β and TNF α) in the colon was not significantly affected after 8 days of HFS diet or metformin treatment (Fig. 4).

Metformin modified the bile acid pool

Bile acid profiling was performed on the caecum at the end of the treatment periods. As shown in Figure 5, 8 days of HFS diet significantly increased total bile acid levels. Even though metformin did not modify total bile acid levels compared the HFS group, the treatment tended to increase primary over secondary bile acid ratio and decreased the hydrophobicity index (Fig. 5).

More specifically, bile acid analysis revealed an increase in cholic acid (CA) levels and a reduction in its main secondary metabolite deoxycholic acid (DCA) upon metformin treatment, whereas the amount of 7-sulfocholic acid was not affected (MET vs HFS) (Fig.5). The murine bile acids, beta-muricholic acid (β -MCA) and omega-muricholic acid (ω -MCA) as well as chenodeoxycholic acid (CDCA) were increased by HFS diet, but were not significantly affected by metformin compared to HFS (Fig. 5). Interestingly, the secondary metabolites of CDCA were significantly affected by metformin. We observed a significant increase in ursodeoxycholic acid (UDCA) and tauroursodeoxycholic acid (TUDCA) while lithocholic acid (LCA) levels were decreased by the treatment (Fig. 5). Of note, the relative abundance of conjugated bile acids (tauro-, or sulfo-conjugated) was not significantly affected by metformin (data not shown).

Metformin modified the luminal microbiota composition in all the regions of the intestine.

The number of operational taxonomic units (OTUs) measured in the luminal effluents in the duodenum, the jejunum and the ileum, was similar between groups, with a trend for a lower number of OTUs in the HFS diet group ($p = 0.049$ vs SD group), not restored by metformin treatment (Sup. Fig. S1). Despite the fact that the bacterial richness appeared globally not modified after 8 days of HFS diet or metformin treatment, we observed a significant impact of metformin at the phylum level in the different regions of the intestinal tract (Fig. 6). While animals fed the HFS diet displayed a slight increase in the proportion of Firmicutes in all segments, especially the duodenum, metformin treatment induced a spectacular increase in the abundance of Verrucomicrobia all along the intestinal tract (Fig. 6). Analysis performed at the genus level revealed that 60% of the sequenced bacteria belonging to this phylum corresponded to *Akkermansia muciniphila*, as shown in Figure 7. Furthermore, additional analyses indicated that HFS diet and metformin treatment were associated with important modifications at the family and genus levels (Fig. 8 and Sup. Fig. S2). We observed an increase abundance of *Clostridium* (belonging to the Clostridiaceae family) in all the region of small intestine upon HFS feeding, which was significantly prevented by metformin (Sup. Fig. S2). This effect of metformin treatment on *Clostridium* was also found in the colon, despite lower abundance of these bacteria in this region (Sup. Fig. S2). Bacteria belonging to the Lachnospiraceae family showed similar trend as *Clostridium*, especially the genus *Dorea*, which was

increased by HFS diet and restored by metformin in all intestinal segments (Sup. Fig S2). Furthermore, in the lumen of the 3 sections of the small intestine, bacteria belonging to the Peptostreptococcaceae family also showed similar trend to *Clostridium*, with metformin reducing their abundance (Sup. Fig. S3).

In contrast, metformin treatment tended to increase *Adlercreutzia* abundance in the jejunum (Sup. Fig. S3). We also observed a positive effect of metformin on *Propionibacterium* (from the Actinobacteria phylum) in the duodenum (Sup. Fig. S3). Finally, there was a marked reduction of the Muribaculaceae family (S24-7) in response to HFS diet in the colon and metformin tended to further decrease the abundance of these bacteria (Sup. Fig. S3).

DISCUSSION

The mechanism of action of the antidiabetic drug metformin is still a matter of discussions although it is now widely accepted that the gut plays an important role¹. Despite major advances, there are still uncertainties regarding the effects of metformin in the intestinal tract, with one major difficulty consisting in evaluating which of the observed changes are direct effects of the drug. Several reports have evidenced modifications of intestinal functions within hours after metformin administration, such as on glucose transport^{16,17} or after only few days, like on gut bile acid profile or on faecal microbiota¹⁸. In the present study, we aimed at gaining more insights into the mechanisms that occur in the different regions of the intestinal tract in response to 8 days of metformin treatment in a mouse model of high-fat high-sucrose (HFS) diet.

Adult male mice fed the HFS diet for 8 days displayed metabolic alterations, as evidenced by mild fasting hyperglycemia and reduced glucose tolerance. Metformin treatment was able to protect against these defects at the whole-body level. Dissecting the molecular changes in the different regions of the gut, we found that metformin counteracted HFS diet-induced overexpression of a network of genes involved in the transport of glucose and fatty acids in the small intestine. The effects of metformin on intestinal glucose transporters showed contradictable results in the literature, although the general consensus is to consider that glucose transport from the intestinal lumen to the circulation is inhibited by metformin, together with an increased uptake and metabolism of glucose by enterocytes¹. Here we observed that mice fed the HFS diet during 8 days

were characterized by increased SGLT1 and GLUT5 mRNA levels in the duodenum, and that metformin treatment abolished these inductions, while it also reduced GLUT5 expression in the ileum and in the colon. These data pointed therefore towards a marked inhibition of sugar, especially fructose, transporter expression after 8 days of metformin treatment in this mouse model, in agreement with a recent report showing reduced intestinal glucose absorption upon metformin ¹⁷.

The impact of metformin on intestinal lipid transport is less documented. We observed marked regulation of the expression of genes for fatty acid transporters (CD36, FATP4, FABP2,) and chylomicron synthesis in both ileum and jejunum, the preferential regions of lipid absorption. Metformin counteracted the overexpression induced by HFS diet or downregulated these genes. Inhibition of MTP expression by metformin, associated to reduced chylomicron production, has been already observed in the *Psammomys obesus* sand rat model ¹⁹. These data supported therefore an inhibition by metformin of HFS diet-induced lipid absorption, which may contribute, together with the reduction of sugar transporter expression and absorption ¹⁷, to the improvement of metabolic homeostasis in treated animals.

Metformin treatment for 8 days also down-regulated the expression of GIP and CCK in the duodenum and of FGF15 in the ileum when compared to the HFS diet group, suggesting that modulation of gut hormones could also contribute to its beneficial action. Fibroblast growth factor 15 (rodent ortholog of the human FGF19) has recently emerged as an important endocrine hormone involved in bile acids, lipid and glucose metabolism ²⁰. In agreement with our gene expression data, metformin was found to reduced serum FGF15 levels in a model of diabetic rats ²¹, and decreased FGF19 levels was recently reported after 3 days of treatment in T2DM patients ¹⁸. Regarding the incretin GIP or the satiety hormone CCK, metformin did not seem to affect their circulating levels in clinical studies, and thus it was difficult to infer a potential role to the changes observed in the present study. In contrast to these hormones, GLP-1 is regarded as one of the main mediators of metformin beneficial action on glucose homeostasis and many studies have reported increased glucagon gene expression in intestinal L-cells as well as higher circulating levels of GLP-1 upon metformin treatment ^{1,9,22}. Rather unexpectedly, we did not observe major modifications of glucagon gene expression in the different regions of the gut, suggesting that the regulation of GLP-1 expression is potentially not a short-term or a primary event in the mechanism of action of metformin in the HFS fed mouse model.

The observed changes in gene expression along the intestinal tract may result from a direct action of metformin via stimulation of the AMPK signalling pathway. However, except for one report suggesting a possible downregulation of SGLT1 in response to AMPK activation²³, a literature survey did not indicate a direct role of AMPK in the regulation of the studied genes and this remains thus to be investigated. The majority of the studies favoured changes in the microbiota community or/and in bile acid pool to explain the modulation by metformin of metabolic functions in the intestinal epithelium^{1,11}.

Regarding microbiota analysis, we evaluated the impact of short-term metformin treatment on the luminal microbiota of each segment of the intestinal tract. The most striking and novel result was a strong induction by metformin of *Akkermansia muciniphila* abundance in all the sections of the gut. The effect of metformin on *A. muciniphila* has been already documented in faeces and stools both in rodent models and in humans^{10,12,14}. The novelty here was to show that this effect of metformin also takes place in all the section of the intestine, including the upper small intestine. *A. muciniphila* classically uses mucins as energy source and leads to the degradation and renewal of the mucus layer, thus maintaining the intestinal barrier function²⁴. However, we did not found modification of MUC2 expression in the different segments of the gut (Sup. Fig. S4), although this marker has been previously associated with mucus layer thickness in mice treated by metformin for 10 weeks¹⁴. This may suggest that other mechanisms could link *A. muciniphila* induction and metformin action. Interestingly, the beneficial metabolic effect of *A. muciniphila*, has been very recently associated with the production in the gut of mono-palmitoyl-glycerol species, members of the endocannabinoid family²⁵. The regulation of these metabolites in the presence of metformin remains to be investigated.

In addition to *A. muciniphila*, we found that metformin reversed the induction by HFS diet of bacteria from the Lachnospiraceae family, including *Dorea*, and from the Clostridiaceae family, especially the genus *Clostridium*, in almost all the sections of the intestine, as well as from the Peptostreptococcaceae family in the small intestine. Reduction in Lachnospiraceae abundance in response to metformin has been already observed in rodent studies²⁶. Similarly, several members of the *Clostridium* genus have been found down-regulated in metformin-treated T2DM patients¹³. Members of these families are generally associated with compromised health conditions, like the Lachnospiraceae that are increased in different diseases, including obesity and diabetes^{27,28}. Our data may

suggest a contribution of members of these families in HFS diet-induced metabolic alterations and in the beneficial effects of metformin.

Metformin treatment seemed also associated with an increase in the abundance of *Adlercreutzia* in the jejunum, with a tendency in the other parts of small intestine, and of *Propionibacterium* in the duodenum. Increase abundance of *Adlercreutzia* has been previously observed in some T2DM patients treated with metformin ²⁹. Interestingly, species of *Adlercreutzia* have been shown to metabolize isoflavonoids to equol that possesses strong antioxidant properties and can favourably affect various metabolic functions ³⁰. Regarding the *Propionibacterium*, several members possess beneficial properties, especially the ability to produce propionate and vitamin B12 ³¹. Increased fecal propionate concentration have been evidenced in T2DM patients treated with metformin ¹³.

Beside *A. muciniphila*, additional specific bacteria were recently proposed as potential mediator of metformin action, but not found in the present study, such as *Lactobacillus* members, that are increased after 1 day of metformin treatment in rat duodenum ¹⁶, and *Bacteroides fragilis* which is decreased after 3 days of metformin in the stools of T2DM patients and that may regulate FXR signalling through the production of the bile acid GUDCA ¹⁸.

The involvement of bile acids in the mechanism of action of metformin has recently emerged ^{11,18,22}. However, the effects of metformin on bile acid pool composition in humans are still rather controversial, with one study showing modifications after 3 days of treatment ¹⁸ while another did not find significant difference after 4 months ¹³. Less is known in animal models and we found here that metformin did not significantly modify total bile acid levels in the caecum of the HFS diet fed mice, but altered their composition. The most striking effect of metformin was a marked decrease in the levels of secondary bile acids DCA and LCA whereas UDCA and TUDCA were increased. DCA and LCA can be produced by gut bacterial 7 α -dehydroxylation (7 α DeOH) of the primary bile acids CA and CDCA. The predominant intestinal species exhibiting 7 α DeOH activity belong to the genus *Clostridium* ^{32,33}. The fact that metformin significantly decreased their abundance in all the sections of the gut, may potentially support the reduction in DCA and LCA levels.

Ursodeoxycholic acid (UDCA) and its taurine conjugated TUDCA are of clinical interest due to their multiple beneficial effects on human health. CDCA can be converted to UDCA in the colon through epimerization by microbial 7 α - and 7 β -hydroxysteroid

dehydrogenases. The bacteria bearing $7\alpha/\beta$ -hydroxysteroid dehydrogenase activities are less characterized, but members of the genera *Clostridium* possess these activities ³⁴. UDCA is then transported from the intestine to the liver through the enterohepatic circulation where it is conjugated with taurine or glycine to produce TUDCA or GUDCA, which are transported back into the gut. We found increased levels of UDCA and TUDCA in the caecum of metformin treated mice, while GUDCA was not detectable (in agreement with the fact that there are very low levels of glycoconjugated bile acids in rodent). Our data in mice agreed therefore with a recent study showing that TUDCA and GUDCA are the most induced bile acids in the stool of T2DM patients treated for 3 days with metformin ¹⁸. These authors also showed that UDCA, TUDCA and GUDCA are inhibitors of the nuclear receptor FXR ¹⁸. Interestingly, DCA and LCA, which were increased in the present study, are also potential inhibitors of FXR ³⁵. Another study demonstrated that metformin could also directly inhibit FXR activity via AMPK activation ³⁶. Supporting an inhibition of FXR activity in metformin treated mice, we found a significant reduction in the expression level of FGF15, a major target gene of FXR in the ileum. Therefore, the contribution of FXR signalling in the mechanism of action of metformin, directly or via bile acid modifications in the gut appeared, to be an important mechanism to take in to consideration.

Altogether the presented data demonstrate that metformin administrated during 8 days is able to counteract important perturbations induced by HFS diet in the different regions of the intestinal tract, including altered expression of key genes of nutrient absorption and increased abundances of some gut bacteria, which have been associated with metabolic disturbances (*f-Lachnospiraceae*, *f-Petostreptococcaceae*, *g-Clostridium*). In addition, metformin promotes a strong increase in the abundance of *A. muciniphila* all along the gut, and beneficial modification of secondary bile acid profile in the caecum, with reduction in DCA and LCA levels and increased abundance of UDCA and TUDCA, potentially leading to FXR inhibition. It will be now important to determine how these different events are interconnected and triggered in a complementary way by metformin. A limitation of the present work is the fact that metformin administration was initiated together with the HFS diet, therefore interfering with the adaptation of the mice to the diet. The results might thus be different in a therapeutic approach with metformin treatment provided in animals with already established metabolic alterations. Although

such studies should be performed in the future, the present work clearly emphasises the role of all the regions of the intestinal tract in the beneficial action of the antidiabetic drug metformin in prediabetic mice.

METHODS

Animals, diet and metformin treatment.

Twelve-week-old C57BL/6J/Ola/Hsd male mice (Envigo, Gannat, France) were maintained in a temperature-controlled ($22\pm 2^{\circ}\text{C}$) facility room with a 12 hours light/dark cycle. Animals were allocated to 3 experimental groups ($n = 5/6$ animals per group) after one week of adaptation. The protocol was independently repeated 4 times. The control group (SD) was fed *ad libitum* with classical chow diet (R16, Genobios, Laval, France), the HFS and HFS-MET groups were fed with a high-fat high-sugar diet (260HF, SAFE, Augy, France) for a period of 8 days while treated daily by intragastric gavage of metformin (300 mg/day/kg body weight) (MET) or water as control (HFS). Housing and experimentations were carried out in compliance with the ARRIVE guidelines and according to the French and European guidelines of laboratory animal care (European Communities Council Directive of 1986, 86/609/EEC). The protocol was approved by local committee for animal care (Comité d'éthique en expérimentation animale de la Région Rhône-Alpes, CECCAP) and registered under the reference CECCAP LS-2017-002.

Body weight was monitored at day 0 and day 8. For glucose tolerance test (ipGTT), mice were fasted for 6 hours then received an intraperitoneal injection of glucose (2 g/kg body weight). Blood glucose was monitored at different time points during 90 minutes at the tip of the tail, using a glucometer (Accu-Check, Roche).

Intestinal tissue sampling.

Animals were killed by cervical dislocation after 6h of fasting and 5h after the last gavage with metformin. Different parts of the intestinal tract (duodenum, jejunum, ileum and the colon from medium part to anus) were sampled. The caecum was also removed and the intestinal segments were cleaned by flushing ice-cold PBS and dipped in liquid nitrogen.

Gene expression analyses in intestinal segments.

Total RNA was extracted with TRI Reagent Solution (Sigma). Target mRNA levels were measured by reverse transcription followed by real-time PCR using a Rotor-Gene (Qiagen). A standard curve was systematically generated with different amounts of purified target cDNA, and each assay was performed in duplicate and normalized using TATA-binding protein (TBP) mRNA level, as previously reported³⁷. The list of the target genes with the PCR primers used for the qPCR assays is in Supplementary Table S1.

Bile acid profiling in caecum.

Bile acid molecular species concentrations were measured by HPLC coupled to tandem mass spectrometry (HPLC-MS/MS) as previously described³⁸. Results were expressed in nmol/g of dried caecum.

Microbiota analysis.

Microbiota composition in the luminal content of the different intestinal sections was determined by 16S rRNA sequencing in 5 animals per group. At sacrifice, the different parts of the intestinal tract (duodenum, jejunum, ileum, colon) were carefully flushed 3 times with 5 ml of ice-cold PBS. The eluents were combined and centrifuged at 1800 rpm during 20 min at 4°C. The supernatants were discarded and the pellets suspended in 300 µl of RNA later (Sigma Aldrich) and stored at -80°C until use. Total DNA was extracted with the QIAamp® PowerFecal® DNA Kit (Qiagen). Library preparation and sequencing were outsourced to the Biomnigene company (<https://www.biomnigene.fr/en/>). A region of approximately 426 bp encompassing the V3 and V4 hypervariable regions of the 16S rDNA gene was targeted for sequencing. For the preparation of the libraries, “Illumina TruseqDNA Sample Preparation v2” was used. Sequencing was performed on Illumina Miseq2000. Quality check was performed by FASTQC and sequences were then trimmed, denoised and chimera filtered using the DADA2 plugin. Then, OTU annotation, taxonomic classification and analyses were performed using the Quantitative Insights Into Microbial Ecology (QIIME2 version 2019.4) platform³⁹, using the fit-classifier-naive-bayes module with the default parameters and the Greengenes database (gg_13_8_otus.tar.gz classifier for 16S rRNA). On the 60 luminal samples prepared (4 intestinal sections of 5 animals per group), 10 samples were removed from the analysis because of low reads numbers after sequencing (< 80,000) and/or aberrant results showing the presence of only one bacteria

family, not found in any other samples, reflecting thus possible amplification artifacts⁴⁰. On the remaining 50 samples, a total of 6 504,328 valid reads were obtained.

Statistical analyses.

Data are presented as mean \pm SEM. For the measured parameters, ordinary one-way ANOVA followed by Tukey's multiple comparison test was used. $p < 0.05$ was considered statistically significant.

Data availability.

The datasets generated and analyzed during this study are available from the corresponding author on reasonable request.

REFERENCES

1. Foretz, M., Guigas, B. & Viollet, B. Understanding the glucoregulatory mechanisms of metformin in type 2 diabetes mellitus. *Nat. Rev. Endocrinol.* **15**, 569–589 (2019).
2. Vial, G., Demaille, D. & Guigas, B. Role of Mitochondria in the Mechanism(s) of Action of Metformin. *Front. Endocrinol.* **10**, 294 (2019).
3. Hundal, R. S. *et al.* Mechanism by which metformin reduces glucose production in type 2 diabetes. *Diabetes* **49**, 2063–2069 (2000).
4. Wilcock, C. & Bailey, C. J. Accumulation of metformin by tissues of the normal and diabetic mouse. *Xenobiotica Fate Foreign Compd. Biol. Syst.* **24**, 49–57 (1994).
5. Sum, C. F. *et al.* The effect of intravenous metformin on glucose metabolism during hyperglycaemia in type 2 diabetes. *Diabet. Med. J. Br. Diabet. Assoc.* **9**, 61–65 (1992).

6. Buse, J. B. *et al.* The Primary Glucose-Lowering Effect of Metformin Resides in the Gut, Not the Circulation: Results From Short-term Pharmacokinetic and 12-Week Dose-Ranging Studies. *Diabetes Care* **39**, 198–205 (2016).
7. Ikeda, T., Iwata, K. & Murakami, H. Inhibitory effect of metformin on intestinal glucose absorption in the perfused rat intestine. *Biochem. Pharmacol.* **59**, 887–890 (2000).
8. Gontier, E. *et al.* High and typical ¹⁸F-FDG bowel uptake in patients treated with metformin. *Eur. J. Nucl. Med. Mol. Imaging* **35**, 95–99 (2008).
9. Bahne, E. *et al.* Metformin-induced glucagon-like peptide-1 secretion contributes to the actions of metformin in type 2 diabetes. *JCI Insight* **3**, (2018).
10. Shin, N.-R. *et al.* An increase in the Akkermansia spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. *Gut* **63**, 727–735 (2014).
11. Sansome, D. J. *et al.* Mechanism of glucose-lowering by metformin in type 2 diabetes: Role of bile acids. *Diabetes Obes. Metab.* **22**, 141–148 (2020).
12. Forslund, K. *et al.* Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature* **528**, 262–266 (2015).
13. Wu, H. *et al.* Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Nat. Med.* **23**, 850–858 (2017).
14. Lee, H. & Ko, G. Effect of metformin on metabolic improvement and gut microbiota. *Appl. Environ. Microbiol.* **80**, 5935–5943 (2014).
15. Adeshirlarijaney, A., Zou, J., Tran, H. Q., Chassaing, B. & Gewirtz, A. T. Amelioration of metabolic syndrome by metformin associates with reduced indices of low-grade inflammation independently of the gut microbiota. *Am. J. Physiol. Endocrinol. Metab.* **317**, E1121–E1130 (2019).

16. Bauer, P. V. *et al.* Metformin Alters Upper Small Intestinal Microbiota that Impact a Glucose-SGLT1-Sensing Glucoregulatory Pathway. *Cell Metab.* **27**, 101-117.e5 (2018).
17. Horakova, O. *et al.* Metformin acutely lowers blood glucose levels by inhibition of intestinal glucose transport. *Sci. Rep.* **9**, 6156 (2019).
18. Sun, L. *et al.* Gut microbiota and intestinal FXR mediate the clinical benefits of metformin. *Nat. Med.* **24**, 1919–1929 (2018).
19. Harmel, E. *et al.* AMPK in the small intestine in normal and pathophysiological conditions. *Endocrinology* **155**, 873–888 (2014).
20. Degirolamo, C., Sabbà, C. & Moschetta, A. Therapeutic potential of the endocrine fibroblast growth factors FGF19, FGF21 and FGF23. *Nat. Rev. Drug Discov.* **15**, 51–69 (2016).
21. Wang, Y. *et al.* The effects of metformin on fibroblast growth factor 19, 21 and fibroblast growth factor receptor 1 in high-fat diet and streptozotocin induced diabetic rats. *Endocr. J.* **64**, 543–552 (2017).
22. Brønden, A. *et al.* Single-Dose Metformin Enhances Bile Acid-Induced Glucagon-Like Peptide-1 Secretion in Patients With Type 2 Diabetes. *J. Clin. Endocrinol. Metab.* **102**, 4153–4162 (2017).
23. Dengler, F., Rackwitz, R., Pfannkuche, H. & Gäbel, G. Glucose transport across lagomorph jejunum epithelium is modulated by AMP-activated protein kinase under hypoxia. *J. Appl. Physiol. Bethesda Md 1985* **123**, 1487–1500 (2017).
24. Ouyang, J. *et al.* The Bacterium *Akkermansia muciniphila*: A Sentinel for Gut Permeability and Its Relevance to HIV-Related Inflammation. *Front. Immunol.* **11**, 645 (2020).

25. Depommier, C. *et al.* Beneficial Effects of Akkermansia muciniphila Are Not Associated with Major Changes in the Circulating Endocannabinoidome but Linked to Higher Mono-Palmitoyl-Glycerol Levels as New PPAR α Agonists. *Cells* **10**, (2021).
26. Ma, W. *et al.* Metformin Alters Gut Microbiota of Healthy Mice: Implication for Its Potential Role in Gut Microbiota Homeostasis. *Front. Microbiol.* **9**, 1336 (2018).
27. Vacca, M. *et al.* The Controversial Role of Human Gut Lachnospiraceae. *Microorganisms* **8**, (2020).
28. Kameyama, K. & Itoh, K. Intestinal colonization by a Lachnospiraceae bacterium contributes to the development of diabetes in obese mice. *Microbes Environ.* **29**, 427–430 (2014).
29. Napolitano, A. *et al.* Novel gut-based pharmacology of metformin in patients with type 2 diabetes mellitus. *PloS One* **9**, e100778 (2014).
30. Setchell, K. D. R. & Clerici, C. Equol: pharmacokinetics and biological actions. *J. Nutr.* **140**, 1363S–8S (2010).
31. Piwowarek, K., Lipińska, E., Hać-Szymańczuk, E., Kieliszek, M. & Ścibisz, I. Propionibacterium spp.-source of propionic acid, vitamin B12, and other metabolites important for the industry. *Appl. Microbiol. Biotechnol.* **102**, 515–538 (2018).
32. Doerner, K. C., Takamine, F., LaVoie, C. P., Mallonee, D. H. & Hylemon, P. B. Assessment of fecal bacteria with bile acid 7 alpha-dehydroxylating activity for the presence of bai-like genes. *Appl. Environ. Microbiol.* **63**, 1185–1188 (1997).
33. Funabashi, M. *et al.* A metabolic pathway for bile acid dehydroxylation by the gut microbiome. *Nature* **582**, 566–570 (2020).
34. Lepercq, P. *et al.* Epimerization of chenodeoxycholic acid to ursodeoxycholic acid by Clostridium baratii isolated from human feces. *FEMS Microbiol. Lett.* **235**, 65–72 (2004).

35. Yu, J. *et al.* Lithocholic acid decreases expression of bile salt export pump through farnesoid X receptor antagonist activity. *J. Biol. Chem.* **277**, 31441–31447 (2002).
36. Lien, F. *et al.* Metformin interferes with bile acid homeostasis through AMPK-FXR crosstalk. *J. Clin. Invest.* **124**, 1037–1051 (2014).
37. Naville, D. *et al.* Chronic exposure to a pollutant mixture at low doses led to tissue-specific metabolic alterations in male mice fed standard and high-fat high-sucrose diet. *Chemosphere* **220**, 1187–1199 (2019).
38. Humbert, L. *et al.* Bile acid profiling in human biological samples: comparison of extraction procedures and application to normal and cholestatic patients. *J. Chromatogr. B Analyt. Technol. Biomed. Life. Sci.* **899**, 135–145 (2012).
39. Bolyen, E. *et al.* Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **37**, 852–857 (2019).
40. Wooley, J. C. & Ye, Y. Metagenomics: Facts and Artifacts, and Computational Challenges*. *J. Comput. Sci. Technol.* **25**, 71–81 (2009).

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AUTHOR CONTRIBUTIONS. C.G., K.M., J.R. and H.V. conceived and designed the study. A.B., C.G, C.D., B.B., K.M. and M.G. conducted the mouse experiments, the metabolic phenotyping and the genomic analyses. C.D. and E.M. performed the microbiota study. B.B. and D.R. performed and interpreted the bile acid analyses. B.B, C.D., M.G. and H.V. analyzed and interpreted the data. H.V. wrote the manuscript with corrections and approval of all the co-authors.

ADDITIONAL INFORMATION. The supplementary data associated with the present paper are available in the separated file.

FIGURE LEGENDS

Figure 1: Metformin improves metabolic parameters and glucose tolerance in high fat high sucrose fed mice. (A) change in body weight after 8 days of standard diet (SD), high fat high sucrose diet without (HFS) or with metformin supplementation (MET). (B) and (C) Fasting plasma glucose and insulin concentrations, respectively. (D) Glycemic curves during intraperitoneal glucose tolerance test (ipGTT) performed on 5 animals per group, and (E) corresponding area under the curves (AUC) of the ipGTT. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, determined by ANOVA followed by Tukey's multiple comparison test.

Figure 2: Effects of HFS diet and metformin treatment on apical glucose transporter gene expression in the different regions of the intestine. GLUT5 and SGLT1 mRNA levels were determined by RT-qPCR in (A) the duodenum, (B) jejunum, (C) ileum and (D) colon of 15 to 18 mice per group (SD : standard diet; HFS : high fat high sucrose diet; MET : HFS diet with metformin supplementation). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, determined by ANOVA followed by Tukey's multiple comparison test.

Figure 3: Effects of HFS diet and metformin treatment on the expression of lipid absorption-related genes in the jejunum and the ileum. The mRNA levels of the targeted genes were determined by RT-qPCR in (A) the jejunum and (B) the ileum of 15 to 18 mice per group (SD : standard diet; HFS : high fat high sucrose diet; MET : HFS diet with metformin supplementation). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, determined by ANOVA followed by Tukey's multiple comparison test.

Figure 4: Effects of HFS diet and metformin treatment on the expression of intestinal hormones and peptides in the different regions of the intestine. The mRNA levels of the targeted genes were determined by RT-qPCR in (A) the duodenum, (B) jejunum, (C) ileum and (D) colon of 15 to 18 mice per group. (SD : standard diet; HFS : high fat high sucrose diet; MET : HFS diet with metformin supplementation). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, determined by ANOVA followed by Tukey's multiple comparison test.

Figure 5 : Metformin treatment impacts bile acid pool in the caecum of the mice.

Bile acid molecular species concentrations were measured by HPLC coupled to tandem mass spectrometry (HPLC-MS/MS) in caecum of 6 mice per group (SD : standard diet; HFS : high fat high sucrose diet; MET : HFS diet with metformin supplementation). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, determined by ANOVA followed by Tukey's multiple comparison test.

Figure 6 : Metformin modifies luminal microbiota composition in the different region of the intestine.

Microbiota composition was determined by 16S rRNA sequencing in luminal samples from the duodenum, jejunum, ileum and colon of animals of the 3 groups (SD : standard diet; HFS : high fat high sucrose diet; MET : HFS diet with metformin supplementation). The figure represents the relative abundance of the main bacterial phyla measured in the luminal samples of individual mice.

Figure 7 : Metformin increases *Akkermansia Muciniphila* abundance in the intestinal lumen all along the gut.

Microbiota composition was determined by 16S rRNA sequencing in luminal samples from the duodenum, the jejunum, the ileum and the colon in the different groups (SD : standard diet; HFS : high fat high sucrose diet; MET : HFS diet with metformin supplementation). The figure represents the relative abundance of *Akkermansia Muciniphila* species. ** $p < 0.01$, *** $p < 0.001$, determined by ANOVA followed by Tukey's multiple comparison test.

Figure 8 : Metformin induces changes of the luminal microbiota composition in the different region of the intestine.

Microbiota composition was determined by 16S rRNA sequencing in luminal samples from the duodenum, jejunum, ileum and colon of animals of the 3 groups (SD : standard diet; HFS : high fat high sucrose diet; MET : HFS diet with metformin supplementation). The figure represents the relative abundance of the main measurable bacterial families and genera in the luminal samples of individual mice.

Figure 1

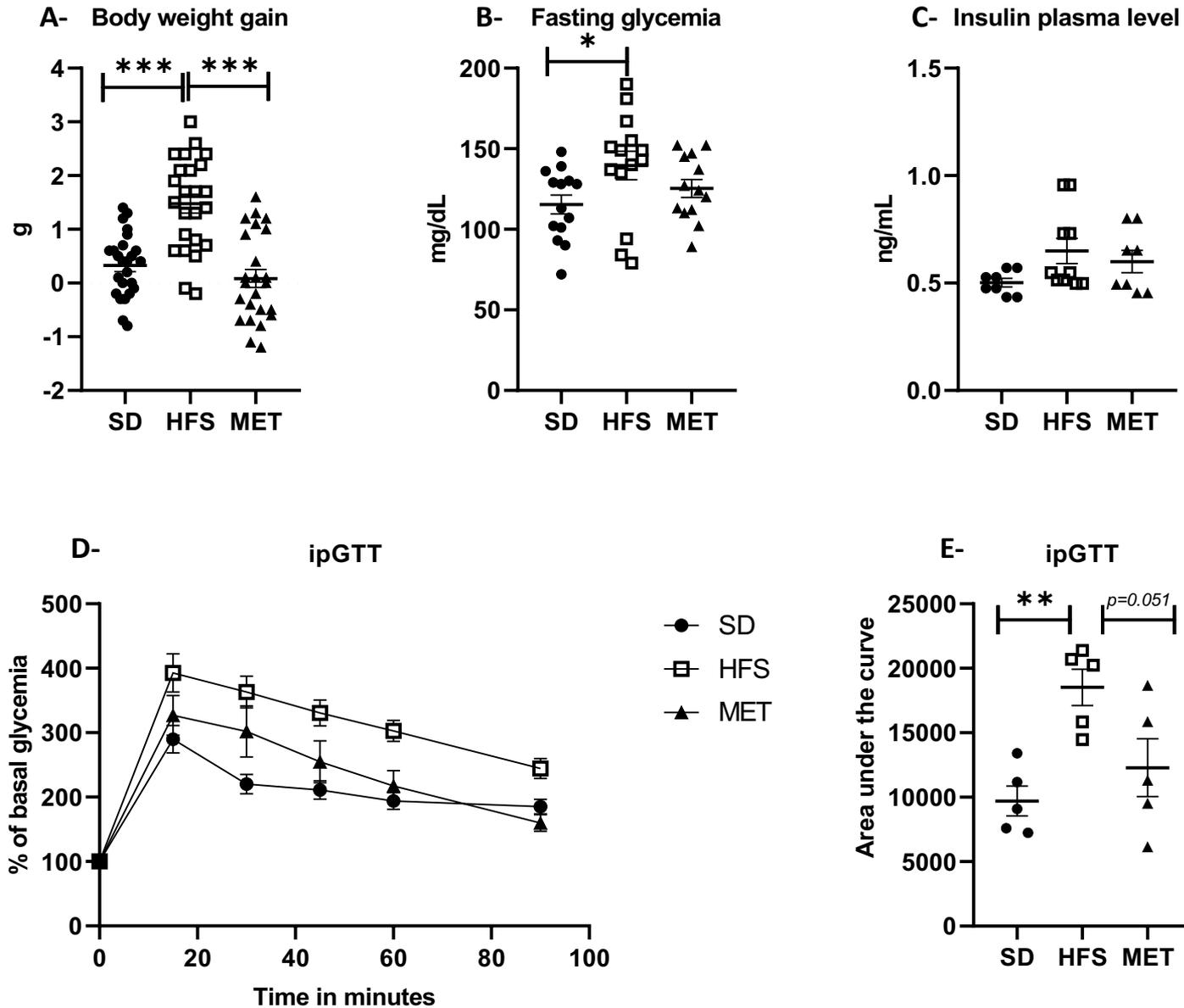


Figure 2

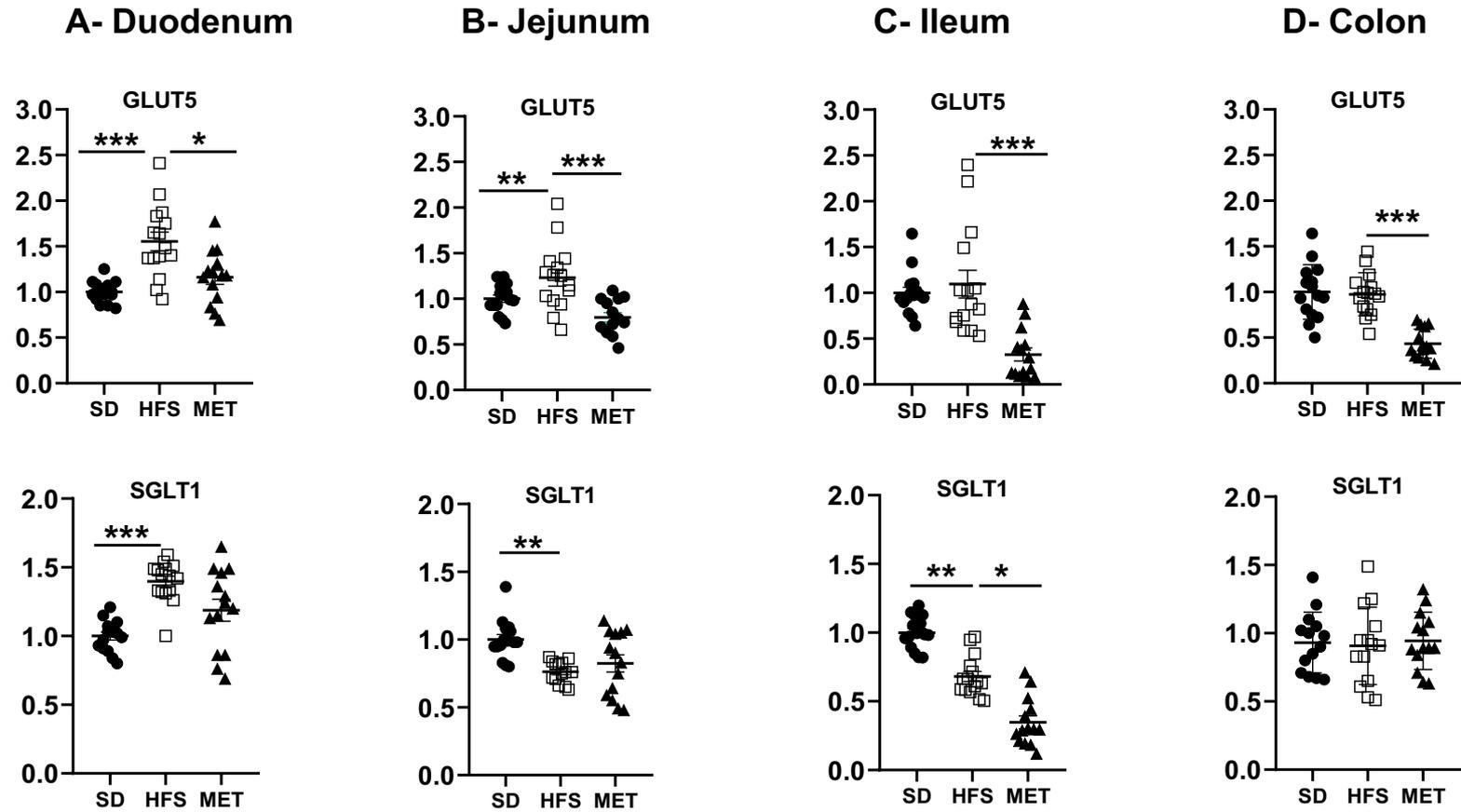
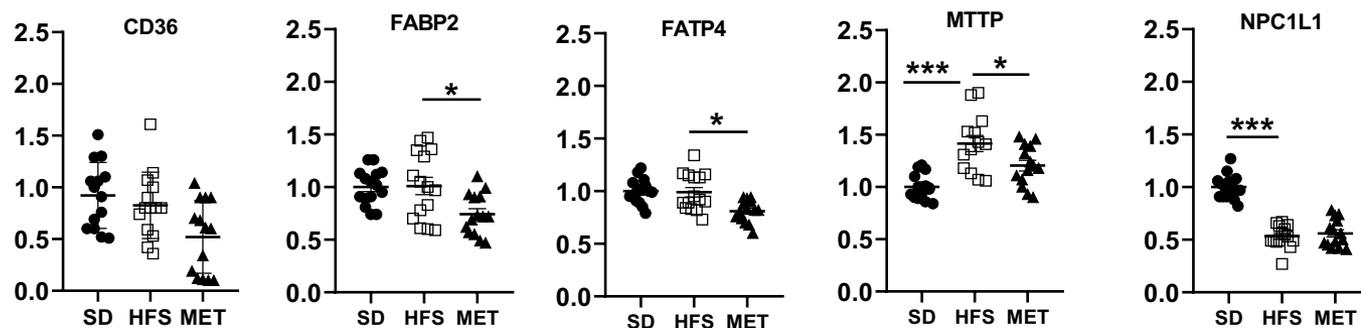


Figure 3

A- Jejunum



B- Ileum

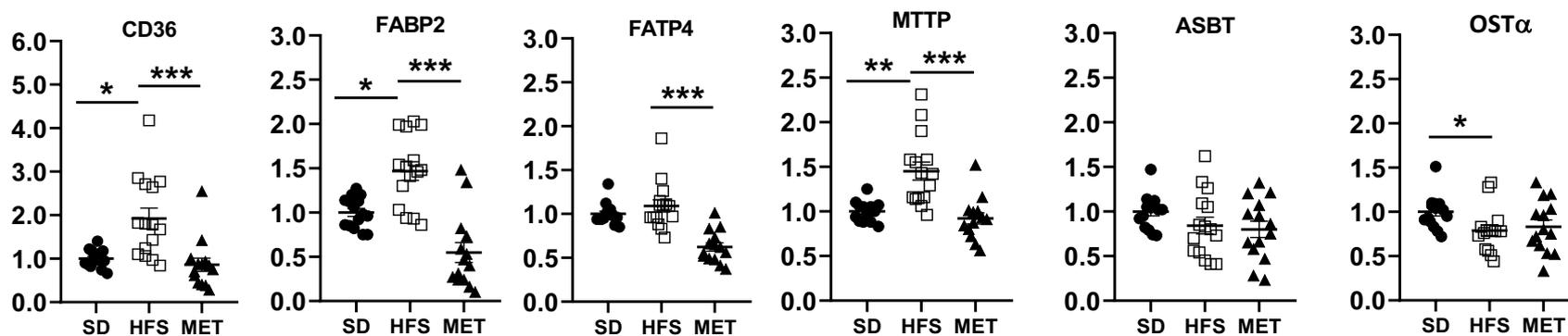


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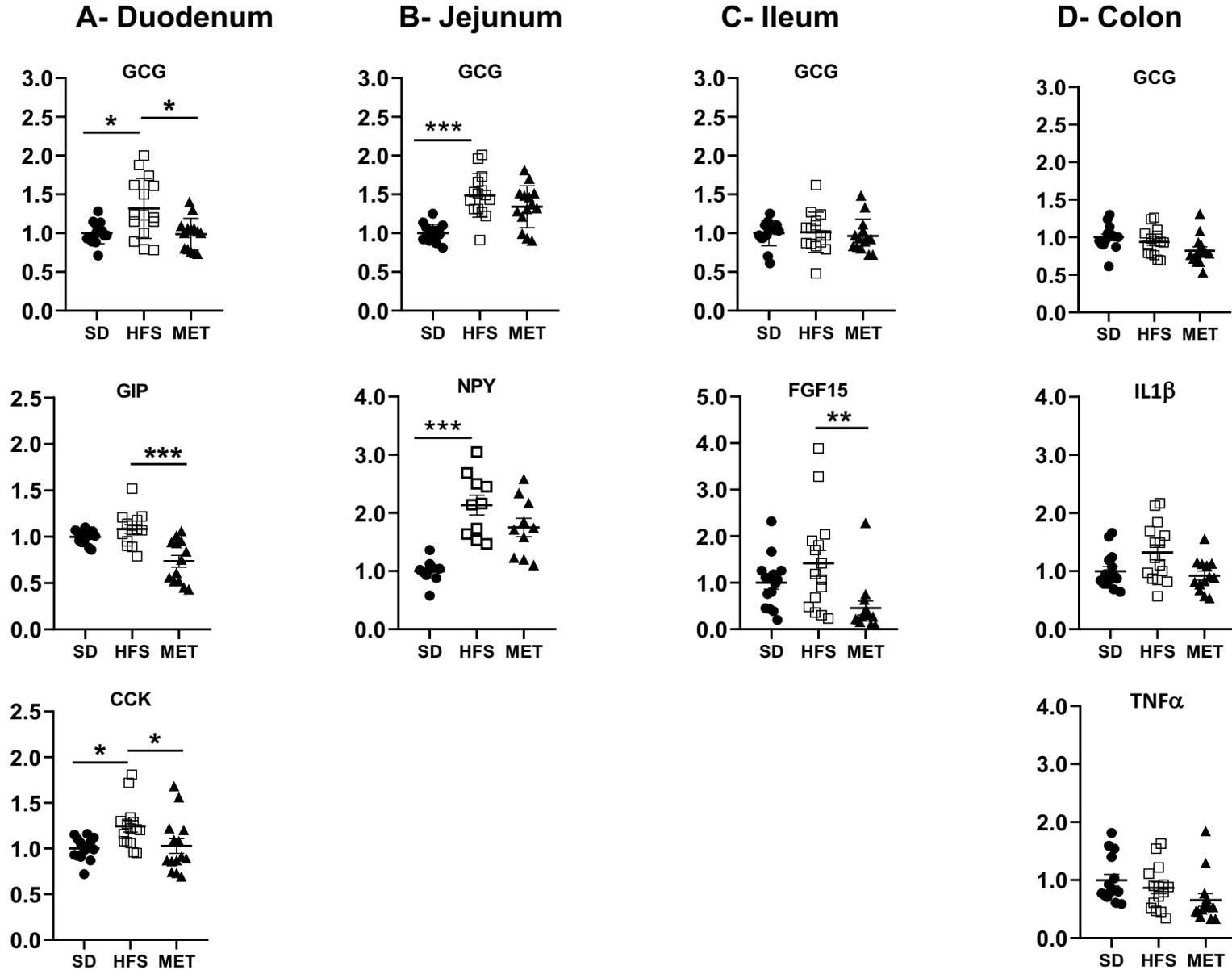


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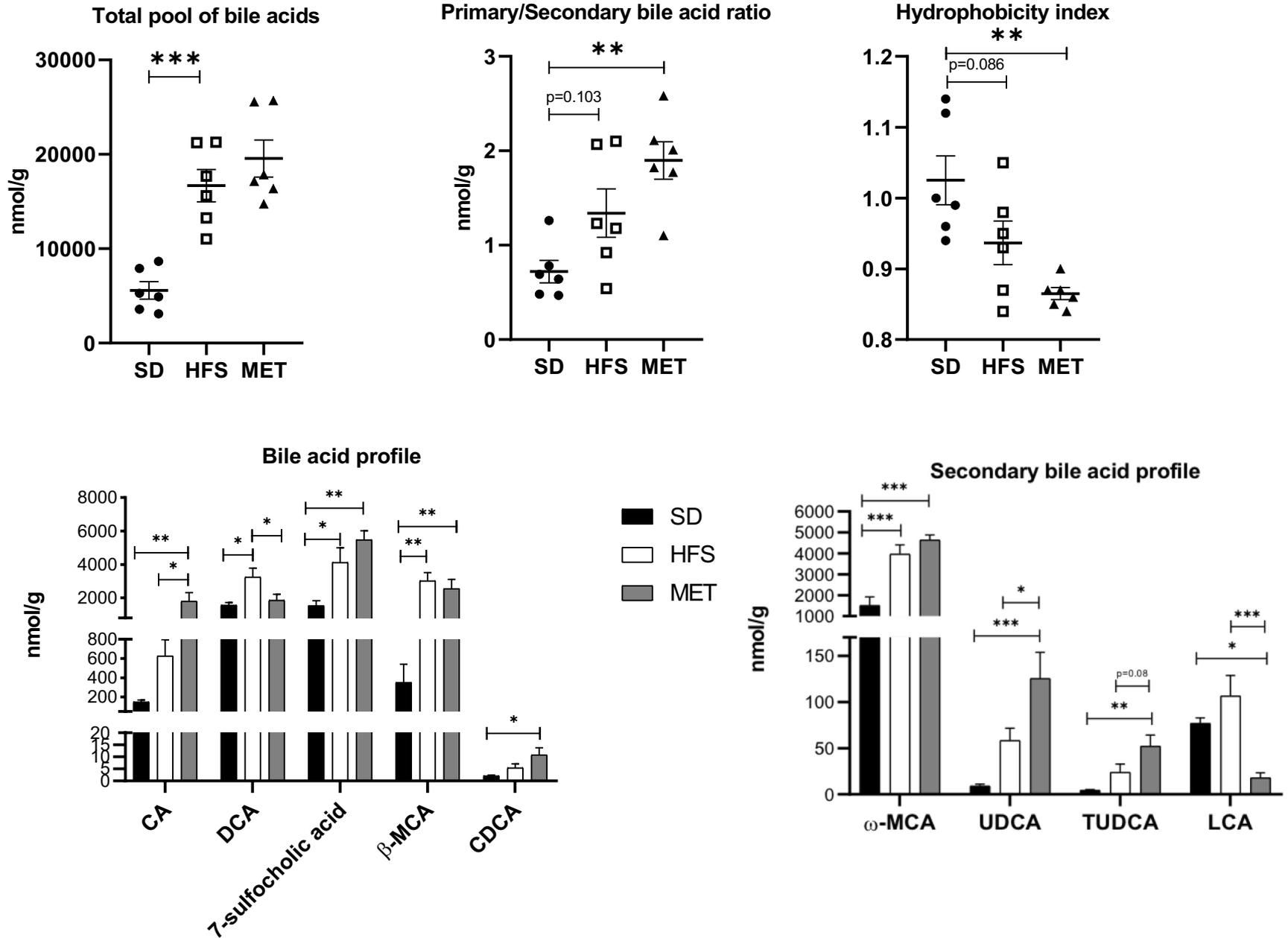


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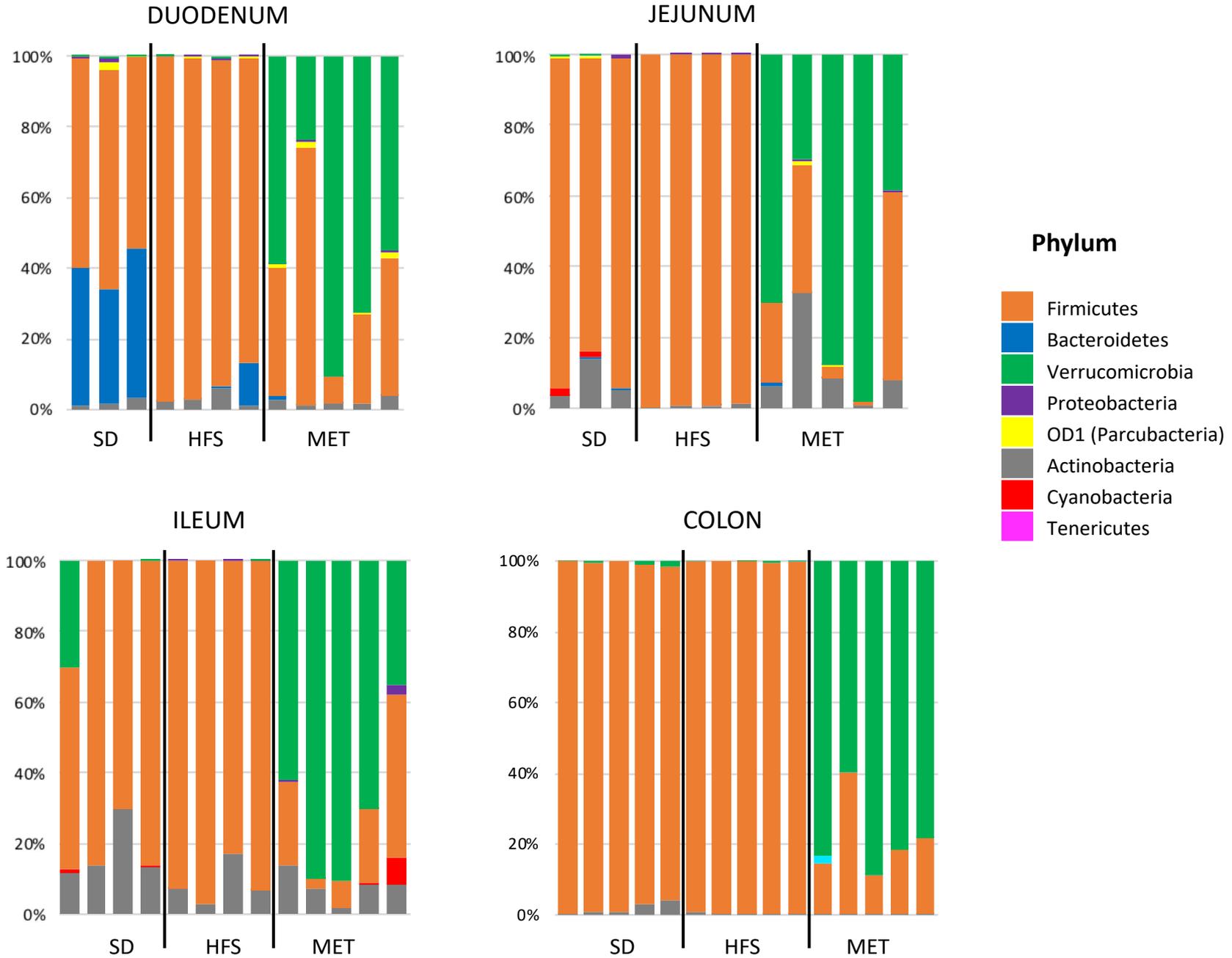


Figure 7

Akkermansia Muciniphila

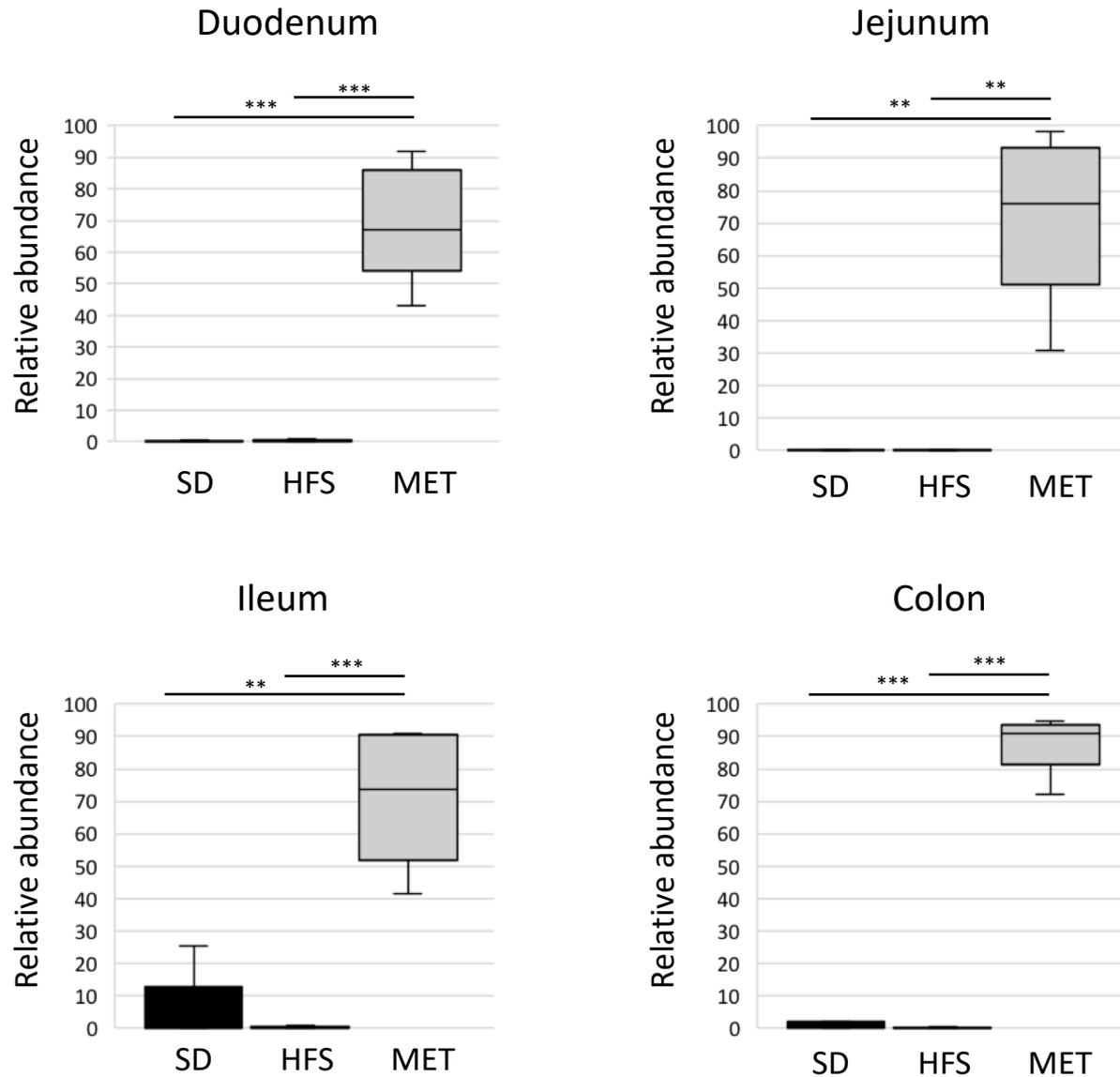
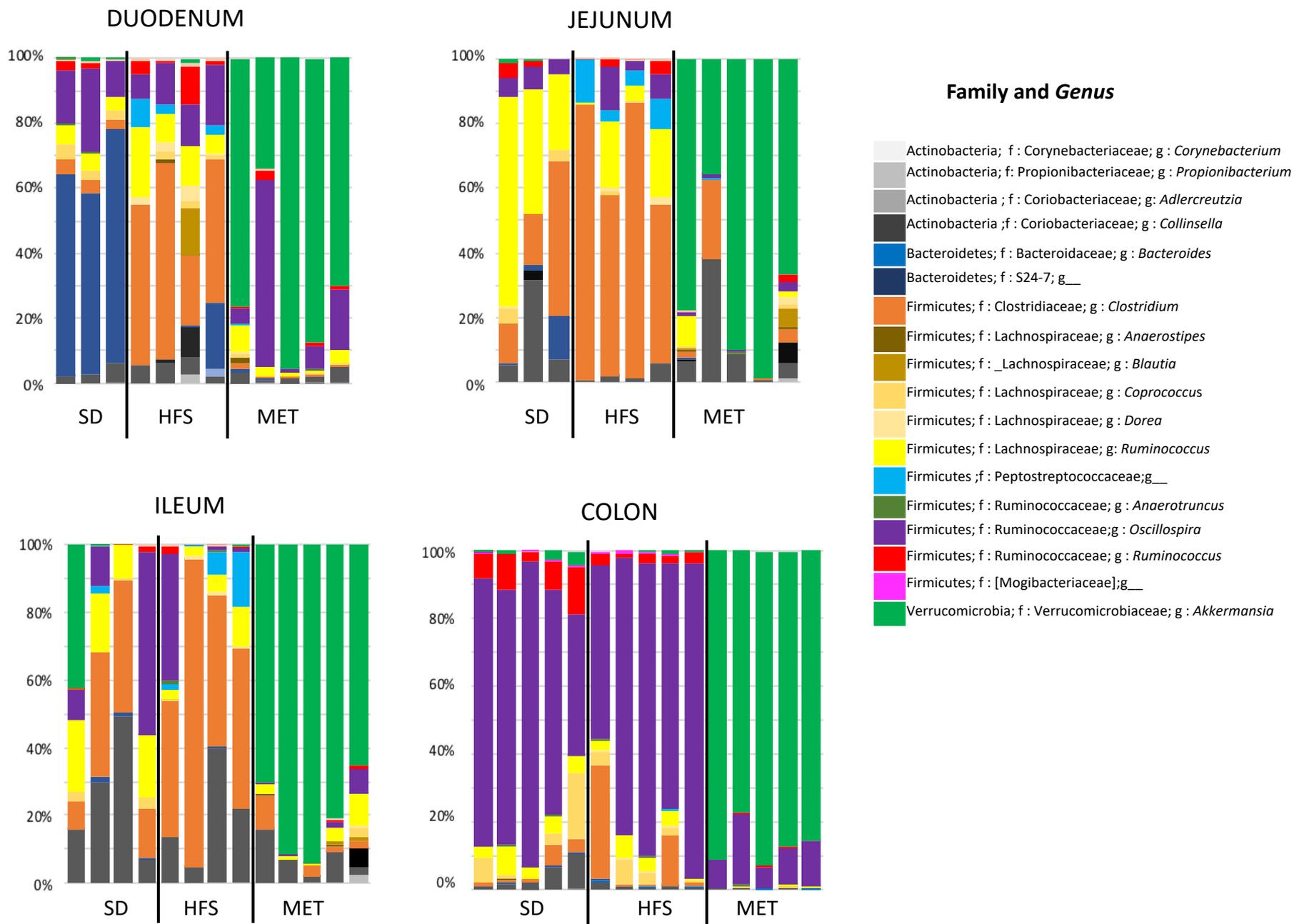


Figure 8



Figures

Figure 1

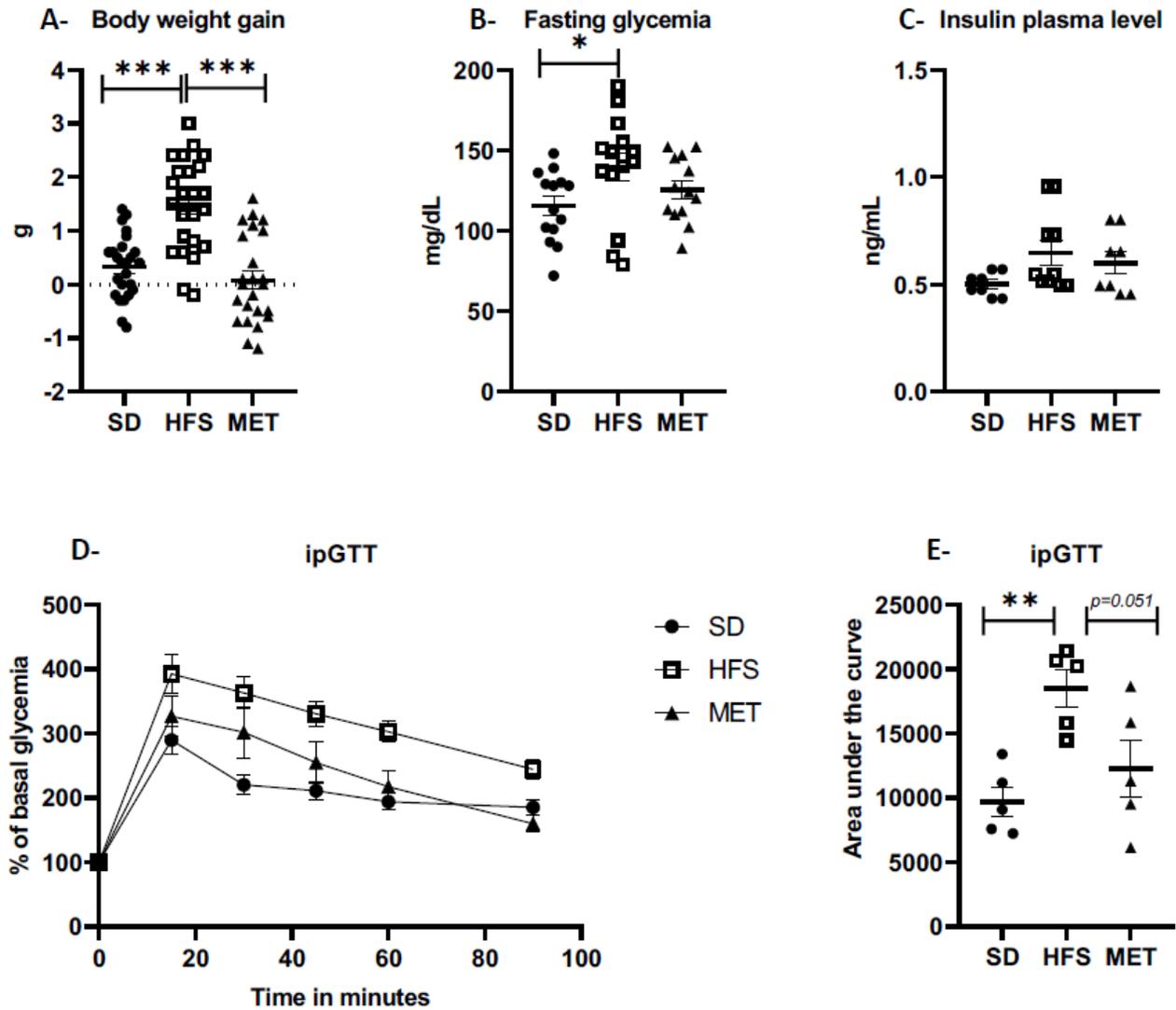


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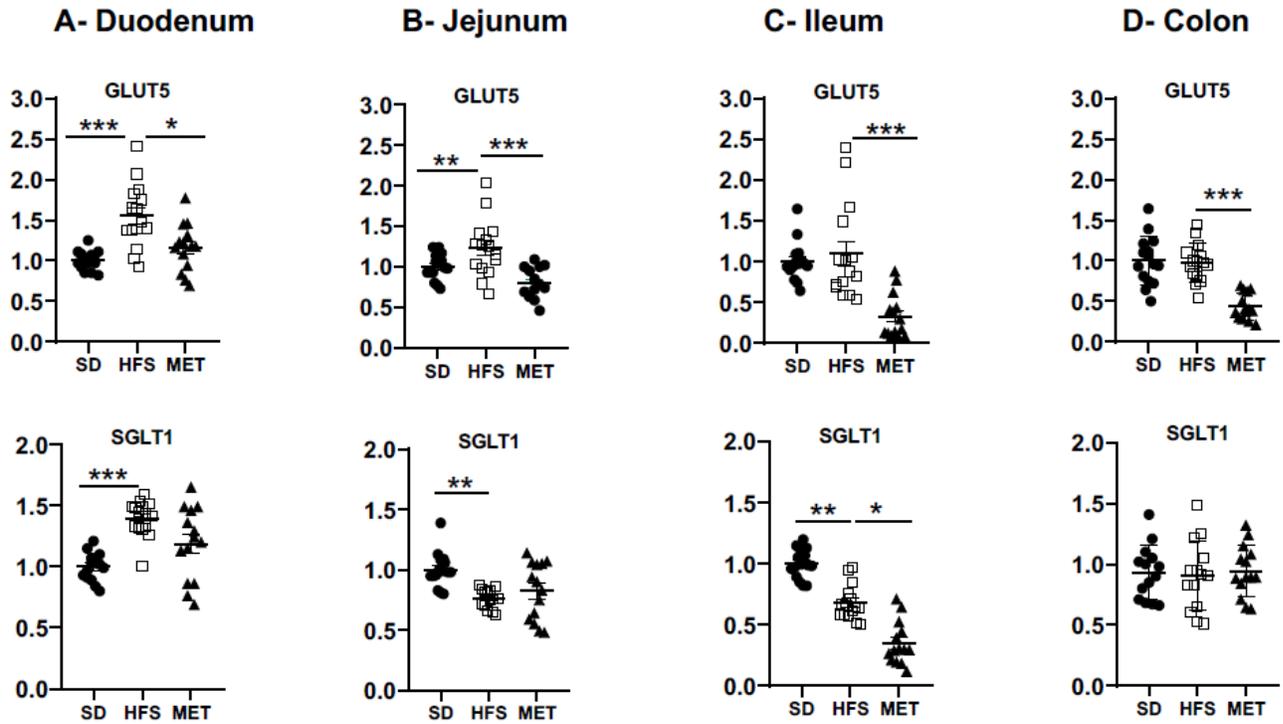


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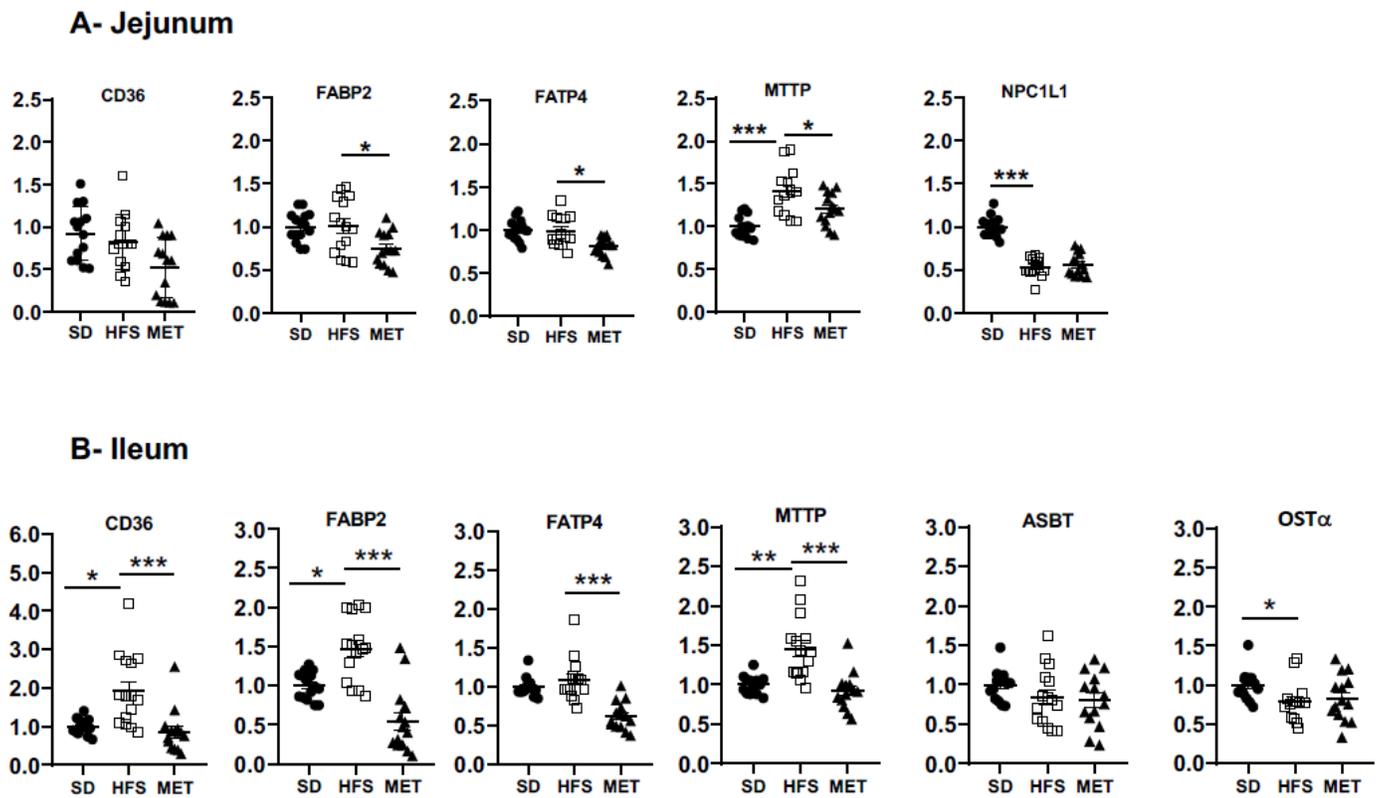


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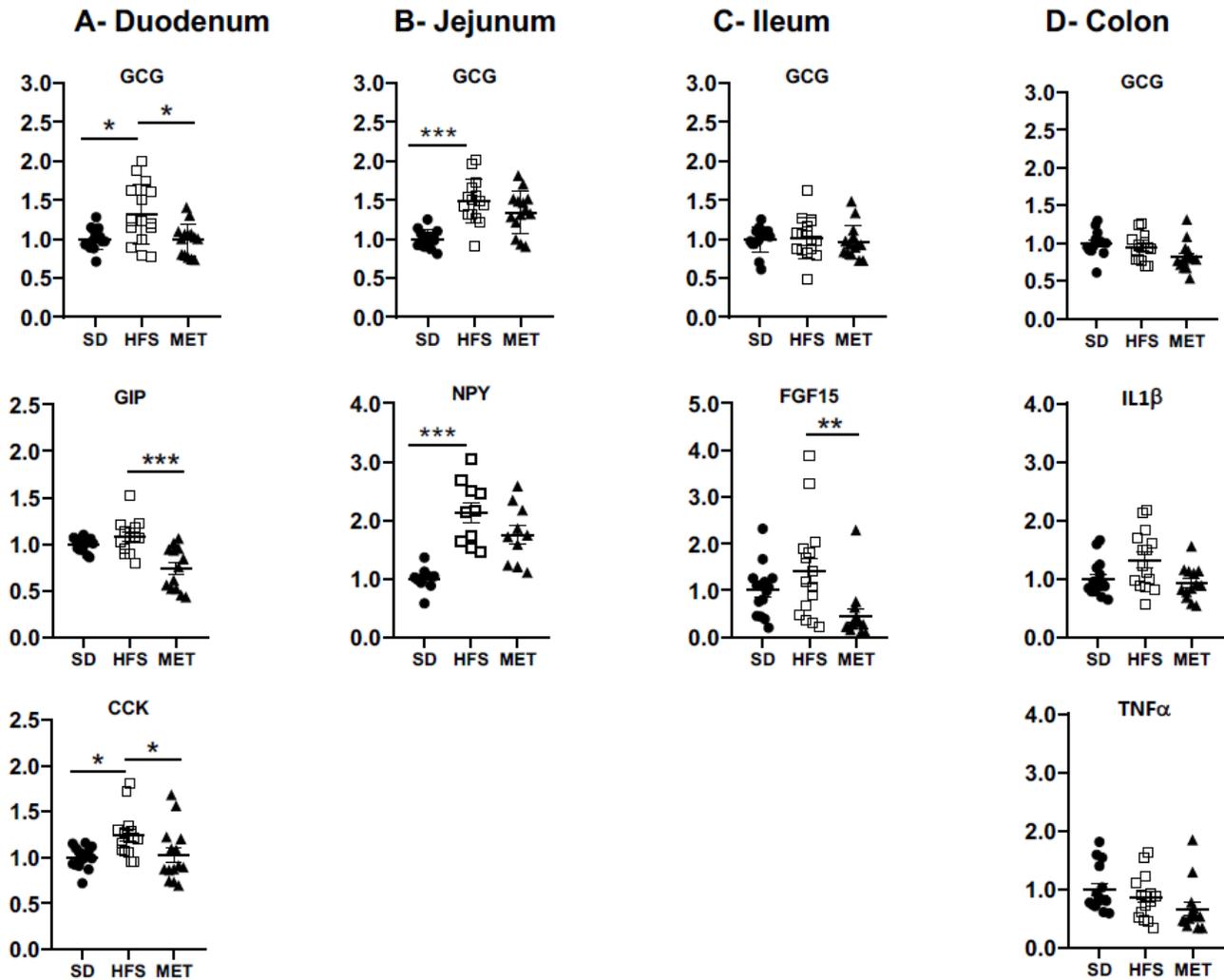


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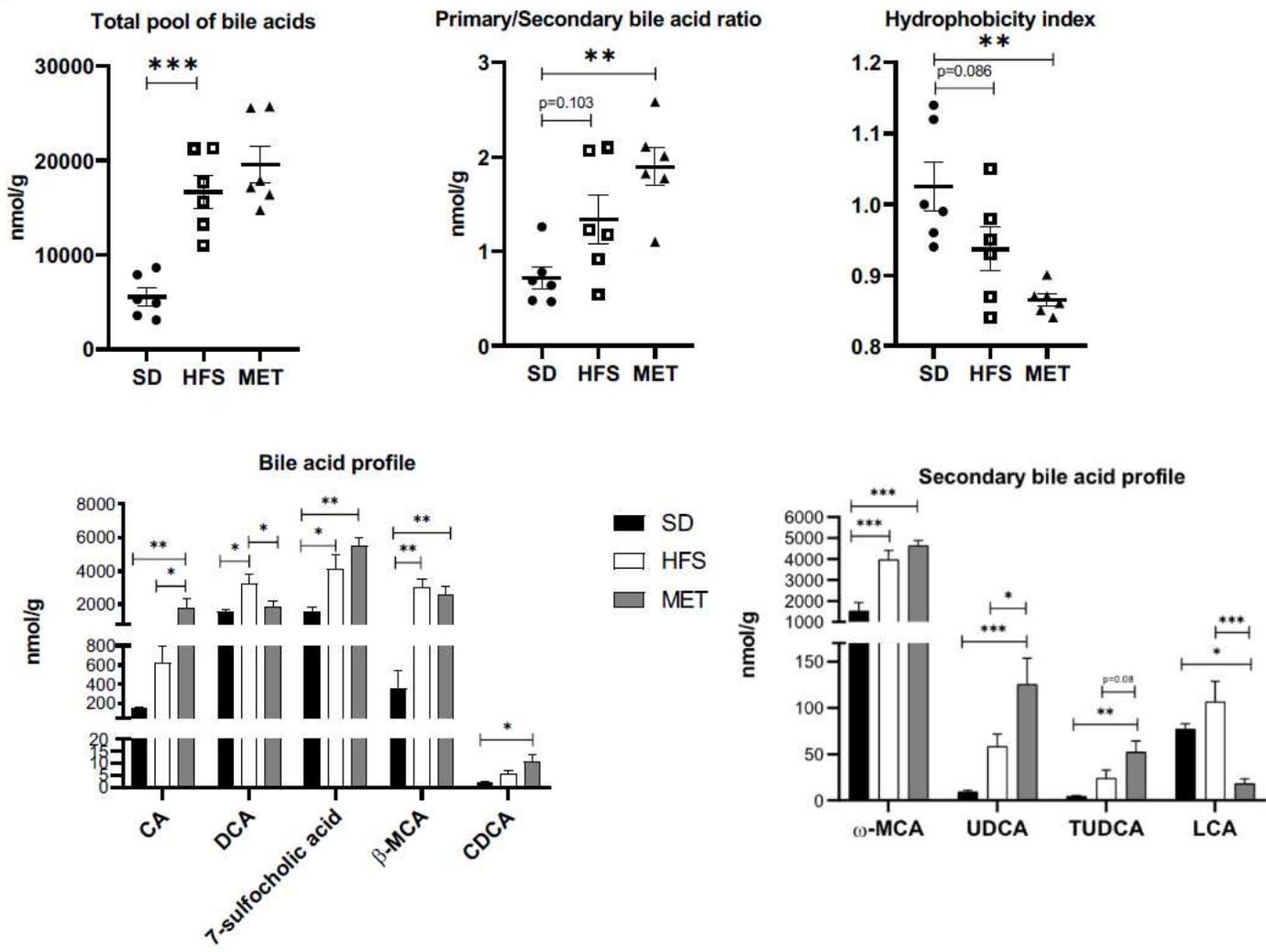


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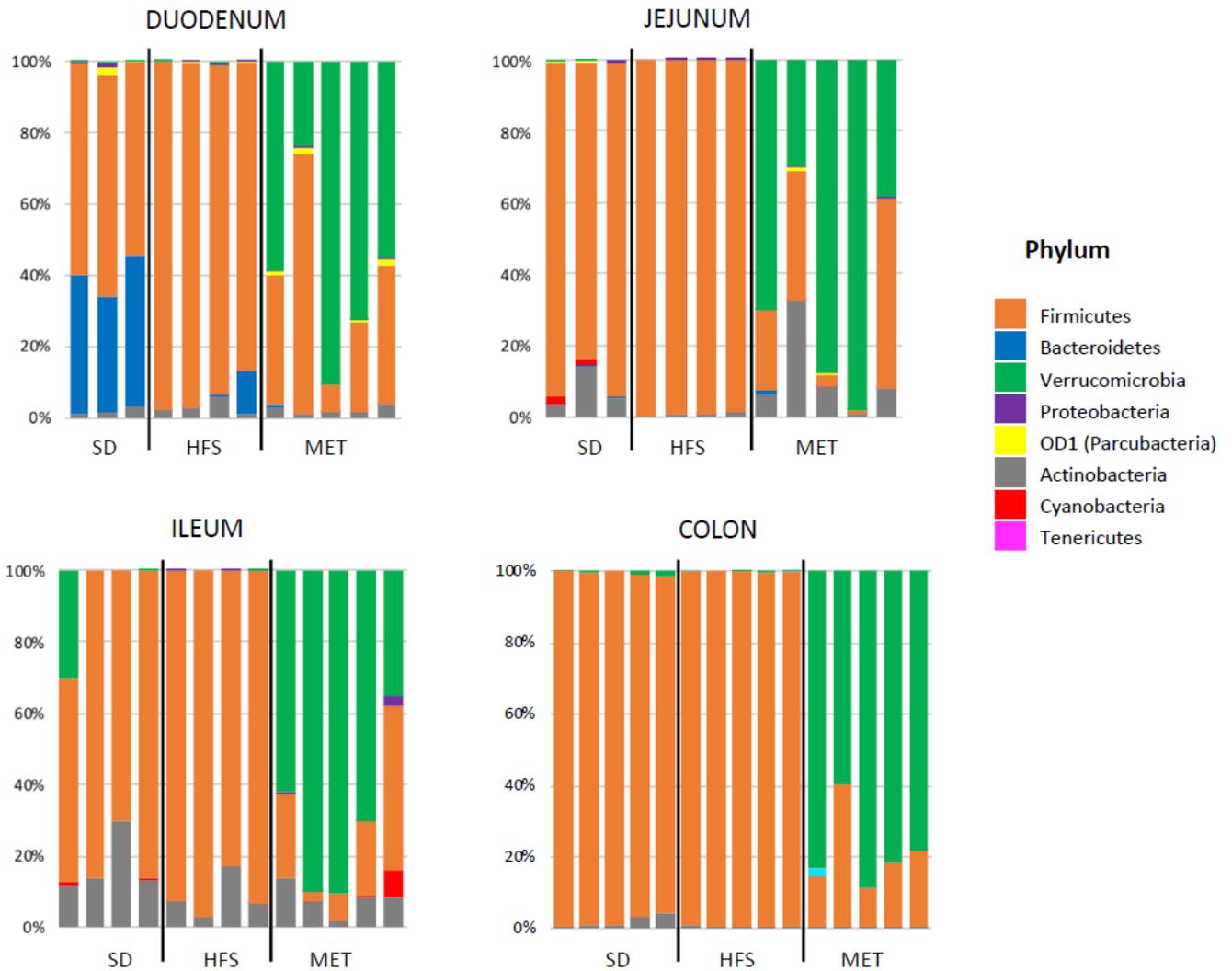


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Figure 7

Akkermansia Muciniphila

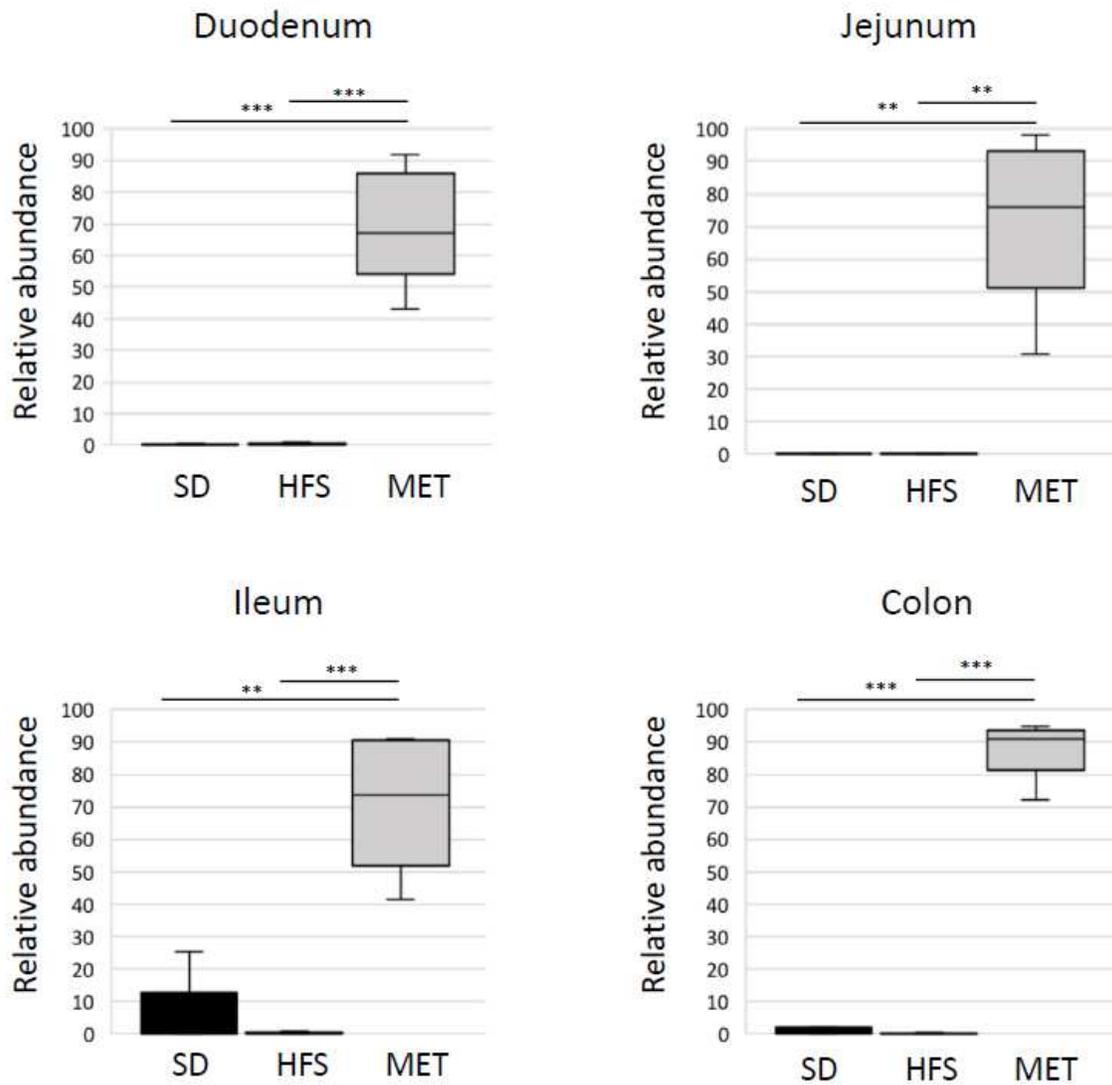


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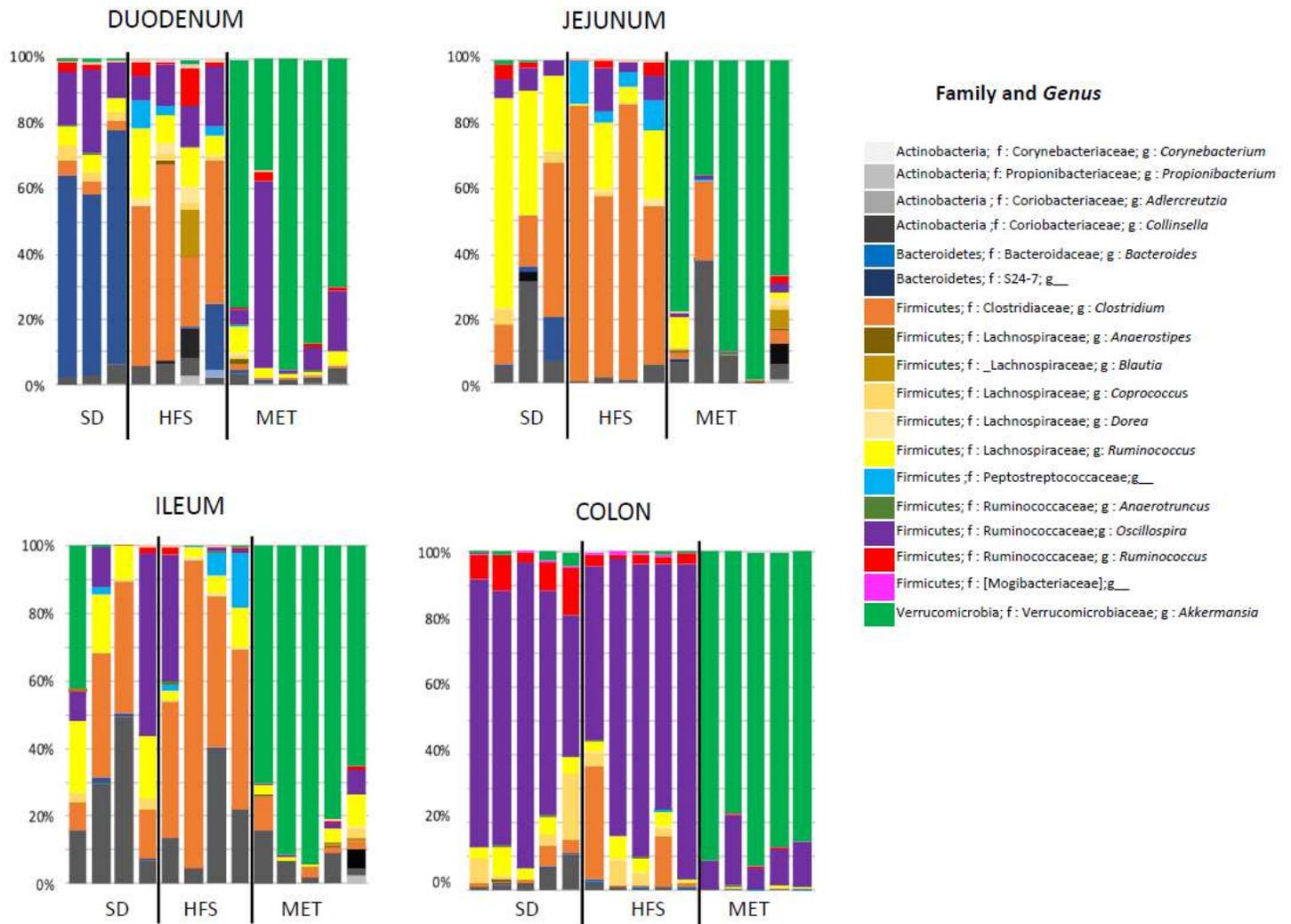


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