Supplemental Discussion

Clinical characteristics of the MetaCardis cohort

As expected, severely obese patients that were characterized as metabolically healthy differed from those presenting metabolic syndrome features or T2D in many aspects (**Supplemental Table 1**): they were younger, more often women, had a lower waist circumference, lower blood pressure, lower levels of fasting blood glucose, glycated hemoglobin, and triglycerides (but higher levels of HDL- cholesterol), lower concentrations of Alanine-aminotransferase (ALT), Aspartate-aminotransferase (AST), and γ-Glutamyl-transferase (GGT), as well as higher leisure-time physical activity. On the other hand, severely obese patients with T2D had a lower BMI, lower percentage of body fat and lower levels of ultra-sensitivity c-reactive protein (us-CRP). For some of the other inflammation markers measured, T2D patients had higher levels as compared to metabolically healthy participants, in particular high-sensitivity interleukin 6 (hs-IL-6), C-X-C motif chemokine ligand 5 (CXCL-5), eotaxin, macrophage inhibitory factor (MIF), and soluble CD14 (sCD14).

Among non-obese and moderately obese participants, relatively similar patterns differences across health groups were observed (**Supplemental Table 2**). However, inflammatory factor profiles were somewhat different. For example, non-obese and moderately obese T2D patients had higher us-CRP than their metabolically healthy counterparts.

Supplemental Table 3 shows general drug treatment characteristics of participants. Among severely obese T2D patients, one quarter did not receive any antidiabetic drug treatment, while this proportion was lower than one tenth in non-obese and moderately obese T2D patients. **Supplemental Table 4** shows detailed drug treatment characteristics for MetaCardis participants with T2D. The majority of these patients (73.1%) received Metformin alone (15.2%) or in combination (57.8%) with another treatment.
Interestingly, in SOB group, despite being under the threshold of lower gene richness, T2D individuals not treated by metformin had increased gene richness compared to other SOB groups **(Supplemental Figure 1a)**. The profile of these individuals was interesting since 67% of them were not treated by other medication vs. 43% among NOB and 44% among MOB suggesting this profile could be influenced by a healthier status. These subjects received also less antidiabetic drugs in comparison with NOB and MOB (Chi2=13.91, P =7.6x10-3; Chi-square test). In contrast, individuals with T2D treated with metformin shows a

more complex medication profile, which increases in severe obese states (**Supplemental Table 4**).

Mouse experiments of HFD and prebiotic supplementation

Prebiotics have been shown to increase gut microbiota diversity and composition, but their effect on serum biotin is unknown. We examined the effect of a prebiotic intervention in C57BL/6J male mice fed a HFD by supplementing drinking water with fructo-oligosaccharides (FOS) (HFD+FOS) for three months. We observed an increase in plasma biotin after one month of treatment compared to non-treated HFD-fed animals (**Supplemental Figure 5c** left, HFD vs. HFD+FOS: Chi2= 2.08, p-value=5.60x10-2, KW with Dunn's multiple comparison test), that reached significance after three months (**Supplemental Figure 5c** right, right HFD vs. HFD+FOS: Chi2= 2.54, p-value=1.65x10-2, KW with Dunn's multiple comparison test). At three months, plasma biotin of the HFD+FOS group was similar to the chow group, suggesting that FOS supplementation alleviates the impact of a HFD on circulating biotin. Using shotgun sequencing, we sequenced the gut microbiota and observed a major shift at the phylum level in the mouse models (**Supplemental Figure 5b**). In contrast with human obesity, we observed a bloom in Firmicutes in the HFD group mainly explained by a major expansion of *Lactococcus lactis* whereas FOS supplementation caused a significant expansion of *Bifidobacterium animalis*, contributing to the increase in Actinobacteria in comparison to Chow and HFD groups (**Supplemental Figure 5b**). Despite these changes, animals fed a chow diet harboured a more diverse microbiome composition at phylum level with a predominance of Bacteroidetes in comparison with HFD and HFD+FOS groups (**Supplemental Figure 5b**). When we quantified the relative abundance of different bacterial groups of biotin producers and transporters, we observed that one month after the intervention, FOS supplementation led to significant increases of biotin producers (bacteria with all genes involved in biotin biosynthesis Pimeloyl-ACP or pimelate and with no biotin transport genes) in comparison with HFD group (FDR=1.13x10-4 Kruskal-Wallis test and FDR=7.20x10-4 pairwise Wilcoxon rank-sum test) in parallel with a significant decrease of biotin transporters (bacteria with incomplete biotin biosynthesis pathway) (FDR=1.13x10-4 Kruskal-Wallis test and FDR=8.68x10-4 pairwise Wilcoxon rank-sum test), with no impact on the group of bacteria capable of biotin synthesis and transport (**Supplemental Figure 5d**). Similar results were observed 3 months after the intervention (**Supplemental Figure 5d**). To note, results were not changed when taking into account the total bacterial abundance (**Supplemental Figure 5e**).