

1 Supplemental Discussion

2 Clinical characteristics of the MetaCardis cohort

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

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

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21 **Supplemental Table 3** shows general drug treatment characteristics of participants. Among
22 severely obese T2D patients, one quarter did not receive any antidiabetic drug treatment,
23 while this proportion was lower than one tenth in non-obese and moderately obese T2D
24 patients. **Supplemental Table 4** shows detailed drug treatment characteristics for
25 MetaCardis participants with T2D. The majority of these patients (73.1%) received Metformin
26 alone (15.2%) or in combination (57.8%) with another treatment.

27 Interestingly, in SOB group, despite being under the threshold of lower gene richness, T2D
28 individuals not treated by metformin had increased gene richness compared to other SOB
29 groups (**Supplemental Figure 1a**). The profile of these individuals was interesting since
30 67% of them were not treated by other medication vs. 43% among NOB and 44% among
31 MOB suggesting this profile could be influenced by a healthier status. These subjects
32 received also less antidiabetic drugs in comparison with NOB and MOB ($\text{Chi}^2=13.91$, P
33 $=7.6 \times 10^{-3}$; Chi-square test). In contrast, individuals with T2D treated with metformin shows a

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

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38 Mouse experiments of HFD and prebiotic supplementation

39 Prebiotics have been shown to increase gut microbiota diversity and composition, but their
40 effect on serum biotin is unknown. We examined the effect of a prebiotic intervention in
41 C57BL/6J male mice fed a HFD by supplementing drinking water with fructo-oligosaccharides
42 (FOS) (HFD+FOS) for three months. We observed an increase in plasma biotin after one
43 month of treatment compared to non-treated HFD-fed animals (**Supplemental Figure 5c** left,
44 HFD vs. HFD+FOS: $\chi^2=2.08$, $p\text{-value}=5.60 \times 10^{-2}$, KW with Dunn's multiple comparison
45 test), that reached significance after three months (**Supplemental Figure 5c** right, right HFD
46 vs. HFD+FOS: $\chi^2=2.54$, $p\text{-value}=1.65 \times 10^{-2}$, KW with Dunn's multiple comparison test). At
47 three months, plasma biotin of the HFD+FOS group was similar to the chow group, suggesting
48 that FOS supplementation alleviates the impact of a HFD on circulating biotin. Using shotgun
49 sequencing, we sequenced the gut microbiota and observed a major shift at the phylum level
50 in the mouse models (**Supplemental Figure 5b**). In contrast with human obesity, we observed
51 a bloom in Firmicutes in the HFD group mainly explained by a major expansion of *Lactococcus*
52 *lactis* whereas FOS supplementation caused a significant expansion of *Bifidobacterium*
53 *animalis*, contributing to the increase in Actinobacteria in comparison to Chow and HFD
54 groups (**Supplemental Figure 5b**). Despite these changes, animals fed a chow diet
55 harboured a more diverse microbiome composition at phylum level with a predominance of
56 Bacteroidetes in comparison with HFD and HFD+FOS groups (**Supplemental Figure 5b**).
57 When we quantified the relative abundance of different bacterial groups of biotin producers
58 and transporters, we observed that one month after the intervention, FOS supplementation
59 led to significant increases of biotin producers (bacteria with all genes involved in biotin
60 biosynthesis Pimeloyl-ACP or pimelate and with no biotin transport genes) in comparison with
61 HFD group ($FDR=1.13 \times 10^{-4}$ Kruskal-Wallis test and $FDR=7.20 \times 10^{-4}$ pairwise Wilcoxon rank-
62 sum test) in parallel with a significant decrease of biotin transporters (bacteria with incomplete
63 biotin biosynthesis pathway) ($FDR=1.13 \times 10^{-4}$ Kruskal-Wallis test and $FDR=8.68 \times 10^{-4}$
64 pairwise Wilcoxon rank-sum test), with no impact on the group of bacteria capable of biotin
65 synthesis and transport (**Supplemental Figure 5d**). Similar results were observed 3 months
66 after the intervention (**Supplemental Figure 5d**). To note, results were not changed when
67 taking into account the total bacterial abundance (**Supplemental Figure 5e**).

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