Abstract title (22/25): A pilot study on the gut microbiome in women with polycystic ovarian syndrome (PCOS) and overweight or obesity during weight loss treatment

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Study question (25/25): Will the gut microbiome change if women with PCOS and overweight/obesity lose weight and will weight loss be different after glucagon-like-peptide-1-agonist (GLP-1 agonist) or inositol?

Summary answer (27/25): Gut microbiome dysbiosis did not decrease significantly, despite 5% weight loss. Although weight reduction was higher with GLP-1-agonist, weight loss after inositol was associated with (baseline-)microbiome composition.

What is known already (99/100): PCOS is a complex, but common endocrine-metabolic disorder in women of reproductive age. Up to 60% of women with PCOS are also overweight or obese and thus, at risk for developing type 2 diabetes and dyslipidemia. If these women would like to conceive, 5-10% weight loss is recommended. Weight is closely linked to the microbiota composition. With the stratification of the gut microbiome into enterotypes, obesity was observed to be tightly linked to the inflammation-associated dysbiotic Bacteroides2 (Bact2) enterotype. Recent research suggests that the gut microbiome, amongst others *Flavonifractor* spp., may influence the body's response to weight loss interventions.

Study design, size, duration (75/75): This was a single centre randomized controlled trial. Eligibility criteria were: age between 18 and 40 years, PCOS according to the Rotterdam criteria, at risk of metabolic syndrome according to the International Diabetes federation consensus definition, obesity, no oral contraceptive pill use, no pregnancy and no contra-indication for the use of GLP-1 agonist. Participants were randomized to GLP-1 agonist or inositol. Faecal samples were collected at baseline and after 4, 8 and 16 weeks.

Participants/materials, settings, methods (74/75): Twelve women initiated an intervention; 6 received GLP-1 agonist, liraglutide (Lira) and 6 inositol, Gynositol® (Ino).
Enterotyping was performed via Dirichlet-Multinomial-Mixture modelling. Quantitative microbiome profiles were obtained combining 16S rRNA gene amplicon sequencing with flow cytometry. Prevalence differences between groups were calculated with Fisher tests and prevalence changes during intervention were tested with logistic mixed-models. Weight loss differences between interventions and its association with (baseline-)microbiome composition were tested with linear mixed-models (R statistical software).

Main results (199/200): All participants had anovulatory cycles, 11 women had clinical and/or biochemical hyperandrogenism and 9 women had polycystic ovarian morphology. Median age and weight of participants was 29.5 years and 93.2 kg, respectively. At baseline, a higher prevalence of Bact2 was observed in participants as compared with females with similar age and weight from a population cohort in the same geographical region (n = 6/13; 46.2% vs. n = 38/203; 18.7%; P=0.028). In total 32 samples were collected; 5 patients had completed the study.
Mean weight change after 16 weeks was -5.43 kg (-5.4%; P<0.001) in the total cohort. Weight change after 16 weeks was greater with Lira than with Ino (-7.85 kg vs. -1.69 kg; P<0.05). Bact2 prevalence decreased over time, but not significantly (-21.2%, beta=-0.91, P=0.236). Greater baseline

quantitative abundances of *Bacteroides* spp. and *Flavonifractor* spp. were accompanied by higher weight loss over the course of the intervention (beta=-1.48, AdjP=0.059 and beta=-1.76, AdjP=0.056, respectively), particularly after Ino (Lira: beta =-0.68, *P*=0.059; beta=-0.25, *P*=0.461, respectively; Ino: beta=-2.30, *P*=0.022; beta=-2.50, *P*< 0.001, respectively). Increasing quantitative abundance of *Flavonifractor* spp. during treatment was associated with steeper weight loss (beta =-0.96, *P*=0.023), especially after Ino (Ino: beta=-2.24, *P*<.001 vs. Lira: beta=0.04, *P*=0.899).

Limitations (43/50): A considerable number of patients dropped out of the study, amongst whom two women stopped Lira treatment prematurely due to side-effects and two women who continued Ino treatment but initiated fertility treatment. Consequently, statistical testing was impacted due to the low sample number.

Wider implications (45/50): Our pilot study suggests a link between weight loss and (baseline-) microbiome composition and implies a role of the gut microbiome in further research on PCOS and obesity and in the development of individualized approaches of weight loss treatment in women with PCOS and obesity.

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