**Effect of hyperglycaemia on the lung microbiome of mouse and human.**

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The human inhales between 0.7 and 7000 bacterial colony forming units (CFU) every minute. The airway epithelium and the airway surface liquid (ASL), play a vital role in the defence against these inhaled organisms. Glucose concentration in the ASL is normally much lower than that of blood (approximately 12.5 times lower) and this may contribute to innate protection against the growth of pathogenic organisms whichcanutilise glucose for growth. However, ASL glucose rises when blood glucose concentration rises (e.g. diabetes) in both humans and animals. We therefore hypothesised that the microbial population of the lung, the microbiome, would change in the diabetic lung.

Seven-week-old female db/db and non-diabetic littermate controls were purchased from Envigo (UK). Mice were maintained in standard animal housing in a 12h light/dark cycle; water and rodent chow available *ad libitum* and allowed to acclimatise for three weeks before lung microbiome collection. Mice were terminated with an overdose of pentobarbital (0.2ml of 100mg/ml i.p.). Blood was collected for glucose measurement. Bronchoalveolar lavage (BAL) was performed and 1 mL of solution was used to extract bacterial DNA using QIAamp DNA Microbiome Kit (Qiagen). In addition, 14 sputum samples were acquired from individuals with Cystic Fibrosis (CF) and those with CF related Diabetes (CFRD) from the Manchester Allergy, Respiratory and Thoracic Surgery (ManARTS) Biobank with informed consent under the licence (IRAS ID 180280, REC reference 15/NW/0409). The V3-V4-region of the 16S rRNA gene was amplified and sequenced using 300 bp paired-end reads on the Illumina MiSeq platform. Bioinformatic analysis was performed using Mothur v1.39.5 as per the MiSeq SOP pipeline. After removing of contaminant sequence reads, downstream statistical analyses were performed using R statistical software.

The bacterial diversity in BAL samples was highly variable within and between diabetic and non-diabetic mice. Hyperglycaemia did not affect the a-diversity of the lung microbiome (Inverse Simpson rating). However, hyperglycaemia had a significant effect on the b-diversity of lung microbiome (analysed with AMOVA, p=0.011, n=9) with the microbiome from diabetic mice clustering together. In individuals with CF, there was a significant association between blood glucose concentration (indicated as HbA1c) and the α-diversity of the lung microbiome (p=0.0125, R2=0.2029, n=28) and the b-diversity (analysed with AMOVA, p=0.002, n=2). At the genus level, bacteria of genus *Staphylococcus* were more abundant in the normoglycaemic mice (n=9, p=0.019) and normoglycaemic CF p=0.024, n=28. *Corynebacterium* which are frequently found in the lung microbiome as commensal organisms were also more abundant in the normoglycaemic mouse lung (p=0.0018, n=9). The genus *Pseudomonas* were more abundant in diabetic mice (n=9, p=0.028) and CFRD (p=0.011, n=28). Azorhizophilus and Porphyromonas were also more abundant in CFRD (p=0.016 and p=0.004 respectively, n=28).

Taken together, these data indicate that sustained hyperglycaemia modifies the lung microbiome, decreasing the abundance of commensal bacteria and promoting the growth of glucose-utilising bacteria such as *Pseudomonas* which may include potential pathogenic species such as *P. aeruginosa.*

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