Microbial translocation in type 2 diabetes: when bacterial invaders overcome host defence in human obesity

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Metabolic endotoxemia, characterised by systemic influx of bacterial components, impairs lipid handling and glucoregulation.[1] Early preclinical studies showed that high fat diet feeding facilitates bacterial translocation to metabolic tissues coinciding with dendritic cell (DC)-mediated inflammation.[2] This process was aggravated in mice lacking either the canonical adaptor for inflammatory signalling pathways downstream of Toll-like receptor (TLR) families, MyD88[2] or the intracellular microbial pattern recognition receptor, NOD2.[3] The first conceptual evidence for bacterial translocation in human type 2 diabetes (T2D) was reported in 2011 when Amar et al showed that 16S rDNA gene content in blood was associated with future T2D risk.[4] These seminal papers pioneered the concept of bacterial translocation in metabolic diseases. Yet, it remains intensely debated whether bacterial translocation to extraintestinal tissues is a true phenomenon or a result of spurious data, as it is notoriously difficult to robustly distinguish biologically relevant bacterial DNA sequences in very low microbial biomass tissues from environmental contaminants.[5 6] Indeed, low bacteria:host cell ratio obviously increases the risk of contamination during surgical procedures, biobanking and wet-laboratory processing of tissue samples. This has revivified the tenet that internal organs are sterile and casted doubt on emerging reports of tissue microbial signatures in a number of diseases, arguing that such findings may be confounded by sample contamination.

In GUT, Massier *et al* challenge again the dogma of sterile internal organs using a strict contamination aware approach corroborating the presence of internal bacteria in 75 weight-matched individuals with morbid obesity.[7] The authors used 16S rRNA sequencing to reveal tissue-specific signatures that were consistent with the expected anatomical route of bacterial translocation across the intestinal barrier. They further showed that tissue-specific microbial profiles could be distinguished between body compartments, and, more importantly, between individuals with normoglycaemia versus T2D.[7] Indeed, tissues from T2D subjects exhibited an over-representation of gram-negative

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bacteria, particularly proteobacteria comprising a vast number of opportunistic pathogens, while normoglycaemia was characterised by an over-representation of gram-positive commensal bacteria. Massier *et al* further demonstrated the presence of intact bacteria in tissue compartments as revealed by catalysed reporter deposition-fluorescence in situ hybridization technique using eubacterial probes.[7] Bacterial load was further linked to the inflammatory tone and this was further substantiated by in vitro experiments where bacterial DNA was found to dose-dependently induce the expression of proinflammatory cytokines (tumour necrosis factor α, interleukin-6 (IL-6) and IL-1) in adipocytes.[7] The findings of tissue-specific microbial signatures, and that T2D status dictates bacterial tissue compartmentalisation in human obesity are consistent with the recent report of Anhê *et al*[8] who also found microbial signatures in adipose tissues and additionally in the liver of morbidly obese persons, using a methodological benchmark approach controlling for potential contamination of biological samples at every step of the process. The fact that very similar results could be duplicated using biological samples from geographically separated cohorts differing in genetics, culture and dietary habits, provide strong new evidence that bacterial tissue compartmentalisation is a factual and reproducible biological trait in human obesity. They also provide compelling evidence that microbial translocation in human obesity is influenced by T2D status.

The underlying mechanism of bacterial translocation in T2D remains elusive and is likely the result of a combination of highly complex and multifaceted factors acting in synergy to orchestrate gut leakiness. One such factor may be disturbed antimicrobial host defence which appears to be a common denominator underlying bacterial translocation, metabolic inflammation and T2D. Indeed, mice lacking NOD2 exhibited increased bacterial translocation and metabolic inflammation,[3] whereas frameshift mutations in NOD2 is linked to compromised secretion of human alpha defensin 5 (HD5),[9] an antimicrobial peptide (AMP) that was recently found to mitigate dyslipidaemia and insulin resistance in diet-induced obese mice.[10] In line with the hypothesis that a perturbed antimicrobial host defence may contribute to bacterial tissue compartmentalisation in obesity and T2D, a recent metaproteomic study revealed substantial alterations in a plethora of AMPs, including HD5, in faecal samples of prediabetic and T2D individuals, which were inversely correlated with severity of dysglycaemia.[11] Similarly, TLR signalling, which mitigate bacterial translocation,[2] is required for human beta defensin 2 to curb DC-mediated inflammation.[12] There is an opportunity for both Anhê *et al*[8] and Massier *et al*[7] to investigate such mechanisms using faecal and tissue biopsies from their cohorts of morbidly obese persons undergoing bariatric surgery. They could examine the transcriptome and/or proteome of faecal, intestinal and bacterially loaded metabolic tissue samples to reveal if dysglycaemia can be linked to altered expression of AMPs and frameshift mutations in extracellular and intracellular pattern recognition receptors in these body compartments. Such future studies would significantly advance our understanding of the underlying mechanism of bacterial translocation. Another important but challenging consideration for future studies is to include lean individuals in order to determine whether bacterial translocation is a trait of obesity per se or mainly driven by T2D status. We critically need to advance knowledge on how translocated live bacteria or bacterial components promote or respond to dysglycaemia.

Another key implication of these findings is that bacterial encroachment may increase the risk for infection by other opportunistic pathogens in patients with T2D. This would not be restricted to bacteria as many viruses also use intestinal adhesion molecules as a corridor for cellular entry. A prime example of this phenomenon includes the current COVID-19 pandemic to which patients with T2D exhibit augmented susceptibility.[13] The hallmark host receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binding and internalisation, ACE2, is expressed in ileum and colon, hence allowing for gastrointestinal entry.[14] Early studies reported increased gastrointestinal symptoms in patients with COVID-19.[15] This patient group also appears to present more severe symptoms, including higher rates of liver injury.[16] Still, more work is needed to understand if and how SARS-CoV-2 enters the gut and may potentially translocate to adipose and hepatic tissues and if these processes are over-represented in patients suffering from T2D compared with their normoglycaemic counterparts.

In conclusion, complementary observations from intercontinental cohorts, using similar benchmark and contamination aware analytical methods, reliably establish that bacterial translocation is an important trait of human obesity and T2D. Future studies are warranted to elucidate the extent of which such translocation is a cause or a consequence of dysglycaemia, how it affects secondary infections and, alternatively, if normoglyacemic subjects with morbid obesity harbours protective communities alleviating or delaying onset of clinical T2D.

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