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Narrative Review

From Obesity to Diabetes: Uncovering Molecular Connections Sketching *Proteomics*, A Narrative Review

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ABSTRACT

Background: Recent research has illuminated the pivotal role of low-grade inflammation in metabolic diseases such as obesity and diabetes, with gut microbiota dysbiosis playing a key role. *Metaproteomics* offers a novel lens to explore these interactions by profiling protein compositions in microbial communities, revealing insights into the metabolic disruptions that accompany these conditions.

Objective: This study aims to compile and analyze existing metaproteomic research related to obesity and both types of diabetes, identifying specific metaproteomic alterations that correlate with these metabolic diseases.

Methods: Adhering to PRISMA guidelines, a rigorous selection process was employed to gather relevant studies on the *metaproteomics* of obesity and diabetes. This involved analyzing microbial and human protein alterations, focusing on patterns consistently observed in these diseases.

Results: Analysis identified unique metaproteomic signatures associated with obesity and diabetes, highlighting both microbial and human proteins. Specifically, alterations in proteins involved in carbohydrate metabolism and inflammation were recurrent. Despite the identification of up- or down-regulated proteins across studies, the limited breadth of comprehensive metaproteomic data restricts definitive conclusions.

Conclusion: *Metaproteomics* has shed light on the intricate metabolic disturbances in obesity and diabetes. However, the discipline remains nascent, necessitating further development of specialized databases and standardized methodologies to deepen our understanding and treatment of these complex diseases.

Keywords: Diabetes, Gut microbiota, Metabolic diseases, *Metaproteomics*, Obesity, *Proteomics*.

INTRODUCTION

The intricate interplay between gut microbiota and human health has emerged as a critical area of medical research, shedding light on the molecular mechanisms underlying various metabolic diseases, including type 1 and type 2 diabetes (T1D and T2D) as well as obesity. These conditions are marked by significant metabolic disruptions, often featuring dysbiosis of gut microbiota—a key factor contributing to their onset and progression. Studies have linked variations in microbial pattern abundance, particularly an imbalance in the Bacteroidetes/Firmicutes ratio, to the production of short-chain fatty acids (SCFAs) like butyrate, propionate, and acetate. Such imbalances may compromise gut epithelial integrity, increase permeability, and disturb immune homeostasis and inflammatory responses, underscoring the role of specific microbial communities in saccharolytic, proteolytic, and lipolytic metabolisms, as well as their influence on enzymatic pathways (1, 3).

While shotgun 16S rRNA marker gene sequencing has provided valuable insights into human microbiota communities, it falls short of capturing the microbiome's plasticity, particularly its adaptation to changing niche conditions (4). In contrast, *metaproteomics* offers a more nuanced understanding by elucidating microbial constituents, their interactions with the gastrointestinal (GI)

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microbiota and the host proteome, signal transduction, and metabolic pathways. This approach enables the detection of functional shifts in microbial and human protein profiles, facilitating the identification of novel diagnostic targets and disease biomarkers (5).

Obesity and diabetes mellitus, characterized by intertwined inflammation across various tissues and organs, present elevated markers of inflammation, such as *C-reactive protein* (*CRP*) in T1D progression, alongside increased production of interleukin (IL)-1β and superoxide radicals by monocytes. These findings highlight the inflammatory underpinnings of insulin resistance in T2D, with obesity serving as a precursor. Inflammatory *cytokines* (*TNF-α, IL-1β, IL-6*), alongside adipocyte-derived metabolites, play critical roles in this process (6).

By leveraging *metaproteomics* in conjunction with other meta-omics approaches, researchers can gain a comprehensive snapshot of the protein landscape in specific health conditions, aiding in the understanding of complex biological processes. However, the field faces methodological challenges, including the management of vast data volumes and the need for standardized analysis protocols to ensure comparability across studies. Addressing these challenges is essential for uncovering the associations between gut microbiota functional variations and obesity/diabetes states (7).

Recent initiatives, such as the Joint Programming Initiative Knowledge Platform-INtesTInalMICrobiomics (JPI *KP-INTIMIC*), have begun to collate meta-data from observational studies on gut microbiomics, enabling federated individual-level meta-analyses through DataSHIELD. This approach promises a more nuanced understanding of the human gut microbial consortium, potentially standardizing omics data across studies (8, 9).

In conclusion, this narrative review aims to critically evaluate the application of *metaproteomics* in studying the onset and progression of diabetes and obesity over the past eleven years. By doing so, it seeks to delineate the molecular connections between gut microbiota and these metabolic diseases, paving the way for the development of targeted interventions. The objective is to synthesize the findings from these studies, offering a standardized framework that enhances our understanding of the complex interactions at play, thereby contributing to improved disease management and therapeutic strategies.

MATERIAL AND METHODS

In accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2015 guidelines, this narrative review adopted a meticulous and selective search strategy across electronic databases such as PubMed and Scholar. The objective was to identify studies that delve into the application of *metaproteomics* for investigating alterations in the human and microbial metaproteome among individuals afflicted with diabetes and obesity. Search terms employed encompassed "*metaproteomics*," "human," "gut microbiota," "obesity," and "diabetes." Specifically, the search strings crafted for PubMed included variations of these terms, namely "(*metaproteomics* OR metaproteomic OR *proteomic*) AND human AND (gut microbiota OR microbiome) AND (obesity OR diabetes)," with an analogous approach adopted for Scholar. This careful selection aimed to retrieve studies pertinent to the review's focus.

During the screening phase, articles were rigorously evaluated for their relevance and eligibility based on predetermined criteria, adhering to the PECOS format (Patients-Exposure-Control-Outcomes-Study design). This approach facilitated the inclusion of studies encompassing patients of any age and gender diagnosed with obesity or Type 1 or Type 2 Diabetes, at various disease stages, and juxtaposing their metaproteome against that of healthy control groups. The primary objective centered on uncovering protein variations associated with these metabolic conditions and elucidating the interaction between microbiota proteins and their metabolic pathways. Furthermore, the review sought to comprehend the functional modifications within gut microbiota in the context of obesity and diabetes, through metaproteomic analysis, either in isolation or in conjunction with other omics technologies.

Inclusion criteria were strictly confined to original observational studies that employed *metaproteomics* to explore the linkage between disease states and gut microbiota, without imposing restrictions on study size or participant demographics. Conversely, studies focusing on animal models, in vitro research, or comprising non-original articles (such as reviews) were systematically excluded. The selection process was diligently executed by two independent reviewers who screened titles and full texts subsequent to de-duplication, with any arising discrepancies resolved through consultation with a third party. This exhaustive process was designed to encompass all relevant studies employing a metaproteomic approach to probe into the association between disease conditions and gut microbiota.

From each included study, data were meticulously extracted, covering aspects such as authors and year, cohort size and composition, participant demographics (sex, age, and country), study design, study scope, applied omics techniques, and noted limitations. To

critically appraise the included studies, the Newcastle-Ottawa Quality Assessment Scale was utilized. This tool evaluates three domains—selection, comparability, and outcomes—allowing for a star-based rating system where a study could garner up to nine stars in total: a maximum of four stars for selection, two for comparability, and three for outcomes. Studies manifesting a high risk of bias, evidenced by the absence of stars in certain domains, were excluded. Given the scarcity of data from the selected studies, the evidence regarding the diversity of GI microbial taxa and their correlation with the progression of metabolic diseases was narratively reported, precluding the possibility of conducting quantitative analyses. This methodological approach underscores the rigorous and systematic effort to synthesize existing knowledge on the interplay between gut microbiota and metabolic diseases through the lens of *metaproteomics*.

Table 1. Baseline characteristics of the included studies and Newcastle-Ottawa Quality Assessment Scale (NOS).

Authors and Year	Size Sample and Characteristics	Subjects Characteristics (Sex, Age, Country)	Scope of Study	Study Design	Metaproteomics Techniques Used	Other "Omics" Techniques Used	Limitations	NOS Score
Zhong et al., 2019	254 subjects: 77 TN-T2D, 80 Pre-DM, and 97 NGT	Suzhou, China 173 females 81 males Age: 41–86	Investigate compositional and functional changes of the gut microbiota to characterize different disease stages	Cross-sectional	iTRAQ-coupled-LC-MS/MS	Metagenomics	MS-based proteomics. Confounding variables: age, drugs (CCB, hypertension, and dyslipidemia), diet, and health conditions.	7
Ferrer et al., 2013	2 subjects: 1 lean, 1 obese	Spain 1 female (lean) and 1 male (obese) Age: 15	Identify and analyze active bacterial members and proteins expressed in lean and obese microbiota	Case-control	1D-gel electrophoresis and UPLC-LTQ Orbitrap-MS/MS	Metagenetics	No information about dietary intake.	7
Kolmeder et al., 2015	29 subjects: 9 lean, 4 overweight, 16 obese	Spain 21 females 8 males Age: 23.1 ± 2.2 (non-obese); 38.6 ± 2.4 (obese)	Characterization of non-obese and obese fecal metaproteome	Case-control	1D-gel electrophoresis RP-HPLC online coupled to MS/MS		Regular medication between obese and non-obese group.	6
Sanchez-Carrillo et al., 2020	40 severely obese adults subjected to BS	Spain Age: 47–60	Investigation the impact of bariatric surgery	Longitudinal study (3 months)	LC-ESI-MS/MS	Metabolomics	Results biased for using pooling strategy.	6
Zhou et al., 2019	106 subjects: healthy and pre-diabetic adults	Stanford, California 55 females and 51 males Age: 25–75	Understand how healthy individuals and those at risk of T2D, change over time, in response to perturbations and in relation to different molecules and microorganism	Longitudinal study (4 years)	SWATH-MS	Metagenomics, metatranscriptomics, and metabolomics	Limited studies of microbial changes. No information about diet and exercise. Heterogeneous data.	6

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Table 2. Summary of metaproteome variation

Authors and Year	Disease	Protein Origin	↑ Proteins	↓ Proteins	Metabolic Pathway/Functionality
Gavin et al., 2018	T1DM	Microbial	1. Enzymes for mucin degradation 2. Elongation factor 3. Ferredoxin reductase	4. Transferases (butyrate synthesis)	3. ↑Ferredoxin catabolism 4. ↑ Butyrate anabolism
		Human	1. Galectin-3 2. Fibrillin	3. CELA-3A, 4. CUZD1 5. CLCA1 6. Neutral ceraminidase 7. IGHA1	6. ↓ Sphingosine (SPH) and sphingosine 1-phosphate (S1P) 3.4.5. ↓ exocrine pancreas functionality 7. ↓ IgA
Pinto et al., 2017	T1DM	Microbial	1. ilvE (BCAA transaminase) 2. Glutamate dehydrogenase (AA degradation) 3. Bifunctional GMP synthase 4. Glutamine amido transferase 5. Chaperonin GroEL	6. Phosphoketolas 7. Glyceraldehyde-3-phosphate dehydrogenase, 8. Transketolase	1.6.8. ↓ Via penthos phosphate → ↑ BCAA synthesis (Shikmic Acid Pathway) ↓ glycolysis 2.↑ NH4+ (Urea) 7. ↓ Glycolysis →↓ Piruvate ↓ SCFAs
		Human	1. Galectin-3 2. Fibrillin	3. CELA-3A, 4. CUZD1 5. CLCA1 6. Neutral ceraminidase 7. IGHA1	6. ↓ Sphingosine (SPH) and sphingosine 1-phosphate (S1P) 3.4.5. ↓ exocrine pancreas functionality 7. ↓ IgA
Pinto et al., 2017	T1DM	Microbial	1. ilvE (BCAA transaminase) 2. Glutamate dehydrogenase (AA degradation) 3. Bifunctional GMP synthase 4. Glutamine amido transferase 5. Chaperonin GroEL	6. Phosphoketolas 7. Glyceraldehyde-3-phosphate dehydrogenase, 8. Transketolase	1.6.8. ↓ Via penthos phosphate → ↑ BCAA synthesis (Shikmic Acid Pathway) ↓ glycolysis 2.↑ NH4+ (Urea) 7. ↓ Glycolysis →↓ Piruvate ↓ SCFAs
		Human	MUC2 precursor	CELA-3A	↑ Intestinal mucin-2 ↓ Exocrine pancreas functionality
Heintz et al., 2016	T1DM	Microbial		Thiamine synthesis Co-factor	↓ Thiamine synthesis
		Human		↓ AMY2A, AMY2B, CPA1, and CUDZ1	↓ Complex sugar degradation
Singh et al., 2017	T1DM	Human urinary proteome	1. LGR1 2. CD14 3. CPE 4. CTSB 5. CTSD 6. NAGA	7. Fibronectin-1 8. Pancreatic α-amylase 9. MUC1 10. PTPRN	1. ↑Inflammatory pathways (TGF-β) 3.↑AA degradation (↑urea production) 8. ↓ Exocrine pancreas functionality and ↓ complex sugar metabolism
				oxidoreductase	transport of sugar in

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RESULTS

The comprehensive search initially identified 4,970 records, ultimately refining this pool to include 20 studies of paramount relevance, as detailed in the review's tables. Spanning from 2017 to 2023, these studies provided insights into the human proteins analyzed from fecal, plasma, and urinary samples. The methodologies employed varied, comprising four case-control, three longitudinal, and three prospective studies that included cross-sectional analyses. Among these, a notable four-year comprehensive prospective study by Zhou et al. stood out, offering an in-depth exploration of changes in the transcriptome, metabolome, serum *cytokine* levels, proteome, and gastrointestinal microbiome pertinent to the early stages of Type 2 Diabetes (32).

Mass spectrometry (MS)-based *metaproteomics* served as the technological foundation for all included studies, with three further augmenting their analysis by integrating gel-free technology alongside either one-dimensional or two-dimensional electrophoresis (1DE and 2DE). The study sizes varied significantly, with three investigations focusing on fewer than 20 participants. A particularly insightful observational study by Ferrer et al. concentrated on a comparative analysis between an obese and a lean subject, while the majority of the studies examined larger cohorts, some of which included over 100 participants (14).

A notable finding across several studies was the significant variation in peptides derived from proteins involved in C5 and C6 carbohydrate metabolism, such as enolase, ribulokinase, xylulokinase, phosphoketolase, and a specific glycoside hydrolase, which were found to be more abundant in non-obese individuals. Conversely, obese subjects exhibited an increased abundance of proteins involved in starch and pectin metabolism, including *glucosidase* and *pectate lyase*. Moreover, research by Hernandez et al. (2013) revealed that the metabolism of fructose, mannose, galactose, and sucrose was upregulated in obese cohorts, evidenced by a heightened total sugar metabolism and an increased activity of glycosidase in stool samples from obese individuals compared to lean counterparts (31).

Additionally, significant alterations in the expression of proteins associated with metabolic derangement, intestinal damage, and a chronic inflammatory state, such as *alkaline phosphatase*, serpins, and α-amylase, were more pronounced in obese patients than in healthy individuals or individuals post-bariatric surgery. Sanchez-Carrillo et al. (2021) also reported variations in protein expression, finding *ferritin* and *ferrous transport protein* present in lean adults while absent in those with severe obesity (17).

Regarding Type 1 Diabetes (T1D), the microbial metaproteome variations were attributed to taxa such as Eubacterium, Faecalibacterium, and Bacteroides, linked mainly to amino acids transport and metabolism, protein turnover, and chaperone activities. The presence of branched-chain amino acid transaminase and glutamate dehydrogenase enzymes was notably higher in T1D subjects, implicating their roles in amino acid transport and metabolism. Furthermore, T1D patients showed increased expression of mucin-2 and a reduction in elastase 3A expression, indicating an elevated synthesis of mucin for gastrointestinal epithelium protection and a diminished exocrine pancreas functionality, respectively.

In the study by Zhong et al. (2019), the focus was on the compositional and functional changes of gut microbiota across pre-diabetic, treatment-naïve T2D, and healthy individuals, uncovering a decrease in pancreatic enzymes in pre-DM and TN-T2D groups, suggesting impaired exocrine pancreas functionality. A significant number of microbial and human proteins associated with pre-DM were identified, with an enrichment in microbial protein modules involved in sugar phosphotransferase systems (PTS), ATP-binding cassette (ABC) transporter of amino acids, and bacterial secretion systems observed in pre-DM compared to normal glucose transport (NGT) individuals (33).

Alterations in human protein production were also highlighted among the different groups, including the exclusive detection of the trimethylamine-N-oxide producing enzyme in the TN-T2D group, alongside a loss of rasGTPase-activating-like protein and unconventional myosin-Ic, implicating disruptions in insulin signaling. Integrating proteins, *cytokine*s, and metabolites data, pathways associated with defense responses, such as interleukin signaling pathways, mTOR signaling, and B and T cell receptor signaling, were identified. Moreover, inflammatory pathways during viral infections were found to be differently altered in insulin-resistant participants compared to insulin-sensitive individuals, suggesting modifications in defense responses.

DISCUSSION

This narrative review aimed to synthesize the latest scientific findings in the realm of *metaproteomics*, offering a comprehensive view of the functional changes brought about by the interactions between gut microbiota and the host in metabolic diseases. The studies selected for this review underscored significant variations in protein expression linked to health conditions, predominantly related to carbohydrate metabolism and inflammation response, indicating the profound impact of host-microbiota interplay in pathological states. This interplay has implications for the activation of peripheral blood mononuclear cells, *cytokine* release, and the production of inflammation-related serum peptides and metabolites(10-14).

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One of the salient points brought forth by the review is the role of gut microbiota in fermenting unabsorbed carbohydrates in obese individuals, leading to an increased uptake of bioavailable short-chain fatty acids (SCFAs) and, consequently, an additional energy source(15,16). This phenomenon aligns with the augmented energy bioavailability in the obese gut, as evidenced by the elevated expression of proteins involved in the refinement of carbohydrates and starch digestion(17,18). Such conditions not only contribute to increased SCFA production, mainly consisting of propionate and butyrate, but also underscore the difference in the metabolic landscape between obese and non-obese subjects (19-21,29,30).

Furthermore, the review highlighted how *metaproteomics* could unravel variations in vitamin production within the gut metaproteome (22), particularly noting the presence of microbial *cobaltochelatases* involved in vitamin B12 synthesis in obese individuals(23-26). This finding ties into broader metabolic processes, including the metabolism of carbohydrates and the inflammation-linked proteins, shedding light on the nuanced metabolic profiles of obese compared to non-obese subjects (27,28).

Despite these insights, the review also acknowledges certain limitations. The field of *metaproteomics*, while promising, has been explored in a relatively limited number of studies (11). The lack of comparability and heterogeneity across study designs hampers the ability to draw conclusive patient- or population-based conclusions. This calls for the development of ad hoc *metaproteomics* databases and standardized analysis protocols to facilitate the comparison of *metaproteomics* data across different studies (24,25).

Strengths of this review include its pioneering effort to consolidate findings from the nascent field of *metaproteomics*, particularly in relation to diabetes and obesity, and its systematic approach following PRISMA guidelines to ensure the relevance and quality of included studies. Moreover, the review's emphasis on the integration of *metaproteomics* with other omics technologies marks a significant stride towards a holistic understanding of the metabolic diseases in question (27).

CONCLUSION

Concluding, this review represents a foundational step towards elucidating the complex interactions between gut microbiota and the host in metabolic diseases through the lens of *metaproteomics*. Future prospects in this field appear promising, especially with the potential for *metaproteomics* to identify specific biomarkers for early diagnosis and targeted therapeutic interventions. However, the path forward necessitates a concerted effort to overcome current limitations through standardized methodologies, enhanced comparability, and the establishment of dedicated databases for pathologic data and related metadata. Such advancements will be crucial in harnessing the full potential of *metaproteomics* to provide deeper insights into the functional proteomic profiles underpinning metabolic diseases and their low-grade inflammatory pathologies.

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