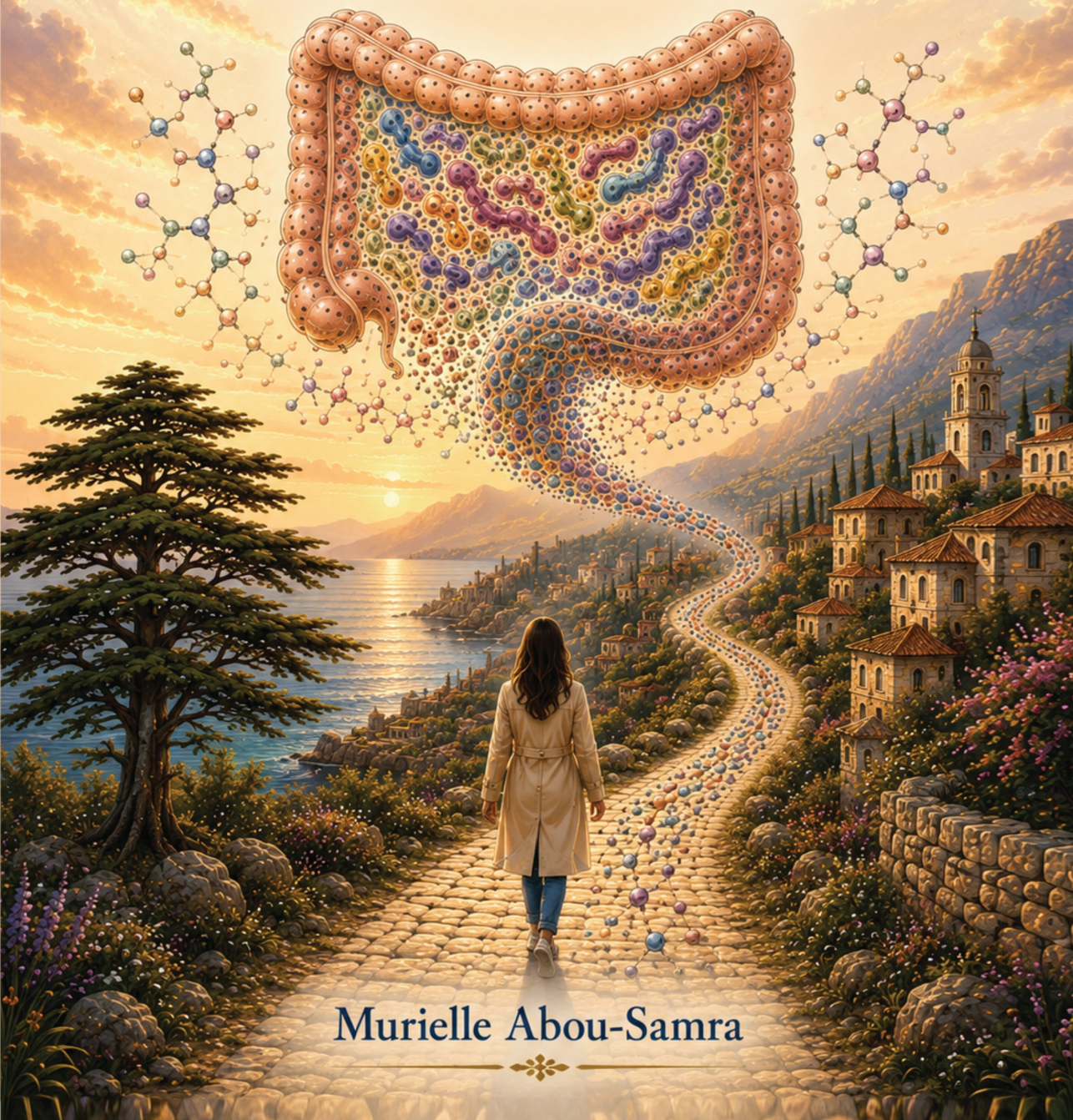
Gut Microbiota and Gut-Derived Peptide Hormones in Obesity

Insights from Middle Eastern Populations



Murielle Abou-Samra

Propositions belonging to the thesis

Gut Microbiota and Gut-Derived Peptide Hormones in Obesity:
Insights from Middle Eastern Populations
By Murielle Abou-Samra

1. Obesity is a heterogeneous disease, and individuals with similar body mass index may exhibit substantially different metabolic and endocrine profiles. (This thesis)

2. Alterations in gut microbiota composition are associated with obesity, but no single microbial signature can universally characterize obesity across populations. (This thesis)

3. Associations between specific bacterial taxa and gut-derived peptide hormones support the concept that microbial and endocrine pathways are interconnected components of metabolic regulation. (This thesis)

4. Population-specific differences in gut microbiota composition highlight the importance of including underrepresented populations in microbiome research rather than extrapolating findings from Western cohorts. (This thesis)

5. Elevated fasting concentrations of anorexigenic hormones in obesity may reflect compensatory physiological responses rather than effective appetite regulation. (This thesis)

6. Understanding microbiota–gut-derived peptide hormone associations may contribute to a more personalized approach to obesity prevention and management. (This thesis)

7. Future obesity research should focus on functional microbial activity and host–microbe interactions rather than taxonomic composition alone.

8. Improving metabolic health requires addressing not only individual behaviors but also the environmental and societal factors that promote obesity.

9. Balancing family life, professional responsibilities, and doctoral research is not a barrier to success; it is often what makes success meaningful.

10. Every clinical recommendation should be preceded by one question: Why?

11. "The good physician treats the disease; the great physician treats the patient who has the disease."— William Osler

Gut Microbiota and Gut-Derived Peptide Hormones in Obesity
Insights from Middle Eastern Populations

Murielle Abou-Samra

2026

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Gut Microbiota and Gut-Derived Peptide Hormones in Obesity
Insights from Middle Eastern Populations

Dissertation

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CHAPTER 1



Chapter 1 – General Introduction

1. Obesity as a Global and Heterogeneous Metabolic Disease

Obesity is a chronic, relapsing metabolic disorder characterized by excessive accumulation of adipose tissue that increases the risk of a wide range of cardiometabolic complications, including insulin resistance, type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), and non-alcoholic fatty liver disease (NAFLD) (Blüher, 2019; Schulze & Stefan, 2024). Over the past several decades, the global prevalence of obesity has risen at an unprecedented rate, becoming one of the most pressing public health challenges of the 21st century. According to the World Health Organization (2024), more than one billion people worldwide are currently living with obesity, while projections from the World Obesity Federation estimate that global obesity prevalence will continue to rise substantially over the coming decades (World Health Organization, 2024; World Obesity Federation, 2025). Long term forecasts from the Global Burden of Disease (GBD) BMI Collaborators further suggest that by 2050, approximately 60% of adults and one third of children and adolescents worldwide may be overweight or obese (Institute for Health Metrics and Evaluation, 2025).The burden of obesity is particularly pronounced in the Middle East and North Africa (MENA) region, where prevalence rates exceed 25–30% in several countries, including Lebanon and the United Arab Emirates (Musaiger, 2011a; World Health Organization, 2016; World Health Organization Regional Office for the Eastern Mediterranean, 2019). Rapid urbanization, transitions toward energy-dense Westernized dietary patterns, reduced physical activity, and sociocultural determinants have collectively contributed to this rise (Musaiger, 2011a; Popkin et al., 2012). Importantly, these environmental and lifestyle changes have occurred over a relatively short period, suggesting that biological systems regulating energy balance may be challenged by rapid external shifts, thereby promoting metabolic dysregulation (Hall et al., 2022; Popkin et al., 2012).

Despite its high prevalence, obesity is increasingly recognized not as a uniform condition, but as a biologically heterogeneous and multifactorial disease (Blüher, 2019). Individuals with similar body mass index (BMI) may exhibit differences in adipose tissue function and body fat distribution metabolic health, including differences in insulin sensitivity, lipid metabolism, inflammatory status, , and cardiovascular risk (Schulze & Stefan, 2024; Smith et al., 2019; Blüher, 2019) . This heterogeneity highlights the limitations of BMI as a sole indicator of metabolic risk and underscores the need to identify

underlying biological mechanisms that differentiate obesity phenotypes (Blüher, 2019; Schulze & Stefan, 2024).

Traditionally, obesity has been conceptualized as a consequence of chronic positive energy balance, whereby energy intake exceeds energy expenditure (Hall et al., 2022). Although this framework remains fundamental, it does not adequately explain the substantial inter-individual variability observed in metabolic responses to excess caloric intake. Increasing evidence suggests that obesity arises from complex interactions between genetic susceptibility, environmental exposures, endocrine signaling pathways, immune responses, adipose tissue remodeling, and intestinal microbial communities (Cani, 2018; Lynch & Pedersen, 2016; Turnbaugh et al., 2006). These interconnected systems form a dynamic regulatory network that governs energy homeostasis and metabolic health (Cani, 2018; Lynch & Pedersen, 2016).

Among these systems, the gut–brain–endocrine axis and the gut microbiota have emerged as critical regulators of metabolic function and energy balance. These systems not only respond to nutritional inputs but also actively modulate host physiology through hormonal signaling, immune interactions, and metabolic pathways (Blaak et al., 2020; Gribble & Reimann, 2019). Importantly, both endocrine responses and microbial composition exhibit substantial inter-individual variability, suggesting that they may contribute to the heterogeneity observed in obesity phenotypes.

Understanding obesity within this integrative framework represents a shift from a purely energy-centric model toward a systems-based perspective of metabolic regulation (Cani, 2018; Cryan et al., 2019). This conceptual transition provides the foundation for investigating how interactions between gut microbiota and endocrine signaling pathways influence appetite regulation, energy homeostasis, and metabolic outcomes. Such an approach is particularly relevant in underrepresented populations, where environmental, dietary, and genetic factors may uniquely shape these regulatory systems.

2. Regulation of Energy Homeostasis: The Gut–Brain–Endocrine Axis

Energy homeostasis is maintained through a highly coordinated and dynamic interaction between peripheral metabolic tissues and the central nervous system, with the hypothalamus serving as the principal integrative center (Morton et al., 2014; Schwartz et al., 2017). This regulatory system integrates neural, hormonal, and nutrient-derived signals

to modulate appetite, satiety, energy expenditure, and glucose metabolism (Schwartz et al., 2017). Signals originating from the gastrointestinal tract, adipose tissue, pancreas, and liver are continuously relayed to the brain, where they are processed to maintain energy balance in response to both short term nutritional status and long term energy stores (Morton et al., 2014; Schwartz et al., 2017).

The gastrointestinal tract functions not only as a digestive organ but also as a major endocrine system through specialized enteroendocrine cells distributed along the intestinal epithelium (Gribble & Reimann, 2019). These cells sense luminal nutrients and secrete a range of peptide hormones that play a central role in the regulation of appetite and metabolic homeostasis (Gribble & Reimann, 2019; Miller, 2019). Among these, ghrelin, glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), peptide YY (PYY), and cholecystokinin (CCK) represent key mediators of short-term energy regulation (Holst, 2007; Miller, 2019).

Ghrelin, predominantly secreted by the stomach during fasting, is the only well established circulating orexigenic hormone and acts to stimulate hunger, increase food intake, and promote energy storage (Cummings et al., 2002; Muller et al., 2015). In contrast, GLP-1 and PYY are anorexigenic hormones released postprandially from enteroendocrine L-cells located primarily in the distal small intestine and colon. These hormones promote satiety, delay gastric emptying, and contribute to reduced caloric intake (Holst, 2007). GLP-1 also functions as an incretin hormone, enhancing glucose-dependent insulin secretion and thereby playing a critical role in glycemic regulation (Drucker, 2018; Holst, 2007). CCK, secreted from I-cells in the proximal small intestine in response to dietary fats and proteins, contributes to meal termination through vagal afferent signaling pathways (Miller, 2019).

In addition to these short-term regulators, long term energy balance is modulated by hormones such as leptin and insulin. Leptin, produced by adipocytes, reflects energy stores and acts centrally to suppress appetite and increase energy expenditure (Friedman & Halaas, 1998). However, in individuals with obesity, elevated circulating leptin levels are often accompanied by leptin resistance, resulting in impaired central signaling and diminished satiety responses (Friedman & Halaas, 1998; Munzberg et al., 2020). Similarly, insulin exerts central effects on appetite regulation and peripheral effects on glucose

metabolism, with insulin resistance representing a hallmark feature of metabolic dysfunction in obesity (Kahn et al., 2006).

Dysregulation of these hormonal pathways is a defining feature of obesity and contributes to impaired appetite control and metabolic imbalance (Blüher, 2019; Schwartz et al., 2017). However, alterations in circulating peptide concentrations are not uniform across individuals with obesity. Variability in fasting and postprandial concentrations of peptides such as ghrelin, GLP-1, and PYY suggests complex dysregulation of enteroendocrine signaling pathways, which may either contribute to, or arise as a consequence of, obesity related metabolic disturbances (Gribble & Reimann, 2019; Miller, 2019). These findings indicate that multiple biological factors, including nutrient availability, neural signaling, inflammatory pathways, and gut microbial activity, may influence appetite regulation and metabolic outcomes (Cryan et al., 2019; Miller, 2019). Evidence from human studies, including findings in Lebanese populations, demonstrates that obesity is associated with distinct alterations in circulating peptide profiles. Individuals with obesity have been shown to exhibit elevated leptin, insulin, and insulin resistance markers, alongside reduced ghrelin concentrations, reflecting disrupted hunger–satiety signaling (Abou-Samra et al., 2022). Furthermore, anorexigenic peptides such as GLP-1 and PYY have been reported to correlate positively with markers of adiposity and insulin resistance, suggesting a potential compensatory endocrine response to metabolic dysfunction (Abou-Samra et al., 2022). However, the direction and magnitude of these associations vary across studies, highlighting the complexity and context dependency of endocrine regulation in obesity.

Collectively, these observations indicate that the gut–brain–endocrine axis is not merely a passive responder to energy imbalance but an active regulatory system influenced by additional biological factors (Schwartz et al., 2017). Increasing attention has therefore focused on identifying upstream modulators of enteroendocrine signaling, including the gut microbiota, which may influence hormone secretion through metabolic, neural, and immune-mediated pathways.

Understanding the factors that modulate gut peptide secretion and signaling is essential for elucidating mechanisms underlying appetite dysregulation and metabolic heterogeneity in obesity. This is particularly relevant given the clinical success of therapies targeting this axis, such as GLP-1 receptor agonists, which have demonstrated substantial

efficacy in weight reduction and glycemic control (Wilding et al., 2021). However, variability in treatment response further underscores the need to identify biological determinants that influence endocrine signaling pathways.

Within this context, the gut microbiota has emerged as a potential upstream regulator of enteroendocrine function, providing a mechanistic link between diet, intestinal physiology, and systemic metabolic regulation (Blaak et al., 2020; Cani, 2018). This interaction forms part of a broader microbiota–gut–brain axis that integrates microbial metabolism with host endocrine and neural signaling pathways.

3. The Gut Microbiota in Metabolic Regulation

The human gastrointestinal tract harbors a highly complex and dynamic microbial ecosystem composed of trillions of microorganisms, including bacteria, archaea, viruses, and fungi, collectively referred to as the gut microbiota (Lynch & Pedersen, 2016). This microbial community possesses a gene repertoire that substantially exceeds that of the human host, enabling a wide range of metabolic functions that are essential for host physiology (Qin et al., 2010; Sender et al., 2016). Through its metabolic activity, the gut microbiota contributes to nutrient metabolism, maintenance of intestinal barrier integrity, modulation of immune responses, and regulation of host energy balance (Rinninella et al., 2019).

At the taxonomic level, the gut microbiota is dominated by a limited number of bacterial phyla, primarily *Bacillota* (formerly *Firmicutes*) and *Bacteroidota* (formerly *Bacteroidetes*), with additional contributions from *Actinomycetota, Pseudomonadota*, and *Verrucomicrobiota* (Oren & Garrity, 2021; Rinninella et al., 2019). While these phyla constitute the majority of the microbial community, substantial inter-individual variability exists at lower taxonomic levels, particularly at the genus and species levels, where functional differences are more pronounced (Lynch & Pedersen, 2016).

One of the primary mechanisms through which the gut microbiota influences host metabolism is the fermentation of nondigestible dietary components, such as dietary fibers, into bioactive metabolites, including short chain fatty acids (SCFAs) such as acetate, propionate, and butyrate (Canfora et al., 2015). These metabolites serve not only as energy substrates but also as signaling molecules that regulate glucose metabolism, lipid

homeostasis, inflammation, and intestinal barrier function (Blaak et al., 2020; Canfora et al., 2019). In addition, microbial metabolites interact with host receptors and endocrine pathways, thereby linking intestinal microbial activity to systemic metabolic regulation (Canfora et al., 2019).

Alterations in gut microbiota composition and function—commonly referred to as dysbiosis—have been associated with obesity and related metabolic disorders (Cani, 2018; Turnbaugh et al., 2006). Human studies have demonstrated that obesity is often associated with reduced microbial diversity, altered abundance of bacterial taxa involved in SCFA production, and changes in microbial metabolic pathways linked to inflammation and energy harvest (Canfora et al., 2019; Cotillard et al., 2013; Le Chatelier et al., 2013). In addition, obesity associated microbial profiles have been characterized by reduced abundance of butyrate producing bacteria and microbial signatures linked to impaired metabolic health (Pinart et al., 2021; Tilg et al., 2020). Collectively, these findings suggest that obesity related dysbiosis may contribute to metabolic dysfunction through alterations in microbial metabolism, intestinal barrier integrity, inflammatory signaling, and host energy homeostasis.

Early studies suggested that obesity may be characterized by shifts in the relative abundance of *Bacillota* and *Bacteroidota*, often described as an increased *Bacillota* to *Bacteroidota* ratio (Turnbaugh et al., 2006). However, subsequent human studies have produced inconsistent findings, with reports of increased, decreased, or unchanged ratios across populations (Magne et al., 2020; Sze & Schloss, 2016). These inconsistencies likely reflect differences in diet, environmental exposures, host genetics, and methodological approaches, highlighting the limitations of relying on phylum level metrics to characterize obesity associated microbial patterns (Magne et al., 2020; Sze & Schloss, 2016). Consequently, current research has shifted toward identifying specific bacterial taxa and functional signatures associated with metabolic health, rather than focusing on broad taxonomic ratios (Pinart et al., 2021). This approach recognizes that metabolic effects of the microbiota are driven by functional capabilities—such as SCFA production, bile acid metabolism, and inflammatory signaling—rather than taxonomic composition alone (Canfora et al., 2019; Pinart et al., 2021).

Evidence from human studies, including research conducted in Lebanese populations, supports the presence of distinct microbial signatures associated with obesity.

In a cross-sectional analysis of Lebanese adults, individuals with obesity exhibited reduced microbial diversity compared to normal weight (NW) counterparts, alongside differential abundance of specific bacterial genera (Abou-Samra et al., 2025). Notably, *Lachnoclostridium* was enriched in individuals with obesity, whereas several genera associated with SCFA production and metabolic health—including *Peptococcus*, *Ruminococcus_2*, members of the *Ruminococcaceae* family, and *Oxalobacter*—were more abundant in NW individuals (Abou-Samra et al., 2025). These taxa are known to contribute to anti-inflammatory processes and improved metabolic function, suggesting a potential protective role against obesity related metabolic disturbances (Blaak et al., 2020; Canfora et al., 2019).

Importantly, these findings highlight that obesity is not associated with a single universal microbial signature (Magne et al., 2020; Sze & Schloss, 2016), but rather with context dependent alterations in microbial communities, influenced by population specific factors such as diet, lifestyle, and environmental exposures (Deschasaux et al., 2018; Rothschild et al., 2018). This is particularly relevant in Middle Eastern populations, where dietary patterns and lifestyle behaviors differ substantially from those observed in Western cohorts (Musaiger, 2011a; Popkin et al., 2012).

Beyond compositional differences, emerging evidence suggests that the metabolic impact of the gut microbiota is mediated through its interaction with host physiological systems (Canfora et al., 2019). Microbial metabolites have been shown to influence insulin sensitivity, lipid metabolism, and systemic inflammation, thereby contributing to the development and progression of metabolic diseases (Canfora et al., 2019). However, the precise mechanisms through which these microbial signals are integrated into host metabolic regulation remain incompletely understood.

Increasingly, attention has focused on the interaction between the gut microbiota and endocrine signaling pathways, particularly those involved in appetite regulation and energy homeostasis. The gut microbiota may influence host metabolism not only through direct metabolic effects but also through modulation of gut-derived peptide hormones (Cryan et al., 2019; Gribble & Reimann, 2019), thereby linking microbial composition to central appetite regulation.

Taken together, these findings position the gut microbiota as a key regulator within a broader metabolic network that integrates dietary inputs, microbial metabolism,

and host physiological responses (Cani, 2018; Cryan et al., 2019). Understanding how microbial composition and function contribute to metabolic regulation is therefore essential for elucidating the biological mechanisms underlying obesity and its heterogeneity.

4. Gut Microbiota–Peptide Crosstalk in Metabolic Regulation

Beyond their independent roles in metabolic regulation, the gut microbiota and the gut–brain–endocrine axis are increasingly recognized as components of an integrated and bidirectional signaling network that influences appetite control, energy homeostasis, and metabolic health. This interaction, often referred to as the microbiota–gut–brain axis, reflects continuous communication between intestinal microorganisms, enteroendocrine cells, and central regulatory pathways (Cryan et al., 2019; Gribble & Reimann, 2019).

A key mechanism underlying this crosstalk involves the production of microbial metabolites, particularly SCFAs, which are generated through fermentation of dietary fibers by gut bacteria. SCFAs—including acetate, propionate, and butyrate—act as signaling molecules that directly influence enteroendocrine cell activity (Blaak et al., 2020; Canfora et al., 2015). These metabolites stimulate the secretion of anorexigenic gut hormones such as GLP-1 and PYY through activation of G protein–coupled receptors, specifically free fatty acid receptor 2 (FFAR2) and FFAR3, expressed on enteroendocrine L-cells (Kimura et al., 2013; Tolhurst et al., 2012). In addition to SCFAs, the gut microbiota extensively modifies bile acid composition through the conversion of primary bile acids into secondary bile acids, including deoxycholic acid and lithocholic acid. These secondary bile acids can activate Takeda G protein coupled receptor 5 (TGR5) expressed on enteroendocrine L-cells, thereby stimulating GLP-1 and GLP-2 secretion (Canfora et al., 2019). Microbial bile acid metabolism may also influence signaling through the farnesoid X receptor (FXR), a nuclear receptor involved in bile acid homeostasis and metabolic regulation, which has been linked to indirect modulation of GLP-1 secretion and glucose metabolism (Canfora et al., 2019). Through these pathways, the gut microbiota can influence satiety signaling, insulin secretion, and glucose homeostasis (Canfora et al., 2015; Canfora et al., 2019).

Other microbial derived metabolites—including lipopolysaccharides, and indole derivatives—have also been implicated in modulating endocrine and metabolic pathways (Canfora et al., 2019; Cani et al., 2008). These metabolites may influence gut barrier integrity, systemic inflammation, and endocrine signaling pathways (Canfora et al., 2019;

Cani et al., 2008). Collectively, these findings suggest that the gut microbiota acts as a metabolic interface between diet and host physiology (Canfora et al., 2019; Cani, 2018), translating dietary inputs into endocrine signals that regulate energy balance.

Conversely, gut-derived peptide hormones can influence the intestinal environment and, indirectly, microbial composition. Hormones such as GLP-1 and PYY regulate gastrointestinal motility and nutrient transit, thereby altering substrate availability for microbial fermentation (Gribble & Reimann, 2019). By slowing gastric emptying and intestinal transit, these hormones may modify the duration and location of nutrient exposure within the gastrointestinal tract, which can influence microbial growth dynamics and metabolic activity. Changes in intestinal transit time and nutrient availability may therefore shape microbial community structure and function, highlighting the bidirectional nature of microbiota–endocrine interactions (Cryan et al., 2019; Gribble & Reimann, 2019). Despite strong mechanistic evidence from animal models, human studies integrating gut microbiota composition with circulating gut peptide profiles remain limited (Canfora et al., 2019; Cryan et al., 2019). Emerging data from population-based studies (Ahmad et al., 2023; Ali Ahmad et al., 2026; Zeevi et al., 2015), including those conducted in Lebanese cohorts, provide initial evidence supporting the existence of microbiota–peptide interactions in humans. In these studies, individuals with obesity exhibited both altered microbial composition and distinct fasting gut peptide profiles, suggesting coordinated dysregulation of these systems (Abou-Samra et al., 2022, 2025).

More specifically, recent findings demonstrate that obesity is characterized by elevated fasting concentrations of anorexigenic peptides such as GLP-1 and PYY, alongside reduced ghrelin levels, reflecting altered appetite signaling (Abou-Samra et al., 2022). At the same time, obesity associated microbial profiles include enrichment of taxa such as *Lachnoclostridium* and reduced abundance of SCFA producing genera, including members of the *Ruminococcaceae* family and *Peptococcus* (Abou-Samra et al., 2025). These compositional changes may influence microbial metabolite production, thereby altering enteroendocrine signaling pathways (Cryan et al., 2019; Gribble & Reimann, 2019).

Importantly, integrative analyses examining associations between microbial taxa and circulating peptide concentrations reveal significant relationships between specific bacterial genera and gut hormones. In particular, inverse associations have been observed

between SCFA production related bacterial taxa—such as *Butyricimonas*, *Alistipes*, *Parasutterella*, and members of the *Ruminococcaceae* family—and anorexigenic peptides including GLP-1, GLP-2, and PYY (Abou-Samra et al., 2026, submitted). These findings suggest that reduced abundance of metabolically beneficial bacteria may be linked to altered peptide signaling, potentially reflecting disruptions in microbiota driven endocrine regulation (Canfora et al., 2019).

The directionality of these associations remains complex and may reflect compensatory physiological responses. For instance, elevated fasting GLP-1 and PYY concentrations in individuals with obesity may represent an adaptive response to chronic energy excess or reduced hormone sensitivity, rather than a direct reflection of enhanced satiety signaling (Miller, 2019). Similarly, reduced ghrelin levels in obesity may indicate altered regulatory setpoints rather than decreased orexigenic drive (Holst, 2007; Muller et al., 2015). These observations underscore the importance of considering both microbial and endocrine factors within a dynamic regulatory framework.

Collectively, these interactions support the concept that gut microbiota, microbial metabolites, and gut-derived peptide hormones form an integrated regulatory network influencing appetite regulation, energy balance, and metabolic homeostasis (Cani, 2018; Cryan et al., 2019). A conceptual overview of these interconnected pathways is presented in Figure 1, illustrating how microbial metabolites interact with enteroendocrine cells to modulate gut peptide secretion and how these signals are subsequently integrated at central and peripheral levels to regulate metabolic function.

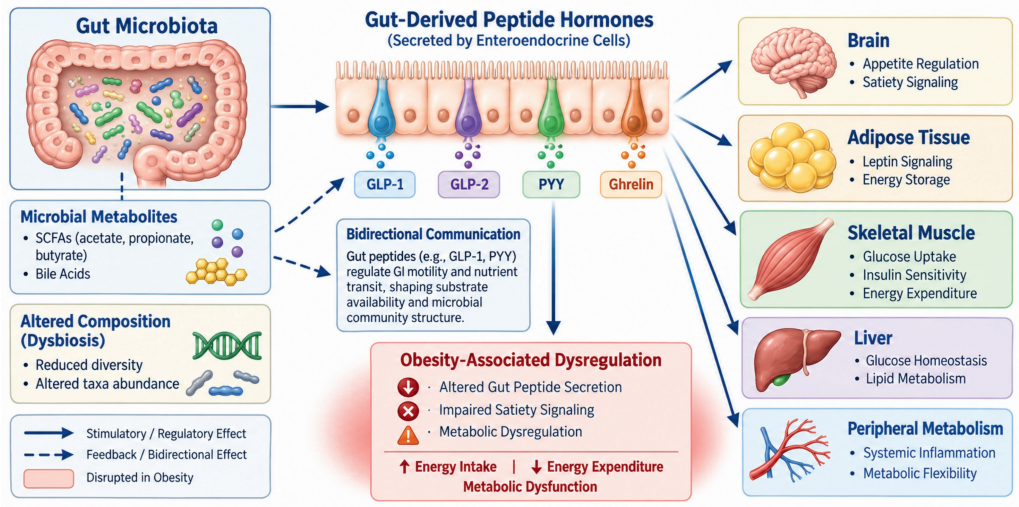

Figure 1. Conceptual framework of gut microbiota–peptide–metabolic interactions in obesity. Gut microbial metabolites, including short chain fatty acids and bile acids, influence enteroendocrine cells and modulate secretion of gut-derived peptide hormones. These hormones subsequently act on central and peripheral tissues, including the brain, adipose tissue, liver, and skeletal muscle, to regulate appetite, insulin sensitivity, glucose homeostasis, lipid metabolism, and energy expenditure. Obesity associated dysregulation may impair these signaling pathways and contribute to metabolic dysfunction.

Taken together, current evidence supports the concept that gut microbiota–peptide crosstalk contributes to the regulation of appetite and metabolic homeostasis, and that disruption of this interaction may play a role in the development and maintenance of obesity (Canfora et al., 2019; Cani, 2018). However, the precise mechanisms underlying these interactions remain incompletely understood, particularly in human populations and within diverse ethnic contexts.

Understanding microbiota–peptide interactions is therefore essential for elucidating the biological basis of metabolic heterogeneity in obesity. This integrated perspective may also provide novel targets for therapeutic intervention, including microbiota modulating strategies and hormone based treatments aimed at restoring metabolic balance (Drucker, 2018; Wilding et al., 2021).

5. Diet as a Shared Driver of Gut Microbiota and Endocrine Signaling

Diet is one of the most influential and modifiable determinants of both gut microbiota composition and host metabolic regulation (Sonnenburg & Bäckhed, 2016; Zhernakova et

al., 2016). Nutritional intake shapes the structure and function of the gut microbiota by altering substrate availability for microbial fermentation, thereby influencing the production of bioactive metabolites such as SCFAs, bile acids, and other microbial derived compounds (David et al., 2014; Sonnenburg & Bäckhed, 2016). These metabolites, in turn, play a critical role in modulating host physiological processes, including energy balance, glucose metabolism, immune responses, and enteroendocrine signaling (Blaak et al., 2020; Canfora et al., 2019).

Dietary patterns characterized by high intake of fiber rich plant based foods promote microbial diversity and the enrichment of SCFA producing bacteria, which are generally associated with beneficial metabolic effects (De Filippo et al., 2010; Makki et al., 2018). In contrast, Westernized dietary patterns—typically high in saturated fats, refined carbohydrates, and low in dietary fiber—have been associated with reduced microbial diversity, increased abundance of pro-inflammatory taxa, and impaired metabolic function (Cani, 2018; Tilg et al., 2020). These dietary influences are particularly relevant in regions undergoing rapid nutritional transition, such as the Middle East, where shifts toward energy dense diets have paralleled rising obesity prevalence (Musaiger, 2011a; Popkin et al., 2012).

Importantly, diet not only influences microbial composition but also interacts with endocrine pathways regulating appetite and metabolism (Cryan et al., 2019; Gribble & Reimann, 2019). Nutrient ingestion directly stimulates enteroendocrine cells to release gut-derived peptide hormones, while microbial metabolites modulate this process through receptor mediated signaling mechanisms (Blaak et al., 2020; Gribble & Reimann, 2019). For example, SCFAs derived from fiber fermentation enhance GLP-1 and PYY secretion, linking dietary intake, microbial metabolism, and satiety regulation (Canfora et al., 2015; Tolhurst et al., 2012).

These interactions position diet as a central modulator of the microbiota–gut–brain axis, influencing both microbial ecology and endocrine signaling (Cryan et al., 2019; Sonnenburg & Bäckhed, 2016). Variability in habitual dietary intake may therefore contribute to inter-individual differences in microbiota composition and gut peptide responses, potentially influencing susceptibility to obesity and metabolic dysfunction (Zeevi et al., 2015; Zhernakova et al., 2016).

Given the strong influence of diet on both microbial and endocrine systems, understanding population specific dietary patterns is essential when interpreting microbiota–metabolic interactions. This is particularly relevant in Middle Eastern populations, where traditional dietary patterns coexist with increasing Westernization, creating unique nutritional exposures that may differentially shape metabolic outcomes (Deschasaux et al., 2018; Popkin et al., 2012).

6. Population Specific Considerations in the Middle Eastern Context

The composition and function of the gut microbiota vary substantially across populations due to differences in diet, lifestyle, environmental exposures, cultural practices, and host genetics (Deschasaux et al., 2018; Rothschild et al., 2018). While host genetic factors contribute to microbial variability, environmental influences—particularly diet—are considered the dominant determinants of microbiota composition at the population level (Rothschild et al., 2018).

To date, the majority of large scale microbiome studies have been conducted in Western populations, limiting the generalizability of findings to other regions with distinct environmental and cultural contexts (Gupta et al., 2017; Rothschild et al., 2018). The Middle East represents a particularly important yet underrepresented region in microbiome research (Deschasaux et al., 2018; Gupta et al., 2017). Countries in this region have undergone rapid economic and nutritional transitions, characterized by increased consumption of processed foods, reduced physical activity, and rising rates of obesity and metabolic disease (Musaiger, 2011a; Popkin et al., 2012).

Despite these trends, microbiome data from Middle Eastern populations remain limited, and region-specific microbial reference profiles are not well established (Deschasaux et al., 2018; Gupta et al., 2017). This lack of representation in global datasets may obscure important population specific microbial signatures associated with health and disease (Rothschild et al., 2018).

Emerging evidence suggests that even within the Middle East, substantial variability exists between populations (Deschasaux et al., 2018; Rothschild et al., 2018). Comparative analyses of Lebanese and Emirati individuals with obesity have demonstrated differences in microbial diversity and composition, influenced by nationality, dietary intake, and demographic factors (Ali Ahmad et al., 2026). Lebanese individuals have been

reported to exhibit higher microbial diversity and distinct taxonomic profiles compared to Emirati counterparts, highlighting the importance of considering population specific factors when interpreting microbiome data (Ali Ahmad et al., 2026; Rothschild et al., 2018).

Furthermore, studies conducted in Lebanese cohorts have identified specific microbial signatures associated with obesity, as well as distinct gut peptide profiles and microbiota–peptide associations (Abou-Samra et al., 2022, 2025). These findings underscore the importance of investigating microbiota–endocrine interactions within regionally relevant populations, as conclusions drawn from Western cohorts may not fully capture the complexity of metabolic regulation in other settings (Gupta et al., 2017; Rothschild et al., 2018).

Understanding these population specific differences is critical for the development of targeted and culturally appropriate strategies for obesity prevention and management (Musaiger, 2011a; Popkin et al., 2012). It also highlights the need for integrative studies that examine microbiota composition, endocrine signaling, and metabolic outcomes within the same well characterized populations.

7. Aim and Objectives of the Thesis

The overall aim of this thesis was to investigate the role of gut microbiota and gut-derived peptide hormones in the context of obesity and metabolic regulation, with a particular focus on their interaction within Middle Eastern populations.

To achieve this aim, the specific objectives were:

1. To examine the association between circulating gut peptide hormones and markers of obesity, glycemia, and lipid metabolism in Lebanese individuals.
2. To characterize the gut microbiota composition of Lebanese normal weight individuals and individuals with obesity and identify bacterial taxa associated with metabolic health.
3. To investigate associations between gut microbiota and circulating gut peptide hormones within the same cohort, with the aim of identifying potential microbiota–peptide interactions contributing to metabolic regulation in obesity.
4. To explore differences in gut microbiota composition in individuals with obesity across Middle Eastern populations (Lebanese and Emirati adults) and assess the influence of nationality and demographic factors on microbial variation.

8. Gap Analysis and Dissertation Overview

Despite substantial advances in understanding the roles of gut microbiota and endocrine signaling in metabolic regulation (Cani, 2018; Cryan et al., 2019), important gaps remain in the literature, particularly regarding their integrated role in human obesity. Although numerous studies have independently examined gut microbiota composition or circulating gut peptide hormones, relatively few have investigated these systems simultaneously within the same human cohort (Canfora et al., 2019). Consequently, the relationship between microbial composition and gut peptide signaling remains insufficiently understood in humans.

In addition, much of the mechanistic evidence linking microbial metabolites to enteroendocrine function has been derived from animal models (Cryan et al., 2019). While these studies provide valuable insights into causal pathways, their translation to human physiology remains limited. Integrative human studies combining microbiome profiling with endocrine measurements are still scarce, particularly in well characterized populations with detailed anthropometric and metabolic phenotyping.

Furthermore, existing research is disproportionately focused on Western populations (Gupta et al., 2017; Rothschild et al., 2018), limiting the generalizability of findings to regions with distinct environmental and cultural contexts. Given that gut microbiota composition is strongly influenced by dietary patterns, lifestyle behaviors, and environmental exposures (Rothschild et al., 2018), the Middle East—characterized by rapid nutritional transition and rising obesity prevalence—represents an important yet underrepresented setting for investigating microbiota–metabolic interactions.

Finally, obesity is increasingly recognized as a heterogeneous condition (Blüher, 2019; Schulze & Stefan, 2024), yet the biological mechanisms underlying inter-individual variability in metabolic responses remain poorly defined. Interactions between gut microbiota and gut-derived peptide hormones may contribute to this heterogeneity, but these relationships have not been comprehensively characterized in human populations.

To address these gaps, the present thesis adopts an integrative approach combining endocrine profiling and microbiome analysis within well defined cohorts of Middle Eastern adults. Figure 2 provides an overview of the study design and analytical framework used throughout the dissertation. By examining gut peptide concentrations, microbial composition, and their associations within the same individuals, this work aims to provide a

more comprehensive understanding of the biological mechanisms underlying obesity and its metabolic variability.

This thesis is structured into seven chapters, each addressing a specific component of the relationship between gut microbiota, gut-derived peptide hormones, and metabolic regulation in obesity.

Chapter 2 examines circulating concentrations of gut-derived peptide hormones and their associations with cardiometabolic risk markers in Lebanese adults with obesity compared to NW controls. Particular emphasis is placed on ghrelin, GLP-1, GLP-2, PYY, CCK, and leptin, and their relationships with adiposity, glycemic parameters, insulin resistance, and lipid profile.

Chapter 3 characterizes gut microbiota composition and diversity in lean and obese Lebanese adults using 16S rRNA gene based amplicon sequencing. This chapter identifies obesity associated microbial signatures and evaluates differences in alpha and beta diversity within this population.

Chapter 4 integrates gut microbiota profiling with circulating gut peptide measurements to explore microbiota–peptide associations in Lebanese adults. The analysis evaluates whether specific bacterial taxa are associated with fasting concentrations of appetite regulating hormones and determines whether these relationships persist after adjustment for adiposity and metabolic confounders.

Chapter 5 extends the investigation to a cross-population comparison between Lebanese and Emirati adults with obesity. This chapter examines nationality associated differences in microbial composition and diversity, and evaluates the influence of dietary intake and demographic factors on microbial variability.

Chapter 6 provides a general discussion in which the findings from all studies are synthesized and interpreted in the context of existing literature. Methodological considerations, strengths and limitations, and implications for future research are discussed.

Chapter 7 discusses the impact and valorization of the research, highlighting the clinical and societal relevance of microbiota–endocrine interactions and their potential application in obesity prevention and management.

Finally, the thesis concludes with a summary of the main findings, followed by acknowledgments.

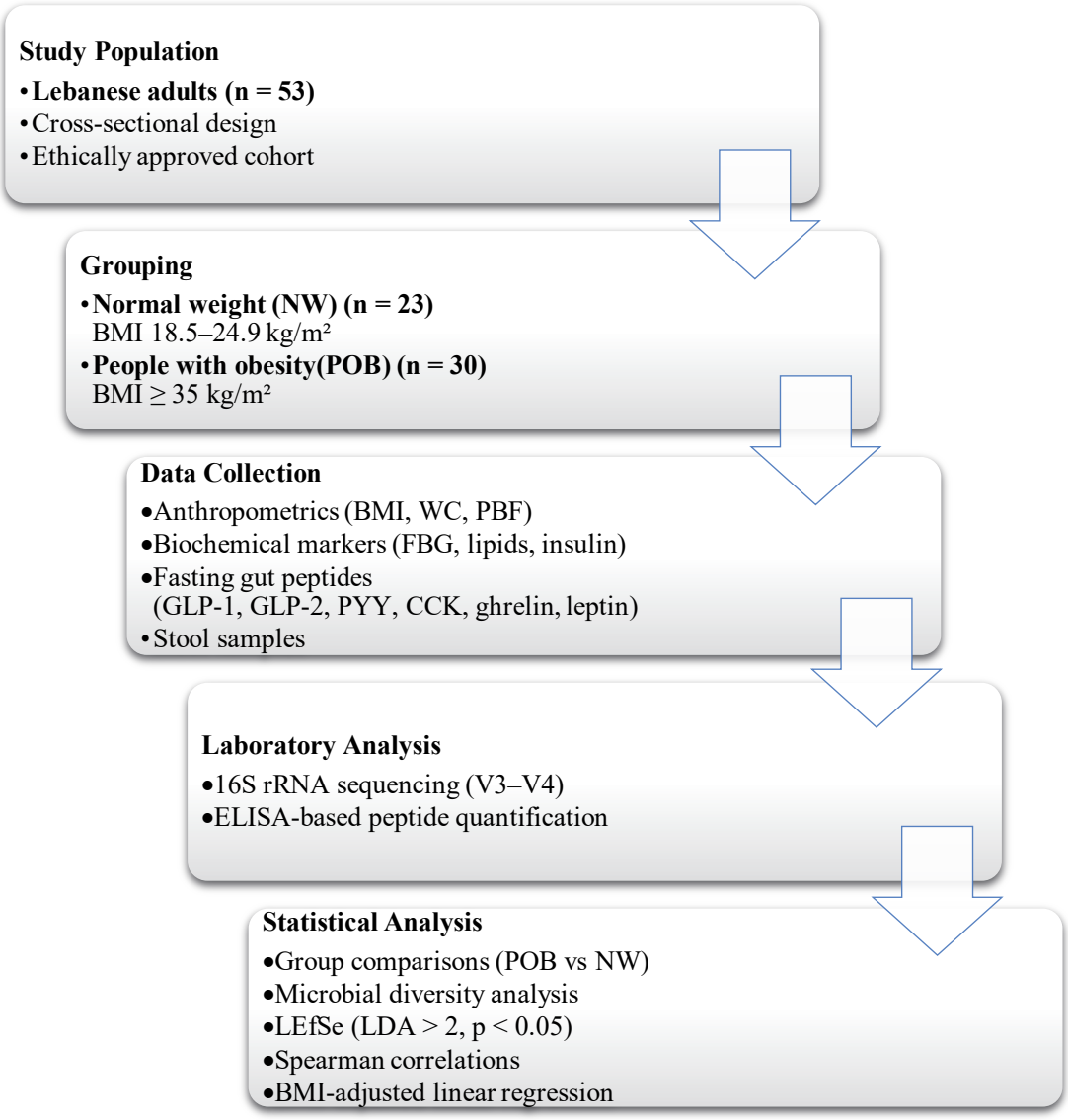

Figure 2. Overview of the study design and analytical framework of the dissertation.

This figure illustrates the cross-sectional study conducted in Lebanese adults, including participant grouping based on body mass index [BMI] (normal weight [NW] *vs*. people with obesity [POB]), data collection procedures, laboratory analyses, and statistical approaches. Anthropometric, biochemical, gut peptide, and microbiota data were integrated to examine associations between gut microbial composition, endocrine signaling, and metabolic outcomes in obesity. Abbreviations: BMI = body mass index; NW = normal weight; POB = people with obesity; WC = waist circumference; PBF = percent body fat; FBG = fasting blood glucose; GLP-1 = glucagon-like peptide-1; GLP-2 = glucagon-like peptide-2; PYY = peptide YY; CCK = cholecystokinin; ELISA = enzyme-linked immunosorbent assay; LEfSe = linear discriminant analysis effect size; LDA = linear discriminant analysis.

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CHAPTER 2



Chapter 2

The Association of Peptide Hormones with Glycemia, Dyslipidemia, and Obesity in Lebanese Individuals

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Abstract: Peptide-hormones, including pancreatic peptide-YY(PYY), glucagon-like peptide-1 (GLP-1), cholecystokinin (CCK), insulin, and leptin function as satiety signals, while ghrelin promotes hunger. These hormones are also involved in glucose homeostasis and body-weight regulation. The aim of this cross-sectional analysis was to examine the association of these peptide-hormones with obesity-markers, insulin-resistance, and dyslipidemia (total-cholesterol (TC), low-density-lipoprotein-cholesterol (LDL-C), high-density-lipoprotein-cholesterol (HDL-C), triglyceride (TG)). Sixteen-obese (OB) adults and 21 normal-weight (NW) age-and gender-matched counterparts were recruited. OB-participants showed significantly higher levels of leptin, insulin, Homeostatic-Model Assessment of Insulin Resistance (HOMA-IR), and TG. NW participants had significantly higher levels of ghrelin. GLP-1 was positively correlated with insulin, HOMA-IR, and obesity-markers except percent body fat. Leptin was positively correlated with all markers (except glucose and dyslipidemia). PYY was positively correlated with BMI, insulin and HOMA-IR. Ghrelin was inversely correlated with all of the markers except glucose, TC, and LDL-C. In the regression analysis model, leptin was positively associated with obesity markers and insulin resistance. Our results indicate a significant difference in peptide hormones among OB and NW Lebanese individuals. Since there is controversial evidence regarding body-weight and peptide-hormones in the literature, this study highlights a step forward towards finding ethnic based strategies to treat obesity and its consequences.
Keywords: obesity; insulin resistance; glucagon-like peptide-1; leptin; ghrelin; pancreatic peptide YY; cholecystokinin; dyslipidemia

1. Introduction

Obesity has become an epidemic, whereby currently overweight/obesity poses more health risks and mortality than underweight/undernourishment [1]. Obesity is a complex condition that is caused by an imbalance between energy intake and energy expenditure, and is defined as a body mass index (BMI) greater than 30 kg/m^2 [2]. Its prevalence is on an ever increasing rise; 42.7% of adults in the United States are obese [3], 30% to 70% in the Eastern Mediterranean region (EMR) [4], 35% in Saudi Arabia followed by 27.8% in United Arab Emirates [5], 27% in Lebanon and 11.7% in Italy [6]. The provision of obesity care has proved challenging in the EMR countries, in particular Lebanon. These challenges include lack of awareness of lifestyle change strategies for obesity treatment among the medical community and overdependence on bariatric surgery [7].

Appetite and energy homeostasis are regulated in humans through the interaction between the central nervous system, in particular the hypothalamus, and peripheral hormones [1, 2, 8, 9]. The hypothalamus regulates energy homeostasis by integrating signals from peptide hormones in the periphery in response to a fluctuation in nutritional status, to modify food intake and metabolic rate [9]. These peripheral hormones are categorized into short term and long-term effectors. The short-term signals regulate energy balance through certain peptide hormones that are released from enteroendocrine cells during fasting or feeding [9]. These gut hormones are divided into appetite/orexigenic and satiety/anorexigenic hormones. Ghrelin is the only potent orexigenic hormone that is released during fasting from the stomach, initiates feeding, and regulates meal frequency [2, 10]. The short term anorexigenic gut peptides, such as cholecystokinin (CCK), pancreatic peptide YY (PYY), glucagon like peptide-1 (GLP-1), and glucagon-like peptide-2 (GLP-2), induce satiety and meal termination [2]. As for the long-term satiety peripheral signals, leptin is secreted by adipose tissue, and insulin by the pancreas can cross the blood–brain barrier to reach the arcuate nucleus in the hypothalamus to control appetite. [1, 2, 8, 11, 12]. Their plasma levels are proportional to body fat; the more fat the higher their level is in circulation [1, 2]. Several lines of research have shown that imbalances in gut hormones are found in those who are overweight or obese, and this is believed to have an effect on the development of insulin resistance, type 2 diabetes, and cardiovascular disease [11-13].

To date and to our knowledge, with the high obesity prevalence among the EMR populations, and in particular Lebanon, there are no studies evaluating the role of these peptide hormones on body weight, glycemia, and lipid profile in Lebanese Individuals. Thus, the aim of our study was to assess the association of peptide hormones with respect to obesity markers and glycemia and lipid profile among normal weight (NW) and obese (OB) Lebanese individuals residing in Lebanon.

2. Materials and Methods

2.1. Study Design

This was a cross sectional study, conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Middle East Institute of Health University Hospital (MEIH-UH) Lebanon (AL-04-02/2018-IRB/MEIH).

2.2. Study Population

The study was conducted at the MEIH-UH between November 2019 and April 2021. Data were retrieved from a larger study focusing on gut microbiota composition as primary outcome profiling the change in the *Firmicutes/Bacteriodetes* ratio pre and post bariatric surgery. Thus, power sample calculations were based on the findings of Damms-Machado et al. (2015) where the mean (SD) *Firmicutes/Bacteriodetes* ratio changed significantly from 5.9 ± 2.1 to 10.4 ± 1.4 in 3 months post surgery [14]. To generate a similar significant effect of a sample size of 2 patients was estimated to achieve 80% power at a 5% alpha [15]. Expecting a drop out effect size in this protocol, we multiplied the number of required patients by 5. Thus, a sample size of 10 Lebanese participants per group was determined to be adequate. Eligibility criteria included stable body weight, free of antibiotic use (last 3 months), adult participants (18–60 years old) from both sexes. The eligibility criteria were assessed by using a screening questionnaire addressing ethnicity, general health, weight fluctuation, alcohol consumption, physical activity, medication, antibiotic and supplement use. Consenting participants with a Body Mass Index (BMI) of ≥35 kg/m^2 were assigned to the obese (OB) study group; afterwards, normal weight (NW) individuals with a BMI of 18.5–24.9 kg/m^2 matched for age and sex with their OB counterparts were assigned to the NW/ control group. A 24-h food diary was given to the subjects and was filled over three days to evaluate energy intake. Exclusion criteria were weight loss ≥5% in last 3 months, pregnancy, ethanol consumption of > ½ ounce (OZ)/day for females and 1 OZ/day for

males [16], patients on medical treatment for diabetes, dyslipidemia and hypertension, and unwilling to provide written consent. OB subjects were recruited from the weight loss program of MEIH-UH by staff over a period of 2 years until sample saturation was achieved. As for the NW subjects, they were recruited from the general community in Lebanon, using direct approach and by word of mouth. All participants gave their informed consent for inclusion prior to participating.

Thirty-seven eligible subjects (10 males, 27 females) consented to join the study. Sixteen were OB (4 males, 12 females) and 21 NW counterparts (6 males, 15 females). Eligible subjects arrived to the MEIH-UH after a 12 h fast and were asked to avoid alcohol consumption, as well as any unusual strenuous exercise 24 h before the screening. On site, venous blood samples were collected by a registered nurse and a total of three vacutainers tubes that contained Ethylenediaminetetraacetic acid (EDTA) were used and one Red Top Blood Collection Tube. Five ml blood was collected in the Red Top Blood Collection Tube for the analysis of glucose, insulin, total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and triglyceride (TG) at the Department of Pathology and Laboratory Medicine at MEIH-UH. In one of the EDTA tubes 20 µL Pefabloc (Sigma Aldrich, St. Louis, MO, USA; 100 mg/mL in distilled water) was added to stabilize ghrelin and in another tube 20 µL DPP IV inhibitor (Sigma Aldrich) was added to 2 mL of blood to prevent the breakdown of PYY. These samples were centrifuged for 15 min at 12,298 g at 3 °C; the separated plasma was stored in aliquots at −20 °C to be used later on for measuring the levels of GLP-1, GLP-2, PYY, CCK, ghrelin, and leptin.

2.3. Anthropometric Measurements

While subjects wore light clothes and no shoes, height and weight were measured using "SECA" balance and BMI was calculated as the ratio of weight (Kg) to height (m) squared (kg/m^2). Waist circumference (WC) (cm) was measured to the nearest 0.1 cm using a flexible, non-stretchable measuring tape at the umbilicus level midway between the lower ribs and the upper iliac crest while standing [17]. Normal WC according to the International Diabetes Federation (IDF) using Europids cut off values of <94 cm for males and <80 cm for females were used [17]. Waist to height ratio (WtHR) was calculated as the ratio of the

waist circumference divided by height (both in cm). A cut-off of 0.5 was used to define abdominal obesity, whereby a WtHR < 0.5 was considered as normal [18]. Body composition was assessed using the Inbody body composition analyzer 720 (InBody Co., Ltd., Seoul, Korea) which uses a segmental multifrequency bioelectrical impedance analysis technique to analyze the percent body fat (%BF) that produces small individual error and can be used as a substitute when dual-energy X-ray absorptiometry is not available [19, 20]. Cutoffs of ≥25% and ≥35% were used to define elevated %BF among men and women, respectively [21].

2.4. Biochemichal Measurements

2.4.1. Glycemia

Concentrations of fasting plasma glucose were determined via an enzymatic colorimetric method using the Roche/Hitachi Cobas® 6000 analyzer (Roche, Basel, Switzerland) at the Department of Pathology and Laboratory Medicine at MEIH-UH.

2.4.2. Insulin

The Department of Pathology and Laboratory Medicine at MEIH-UH determined the serum concentration of insulin via a sandwich ELISA principle using the Elecsys insulin kit (Roche, Basel, Switzerland) by using Roche/Hitachi Cobas® 6000 analyzer.

2.4.3. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR)

Insulin resistance was calculated using the HOMA index defined as (fasting immunoreactive insulin in microunits per milliliter (μU/mL) × fasting plasma glucose in milligrams per deciliter (mg/dL))/ 405 [22]. A cut off value of 2.32 was used as the value for healthy individuals [23].

2.4.4. Blood Lipids (Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C), High Density Lipoprotein Cholesterol (HDL-C), and Triglyceride (TG))

Concentrations of fasting blood lipids (TC, LDL-C, HDL-C, and TG) were determined via an enzymatic colorimetric method using the Roche/Hitachi Cobas® 6000 analyzer (Roche, Basel, Switzerland) at the Department of Pathology and Laboratory Medicine at MEIH-UH.

2.4.5. GLP-1, GLP-2, PYY, CCK, Ghrelin, Leptin, and Leptin/Ghrelin Ratio

Concentrations of serum GLP-1, GLP-2, PYY, ghrelin, and leptin were measured by the use of ELISA kits from Diametra Millipore (Billerica, MA, USA), following the manufacturer's instruction. The enzyme activity was measured spectrophotometrically by the Thermo Scientific™ Varioskan™ LUX multimode microplate reader (Thermofisher Scientific Inc., Waltham, MA, USA) set to 450 nm.

CCK was measured by use of ELISA kit from ABclonal Technology (Wuhan, China), following the manufacturer's instruction. Samples were measured using the same microplate reader set to 450 nm.

For leptin/ghrelin ratio, serum leptin concentrations were converted from ng/m to pg/mL by multiplying by the value with 1000 and then serum leptin level was divided by the serum ghrelin levels.

2.5. Statistical Analysis

All analyses were performed using the Statistical Package for the Social Sciences software version 20 (SPSS Inc. Chicago, IL, USA). The normality of the data was tested using the Skewness and Kurtosis. Normally distributed data are expressed as mean (M) ± standard deviation (SD), and skewed data as median (Mdn) and interquartile range (IQR). Depending on the data distribution differences between the groups were assessed using independent samples t-test for normally distributed continuous variables or nonparametric test using Mann–Whitney U-test for skewed continuous variables and Chi-square for categorical values. Correlations between the gut peptides (GLP-1, GLP-2, PYY, ghrelin, leptin and insulin), obesity markers (WC, %BF, BMI, WtHR), glucose homeostasis indicators (insulin, HOMA-IR, glucose) to check for insulin resistance, and lipid profile (TC, LDL-C, HDL-C, TG), were determined by Pearson's correlation analysis for normally distributed variables, and Spearman correlation for skewed variables. Binary logistic regression analyses were conducted using the forward method with categories of weight status, HOMA-IR, WC, WtHR, and %BF being the dependent variables and the gut peptides being the independent variables. The number of independent variables entered into each model was calculated as 10% of the group with the outcome and based on the results

of the bivariate analyses ($p < 0.25$), and scientific literature. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Participant Characteristics

The participant characteristics are presented in Table 1. A total of 21 NW control subjects (BMI between 18.5–24.9 kg/m^2) and 16 OB (BMI ≥ 35kg/m^2) were enrolled in this study. Majority of the subjects were females who represented 71.4% and 70.6% in the NW and OB respectively. Anthropometric measurements showed that WC, WtHR, and %BF were significantly higher in the OB group, as expected. As for energy intake there was no significant difference between the two groups ($p = 0.123$).

Table 1. Participant characteristics.

	Normal Weight	Obese	*p*
N	21	16	
Age (M ± SD)	39.10 ± 9.86	35.66 ± 10.37	0.31
Female (n (%))	15 (71.4)	12 (70.6)	0.81
Anthropometric data			
BMI (kg/m^2) (M ± SD)	22.07 ± 1.81	38.79 ± 3.72	**<0.001 ***
WC (cm) (M ± SD)†	82.14 ± 9.39	117.34 ± 7.88	**<0.001 ***
WtHR (M ± SD)†	0.37 ± 0.04	0.64 ± 0.06	**<0.001 ***
Percent Body Fat (%) (M ± SD)	24.38 ± 6.18	45.75 ± 5.88	**<0.001 ***
Energy Intake (Kcal) (M ± SD)	1641.95 ± 522.96	2042.67 ± 884.40	0.123

* The statistical significance was evaluated by t test for independent samples for continuous variables and by Pearson's chi-squared test for categorical variables. Bold values denote statistical significance ($p < 0.05$); † Clinical characteristics of study participants. Normal Waist Circumference according to IDF using Europids cut off values of <94 cm for males and <80 cm for females; Waist to Height Ratio normal refers to <0.5. Abbreviations: BMI: Body mass index; WC: waist circumference; WtHR: waist to height ratio, M ± SD: Mean ± Standard Deviation.

3.2. Biochemical and Gut Peptide Analyses

The biochemical and gut peptide analyses of the participants are shown in Tables 2 a, b. Table 2a indicates the normally distributed variables and Table 2b for the skewed variables. OB participants showed significantly higher levels of leptin ($p < 0.001$), leptin/ghrelin ratio ($p < 0.001$), insulin ($p < 0.001$), HOMA-IR ($p < 0.001$), and TG ($p = 0.017$). NW participants had significantly higher levels of ghrelin ($p = 0.002$) and HDL-C ($p = 0.001$) compared to the OB. No significant differences were noted regarding PYY, GLP-1, CCK, GLP-2, fasting plasma glucose concentration, TC, and LDL-C.

Table 2. (**a**) Biochemical and gut peptide analyses in normal weight vs. obese participants (mean ± SD for normally distributed data). (**b**) Biochemical and gut peptide analyses in normal weight vs. obese participants (median (IQR) for non-normally distributed data).

(a)			
Variable	**NW (M ± SD) n = 21**	**OB (M ± SD) n = 16**	***p***
Leptin (ng/mL)	14.28 ± 8.64	64.03 ± 32.79	**<0.001 ***
Ghrelin (pg/mL)	449.40 ± 203.11	246.82 ± 150.85	**0.002 ***
PYY (pg/mL)	60.42 ± 31.48	74.22 ± 47.51	0.296
GLP-1 (pM)	24.95 ± 9.59	30.98 ± 11.84	0.096
GLP-2 (ng/mL)	3.41 ± 1.37	3.89 ± 1.21	0.273
HDL-C (mg/dL)	61.38 ± 16.61	44.69 ± 9.75	**0.001 ***
TG (mg/dL)	105.43 ± 50.38	149.81 ± 57.65	**0.017 ***
(b)			
Variable	**NW (Median (IQR)) n = 21**	**OB (Median (IQR)) n = 16**	***p***
Leptin/Ghrelin ratio	24.26 (10.79–142.76)	320.10 (81.41–908.36)	**<0.001 ***
CCK (pg/mL)	36.54 (21.20–152.45)	38.05 (20.40–66.68)	0.927
Glucose (mg/dL)	97.00 (80–116)	98.50 (93–119)	0.133
Insulin (µU/mL)	7.77 (4.46–13.35)	20.15 (7.74–44.15)	**<0.001 ***
HOMA-IR	1.85 (1.05–3.82)	4.78 (1.97–11.77)	**<0.001 ***
TC (mg/dL)	182.00 (131–364)	179.00 (133–358)	0.902
LDL-C (mg/dL)	101.00 (50–299)	109.00 (70–290)	0.462

(a) * Bold values denote statistical significance ($p < 0.05$) between the 2 groups. All normally distributed numerical variables were calculated using Independent-Samples *t*-test, Abbreviations: PYY: pancreatic peptide YY, GLP-1: glucagon like peptide-1; GLP-2: glucagon-like peptide-2; HDL: high density lipoprotein cholesterol; TG: triglyceride; OB: obese; NW: normal weight; M ± SD: Mean ± Standard Deviation. (b) * Bold values denote statistical significance ($p < 0.05$) between the 2 groups. All skewed numerical variables were calculated using nonparametric test using Mann–Whitney U-test, Abbreviations: CCK: Cholecystokinin; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; TC: total cholesterol; LDL-C: low density lipoprotein cholesterol; OB: obese; NW: normal weight; IQR: Interquartile range.

3.3. Percent Body Fat and Gut Peptide Differences between the Sexes

Percent body fat and gut peptide differences between the sexes are shown in Table 3. %BF was higher in females in both lean ($p = 0.01$) and obese ($p = 0.02$) compared to males. Female lean and obese participants showed significantly higher levels of leptin ($p = 0.001$) compared to lean and obese males respectively. No significant differences were noted regarding the remaining gut peptides between the sexes.

Table 3. Effect of Sex on Percent body fat and gut peptide.

Variable	Females Lean (M ± SD) n = 15	Males Lean (M ± SD) n = 6	*p*	Females Obese (M ± SD) n = 12	Males Obese (M ± SD) n = 4	*p*
%BF	26.44 ± 6.09	19.22 ± 2.07	**0.001** *	48.10 ± 3.51	38.70 ± 6.28	**0.002** *
Leptin (ng/mL)	17.21 ± 8.43	6.98 ± 3.10	**0.001** *	77.55 ± 25.05	23.47 ± 12.22	**0.001** *

* Bold values denote statistical significance ($p < 0.05$) between the 2 groups. Independent-Samples *t*-test was used. Abbreviations: %BF: percent body fat; M ± SD: Mean ± Standard Deviation.

3.4. Gut Peptides, Obesity Markers, Lipid Profile, and Glucose Homeostasis Indicators

Correlations between gut peptides, obesity markers (WC, %BF, BMI, and WtHR), glucose homeostasis indicators (insulin, HOMA-IR, glucose) and lipid profile (TC, LDL-C, HDL-C, and TG) are presented in Table 4.

A positive correlation was found between the following obesity markers and peptide hormones: BMI with insulin ($r = 0.664$, $p < 0.001$), leptin ($r = 0.800$, $p < 0.001$), GLP-1 ($r = 0.365$, $p = 0.026$), PYY ($r = 0.333$, $p = 0.044$), and leptin/ghrelin ratio ($r = 0.809$, $p < 0.001$); WC and WtHR with insulin ($r = 0.757$; $p < 0.001$), ($r = 0.664$, $p < 0.001$), leptin ($r = 0.664$; $p < 0.001$), ($r = 0.753$, $p < 0.001$), GLP-1 ($r = 0.363$; $p = 0.027$), ($r = 0.334$, $p = 0.043$) and leptin/ghrelin ratio ($r = 0.813$; $p < 0.001$), ($r = 0.764$, $p < 0.001$) respectively; and %BF with insulin ($r = 0.734$, $p < 0.001$), leptin ($r = 0.874$, $p < 0.001$), and leptin/ghrelin ratio ($r = 0.871$, $p < 0.001$).

As for markers of glucose homeostasis; leptin ($r = 0.653$, $p < 0.001$), GLP-1 ($r = 0.382$, $p = 0.020$), PYY ($r = 0.333$, $p = 0.044$), and leptin/ghrelin ratio ($r = 0.762$, $p < 0.001$) were positively correlated with insulin. Moreover, leptin ($r = 0.658$, $p < 0.001$), GLP-1 ($r = 0.355$, $p = 0.031$), PYY ($r = 0.364$, $p = 0.027$), and leptin/ghrelin ratio ($r = 0.787$, $p < 0.001$) showed a positive correlation with HOMA-IR.

Ghrelin was negatively correlated with BMI (r = −0.521, $p = 0.001$), WC (r = −0.575; $p < 0.001$), WtHR (r = −0.530, $p = 0.001$), %BF (r = −0.362, $p = 0.028$), insulin (r = −0.554, $p < 0.001$), and HOMA-IR (r = −0.582, $p < 0.001$).

None of the gut peptides were correlated with TC and LDL-C, and there was no significant correlation between CCK, obesity markers, glucose homeostasis indicators and lipid markers. There was a negative correlation between HDL-C, insulin (r = −0.475, $p = 0.003$) GLP-1 (r = −0.365, $p = 0.026$), and leptin/ghrelin ratio (r = −0.460, $p = 0.004$); and a positive association for ghrelin (r = 0.537, $p = 0.001$). As for TG positive correlations were seen with insulin (r = 0.363, $p = 0.027$) and GLP-2 (r = 0.404, $p = 0.013$); and negative correlation with ghrelin (r = −0.346, $p = 0.036$).

Table 4. Correlations between gut peptides, obesity markers, lipid profile, and glucose homeostasis indicators.

	Insulin	Leptin	GLP1	GLP2	PYY	CCK	Ghrelin	Leptin/Ghrelin Ratio
BMI	r **0.664** [b]	**0.800** [a]	**0.365** [a]	0.229 [a]	**0.333** [a]	0.111 [b]	**−0.521** [a]	**0.809** [b]
	p **<0.001** **	**<0.001** **	**0.026** *	0.172	**0.044** *	0.514	**0.001** **	**<0.001** **
WC	r **0.757** [b]	**0.664** [a]	**0.363** [a]	0.296 [a]	0.279 [a]	−0.117 [b]	**−0.575** [a]	**0.813** [b]
	p **<0.001** **	**<0.001** **	**0.027** *	0.075	0.094	0.490	**<0.001** **	**<0.001** **
WtHR	r **0.664** [b]	**0.753** [a]	**0.334** [a]	0.219 [a]	0.306 [a]	0.042 [b]	**−0.530** [a]	**0.764** [b]
	p **<0.001** **	**<0.001** **	**0.043** *	0.192	0.065	0.806	**0.001** **	**<0.001** **
%BF	r **0.734** [b]	**0.874** [a]	0.277 [a]	0.199 [a]	0.303 [a]	0.043 [b]	**−0.362** [a]	**0.871** [b]
	p **<0.001** **	**<0.001** **	0.097	0.238	0.068	0.802	**0.028** *	**<0.001** **
Glucose	r **0.334** [b]	0.217 [a]	0.142 [a]	−0.013 [a]	0.281 [a]	−0.083 [b]	**−0.423** [a]	**0.402** [b]
	p **0.043** *	0.196	0.400	0.940	0.092	0.624	**0.009** **	**0.014** *
Insulin	r –	**0.653** [b]	**0.382** [b]	0.269 [b]	**0.333** [b]	−0.092 [b]	**−0.554** [b]	**0.762** [b]
	p –	**<0.001** **	**0.020** *	0.108	**0.044** *	0.589	**<0.001** **	**<0.001** **
HOMA-IR	r **0.991** [b]	**0.658** [b]	**0.355** [b]	0.232 [b]	**0.364** [b]	−0.104 [b]	**−0.582** [b]	**0.787** [b]
	p **<0.001** **	**<0.001** **	**0.031** *	0.166	**0.027** *	0.542	**<0.001** **	**<0.001** **

TC	r 0.107 [b]	−0.039 [b]	0.086 [b]	0.212 [b]	−0.122 [b]	−0.128 [b]	0.044 [b]	−0.707 [b]
	p 0.529	0.820	0.611	0.208	0.471	0.452	0.794	0.679
LDL-C	r 0.163 [b]	−0.044 [b]	0.112 [b]	0.217 [b]	−0.148 [b]	−0.038 [b]	−0.011 [b]	−0.034 [b]
	p 0.334	0.797	0.509	0.197	0.382	0.821	0.949	0.843
HDL-C	r **−0.475** [b]	−0.294 [a]	**−0.365** [a]	−0.293 [a]	−0.186 [a]	−0.085 [b]	**0.537** [a]	**−0.460** [b]
	p **0.003** **	0.078	**0.026** *	0.079	0.271	0.618	**0.001** **	**0.004** **
TG	r **0.363** [b]	0.266 [a]	0.315 [a]	**0.404** [a]	0.234 [a]	−0.127 [b]	**−0.346** [a]	0.310 [b]
	p **0.027** *	0.111	0.058	**0.013** *	0.163	0.452	**0.036** *	0.062

Spearman correlation was conducted for correlations involving insulin, CCK, HOMA-IR, total cholesterol, leptin/ghrelin ratio, and LDL-C; while Pearson correlation was conducted for correlations involving other variables: [a] Pearson correlation, [b] Spearman correlation. Abbreviations: WC: waist circumference, %BF: percent body fat; BMI: Body mass index; WtHR: waist to height ratio; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; TG: triglyceride; GLP-1: glucagon like peptide-1; GLP-2: glucagon-like peptide-2; CCK: cholecystokinin; PYY: pancreatic peptide YY. r = correlation coefficient * Bold values denote correlation is significant at the 0.05 level (2-tailed). ** Bold values denote correlation is significant at the 0.01 level (2-tailed).

In the binary logistic regression analysis, reported in Table 5, leptin was positively associated with HOMA-IR (odds ratio (OR) = 1.056 and 95% confidence interval (CI) = 1.006–1.108), weight status (as being obese; OR = 1.219, and 95% CI = 1.013–1.467), WC (OR =1.151, and 95% CI = 1.022–1.297), WtHR (OR =1.139, and 95% CI = 1.038–1.251), and %BF (OR = 1.202, and 95% CI = 1.034–1.398). No significant associations were noted with ghrelin, and %BF, and weight status. On the other hand, ghrelin was negatively associated with HOMA-IR (OR = 0.991, and 95% CI = 0.984–0.998) and WC (OR = 0.987, and 95% CI = 0.976–0.998). No significant associations were noted with the blood lipid markers and the gut peptides and therefore these were not included in the binary logistic regression analysis.

Table 5. Binary logistic regression of predictors of markers of obesity and glucose homeostasis.

Logistic Regression	Predictors	OR	*p*	R^2	95% CI
HOMA-IR [a]	Leptin	1.056	0.027	0.652	1.006–1.108
	Ghrelin	0.991	0.007		0.984–0.998
Weight status [a]	Leptin	1.219	0.036	0.882	1.013–1.467
WC [a]	Leptin	1.151	0.021	0.818	1.022–1.297
	Ghrelin	0.987	0.020		0.976–0.998
WtHR [b]	Leptin	1.139	0.006	0.759	1.038–1.251
%BF [a]	Leptin	1.202	0.017	0.816	1.034–1.398

[a] for HOMA-IR, weight status, WC, %BF: predictors entered in the model: leptin, ghrelin. [b] for, WtHR predictors entered in the model: leptin. Abbreviations: HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; WC: waist circumference, WtHR: waist to height ratio; %BF: percent body fat. Reference category: HOMA-IR < 2.32 vs. HOMA-IR ≥ 2; Weight status: lean vs. obese Waist Circumference: appropriate WC vs. increased WC; Waist to Height Ratio: normal WtHR vs. high WtHR.

4. Discussion

Obesity is one of the main risk factors for the development of type 2 diabetes, cardiovascular disease, and other non-communicable diseases, that are the leading cause of death worldwide [24]. Several lines of research have shown a link between an imbalance in gut peptide in overweight or obese individuals that may explain their underlying condition [2]. To our knowledge, the relationship between gut peptide hormones and obesity related health conditions have received little attention in the scientific literature when it comes to the Lebanese population, thus demonstrating the need for this research. Lebanon is part of the Eastern Mediterranean region and it is of surprise to find that Lebanon has a high percentage of obesity compared to other Eastern Mediterranean countries [6].

In our study, we noted a statistically significant difference in leptin, and ghrelin between OB and NW individuals, but no significant difference with respect to fasting GLP-1, GLP-2, PYY, and CCK. Our results confirm the dysregulation for some peptide hormones (leptin and ghrelin) and point out further controversy for other hormones with respect to obesity, insulin resistance and dyslipidemia [2]. A similar study to ours was conducted among lean and obese individuals in the United Arab Emirates (UAE) utilizing the same peptide hormones along with obesity markers and glycemia [25]. According to the latter study, conducted in UAE, the authors found a statistically significant difference in GLP-1, GLP-2, insulin, leptin, ghrelin, and CCK between obese and lean participants [25] and as a result it further confirmed the need for population-based research due to the difference in results.

Ghrelin is the gut peptide, which stimulates appetite, lipogenesis, and insulin resistance [1]. Plasma ghrelin levels peak during fasting and prior to a meal [26], and this induces eating. In accordance to the literature, [26-28] our study also showed fasting plasma ghrelin levels to be lower in OB than in NW individuals. In addition to this, ghrelin was negatively correlated with obesity markers (WC, %BF, BMI, and WtHR). These findings suggest that ghrelin levels fluctuate in accordance to a change in body weight [29]. Fasting ghrelin levels decrease with obesity and increase with weight loss [29]. This decrease leads to a physiological adaptation to the positive energy balance, rather than a causal effect [29]. Furthermore, obese individuals lack a decline in ghrelin after food intake, and as a result appetite will not be reduced and further weight gain will be observed [28, 30]. In addition, we also found a negative correlation between ghrelin, insulin, HOMA-IR, and TG and a positive association with HDL-C. This negative correlation between fasting ghrelin and insulin has also been reported in previous studies [28, 31, 32], and our data are in line with these results and this negative correlation could eventually lead to type 2 diabetes and cardiovascular disease in obese individuals [31]. With respect to TG and ghrelin, OB individuals were found to have higher fasting TG and lower ghrelin compared to the NW. In agreement with our results, a study that was conducted on rodents, showed the inverse relationship between triglyceride and ghrelin [33]. High triglyceride levels were found to have opposite effects on leptin and ghrelin transport across the blood–brain barrier. Furthermore, triglycerides were found to decrease the transport of leptin while increasing the uptake of ghrelin into the brain and as a result this stimulated appetite and feeding [33].

In addition to our findings, hypertriglyceridemia is a risk factor for cardiovascular disease and is associated with obesity and insulin resistance [34, 35]. Furthermore, when there is an increase in TG there is a reduction in HDL-C [34]. This was seen in our study where fasting TG and insulin were higher and HDL was lower in the OB compared to the NW individuals. With respect to the peptide hormones there was a positive correlation with GLP-2 and TG. It is known that GLP-1 and GLP-2 are co-secreted in response to the ingestion of the same nutrients [35]. Several studies regarding post-prandial GLP-1 and GLP-2 secretion and the production of triglyceride rich lipoprotein (TRL) have been conducted in which GLP-1 and GLP-2 have opposite effects. GLP-1 was found to decrease

TRL in plasma and GLP-2 was found to do the opposite [34, 35]. Based on these results and to further understand the underlying mechanism, it is important to do additional research involving GLP-2 and its effect on TG during fasting and in the postprandial state in OB and NW individuals.

As for the link between obesity and GLP-1, there is mixed evidence, with some studies showing that those who are obese have lower circulating levels of this hormone than those of normal weight, while others have not found this [25, 36]. In our study, fasting GLP-1 was not found to be statistically different between the two groups. BMI, WC, and WtHR were significant predictors of fasting GLP-1 concentrations in OB subjects. Furthermore, fasting GLP-1 concentrations were positively associated with HOMA-IR and insulin. These observations may be essential features of obesity and require further investigation to elucidate the mechanisms. One can envisage higher fasting GLP-1 may increase circulating insulin, which may lead to insulin resistance among obese patients [37]. Increased adiposity as a result of high insulin has been well documented through several known mechanisms, including reduced lipolysis, increased lipogenesis and adipogenesis [38]. On the other hand, GLP-1 is secreted in response to food intake and it has a known role in the regulation of glucose homeostasis, increasing satiety, reducing gastric emptying, inducing weight loss, and as a result, improving insulin sensitivity [39]. However, GLP-1 secretion seems to be affected by the meal composition, type of nutrient and sequence consumed, and the preceding meal [39]. Therefore, it is important to also evaluate GLP-1 postprandially and its association with HOMA-IR.

Regarding PYY, it is considered to be an appetite suppressing hormone and plays a role in energy homeostasis. However, the evidence in the literature with respect to the involvement of PYY in the development of obesity is controversial and unclear. In our study there was no significant difference between the groups. On the other hand, we also found a positive correlation between PYY, BMI, and HOMA-IR. Studies conducted on humans showed that PYY concentrations were lower in obese compared to normal weight individuals, suggesting that this appetite suppressing gut peptide is associated with the development of obesity [40-42]. However, other human based studies have failed to reproduce this inverse relationship between PYY and adiposity [43-45]. It has also been shown that there is a different response in the secretion of PYY in the fasting versus the fed

state in obese individuals compared to lean subjects with the obese having a lower secretion following a meal [46]. In addition to this, PYY concentrations are affected postprandially with the type of macronutrient consumed with protein having the highest PYY response followed by fat and the least by carbohydrate [46]. The mechanisms by which obese individuals have an altered PYY profile remain unknown and it might be related to a change in the synthesis, release or removal of PYY [46]. Therefore, further studies that monitor the fasting and postprandial response of PYY to different diets should be performed.

Leptin on the other hand is not a gut hormone but rather an adiposity hormone produced by adipose tissue [8]. Leptin secretion is directly proportional to lipid content in adipose tissue [47]. This was also seen in our current study with higher concentrations of fasting leptin in obese individuals compared to NW counterparts. Furthermore, as observed and well-known before, %BF between males and females differed in both groups (Table 3). Furthermore, leptin was significantly different between sexes in both groups, with higher concentrations in females than in males, as has also been observed before [48]. These two observations are related, as one of the mechanisms of higher leptin in females has been proposed to be a higher proportion of adipose tissue and increased production rate of leptin per unit mass of adipose tissue in women than in men [48]. However, since leptin is an anorexigenic hormone that promotes satiety, there are numerous studies that support the fact that obese individuals exhibit leptin resistance at the level of the brain thus disrupting the satiety response [11, 25, 47]. Further to this point, excessive weight loss might resolve leptin resistance [12]. In our study, in addition to the result that leptin was found to positively correlate with all obesity markers, OB subjects had higher insulin and HOMA-IR values compared to NW subjects, and a positive correlation was seen between leptin, insulin and HOMA-IR. Studies have suggested that insulin resistance can cause leptin resistance and therefore lead to obesity, and its comorbidities [11, 12, 49]. In addition to this, obesity leads to high levels of leptin, which acts as a cause for the release of pro-inflammatory cytokines and an exaggerated release of insulin leading to insulin resistance [49]. These findings shed light to understanding the pathophysiology of leptin resistance in humans.

Due to the inconsistency among different results of gut peptides related to leptin and ghrelin, new parameters have been evaluated in which one of them is leptin/ghrelin

ratio [50]. This ratio appears to be associated with regulating hunger, in the sense that a lower ratio is associated with reducing hunger [50]. Our results are in accordance with other studies in which obese subjects had a higher leptin/ghrelin ratio in the fasting state compared to the lean counterparts [50],[51]. This was evident among all of the obesity markers (BMI, WC, WtHR, and %BF). In addition to this, diabetics were found to have a higher ratio of leptin/ghrelin [51]; in our study we noticed a positive correlation with the HOMA-IR. Since both leptin and ghrelin are involved in glucose metabolism, diabetics tend to have lower ghrelin levels and higher serum leptin [52] and as a result the leptin/ghrelin ratio would be higher in diabetics. However, further studies are required on a bigger sample size with the consideration of the effect of postprandial meal on the leptin/ghrelin ratio and also comparing diabetics to nondiabetics (obese) individuals to further understand the relationship.

Strengths of the present study include focusing on Lebanese individuals that were residing in Lebanon, while the study design involved using validated obesity and glucose homeostasis markers in order to explore the association between gut peptides and obesity and insulin resistance. However, this study had limitations, one of them being a small number of participants and this might alter our results, therefore prospective studies with a large sample size should be carried out to validate our primary findings. Furthermore, since our analysis only focused on fasting peptide hormone concentrations, looking at postprandial concentrations can provide additional insight to the relationship with obesity and impaired glucose homeostasis and dyslipidemia. Furthermore, further analysis and prospective studies involving all parameters of the metabolic syndrome and the involvement of the gut peptides should be taken into consideration to identify if these differences are a consequence or cause of obesity.

5. Conclusions

To our knowledge this is the first study conducted in the EMR, on fasting levels of peptide hormones in OB and NW Lebanese individuals. In this study we observed significant difference in the fasting plasma peptide hormones among the obese and normal weight Lebanese individuals with a notable relationship between leptin, glucose, and obesity markers. Since there are controversial results in the literature concerning peptide hormones among obese individuals, our results further highlight a need for finding strategies that alter

peptide hormones in the fasting and postprandial state in the treatment of obesity and its consequences that might be related to different ethnic backgrounds. More long-term research is required while taking into consideration the ethnic groups and the emerging role of gut peptides in obesity therapy.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The raw data presented in this study are openly available in Mendeley Data repository: https://data.mendeley.com/datasets/yd5d2gpv4c/3 (accessed on 21 October 2022) at https://doi.org/ 10.17632/yd5d2gpv4c.3

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CHAPTER 3



Chapter 3

Gut Microbiota Composition of Lean and Obese Lebanese Individuals

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Abstract

An altered gut microbiota has been shown to contribute to the development of metabolic diseases such as obesity. In this study gut microbiota profile of 30 obese and 23 lean Lebanese individuals was performed via DNA isolation and sequencing of the V3–V4 region of the 16S rRNA of fecal samples. The abundance of the phylum *Verrucomicrobiota* was higher in lean subjects and there was no significant difference in the *Bacillota/ Bacteroidota* ratio between the obese and lean groups. The evenness and Shannon alpha diversity indices were significantly higher in the lean group than in the obese group (q = 0.012 and q = 0.030, respectively). Beta diversity was higher in the obese group based for unweighted UniFrac distance variability (q = 0.047). *Lachnoclostridium* was the only genus that was higher in obese (q = 0.013) and it is linked to diet induced obesity, while the abundance of the genera *Peptococcus*, *Ruminococcus_2*, *Lachnospiraceae UCG-001*, *Ruminiclostridium 6*, the uncharacterized taxon within *Coriobacteriaceae*, *Ruminococcaceae UCG-005*, *Ruminococcaceae UCG-010* and *Oxalobacter*, were significantly higher in lean subjects. These bacterial species that were higher in lean people, possess anti-inflammatory properties through the production of short chain fatty acids and are linked with lower BMI, promote satiety and weight loss and may play a role in the protection against obesity and type 2 diabetes. Further research to generate a clear understanding of the interaction of the gut microbiota and health is needed.

Key words

Gut microbiota, obesity, *Bacillota*, *Bacteroidota*, 16S rRNA

1. Introduction

Obesity has become a global epidemic, whereby currently overweight/ obesity poses more health risks and mortality than underweight/ undernourishment (Mishra et al., 2016). Its prevalence is on an ever increasing rise; 42.7% of adults in the United States are obese (federation, 2017), 30% to 70% in the Eastern Mediterranean region (EMR) (Musaiger, 2011), 35% in Saudi Arabia followed by 27.8% in United Arab Emirates (AlAbdulKader et al., 2020), 27% in Lebanon and 11.7% in Italy (World Health Organization, 2017). The provision of obesity care has proved challenging in the EMR countries, including Lebanon. Obesity is a complex condition that is caused by an imbalance between excessive energy intake coupled with decreased energy expenditure (Morgen and Sorensen, 2014) with new factors emerging into the formula, ranging from genetics to endocrine disruptors, pharmaceutical effects, and epigenetics (McAllister et al., 2009) and is defined as a body mass index (BMI) greater than 30 kg/m^2 (Alhabeeb et al., 2021; Morgen & Sorensen, 2014).

The gut microbiota is a collection of microorganisms that inhabit the human intestines and is composed of different species that include bacteria, viruses, fungi, and parasites (Arumugam et al., 2011; Ballini et al., 2020; Bouter et al., 2017). The bacteria are taxonomically classified into phyla, classes, orders, families, genera, and species (Arumugam et al., 2011). The dominant gut microbial phyla are known as *Bacillota, Bacteroidota, Actinomycetota, Pseudomonadota, Fusobacteriota*, and *Verrucomicrobiota* (Oren & Garrity, 2021). Approximately 90 percent of the gut microbiota is composed of the two phyla *Bacillota* and *Bacteroidota* (Arumugam et al., 2011; Rinninella et al., 2019). The ratio between the *Bacteroidota* and *Bacillota* has been shown to play an important role in health and disease (Adak & Khan, 2019), although this also seems to depend on geographical location (Guida & Venema, 2015). The gut microbiota is involved in maintaining health due to its important role in nutrient metabolism, mucosal gut barrier structural integrity, immunity against pathogens, and immunomodulation (Arumugam et al., 2011; Rinninella et al., 2019). Studies performed in humans and animals have depicted this relationship but with contrasting results (Chan et al., 2013; Cuevas-Sierra et al., 2019; DeGruttola et al., 2016). Dysbiosis is the condition of having an imbalance in gut bacteria associated with pathology (Cuevas-Sierra et al., 2019). In accordance with this, it has been shown in mice that obesity can be associated with dysbiosis (Cuevas-Sierra et al., 2019) and there has been successful reduction of weight in interventions when microbiota was manipulated. In addition to this, there are

differences in composition and function in the gut microbiota of obese versus healthy individuals (Tremaroli & Backhed, 2012) in the sense that there is more abundance in key bacteria— *Bacteroidota*, *Prevotella* and members of the *Clostridiales* order in healthy individuals compared to obese (Adak & Khan, 2019; Tremaroli & Backhed, 2012). Many factors such as the environment, diet, use of antibiotics, and host genetics are contributors to this big difference in the gut microbiota profile (Adak & Khan, 2019; Plummer et al., 2020; Rinninella et al., 2019).

In the face of high obesity prevalence among the EMR populations, there is insufficient amount of knowledge on the gut microbiota in healthy and obese, and Lebanon is not exempt from this situation. Therefore, a more detailed knowledge of the structure of the microbiota composition, species relationships, and metabolic functions could aid in counteracting the onset of obesity, globally and in the EMR. Due to the discrepant results regarding the gut microbiota that are present among different countries (Pinart et al., 2021), identifying key bacterial species among the Lebanese population will allow us to create a database of beneficial bacteria, making it possible to target the gut microbiota in clinical care as treatment for obesity and its comorbidities.

2. Materials and Methods

Study design

The study had a cross sectional design, conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Middle East Institute of Health University Hospital (MEIH-UH) Lebanon (AL-04-02/2018-IRB/MEIH).

Study population

The study was conducted at the MEIH-UH between November 2019 and April 2021. Sample size was determined according to change in *Bacteroidota*/*Bacillota* ratio, the main objective outcome of this study. Power calculations were based on the data retrieved from Damms-Machado *et al.* (2015) where fecal *Bacteroidota*/*Bacillota* ratio changed significantly from 5.9 ±2.1 to 10.4± 1.4 in three months post bariatric surgery (Damms-Machado et al., 2015). To generate a similar significant effect a sample size of two patients was estimated to achieve

80% power at a 5% alpha. Expecting a drop out of 30 percent in effect size in this protocol, therefore we multiplied the number of required patients by 10. Thus, a sample size of 20 Lebanese participants per group was determined to be adequate. Eligibility criteria included adult participants (18–60 years old) from both sexes that had stable body weight and free of antibiotic use for the last three months. Eligibility criteria was assessed by using a screening questionnaire addressing ethnicity, general health, weight fluctuation, alcohol consumption, physical activity, medication, antibiotic use, supplement use (including probiotic/fiber/prebiotic supplements), and smoking. Consenting participants with a BMI ≥35 kg/m^2 were assigned to the obese (OB) study group; afterwards, lean individuals with a BMI of 18.5–24.9 kg/m^2 were matched for age and sex with their OB counterparts and assigned to the lean/ control group. A 24-hour (h) food diary was filled over three nonconsecutive days (two weekdays and one weekend) to evaluate energy and fiber intake. Exclusion criteria were weight loss ≥5% in last three months, pregnancy, alcohol consumption (> ½ ounce (OZ)/day for females;1 OZ/day for males (Snetselaar et al., 2021)), and subjects unwilling to consent to the study. OB subjects were recruited from the weight loss program of MEIH-UH by staff over a period of two years. As for the lean subjects, they were recruited from the general community in Lebanon, using direct approach and by word of mouth. All participants gave their informed consent for inclusion prior to participating.

Fifty-three eligible subjects (16 males, 37 females) consented to join the study. Thirty were OB (10 males, 20 females) and twenty-three were lean (6 males, 17 females). Eligible subjects arrived at the MEIH-UH after a 12 h fast and were asked to avoid alcohol consumption, as well as any unusual strenuous exercise 24 h prior to the screening.

Anthropometric measurements

While subjects wore light clothes and no shoes, height and weight were measured using "SECA" balance and BMI was calculated as the ratio of weight (Kg) to height (m) squared (kg/m²). Waist circumference (WC) (cm) was measured to the nearest 0.1 cm using a flexible, non-stretchable measuring tape at the umbilicus level midway between the lower ribs and the upper iliac crest while standing (Alberti et al., 2006). WC was defined as normal or 'at risk' according to the International Diabetes Federation (IDF) using Europids cut off values for normal of <94 cm for males and <80 cm for females (Alberti et al., 2006). Waist to height

ratio (WtHR) was calculated as the ratio of the waist circumference divided by height (both in cm). A cut-off of 0.5 was used to define abdominal obesity, whereby a WtHR < 0.5 was considered as normal (Gibson & Ashwell, 2020). Body composition was assessed using the Inbody body composition analyzer 720 (InBody Co., Ltd., Seoul, Korea) which uses a segmental multifrequency bioelectrical impedance analysis technique to analyze the percent body fat (%BF) that produces small individual error and can be used as a substitute when dual-energy X-ray absorptiometry is not available (Hurt et al., 2020; McLester et al., 2020). Cutoffs of ≥25% and ≥35% were used to define elevated %BF among men and women, respectively (Nuttall, 2015).

Stool collection

Stool was collected using a fecal specimen collector along with the Zymo Deoxyribonucleic acid /Ribonucleic acid (DNA/RNA) Shield™ Fecal Collection Tube and subjects were asked to follow the manual instructions.

DNA isolation and sequencing of the V3–V4 region of the 16S rRna gene

To identify the different taxa present in the Lebanese lean and obese sample, DNA isolation and sequencing of the barcoded amplicons of the V3–V4 region of the 16S rRNA gene were performed according to the established protocols provided by Illumina (Illumina, Eindhoven, The Netherlands) as previously described (Surono et al., 2022). The sequencing was performed using the Illumina MiSeq system (San Diego, CA, USA) using barcodes and the paired-end 2x 300 bp protocol. The sequencing run was analyzed with the QIIME 2 (https://qiime2.org/)(Bolyen et al., 2019) with demultiplexing based on sample-specific indexes. The sequences were classified using the Silva database (version 132) as a reference 16S rRNA gene database.

Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences software version 20 (SPSS Inc. Chicago, IL, USA) and software package R (4.2.1) (R Core Team, http://www.R-project.org) in RStudio. The normality of the data using SPSS was tested using the Skewness and Kurtosis. Normally distributed data are expressed as mean (M) ± standard deviation (SD), and skewed data as median (Mdn) and interquartile range (IQR). Depending on the data distribution, differences between the groups were assessed

using independent samples t-test for normally distributed continuous variables or nonparametric test using Mann–Whitney U-test for skewed continuous variables and Chi-square for categorical values. A value of $p < 0.05$ was used to denote statistical significance.

Correlations between the amplified sequence variants (ASVs) and the different categorical variables such as lean vs. OB were investigated using the non-parametric Kruskal–Wallis test corrected with the Benjamini–Hochberg false discovery rate (FDR) for multiple comparisons by using the software package R in RStudio. Non-parametric Spearman's rank-order correlations were obtained between the ASVs and continuous variables such as weight, BMI, WC, WtHR, and %BF. The *q*-values (adjusted *p*-values after FDR correction) were considered significantly different at a strict cut-off of $q < 0.05$. Taxonomic analysis was visualized using Krona plots at the phylum level for both groups. *Bacillota* to *Bacteroidota* ratio was calculated by dividing the relative abundances of *Bacillota* and *Bacteroidota*.

Linear discriminant analysis (LDA) effect size (LEfSe) were done on Galaxy website (version 1) (Galaxy/Hutlab) to explore microbial taxa with differential abundance between lean and OB. Species enriched in each group with an LDA score >2 at a value of $p < 0.05$ were considered (Segata et al., 2011).

Alpha and beta diversity

The used alpha diversity measurements were Faith's phylogenetic diversity (PD) (Faith, 2007), observed number of amplicon sequence variants (ASVs) (Callahan et al., 2017), Chao1(Chao, 1984), Pielou's evenness and Shannon's indices (Magurran, 2021). Alpha diversity indices were calculated with QIIME 2 and shown using the R software (4.2.1) in RStudio. The rarefaction curves were generated using the ggplot2 package for R. Rarefication depth was set at 3800.

Beta diversity measurements were visualized using Principal Coordinate Analysis (PCoA) plots based on the Bray–Curtis (BC) dissimilarity (Bray & Curtis, 1957), Jaccard (Jaccard, 1912), unweighted unique fraction metric (UniFrac) (Lozupone and Knight (2005), and weighted UniFrac (Lozupone et al., 2007). The statistical significance of beta diversity between the two groups (lean *vs.* OB) was determined using Permutational Multivariate Analysis of Variance (PERMANOVA).

3. Results

Participant characteristics

The participant characteristics are presented in Table 1. A total of 23 lean control subjects (BMI between 18.5-24.9 kg/m^2) and 30 OB (BMI ≥35kg/m^2) were enrolled in the study. Majority of the subjects were females who represented 73.9% and 66.7% in the lean and OB, respectively (p = 0.57). Anthropometric measurements showed that WC, WtHR, and %BF, were significantly higher in the OB group, as expected. As for energy and fiber intake there was no significant difference between the two groups (p = 0.23; p = 0.79 respectively), nor for smoking (p = 0.93). None of the participants were on probiotic, prebiotic, or fiber supplements.

Table 1. Participant characteristics

	Lean	**Obese**	***p***
N	23	30	
Age (mean±SD)	40.13±10.08	41.05±10.72	0.75
Female (n (%))	17 (73.9)	20 (66.7)	0.57
Anthropometric data			
BMI (kg/m^2) (mean±SD)	22.02±1.91	38.83±3.49	**<0.001***
WC (cm) (mean±SD)	81.76±9.88	118.73±8.52	**<0.001***
WtHR (mean±SD)	0.37±0.04	0.63±0.07	**<0.001***
Percent Body Fat (%) (mean±SD)	24.46±6.21	45.44±5.69	**<0.001***
Fiber Intake (g) (mean±SD)	17.61±7.31	18.19±8.41	0.79
Energy Intake (Kcal) (mean±SD)	1612.57±508.59	1822.08±745.82	0.23
Smoker Yes (n, %)	11 (47.8)	14 (46.7)	0.93
Probiotic Supplement Yes (n, %)	0 (0)	0 (0)	-
Fiber Supplement Yes (n, %)	0 (0)	0 (0)	-
Prebiotic Supplement Yes (n, %)	0 (0)	0 (0)	-

* The statistical significance was evaluated by t-test for independent samples for continuous variables and by Pearson's chi-squared test for categorical variables. Bold values denote statistical significance ($p < 0.05$); Normal waist circumference according to IDF using Europids cut off values of <94 cm for males and <80 cm for females; waist to height ratio normal refers to <0.5. Abbreviations: BMI: body mass index; WC: waist circumference; WtHR: waist to height ratio, SD: standard deviation.

Taxonomic analysis

Taxanomic analysis was perfomed and visualized using Krona plots at the phylum level for both groups (Figure 1) with 49% *Bacillota*, 41% *Bacteroidota*, 9% *Pseudomonadota*, and less than 1% for others for the OB group and 51% *Bacillota*, 41 % *Bacteroidota*, 6% *Pseudomonadota*, and less than 1% for others for the lean group. The only phylum that showed a significant difference between lean and OB was *Verrucomicrobiota* (Figure 2; $q = 0.044$) with a higher content in lean compared to OB individuals. As for the *Bacteroidota/Bacillota* ratio, it was not significantly different between lean and OB with a q value of 0.451.

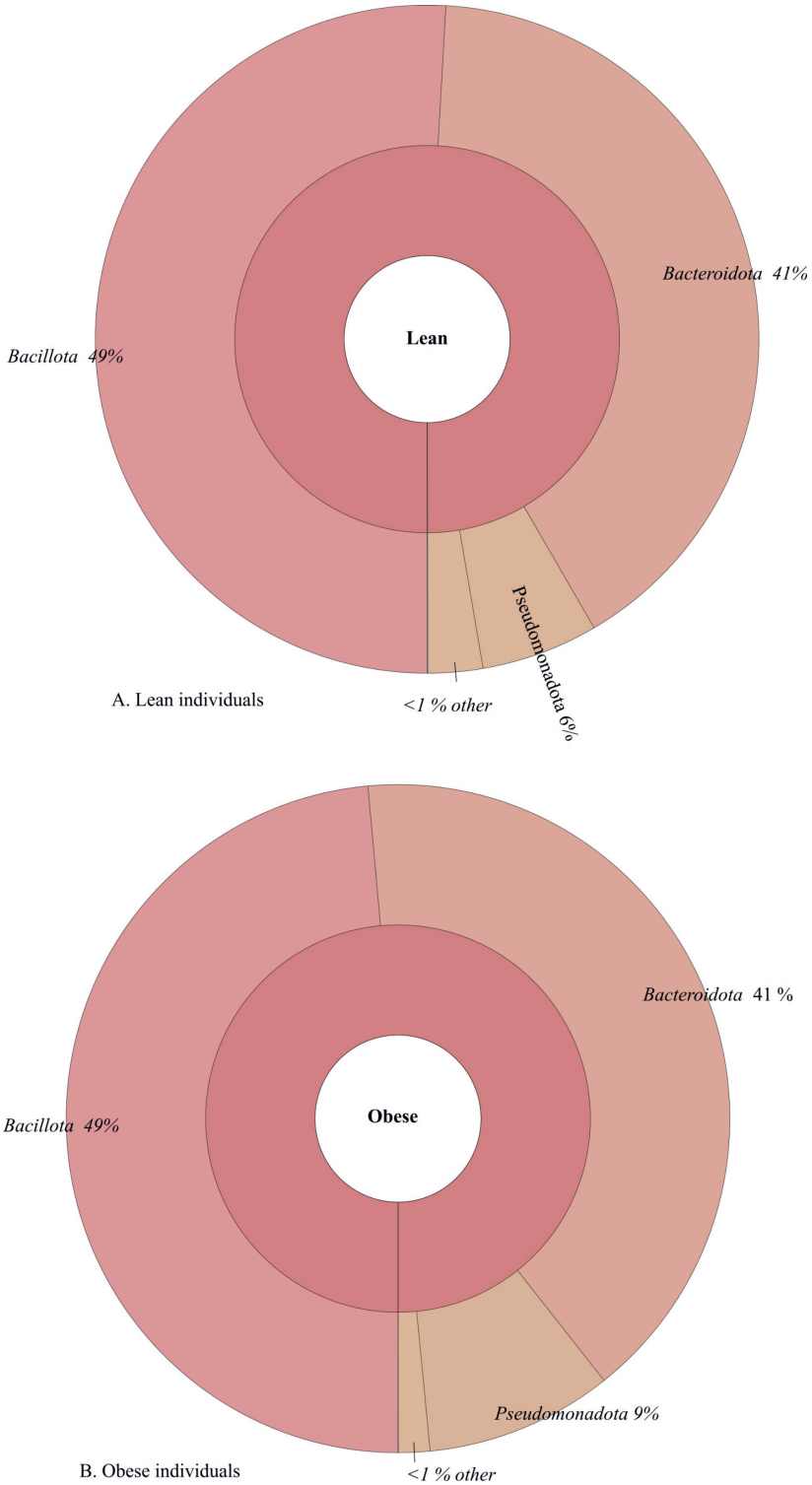

Figure 1. Krona plots

Krona plots of phyla detected in datasets of lean (a) *vs*. obese (b) Lebanese individuals. Relative abundances (percentages) of the phyla are displayed.

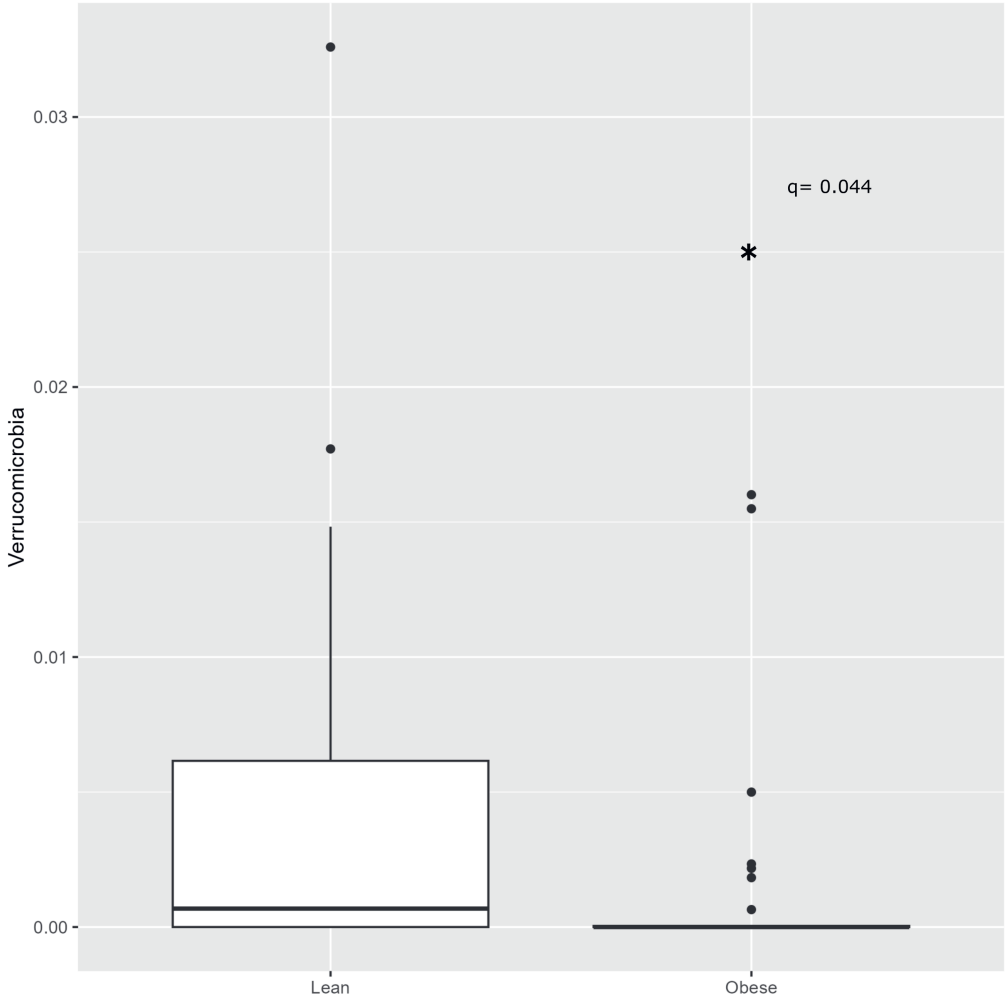

Figure 2. Taxonomic composition box plot

Taxonomic composition boxplot showing the gut microbial phylum *Verrucomicrobiota*, for lean and obese individuals. The relative abundance of the phyla is shown on the y-axis and the groups (lean or obese) are on the x-axis. Boxes denote the interquartile range (IQR) between the first and third quartiles, and the horizontal line defines the median. Whiskers represent the smallest (ymin) and largest (ymax). Outliers are indicated by black circles. Kruskal Wallis test was performed. * Indicates significant difference between lean and obese with a q value <0.05.

Alpha diversity

To investigate whether the gut microbial diversity and structure differed between OB and lean individuals, we first assessed the microbial diversity within each individual as seen in figure 3 by looking at the Observed Operational Taxonomical Units (OTUs), Chao 1 Diversity, Faith's phylogenetic diversity, Shannon index, and Pielou's evenness.

Rarefaction curves flattened (not shown), which suggests that the sequencing depth was enough to catch all the diversity present.

In figure 3, the lean group showed more OTUs (or richness) than the OB group. For both samples (OB and lean), most of the samples have a richness between 180 and 320 OTUs. However, these values were not determined to be statistically significant by Kruskal-Wallis testing with a q value of 0.36. Also the richness for the Chao1 lies between 180 and 380 making it very similar in its richness. Furthermore, these values were not determined to be statistically significant by Kruskal-Wallis testing with a q value of 0.36. Furthermore Faith's phylogenetic diversity (PD) was higher in the lean group than the OB group (lean samples have more diverse gut microbiota profile than the OB samples), but were not determined to be statistically significant by Kruskal-Wallis testing with a q value of 0.07.

As for Pielou's evenness and Shannon indices, the intra-variability between samples is lower for the OB samples than in the lean. Evenness and Shannon are lower in OB (i.e. around 0.85 and 6.8, respectively) than in the lean (i.e. around 0.9 and 7.1, respectively) and these values were determined to be statistically significant by Kruskal-Wallis testing (i.e. $q = 0.012$ and $q = 0.030$, respectively).

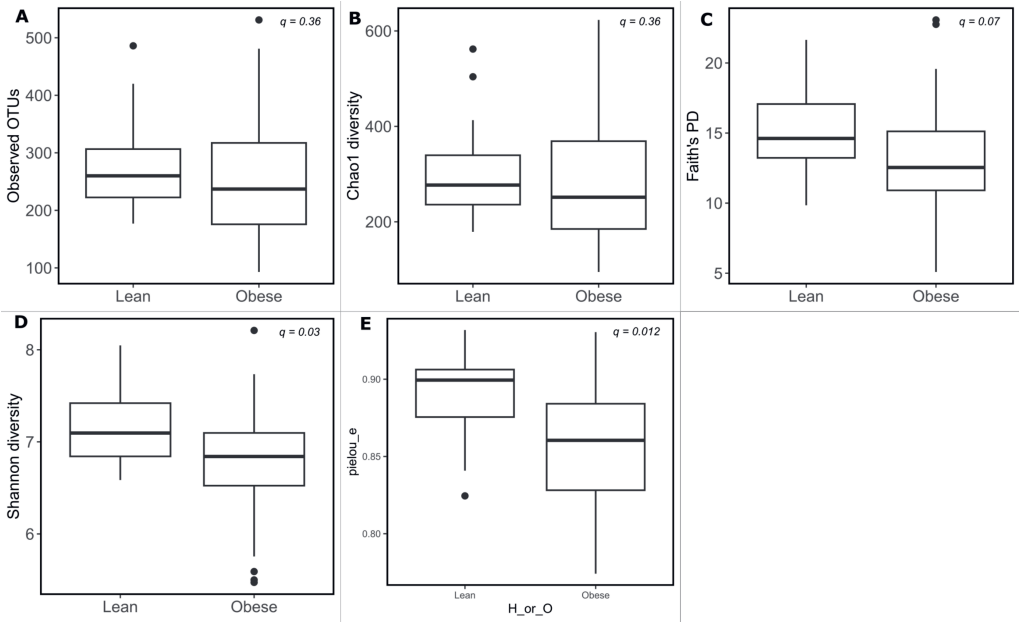

Figure 3. Alpha diversity boxplots

Alpha diversity boxplot showing Observed operational taxonomical units (OTUs) (A), Chao1diversity (B), Faith's phylogenetic diversity (Faith's PD) (C), Pielou's evenness (D) and Shannon indices (E) for lean and obese individuals. For each sample, the alpha diversity indices are shown on the y-axis and the groups (lean or obese) are on the x-axis. Boxes denote the interquartile range (IQR) between the first and third quartiles, and the horizontal line defines the median. Whiskers represent the smallest (ymin) and largest (ymax). Outliers indicated by black circles. Kruskal Wallis test was performed. A q-value <0.05 indicates significant difference between lean and obese.

Beta diversity (bray curtis index, jaccard index, unweighted and weighted unifrac distance)

The Beta diversity metrics (bray curtis index, jaccard index, unweighted and weighted unifrac distance) (figure 4) The Bray-Curtis dissimilarity is based on occurrence data (abundance) (Bray & Curtis, 1957). As seen in figure 4 by looking at the principal coordinate analysis (PCoA) plot no differences were observed between lean and OB based on the bray-curtis index. Permanova analysis confirmed this observation with no overall significant variation among OB and lean individuals (q = 0.067), but with a trend for differences.

The Jaccard index was performed to analyze differences in the composition of the different microbiomes. The Jaccard index is a similarity coefficient which relies on the presence/absence of OTUs (i.e. the composition of the microbiome) (Jaccard, 1912).

According to our results no differences were observed between lean and OB based on Jaccard index. Permanova analysis confirmed this observation with no overall significant variation among OB and lean individuals ($q = 0.061$), but also with a trend for differences.

UniFrac uses phylogenetic information to compare environmental samples by taking into account the occurrence table and the phylogeny diversity (sequence distance) (Lozupone & Knight, 2005). Weighted-Unifrac considers the abundance of OTUs shared between samples, whereas unweighted-Unifrac only considers presence/absence of species (samples sharing more OTUs are given greater similarity) (Lozupone et al., 2007). Our results showed that the OB group had greater UniFrac diversity than did lean due to the greater spread of samples across the plot. Based on permanova analysis there was a significant effect on unweighted UniFrac distance variability ($q = 0.047$) but not for weighted UniFrac distances ($q = 0.237$).

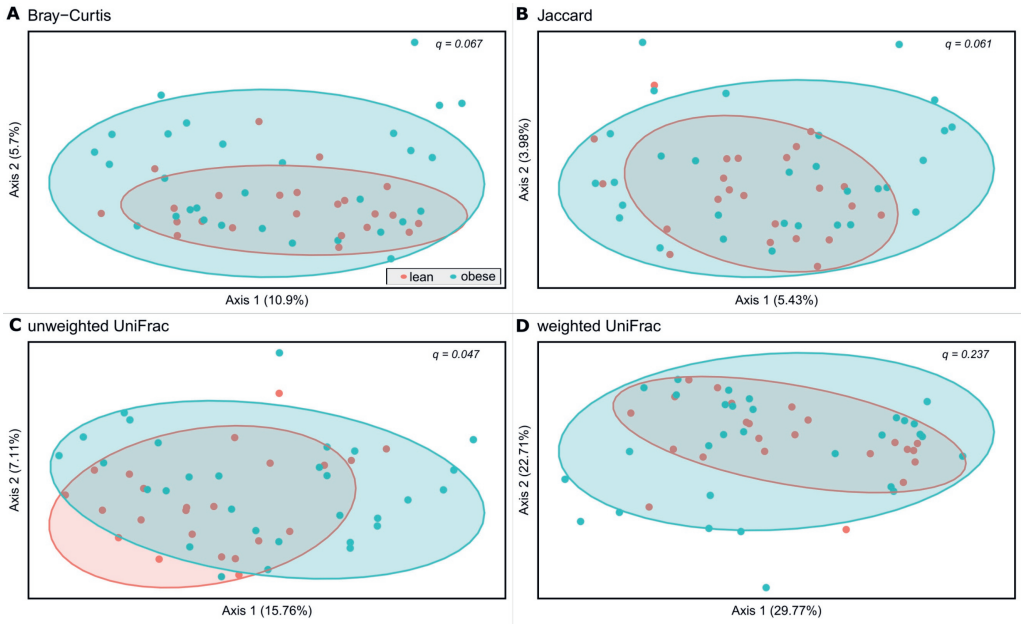

Figure 4. Beta diversity principal coordinate analysis (PCoA) plots

Principal coordinate analysis (PCoA) plot of bray curtis index (A), jaccard index (B), unweighted (C) and weighted unifrac (D) distance between lean and obese Lebanese individuals. Lean is red and obese is blue. A q-value <0.05 denotes significant difference between lean and obese.

Linear discriminant analysis (LDA) of effect size (LEfSe)

Based on the relative abundances of the gut bacteria in the OB *vs*. lean groups, we used a linear discriminant analysis effect size (LEfSe) algorithm, followed by Benjamini–Hochberg correction (*q*-values), to determine the bacteria that might explain the differences between the groups. The LDA score represents the effect size of each abundant species (Harakeh et al., 2020). Species enriched in each group with an LDA score >2 are considered (Harakeh et al., 2020).

In figure 5, for the OB group there is a positive LDA value for the genus *Lachnoclostridium* (*Bacillota* phylum) which means that there is an increase in the relative abundance of that genus in the OB group compared to the lean group. For the negative LDA values, there is an increased relative abundance of 15 genera in the lean group compared to the OB which are: *Peptococcus*, *Lachnospiraceae UCG-001*, *Ruminococcaceae UCG-008, Ruminiclostridium 6, , Ruminococcus 2, Ruminococcaceae UCG-010*, *Christensenellaceae R -7 group*, and *Ruminococcaceae UCG-005, (*all *Bacillota* phylum*); Paraprevotella*, *Alistipes (*all *Bacteroidota* phylum*); Oxalobacter (Pseudomonadota* phylum*);* and uncharacterized taxa within *Gastranaerophilales* (*Melainabacteria* phylum)*, Clostridiales_vadinBB60, Erysipelotrichaceae (*both *Bacillota* phylum*),* and *Coriobacteriaceae (Actinomycetota* phylum*).*

According to the Kruskal-Wallis analysis with Benjamini–Hochberg correction at genus level as seen in table 2, there is an overlap with some of the results from the LEfSe. In accordance to the LEfSe results, *Lachnoclostridium* was the only genus that was higher in OB (q=0.013). As for the lean group there is an increased relative abundance of 8 out of the 15 genera from the LEfSe which are *Peptococcus* (q = 0.023), *Ruminococcus_2* (q = 0.008), *Lachnospiraceae UCG-001* (q= 0.009), *Ruminiclostridium 6* (q = 0.019), the uncharacterized taxon within *Coriobacteriaceae* (q = 0.021), *Ruminococcaceae UCG-005* (q = 0.016), *Ruminococcaceae UCG-010* (q = 0.028) and *Oxalobacter* (q = 0.036) compared to the OB group.

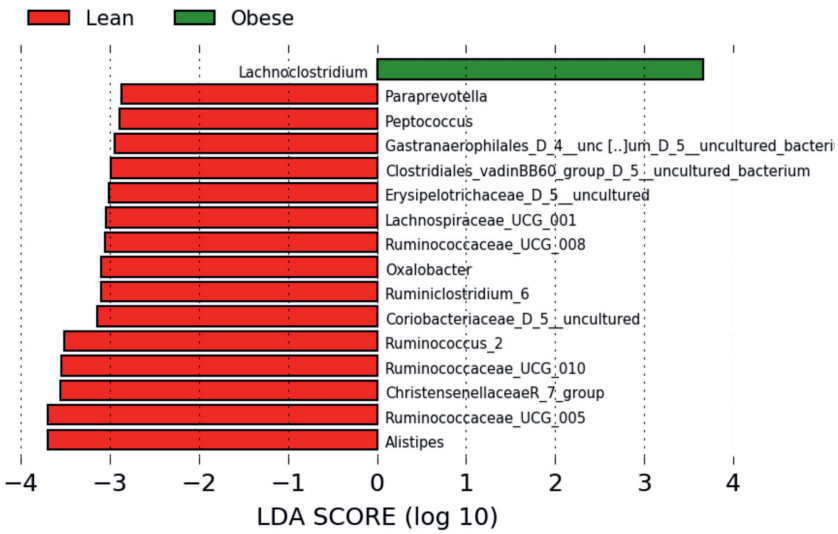

Figure 5. Linear discriminant analysis effect size (LEfSe) algorithm

The genera enriched in obese and lean are colored by green and red respectively. Histogram of the LDA scores computed for features differentially abundant among lean and obese. Only class meeting an LDA significant threshold >2 and $p < 0.05$ are shown.

Table 2. Significant Differences in the Relative Abundance of Bacteria at Genus Level Between Obese and Lean Individuals

Genus	Lean (Median (IQR)) n = 23	OB (Median (IQR)) n = 30	p	q
Ruminococcus 2	0.00576 (0.00745)	0.00157 (0.00377)	0.003	**0.008***
Lachnospiraceae UCG-001	0.00406 (0.00323)	0.000850 (0.00250)	0.003	**0.009***
Lachnoclostridium	0.00988 (0.00826)	0.0167 (0.0175)	0.005	**0.013***
Ruminococcaceae UCG-005	0.0158 (0.0200)	0.00761 (0.0109)	0.006	**0.016***
Ruminiclostridium 6	0.00424 (0.00485)	0.000464 (0.00285)	0.008	**0.019***
Uncharacterized taxon within *Coriobacteriaceae*	0.000411 (0.000815)	0.00 (0.000270)	0.009	**0.021***
Peptococcus	0.000359 (0.000737)	0.00 (0.000255)	0.010	**0.023***
Ruminococcaceae UCG-010	0.00430 (0.00895)	0.00100 (0.00444)	0.012	**0.028***
Oxalobacter	0.000217 (0.000637)	0 (0.000207)	0.015	**0.036***
Alistipes	0.0166 (0.0148)	0.00598 (0.0143)	0.022	0.051
Uncharacterized taxon within *Clostridiales.vadinBB60*.group	0.00167 (0.00460)	0.00 (0.00200)	0.033	0.075
Ruminococcaceae UCG-008	0.00 (0.000338)	0.00 (0.00)	0.035	0.079
Uncharacterized taxon within *Erysipelotrichaceae*	0.000153 (0.00160)	0 (0.000330)	0.036	0.081
Uncharacterized taxon within *Gastranaerophilales*	0.00 (0.000707)	0.00 (0.00)	0.039	0.088
Christensenellaceae R-7 group	0.0108 (0.0170)	0.00398 (0.0123)	0.041	0.092
Paraprevotella	0.00287 (0.00428)	0.000299 (0.00238)	0.043	0.095

* Indicates $q < 0.05$ for kruskal-wallis with FDR correction. Eight taxa are significant according to these criteria. The other taxa that showed significance in LEfSe are listed as well.

Correlation between gut microbiota and anthropometric measurements

No correlations were found between the gut microbiota at the genus level and the anthropometric measurements. Furthermore, there was no correlation with gender.

4. Discussion

The study had a cross sectional design with the intention to compare the gut microbiota of lean and OB Lebanese individuals residing in Lebanon. Concerning Lebanon which is in the EMR, the Lebanese diet is known for its healthy traditional cuisine that includes an abundance of vegetables, fruits, whole grains, legumes, nuts, fresh fish and seafood and minimally processed vegetarian recipes (Hwalla & El Khoury, 2008). Olive oil is the principle fat used and poultry is eaten more than red meat (which is usually lamb and goat) (Hwalla & El Khoury, 2008). With modernization, consumer tastes have had an effect that caused deviations from the traditional Lebanese foods to a more Westernized diet high in saturated fat, sugar, and red meat that is known to be associated with the increased risk of obesity, cardiovascular disease, and diabetes (Hwalla & El Khoury, 2008).In previous studies it has been shown that an altered gut microbiota has been linked to the development of metabolic diseases and in particular obesity (Bouter et al., 2017). However, there are multiple studies with conflicting results that were performed on individuals in the West and East (Escobar et al., 2014; R. Gao et al., 2018; Jiang et al., 2015; Kaplan et al., 2019; Riva et al., 2017; Shen et al., 2017; Wang et al., 2016; Zhu et al., 2013)and to our knowledge there is limited studies concerning the Eastern Mediterranean Region and in particular Lebanon.

Our study confirmed some prior results that there is a significant difference in the alpha-diversity measures (Pielou's evenness and Shannon index), with these being significantly greater in lean subjects compared with OB, indicating that lean individuals have more diverse gut microbiota than OB individuals. Moreover, with respect to the observed OTUs, Chao1, and Faith's PD, these were slightly higher in lean than OB but not significant (but with a trend for Faith's PD). However, there is a discrepancy in the results found in the literature with respect to the Shannon index and obesity. Some studies, like ours, showed a higher Shannon index in non-OB compared to OB subjects (Andoh et al., 2016; Beaumont et al., 2016; de la Cuesta-Zuluaga et al., 2018; X. Gao et al., 2018; Kaplan et al., 2019; Osborne et al., 2020; Ozato et al., 2019; Salah et al., 2019; Yasir et al., 2015). Other studies showed the contrary with OB having higher Shannon index (Kasai et

al., 2015; Salah et al., 2019), and some studies showed no significant differences among OB and lean individuals (Chavez-Carbajal et al., 2019; Davis et al., 2020; Finucane et al., 2014; Galle et al., 2020; Harakeh et al., 2020; Loftfield et al., 2020; Oduaran et al., 2020; Patil et al., 2012; Peters et al., 2018; Rahat-Rozenbloom et al., 2014; Yasir et al., 2015). As for beta-diversity, our OB subjects had a greater unweighted UniFrac compared to lean indicating that, in the OB group, a unique set of microbial taxa is found that is not present in the lean, but at low levels.

Moreover, we also found evidence that there is a difference in the composition of the gut microbiota of OB and lean Lebanese individuals at the phylum and genus levels. There are several human studies that have linked the human gut microbiota to obesity with the explanation that an increased *Bacteroidota/Bacillota* ratio may play a role in this pathophysiology (Chavez-Carbajal et al., 2019; Kaplan et al., 2019; Kasai et al., 2015; Rahat-Rozenbloom et al., 2014). However, our results do not support this hypothesis since there was no significant difference in the *Bacteroidota/Bacillota* ratio ($q = 0.451$), like many other studies (Galle et al., 2020; Loftfield et al., 2020; Peters et al., 2018; Salah et al., 2019; Whisner et al., 2018). It was shown that mice that were fed a high fat diet exhibited a high *Bacteroidota/Bacillota* ratio (Bisanz et al., 2019), however our subjects in both groups had similar energy and fiber intake. Nevertheless, we noted that the phylum *Verrucomicrobiota* was higher in lean compared to OB individuals. *Verrucomicrobiota* contain the genus *Akkermansia*, which is well known to play a role in maintaining the anti-inflammatory state of the gut and regulating glucose metabolism by improving insulin sensitivity and this genus was shown to be absent in OB individuals with type 2 diabetes (Ahmad et al., 2019; Fujio-Vejar et al., 2017; Shin et al., 2014).

Our results on bacterial diversity suggest that it is important to analyze the type and function of bacteria that compose the microbiota. Therefore, we analyzed the data using the LEfSe and Kruskal-Wallis with Benjamini–Hochberg correction, obtaining relevant bacteria that were significantly enriched in lean and OB groups. For the OB group, *Lachnoclostridium* was the only genus that was more abundant compared to the lean group. Several studies have reported *Lachnoclostridium* to be linked to diet induced obesity (Li & Vance, 2008; Sun et al., 2020; Zhao et al., 2017) and it might negatively impact obesity and type 2 diabetes (Nogal et al., 2021). On the other hand, only eight genera were significantly enriched in the lean group post FDR correction which were: *Peptococcus*,

Ruminococcus_2, *Lachnospiraceae UCG-001*, *Ruminiclostridium 6*, the uncharacterized taxon within *Coriobacteriaceae*, *Ruminococcaceae UCG-005*, *Ruminococcaceae UCG-010* and *Oxalobacter*. In line with our results, *Ruminiclostridium* has been found to improve intestinal barrier function, regulate lipid metabolism, produce short-chain fatty acids (SCFAs), and reduce inflammation (Song et al., 2021; Wang et al., 2020). Also, in our study we noted that the genus *Ruminococcus 2* was present among the lean subjects. Conflicting results regarding *Ruminococcus* and obesity were found in studies from the West with it being an obesity-associated and non-obesity associated genus (Escobar et al., 2014; Kaplan et al., 2019; Riva et al., 2017; Zhu et al., 2013), but with respect to studies in the East, *Ruminococcus* has been shown to be a lean-associated genera (R. Gao et al., 2018; Jiang et al., 2015; Shen et al., 2017; Wang et al., 2016) and this is in line with our results since Lebanon is a country in the EMR. *Ruminococcus* is involved in the degradation of fiber in particular cellulose and hemicellulose that are further fermented to produce SCFAs that are used as energy by the host (Jiang et al., 2015; Xu et al., 2022). In addition to this the SCFAs play a role in gut protection against inflammation, which is known to be a contributing factor in obesity (Jiang et al., 2015).

The *Lachnospiraceae UCG-001* genus belongs to the family *Lachnospiraceae*, *Ruminococcaceae* UCG-005 and *Ruminococcaceae* UCG-010 belonging to the family *Ruminococcaceae*, both of which have been reported to be important SCFA-producing bacteria that are known to have anti-inflammatory properties (Mancabelli et al., 2017; Tsai et al., 2020) and are involved in the release of glucagon-like peptide 1 that promotes satiety and weight loss (Oliphant & Allen-Vercoe, 2019) and may play a role in the protection against type 2 diabetes (Gryaznova et al., 2022; Wei et al., 2018).

In addition to this *Coriobacteriaceae* in our study was significantly higher in the lean group. The family *Coriobacteriaceae* within *Actinomycetota* has been shown to be involved in glucose homeostasis, bile acid and lipid metabolism (Kim et al., 2020). Also, it has been shown to be associated with good metabolic health in OB and overweight populations (Kim et al., 2020). In addition to this, in post bariatric surgery, in particular Roux-en-Y gastric bypass (RYGB), there is an improvement in glucose metabolism and a change in the gut microbiota with an increase in *Coriobacteriaceae* that may mediate the beneficial effects of RYGB on type 2 diabetes (Liu et al., 2018).

Furthermore, our lean subjects had a significantly higher content of *Oxalobacter. Oxalobacter, and* in particular *Oxalobacter formigenes* is beneficial to overall health since it has a specific role in the degradation of dietary oxalates and regulating blood oxalate levels and thus preventing calcium oxalate kidney stone formation (PeBenito et al., 2019).

Even though this study was an observational study and it is limited by its relatively small sample size, it provides preliminary data on the microbiota of lean *vs.* OB Lebanese individuals that are residing in Lebanon using comprehensive microbiome analysis. Furthermore, we did not adjust for dietary intake which is a potential confounding factor, that could alter our ability to link gut microbiota profiles with different diets. A lot of controversial evidence is observed in the literature about associations of microbial taxa and obesity, and this might be due to many factors such as ethnicity, environment, diet, lifestyle, gender, method of DNA extraction and sequencing (Pinart et al., 2021). 16S rRNA sequencing was utilized to provide more taxonomic resolution than metagenomic sequencing in this case, but further testing using shotgun metagenomics to go beyond the genus level as well as metabolomics to study the metabolites to monitor the progression of metabolic diseases such as obesity and diabetes is warranted in future research. In addition, to16S rRNA sequencing, phylogenetic investigation of communities by reconstruction of unobserved states (PICRUST) to predict functional profiles from 16S rRNA data would be of interest in future research in larger groups. Finally, our results should be interpreted cautiously as they are based on observational data, therefore there is a need for more comprehensive, larger, and longitudinal studies to be added to the preliminary results obtained in this study.

5. Conclusion

Overall, there are discrepant results in the gut microbiota composition of OB *vs*. lean individuals among different (geographical) studies at the phylum and genus level. In the current study, the difference in the *Bacteroidota/Bacillota* ratio was not significant in the Lebanese population being studied and therefore we cannot rely on it to be an obesity indicator/marker. However, we did find a significant difference in gut microbiota post FDR correction between lean and OB Lebanese subjects with certain bacterial taxa (at the genus level) being present in the lean group and not in the OB group such as *Peptococcus*, *Ruminococcus_2*, *Lachnospiraceae UCG-001*, *Ruminiclostridium 6*, an uncharacterized taxon within *Coriobacteriaceae*, *Ruminococcaceae UCG-005*, *Ruminococcaceae UCG-010*

and *Oxalobacter*, of which most have been linked with lower BMI and are reported to be SCFA-producing bacteria that possess anti-inflammatory properties (Mancabelli et al., 2017; Tsai et al., 2020), promote satiety and weight loss (Oliphant & Allen-Vercoe, 2019) and play a role in the protection against type 2 diabetes (Gryaznova et al., 2022; Liu et al., 2018; Wei et al., 2018). This study provides new perspectives in the field of obesity among one of the Eastern Mediterranean populations. However, future studies involving larger groups and using additional techniques (such as shotgun metagenomics and metabolomics) should be performed to determine whether modulating the gut microbiota can be a strategy for treating obesity.

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CHAPTER 4



Chapter 4

Associations Between Gut Microbiota Composition and Fasting Gut Peptides in Lebanese Adults with Obesity

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Abstract

Context

Obesity is associated with metabolic dysfunction and alterations in gut microbiota composition. Evidence suggests that gut microbes influence host metabolism through interactions with gut peptides; however, human data remain limited, particularly in Middle Eastern populations.

Objective

To examine gut microbiota composition and its associations with fasting gut peptides in Lebanese adults with obesity and normal-weight controls.

Methods

In this cross-sectional study, 53 Lebanese adults (30 with obesity [POB], 23 normal-weight [NW]) underwent anthropometric and biochemical assessment, stool16S rRNA gene sequencing, and fasting measurement of glucagon-like-peptide-1 (GLP-1), glucagon-like-peptide-2 (GLP-2), peptide YY (PYY), cholecystokinin (CCK), ghrelin, and leptin. Differential abundance was assessed using LEfSe. Spearman correlations and BMI-adjusted regression models evaluated microbiota–peptide associations.

Results

Lachnoclostridium was enriched in POB, whereas *Peptococcus* and several *Ruminococcaceae* taxa were more abundant in NW participants. GLP-1 and PYY concentrations were higher in POB, while ghrelin was higher in NW. Several bacterial taxa were inversely associated with GLP-1, GLP-2, and PYY concentrations, and some associations remained significant after BMI adjustment.

Conclusion

Obesity was characterized by distinct microbial profiles and altered fasting gut peptide concentrations. These exploratory findings support associations between gut microbiota and gut peptides and warrant confirmation in longitudinal and interventional studies.

Keywords: Gut microbiota; Gut peptides; Obesity; GLP-1; PYY; Ghrelin

1. Introduction

Obesity is a major public health challenge worldwide, strongly associated with metabolic complications such as insulin resistance, type 2 diabetes, and cardiovascular disease [1,2]. According to the World Health Organization, the prevalence of obesity has nearly tripled since 1975, affecting individuals across diverse age groups and regions [1,2]. The Middle East, including Lebanon, has experienced a rapid increase in obesity rates, driven by sedentary lifestyles, Westernized dietary patterns, and genetic predisposition [3].

In recent years, the gut microbiota has emerged as a key player in the pathophysiology of obesity and its metabolic comorbidities [4,5]. The gut harbors trillions of microorganisms that participate in nutrient absorption, immune regulation, and energy balance [6,7]. Dysbiosis—defined as an imbalance in microbial diversity and composition—has been linked to enhanced energy harvest, systemic inflammation, and impaired metabolic regulation [6,7]. Several studies have identified taxonomic shifts in individuals with obesity, including higher abundances of Bacillota (formerly Firmicutes) and lower abundances of Bacteroidota (formerly Bacteroidetes) [7]. However, findings remain inconsistent across populations due to differences in genetics, diet, and environmental exposures [6,7].

Beyond microbial composition, the gut microbiota exerts metabolic effects through interactions with host-derived gut peptides that regulate appetite and energy intake [8-11] . These peptides, secreted by enteroendocrine cells in response to nutrient stimuli, include ghrelin, glucagon-like peptide-1 (GLP-1), and peptide YY (PYY) [9]. Ghrelin is orexigenic, stimulating appetite and promoting food intake, whereas GLP-1 and PYY are anorexigenic hormones that enhance satiety and slow gastric emptying [10]. Leptin, secreted primarily by adipose tissue, acts as a long-term regulator of energy balance by signaling nutrient sufficiency to the hypothalamus and suppressing appetite. Alterations in leptin sensitivity are common in obesity, and emerging evidence suggests that gut microbiota composition may influence circulating leptin levels and leptin resistance[8 9,11-13].

Microbial metabolites such as short-chain fatty acids (SCFAs), bile acids, and indole derivatives modulate gut-peptide secretion and signaling [8]. For example, butyrate and propionate stimulate GLP-1 and PYY release, whereas microbial-derived lipopolysaccharides impair peptide signaling and promote metabolic inflammation [12]. Despite growing evidence for this bidirectional gut–peptide axis, few studies have jointly

characterized gut microbial composition and gut peptides in human populations, particularly within Middle Eastern cohorts [8,9,11-13].

Lebanon represents a unique setting for such investigations due to its heterogeneous population and Mediterranean-influenced diet, which differs from typical Western dietary patterns [14]. Understanding how gut microbial composition relates to gut peptide and leptin regulation in this population may yield insights into obesity-related metabolic mechanisms.

Therefore, this study aimed to examine associations between specific bacterial taxa and fasting concentrations of appetite-regulating peptides, including ghrelin, GLP-1, glucagon-like peptide-2 (GLP-2), cholecystokinin (CCK), PYY, and leptin. We hypothesized that obesity would be characterized by distinct microbial signatures and that certain bacterial taxa would be associated with altered gut peptide levels, reflecting potential disruptions in gut–peptide interactions contributing to obesity-related metabolic dysregulation.

2. Materials and Methods

2.1. Study design

This investigation was designed as a cross-sectional study including Lebanese participants residing in Lebanon. All procedures adhered to the ethical principles of the Declaration of Helsinki and received approval from the Institutional Review Board of the Middle East Institute of Health University Hospital (MEIH-UH), Lebanon (AL-04-02/2018-IRB/MEIH). The study was registered at ClinicalTrials.gov (Identifier: NCT04200521).

2.2. Study population

Participant recruitment occurred between November 2019 and April 2021.

Sample size estimation was initially based on anticipated differences in major microbial phyla between obesity and normal-weight groups. However, the present analyses focus primarily on exploratory associations between gut peptide concentrations and bacterial genera. Given the absence of prior human data quantifying expected correlation effect sizes between fasting incretins and specific gut taxa, formal a priori power calculation for correlation analyses was not feasible.

Post hoc sensitivity analysis indicated that with a total sample of 53 participants, the study had 80% power to detect correlation coefficients of $r \geq 0.38$ at $\alpha = 0.05$. Therefore, the study was adequately powered to detect moderate-to-large associations but may have been underpowered for small effect sizes. Accordingly, results should be interpreted as exploratory and hypothesis-generating.

Inclusion criteria were: stable body weight, absence of antibiotic treatment during the past three months, age 18–60 years, and both sexes. Eligibility was assessed via a screening questionnaire covering nationality (only Lebanese nationals with two Lebanese parents and living in Lebanon were included), overall health, weight history, alcohol use, physical activity, medication and supplement intake, probiotic/prebiotic/fiber use, and smoking status. Restricting the cohort to Lebanese nationals reduced heterogeneity in factors known to influence gut microbiota composition[15,16]. Physical activity was assessed via self-report and examined descriptively but was not included as a covariate in regression models. Individuals with obesity (Body Mass Index (BMI) ≥35 kg/m²) that were assigned to the POB group, were recruited from the MEIH-UH bariatric surgery program prior to undergoing bariatric surgery and before any pre-operative dietary intervention. This threshold reflects standard bariatric surgery eligibility criteria and provided a clinically well-defined severe obesity phenotype. Age- and sex-matched individuals of NW (BMI 18.5–24.9 kg/m²) were recruited as controls from the general community through personal contacts and word of mouth.

Exclusion criteria included weight reduction ≥5% in the previous three months, pregnancy, alcohol intake above ½ ounce/day for women or 1 ounce/day for men [17], and refusal to provide informed consent.

In total, 53 participants (16 men and 37 women) were enrolled: 30 POB (10 men, 20 women) and 23 NW (6 men, 17 women).

2.3. Anthropometric measurements

Participants attended the MEIH-UH testing site following a 12-hour (h) overnight fast. They were instructed to avoid alcohol, caffeine, and vigorous activity during the preceding 24 h. Height and weight were measured with light clothing and no shoes using a SECA balance, and BMI was calculated as weight (kg)/height (m²). Waist circumference (WC) was measured to the nearest 0.1 cm using a non-stretchable tape at the umbilical level

midway between the lower ribs and the iliac crest [18]. WC cut-offs for increased risk were those recommended by the International Diabetes Federation (IDF): <94 cm for men and <80 cm for women. Waist-to-height ratio (WtHR) was determined by dividing WC (cm) by height (cm), with ≥0.5 indicating abdominal obesity [19]. Body composition was measured by bioelectrical impedance using the InBody 720 analyzer (InBody Co., Ltd., Seoul, Korea), and elevated percent body fat (PBF) was defined as ≥25% for men and ≥35% for women [20].

2.4 Dietary assessment

Habitual dietary intake was assessed using a 3-day food diary consisting of two weekdays and one weekend day. Participants were instructed to record all foods and beverages consumed, including portion sizes, preparation methods, and brand names where applicable. Written and verbal instructions were provided to improve accuracy and completeness.

Dietary records were reviewed by trained research personnel for clarification where necessary. Total energy and fiber intake were calculated using food reference tables from *Understanding Nutrition* [21]. Mean daily energy and fiber intake were derived from the three recorded days and used for descriptive and comparative analyses.

2.5 Biochemical analyses

Venous blood was drawn by a registered nurse after an overnight fast. Fasting blood glucose (FBG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were analyzed enzymatically using the Roche/Hitachi Cobas® 6000 analyzer (Roche Diagnostics, Basel, Switzerland). Fasting insulin was quantified by electrochemiluminescence immunoassay (sandwich ELISA) with the Elecsys kit (Roche) on the same platform at the Department of Pathology and Laboratory Medicine, MEIH-UH. Insulin resistance was estimated using the homeostatic model assessment for insulin resistance (HOMA-IR) equation: (fasting insulin [μU/mL] × fasting glucose [mg/dL]) ÷ 405 [22], with a cut-off value of 2.32 to indicate insulin resistance [23].

For gut peptide analyses (GLP-1, GLP-2, PYY, CCK, ghrelin, leptin), blood samples were stabilized with Pefabloc (Sigma Aldrich, St. Louis, MO, USA; 100 mg/mL) and DPP IV inhibitor (Sigma Aldrich) to prevent peptide degradation. Serum

concentrations of GLP-1, GLP-2, PYY, ghrelin, and leptin were determined using ELISA kits (Diametra Millipore, Billerica, MA, USA), and CCK was measured with an ELISA kit from ABclonal Technology (Wuhan, China). Absorbance was read at 450 nm using a Thermo Scientific™ Varioskan™ LUX multimode microplate reader (Thermo Fisher Scientific, MA, USA). Peptides were measured in the fasting state to standardize metabolic conditions and reduce postprandial variability. While postprandial measurements may amplify hormonal responses, fasting levels reflect basal enteroendocrine tone and allow between-group comparison under controlled conditions.

2.6 Stool collection

Stool samples were collected using Zymo DNA/RNA Shield™ fecal collection kits (Zymo Research Corp., Irvine, USA). Participants followed written instructions to obtain two samples, one used for analysis and the other retained for verification. Each collection tube contained 9 mL of DNA/RNA Shield™ and a spoon attachment for transferring approximately 1 g of feces. This preservative ensured stability of DNA at room temperature for >1 year and RNA for up to 1 month.

2.7 DNA Isolation and Sequencing of the V3–V4 Region of the 16S rRNA Gene

Genomic DNA was extracted, and amplicons of the V3–V4 region of the 16S rRNA gene were prepared using Illumina protocols (Illumina, Eindhoven, The Netherlands) [24]. Sequencing was performed on an Illumina MiSeq platform (San Diego, CA, USA) using 2 × 300 bp paired-end reads with sample-specific barcodes. Data were processed in QIIME2 (https://qiime2.org) [25], with taxonomic assignment against the SILVA reference database (version 132).

2.8 Statistical analyses

Data analysis was performed using SPSS version 20 (IBM SPSS, Chicago, IL, USA) and R version 4.2.1 (R Core Team, http://www.r-project.org) in RStudio. Normality was tested by skewness and kurtosis. Normally distributed variables are reported as mean ± standard deviation (SD), while skewed data are shown as median (Mdn) with interquartile range (IQR). Between-group differences were analyzed using independent-samples t-tests for

normally distributed data, Mann–Whitney U tests for skewed data, and chi-square tests for categorical variables. A p-value <0.05 was considered statistically significant.

Prior to microbiota analyses, taxa present in fewer than 20% of samples were excluded to reduce data sparsity and improve the robustness of downstream analyses. Differences in microbial taxa abundance between NW and POB groups were assessed using the Kruskal–Wallis test, with correction for multiple comparisons using the Benjamini–Hochberg false discovery rate (FDR). Linear discriminant analysis effect size (LEfSe) was performed through the Galaxy platform [26] to identify differentially abundant taxa. Microbial taxa with an LDA score >2 and p <0.05 were considered significant [26].

Spearman's correlations were performed to evaluate relationships between microbial taxa and metabolic parameters, including gut peptide concentrations. Correlation coefficients (ρ) were calculated to estimate the strength and direction of associations, and results were visualized in heatmaps generated using the R ggplot2 package. To account for multiple testing, p-values were adjusted using the Benjamini–Hochberg FDR procedure. FDR-adjusted q-values <0.05 were considered statistically significant.

For microbial taxa demonstrating significant correlations with gut peptide concentrations, separate multiple linear regression models were constructed to evaluate whether the observed associations remained after adjustment for BMI. In these analyses, the relative abundance of the bacterial taxon was entered as the dependent variable, while the corresponding fasting peptide concentration (GLP-1, GLP-2, or PYY) was included as the independent variable. BMI was included as a covariate to assess its potential confounding effect. Regression coefficients, 95% confidence intervals (CIs), p-values, and adjusted R^2 values were reported. The percentage change in the regression coefficient following BMI adjustment was calculated to quantify the magnitude of confounding, with a change of ≥10% considered indicative of meaningful confounding [27].

Given the exploratory nature and modest sample size of the study, analyses focused on pairwise correlations and BMI-adjusted regression models to identify specific microbiota–peptide associations while minimizing overfitting.

3. Results

3.1 Participant characteristics

The baseline characteristics of participants are summarized in Table 1 (data presented in a previous study [28]). The study enrolled 23 NW (BMI 18.5–24.9 kg/m²) individuals and 30

POB (BMI ≥35 kg/m²). Women comprised the majority of both groups, accounting for 73.9% of the NW group and 66.7% of the POB group, with no significant difference in sex distribution ($p = 0.57$).

As expected, anthropometric indicators including WC, WtHR, and PBF were significantly greater among POB. Regarding lifestyle factors, energy intake, fiber intake, smoking status, and use of probiotic, prebiotic, or fiber supplements did not differ significantly between groups. Notably, however, the NW group reported higher alcohol consumption ($p = 0.03$) and greater intake of probiotic-containing foods ($p < 0.001$) compared with the POB group.

Table 1. Participant characteristics [28]

	NW n=23	**POB n=30**	**p**
Age (M±SD)	40.13±10.08	41.05±10.72	0.75
Female (n (%))	17 (73.90)	20 (66.70)	0.57
Anthropometric data			
BMI (kg/m^2) (M±SD)	22.02±1.91	38.83±3.49	**<0.001***
WC (cm) (M±SD)†	81.76±9.88	118.73±8.52	**<0.001***
WtHR (M±SD)†	0.37±0.04	0.63±0.07	**<0.001***
Percent Body Fat (M±SD)	24.46±6.21	45.44±5.69	**<0.001***
Lifestyle factors			
Fiber Intake (g) (M±SD)	17.61±7.31	18.19±8.41	0.79
Energy Intake (Kcal) (M±SD)	1612.57±508.59	1822.08±745.82	0.23
Smoker Yes (n,%)	11 (47.80)	14 (46.70)	0.93
Alcohol Yes (n,%)	16 (69.60)	12 (40.00)	**0.03***
Probiotic Supplement Yes (n,%)	0 (0)	0 (0)	-
Fiber Supplement Yes (n,%)	0 (0)	0 (0)	-
Prebiotic Supplement Yes (n,%)	0 (0)	0 (0)	-
Probiotic Food Consumption Yes (n,%)	19 (82.60)	8 (26.70)	**<0.001***

* The statistical significance was evaluated by t test for independent samples for continuous variables and by Pearson's chi-squared test for categorical variables Bold values denote statistical significance ($p < 0.05$).
Abbreviations: NW: normal weight; POB: people with obesity; BMI: body mass index; WC: waist circumference; WtHR: waist to height ratio, M±SD: Mean ± Standard Deviation.
† Clinical characteristics of study participants. Normal Waist Circumference according to IDF using Europids cut off values of <94 cm for males and <80 cm for females; Waist to Height Ratio normal refers to <0.5.

3.2 Biochemical and gut peptide analyses

The biochemical and gut peptide analyses of the participants are shown in Table 2. POB showed significantly higher levels of leptin (p<0.001), PYY (p=0.036), GLP-1 (p=0.022), insulin (p<0.001), HOMA-IR (p<0.001) and, TG (p=0.04). NW participants had significantly higher levels of ghrelin (p<0.001) and HDL-C (p<0.001) compared to POB. No significant differences were noted regarding CCK, GLP-2, FBG, TC, and LDL-C concentrations.

Table 2. Biochemical and gut peptide analyses in POB and NW individuals

Variable	NW n=23	POB n=30	p
Leptin (ng/ml) (M±SD)	13.92 ±8.67	62.54±28.76	**<0.001***
Ghrelin (pg/ml) (M±SD)	431.70±202.30	231.7±130.1	**<0.001***
PYY (pg/ml) (M±SD)	57.60±31.66	82.44±51.81	**0.036***
GLP-1 (pM) (Mdn±IQR)	23.30 ± 13.20	27.60±13.80	**0.022***
GLP-2 (ng/ml) (M±SD)	3.43±1.35	4.19±1.50	0.063
CCK (pg/ml) (Mdn±IQR)	36.40±18.10	36.90±9.89	0.538
FBG (mg/dl) (Mdn±IQR)	97.00 ±8.50	98.50±11.50	0.211
Insulin (µU/ml) (M±SD)	8.01±2.47	20.91±10.32	**<0.001***
HOMA-IR (M±SD)	1.95±0.70	5.43±2.63	**<0.001***
Total Cholesterol (mg/dl) (Mdn±IQR)	182.00±50.50	177.00±55.80	0.911
LDL-C (mg/dl) (Mdn±IQR)	101.00±42.00	109.00±49.00	0.636
HDL-C (mg/dl) (M±SD)	62.65±19.17	44.63±11.25	**<0.001***
Triglyceride(mg/dl) (M± SD)	107.09±51.67	149.67±50.23	**0.04***

* Significant difference at p<0.05 between the two groups

All numerical variables were calculated using Independent-Samples t-test; except for FBG; GLP-1; CCK, total cholesterol, LDL-C (nonparametric test using Mann-Whitney U-test), Abbreviations: NW: normal weight; POB: people with obesity CCK: cholecystokinin; PYY: peptide YY, GLP-1: glucagon like peptide-1; GLP-2: glucagon-like peptide-2; HOMA-IR: homeostatic model assessment for insulin resistance; LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol; FBG: fasting blood glucose; M±SD: mean ± standard deviation; Mdn±IQR: median ± interquartile range

3.3 Relative abundance of bacteria between POB and NW individuals

To explore differences in bacterial taxa between groups, relative abundances of gut microbes in the POB and NW participants were analyzed using the LEfSe algorithm. Notably, several significant taxa were of relatively low median abundance. To minimize false discovery signals, all differential abundance and correlation analyses were corrected using the Benjamini–Hochberg false discovery rate procedure (Table 3) (data presented in a previous study [28]). Nevertheless, given the compositional and sparse nature of microbiome data, associations involving low-abundance taxa should be interpreted cautiously. Replication in larger cohorts is warranted to confirm these signals. The analysis revealed that *Lachnoclostridium* was the only genus significantly enriched in the POB group (q = 0.013).

In contrast, eight genera were more abundant in NW participants: *Peptococcus* (q = 0.023), *Ruminococcus-2* (q = 0.008), *Lachnospiraceae UCG-001* (q = 0.009), *Ruminiclostridium 6* (q = 0.019), an unclassified taxon within the *Coriobacteriaceae* family (q = 0.021), *Ruminococcaceae UCG-005* (q = 0.016), *Ruminococcaceae UCG-010* (q = 0.028), and *Oxalobacter* (q = 0.036).

Table 3. Significant differences in the relative abundance of bacteria between POB and NW individuals [28]

Bacteria	NW (Median (IQR) n = 23	POB (Median (IQR) n = 30	p	q
Ruminococcus 2	5.76×10^{-3} (7.45×10^{-3})	1.57×10^{-3} (3.77×10^{-3})	0.003	**0.008***
Lachnospiraceae UCG-001	4.06×10^{-3} (3.23×10^{-3})	8.50×10^{-4} (2.50×10^{-3})	0.003	**0.009***
Lachnoclostridium	9.88×10^{-3} (8.26×10^{-3})	1.67×10^{-2} (1.75×10^{-2})	0.005	**0.013***
Ruminococcaceae UCG-005	1.58×10^{-2} (2.00×10^{-2})	7.61×10^{-3} (1.09×10^{-2})	0.006	**0.016***
Ruminiclostridium 6	4.24×10^{-3} (4.85×10^{-3})	4.64×10^{-4} (2.85×10^{-3})	0.008	**0.019***
Uncharacterized taxon within *Coriobacteriaceae*	4.11×10^{-4} (8.15×10^{-4})	0.00 (2.70×10^{-4})	0.009	**0.021***
Peptococcus	3.59×10^{-4} (7.37×10^{-4})	0.00 (2.55×10^{-4})	0.010	**0.023***
Ruminococcaceae UCG-010	4.30×10^{-3} (8.95×10^{-3})	1.00×10^{-3} (4.44×10^{-3})	0.012	**0.028***
Oxalobacter	2.17×10^{-4} (6.37×10^{-4})	0.00 (2.07×10^{-4})	0.015	**0.036***

* Indicates $q < 0.05$ for Kruskal-Wallis with FDR correction

Abbreviations: NW: normal weight; POB: people with obesity; IQR: interquartile range

3.4 Spearman correlation between gut microbiota and clinical and biochemical variables in POB and NW individuals and relative abundance of these bacteria

Spearman correlation analysis between gut microbiota and clinical and biochemical variables in POB and NW can be seen in the heatmap in Figure 1. Based on Figure 1 and Table 4, the bacteria that were negatively correlated with GLP-1 were *Butyricimonas (q*<0.001*, rho*=-0.55) an uncharacterized taxon within *Ruminococcaceae (q*<0.001*, rho*=-0.53*), Alistipes (q*<0.001*, rho*= -0.51*),* an uncharacterized taxon within *Coriobacteriaceae (q*=0.002*, rho*=-0.44*), Ruminococcaceae UCG-005 (q*=0.003*, rho*=-0.44*), Ruminococcaceae UCG-002 (q*=0.005*, rho*=-0.41*)*.

As for GLP-2, a negative correlation was found with the following bacteria *Butyricimonas (q*<0.001*, rho*=-0.55) *and* an uncharacterized taxon within *Ruminococcaceae (q*<0.001*, rho*=-0.48*).*

For PYY, *Eubacterium ventriosum* group *(q*<0.001*, rho*=-0.51*), Parasutterella (q*<0.001*, rho*=-0.49*),* a 2nd uncharacterized taxon within *Ruminococcaceae (q*=0.002*, rho*=-0.44*), Subdoligranulum (q*=0.004*, rho*=-0.43*),* and *Alistipes (q*=0.004*, rho*=-0.42) were negatively correlated. As shown in Table 5, several taxa that were inversely associated with gut peptides were also more abundant in NW participants, although only the uncharacterized taxon within *Coriobacteriaceae (q*=0.021**)** *and Ruminococcaceae UCG-005* (*q*= 0.016) remained significantly different after FDR correction.

In our findings, only age was positively correlated with *Ruminiclostridium 6*. On the other hand, no correlation was found for weight, BMI, WC, WtHR, PBF, insulin, glucose, HOMA-index, TC, LDL, HDL, TG, leptin, CCK, and ghrelin.

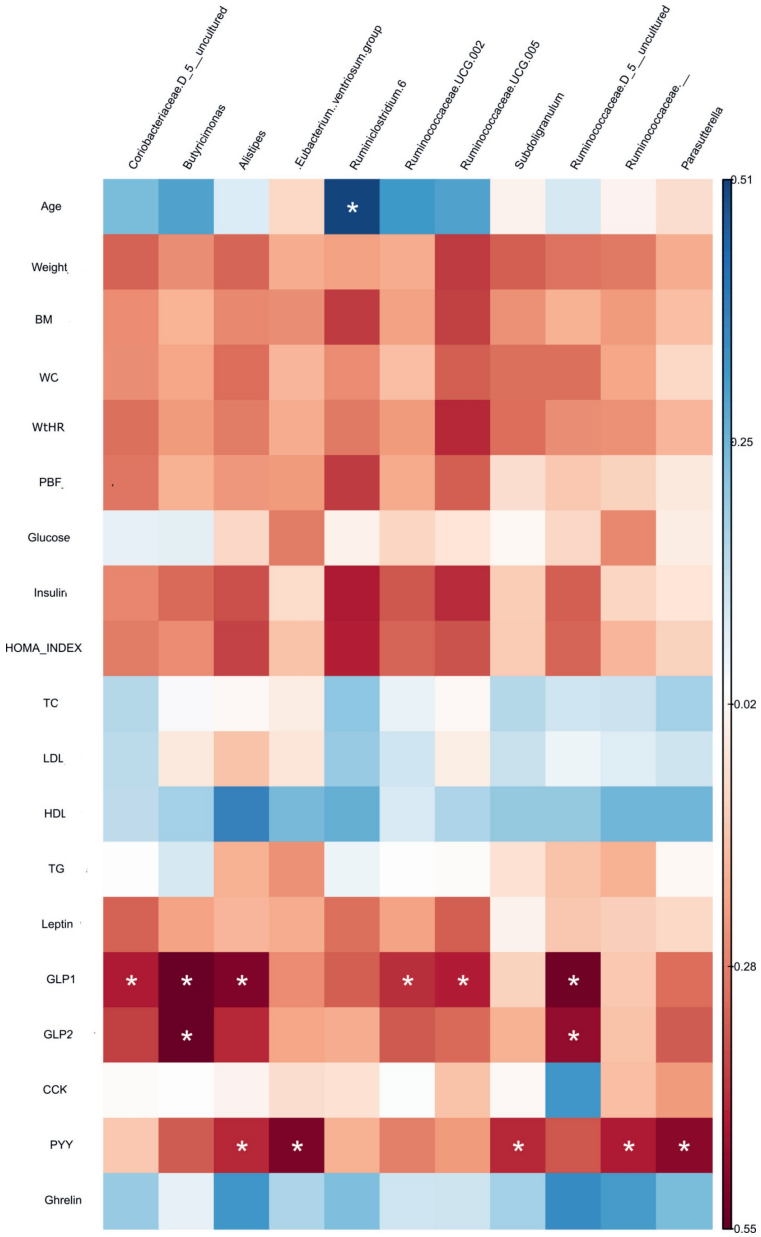

Figure 1. Spearman correlation analysis between gut microbiota and clinical variables in POB and NW Lebanese individuals. In the heatmap, blue squares indicate significant positive correlations and red squares indicate significant negative correlations. * Significant correlation at q<0.05 (FDR-correction) between POB and NW and relevant clinical variable. Abbreviations: CCK: cholecystokinin; PYY: peptide YY, GLP-1: glucagon-like peptide-1; GLP-2: glucagon-like peptide-2; LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol; TG: triglyceride; BMI: Body mass index; WC: waist circumference; WtHR: waist to height ratio, PBF: percent body fat

Table 4. Correlation analysis between gut microbiota and GLP-1, GLP-2 and PYY in POB and NW Lebanese individuals

Bacteria	Gut peptide	q	rho
	GLP-1		
Butyricimonas		**<0.001***	-0.55
an uncharacterized taxon within *Ruminococcaceae*		**<0.001***	-0.53
Alistipes		**<0.001***	-0.51
an uncharacterized taxon within *Coriobacteriaceae*		**0.002***	-0.44
Ruminococcaceae UCG-005		**0.003***	-0.44
Ruminococcaceae UCG-002		**0.005***	-0.41
	GLP-2		
Butyricimonas		**<0.001***	-0.55
an uncharacterized taxon within *Ruminococcaceae*		**<0.001***	-0.48
	PYY		
Eubacterium ventriosum group		**<0.001***	-0.51
Parasutterella		**<0.001***	-0.49
a 2nd uncharacterized taxon within *Ruminococcaceae*		**0.002***	-0.44
Subdoligranulum		**0.004***	-0.43
Alistipes		**0.004***	-0.42

* Indicates significant correlation q < 0.05 for Spearman correlation analysis with FDR correction.

Abbreviations: PYY: peptide YY, GLP-1: glucagon like peptide-1; GLP-2: glucagon-like peptide-2; NW: normal weight; POB: people with obesity; rho: spearman correlation

Table 5. Relative abundance of bacteria between POB and NW Individuals

Bacteria	NW (Median (IQR) n = 23	POB (Median (IQR) n = 30	q
Butyricimonas	1.52×10^{-3} (2.28×10^{-3})	9.66×10^{-4} (2.52×10^{-3})	0.277
an uncharacterized taxon within *Ruminococcaceae*	2.23×10^{-3} (2.46×10^{-3})	1.47×10^{-3} (2.47×10^{-3})	0.137
Alistipes	1.66×10^{-2} (1.48×10^{-2})	5.98×10^{-3} (1.43×10^{-2})	0.051
an uncharacterized taxon within *Coriobacteriaceae*	4.11×10^{-4} (8.15×10^{-4})	0.00 (2.70×10^{-4})	**0.021***
Ruminococcaceae UCG-005	1.58×10^{-2} (2.00×10^{-2})	7.61×10^{-3} (1.09×10^{-2})	**0.016***
Ruminococcaceae UCG-002	2.41×10^{-2} (1.56×10^{-2})	2.10×10^{-2} (2.41×10^{-2})	0.364
Eubacterium ventriosum group	4.71×10^{-4} (2.10×10^{-3})	7.71×10^{-5} (6.43×10^{-4})	0.242
Parasutterella	8.90×10^{-4} (3.76×10^{-3})	4.28×10^{-4} (2.48×10^{-3})	0.516
a 2nd uncharacterized taxon within *Ruminococcaceae*	8.84×10^{-3} (7.70×10^{-3})	6.17×10^{-3} (5.82×10^{-3})	0.393
Subdoligranulum	2.40×10^{-3} (3.74×10^{-3})	1.23×10^{-3} (2.24×10^{-3})	0.247

*Indicates $q < 0.05$ for Kruskal-Wallis with FDR correction

Abbreviations: NW: normal weight; POB: people with obesity; IQR: interquartile range

3.5 Linear regression analysis

Table 6 present the associations between gut microbial taxa and gut peptides (GLP-1, GLP-2, and PYY) before and after adjustment for BMI. Results from both the unadjusted and BMI-adjusted models are shown to evaluate the potential influence of BMI on the observed associations. Significant findings ($p < 0.05$) are highlighted, and the percentage change in regression coefficients following BMI adjustment is reported as an indicator of the magnitude of confounding.

Based on the linear regression analysis between GLP-1 and gut microbiota in Table 6, in the unadjusted models, significant negative associations were observed between GLP-1 and the abundance of *Butyricimonas*, *Alistipes*, uncharacterized taxon within *Ruminococcaceae*, *Ruminococcaceae UCG-005*, *Ruminococcaceae UCG-002*, and uncharacterized taxon within *Coriobacteriaceae* (all $p < 0.05$). After adjustment for BMI, these associations remained negative, though attenuated, with varying degrees of change in effect size. Notably, *Ruminococcaceae UCG-005* demonstrated the strongest evidence of confounding, with a 29.45% reduction in the GLP-1 coefficient and a significant BMI association ($p = 0.012$), indicating that BMI partially explained the relationship.

Coriobacteriaceae (20.3%) and *Alistipes* (15.1%) also exhibited moderate confounding effects. Conversely, *Butyricimonas* and an uncharacterized taxon within *Ruminococcaceae* showed minimal changes (<6%), suggesting a more robust association with GLP-1 independent of BMI.

These findings indicate that GLP-1 concentrations were associated with specific bacterial profiles and that BMI partially mediates or confounds these associations, particularly within members of the *Ruminococcaceae* family.

Also, as can be seen in Table 6, the analysis between GLP-2 and gut microbiota, two bacteria, *Butyricimonas* and an uncharacterized taxon within *Ruminococcaceae*, showed statistically significant negative associations with GLP-2 in both unadjusted and BMI-adjusted models. For *Butyricimonas*, the unadjusted beta was $–5.18 \times 10^{-4}$ ($p < 0.001$), and remained significant after adjustment ($\beta = –4.98 \times 10^{-4}$, $p < 0.001$), with only a 3.86% reduction in effect size, indicating minimal confounding by BMI. Similarly, the uncharacterized taxon within *Ruminococcaceae* was significantly negatively associated with GLP-2 ($\beta = –7.08 \times 10^{-4}$, $p < 0.001$), and the association persisted after adjustment ($\beta = –6.82 \times 10^{-4}$, $p < 0.001$), with a 3.67% reduction in effect size. These consistent findings suggest that higher relative abundances of *Butyricimonas* and *Ruminococcaceae* are independently associated with lower GLP-2 levels, regardless of BMI.

In the unadjusted models, significant negative associations were observed between PYY and *Eubacterium ventriosum* group, *Parasutterella*, *Subdoligranulum*, and *Alistipes* (all $p < 0.05$). After adjusting for BMI, these associations remained negative; however, the effect sizes were attenuated in most cases. *Eubacterium ventriosum* group showed a 27.8% reduction in effect size post-adjustment, and the association became non-significant ($p = 0.134$), suggesting that BMI may confound this relationship. *Subdoligranulum* and *Alistipes* also demonstrated moderate confounding effects, with 15.2% and 16.1% reductions in the regression coefficients, respectively, though their associations remained statistically significant. Conversely, *Parasutterella* retained a strong, statistically significant association with PYY in both models, with minimal change in effect size (2.5%), indicating an independent relationship. A 2nd uncharacterized taxon within *Ruminococcaceae* showed a borderline association in the unadjusted model ($p = 0.066$), which strengthened after adjustment ($p = 0.052$), suggesting a potential suppressor effect of BMI. These findings

indicate that PYY levels are inversely associated with the abundance of several gut microbial bacteria, particularly *Parasutterella*, and that BMI plays a moderate confounding role in select associations.

Table 6. Associations between gut peptide hormones (GLP-1, GLP-2, and PYY) and bacterial taxa before and after adjustment for BMI

Bacteria	Peptide	Estimate (Unadj.)	95% CI **(Unadj.)**	p	Estimate (Adj.)	95% CI **(Adj.)**	p	% Change	BMI p	Adj. R^2
Butyricimonas	GLP-1	-5.38×10^{-5}	$(-8.0 \times 10^{-5}, -2.8 \times 10^{-5})$	<0.001*	-5.13×10^{-5}	$(-8.1 \times 10^{-5}, -2.2 \times 10^{-5})$	0.001*	4.65 %	0.610	0.221
an uncharacterized taxon within *Ruminococcaceae*	GLP-1	-6.92×10^{-5}	$(-1.1 \times 10^{-4}, -2.8 \times 10^{-5})$	0.001*	-6.55×10^{-5}	$(-1.1 \times 10^{-4}, -2.4 \times 10^{-5})$	0.002*	5.35 %	0.596	0.181
Alistipes	GLP-1	-4.70×10^{-4}	$(-7.6 \times 10^{-4}, -1.8 \times 10^{-4})$	0.001*	-3.99×10^{-4}	$(-6.9 \times 10^{-4}, -1.1 \times 10^{-4})$	0.007*	15.11 %	0.146	0.194
an uncharacterized taxon within *Coriobacteriaceae*	GLP-1	-1.48×10^{-5}	$(-2.5 \times 10^{-5}, -4.0 \times 10^{-6})$	0.005*	-1.18×10^{-5}	$(-2.2 \times 10^{-5}, -1.2 \times 10^{-6})$	0.030*	20.27 %	0.101	0.157
Ruminococcaceae UCG-005	GLP-1	-3.26×10^{-4}	$(-5.4 \times 10^{-4}, -1.1 \times 10^{-4})$	0.003*	-2.30×10^{-4}	$(-4.4 \times 10^{-4}, -1.7$	0.036*	29.45 %	0.012*	0.228

						× 10^{-5})				
Ruminococcaceae UCG-002	GLP-1	–3.84 × 10^{-4}	(–6.4 × 10^{-4}, –1.3 × 10^{-4})	0.003*	–3.44 × 10^{-4}	(–6.1 × 10^{-4}, –7.9 × 10^{-5})	0.012*	10.42 %	0.369	0.140
Butyricimonas	GLP-2	–5.18 × 10^{-4}	(–7.5 × 10^{-4}, –2.9 × 10^{-4})	<0.001*	–4.98 × 10^{-4}	(–7.4 × 10^{-4}, –2.5 × 10^{-4})	<0.001*	3.86 %	0.612	0.258
an uncharacterized taxon within *Ruminococcaceae*	GLP-2	–7.08 × 10^{-4}	(–1.0 × 10^{-3}, –3.8 × 10^{-4})	<0.001*	–6.82 × 10^{-4}	(–1.0 × 10^{-3}, –3.3 × 10^{-4})	<0.001*	3.67 %	0.650	0.244
Eubacterium ventriosum group	PYY	–9.56 × 10^{-6}	(–1.8 × 10^{-5}, –8.5 × 10^{-7})	0.032*	–6.90 × 10^{-6}	(–1.6 × 10^{-5}, 2.2 × 10^{-6})	0.134	27.8 %	0.090	0.104
Parasutterella	PYY	–6.70 × 10^{-5}	(–1.1 × 10^{-4}, –2.6 × 10^{-5})	0.002*	–6.87 × 10^{-5}	(–1.1 × 10^{-4}, –2.4 × 10^{-5})	0.003*	2.5%	0.822	0.140
A 2nd uncharacterized taxon within *Ruminococcaceae*	PYY	–5.43 × 10^{-5}	(–1.1 × 10^{-4}, 3.8 × 10^{-6})	0.066	–6.16 × 10^{-5}	(–1.2 × 10^{-4}, 5.2 × 10^{-7})	0.052	13.4 %	0.490	0.036

Subdoligranulum	PYY	–1.98 × 10^{-5}	(–3.2 × 10^{-5}, –8.0 × 10^{-6})	0.001 *	–1.68 × 10^{-5}	(–2.9 × 10^{-5}, –4.4 × 10^{-6})	0.009 *	15.2 %	0.166	0.183
Alistipes	PYY	–1.37 × 10^{-4}	(–2.2 × 10^{-4}, –5.3 × 10^{-5})	0.002 *	–1.15 × 10^{-4}	(–2.0 × 10^{-4}, –2.6 × 10^{-5})	0.012 *	16.1 %	0.135	0.179

* Indicates significant correlation $p < 0.05$ for linear regression analysis. Abbreviations: PYY: peptide YY; BMI: body mass index; CI: confidence interval

4. Discussion

The present study examined associations between gut microbial composition and fasting gut peptide concentrations in Lebanese adults with obesity compared to normal-weight controls. Distinct microbial signatures were observed between groups, and several bacterial genera were inversely associated with GLP-1, GLP-2, and PYY concentrations. Because of the cross-sectional design, directionality cannot be determined.

Consistent with previous reports linking gut dysbiosis to obesity and metabolic dysfunction [29,30], individuals with obesity in this cohort exhibited enrichment of *Lachnoclostridium* and reduced abundance of several genera previously associated with metabolic health, including members of the *Ruminococcaceae* family. Population-level microbiome analyses have demonstrated that microbial composition varies substantially across ethnic and environmental contexts [15,16]. By focusing on a relatively homogenous Lebanese cohort, the present study aimed to reduce heterogeneity related to ethnicity and diet; nevertheless, microbial signatures in obesity remain context-dependent and should not be generalized without caution.

Participants with obesity exhibited higher fasting GLP-1 and PYY concentrations. Although GLP-1 and PYY are classically anorexigenic hormones[9,10], elevated fasting levels have been observed in obesity and may reflect compensatory adaptations to insulin resistance or reduced receptor sensitivity rather than enhanced satiety signaling[31,32]. In the present study, several bacterial genera—including *Butyricimonas*, *Alistipes*, and

uncharacterized taxa within *Ruminococcaceae*—were inversely associated with GLP-1 concentrations. Similar inverse associations were observed with GLP-2 and PYY for selected taxa.

Members of the *Ruminococcaceae* family and related SCFA-producing bacteria have been implicated in metabolic regulation and enteroendocrine stimulation[8,33]. Experimental evidence suggests that SCFAs may enhance GLP-1 and PYY secretion via activation of G-protein–coupled receptors in enteroendocrine cells [8,34]. However, human data remain inconsistent. The inverse associations observed here may reflect complex metabolic adaptations in obesity rather than direct effects on hormone secretion.
GLP-2 concentrations did not differ significantly between groups but were negatively associated with *Butyricimonas* and an uncharacterized *Ruminococcaceae* taxon. GLP-2 plays a role in intestinal barrier integrity and mucosal growth [35,36]. Whether the observed associations reflect microbial modulation of intestinal function or adaptive host responses cannot be determined in the current design.

Fasting ghrelin concentrations were significantly lower in individuals with obesity, consistent with established literature demonstrating suppressed ghrelin levels in positive energy balance states [37-39]. No significant associations between ghrelin and microbial taxa were detected. While gut microbiota have been suggested to influence ghrelin under certain conditions [40,41], fasting ghrelin regulation appears primarily driven by energy status and adiposity.

CCK concentrations did not differ significantly between groups and showed no associations with microbial abundance. Because CCK is primarily stimulated postprandially[42,43], fasting measurements may not reflect dynamic secretion patterns, potentially limiting detection of microbiota-related effects.

Leptin concentrations were markedly elevated in individuals with obesity, reflecting increased adiposity [14]. No associations between leptin and gut microbiota were identified. Experimental studies suggest that leptin can modulate microbial composition [44,45], but in humans, leptin resistance in obesity may operate largely independent of basal microbial abundance.

It is important to note that several taxa associated with gut peptides were of relatively low median abundance. Although false discovery rate correction was applied to reduce type I error, compositional microbiome data are inherently sparse and

interdependent. Therefore, associations involving low-abundance genera should be interpreted cautiously and require validation in larger cohorts employing compositional data analysis and network-based modeling approaches.

Several limitations warrant consideration. First, the cross-sectional design precludes determination of temporal or causal relationships. Second, although post hoc sensitivity analysis indicated adequate power to detect moderate correlations, smaller effect sizes may have gone undetected. Third, microbial taxa were analyzed individually rather than through ecological network analysis, potentially oversimplifying interrelated microbial dynamics. Fourth, peptides were measured exclusively in the fasting state. Because GLP-1, PYY, and CCK are primarily nutrient-stimulated hormones[34,42,43], fasting measurements may underestimate dynamic gut–microbiota interactions. Postprandial testing using standardized meal challenges could reveal amplified or temporally distinct associations and should be considered in future mechanistic studies. Fifth, dietary intake and lifestyle factors were self-reported, and objective energy expenditure was not assessed. Additionally, probiotic-containing food intake differed significantly between groups and was not included as a covariate in regression models. Although no consistent direct associations between probiotic intake and gut peptides were observed descriptively, residual confounding cannot be excluded. Finally, residual confounding by unmeasured metabolic or hormonal variables cannot be excluded.

Despite these limitations, this study provides novel data integrating gut microbial profiling and fasting gut peptides in a Middle Eastern population, a region underrepresented in microbiome research.

In summary, obesity in this Lebanese cohort was accompanied by alterations in gut microbial composition and fasting enteroendocrine peptide profiles. Several inverse microbe–peptide associations persisted after BMI adjustment. Further investigation of the gut microbiota–peptide axis in obesity is warranted.

5. Conclusion

This study demonstrates that obesity in a Lebanese adult cohort is associated with distinct gut microbial signatures and altered fasting concentrations of key appetite- and metabolism-regulating peptides. Specifically, individuals with obesity exhibited enrichment of *Lachnoclostridium*, while several genera—including *Peptococcus*, members of the

Ruminococcaceae family, and other taxa—were more abundant in normal-weight participants.

Inverse associations were identified between specific bacterial genera and fasting concentrations of GLP-1, GLP-2, and PYY, with several relationships remaining significant after adjustment for BMI. Notably, associations were observed even for taxa not significantly different in abundance between groups, underscoring that microbial–hormonal interactions may occur independently of gross compositional shifts. However, given the modest sample size, fasting-only peptide assessment, and potential residual confounding—including differences in probiotic-containing food intake—these results should be interpreted as exploratory and hypothesis-generating.

Overall, the findings support an association between gut microbial composition and fasting gut peptide profiles in obesity and highlight the need for longitudinal and interventional studies to clarify potential causal pathways and determine whether modulation of the gut microbiota influences metabolic hormone regulation.

Data Availability: The datasets generated and analyzed during the current study are publicly available in the Mendeley Data repository: https://data.mendeley.com/datasets/nkbgydybr4/1 DOI: 10.17632/nkbgydybr4.1, in accordance with FAIR data principles.

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CHAPTER 5



Chapter 5

Obesity and the Gut Microbiota in The Middle East: A Cross-Cultural Study of Lebanese and Emirati Adults

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Abstract

Obesity is a growing public health concern in the Middle East and North Africa (MENA) region, yet limited research has explored how gut microbiota varies between Arab populations. This study compared the gut microbiota composition and diversity of Emirati and Lebanese adults with obesity and assessed the role of age and nationality in shaping microbial variation. A total of 43 Emirati and 30 Lebanese individuals with obesity (body mass index (BMI) ≥35 kg/m²) were recruited. Participants provided anthropometric and biochemical data, dietary records, and stool samples for 16S rRNA sequencing. The analysis revealed significantly higher BMI, weight, and fat mass in Emirati participants, while Lebanese individuals reported higher fiber intake. Taxonomic profiling showed higher relative abundance of *Pseudomonadota, Mycoplasmatota, Cyanobacteriota*, and *Lentisphaerota* in the Lebanese group, whereas *Bacteroidota* was more abundant among Emiratis. Lebanese participants also exhibited significantly greater microbial alpha diversity. Beta diversity analysis confirmed clear distinctions in microbial community structure between the two groups. Linear Discriminant Analysis Effect Size (LefSe) (LDA score $>^{10}\log 2$) and regression models ($p< 0.05$) identified specific bacterial genera associated with nationality, although these associations were attenuated after adjusting for age. These findings suggest that gut microbiota in Arab populations is influenced by demographic, dietary, and environmental factors, emphasising the need for culturally tailored microbiota-based strategies to manage obesity and related metabolic conditions.

Keywords

gut microbiota, 16S rRNA, microbial diversity, ethnicity, anthropometrics

1. Introduction

Obesity has emerged as one of the most pressing global health challenges of the 21st century. According to the World Health Organization (2024), more than one billion people—approximately one in eight worldwide—are currently living with obesity (World Health Organization, 2024). The World Obesity Federation (2025) projects that by 2030, adult obesity prevalence will have increased by over 115% compared with 2010, reaching roughly 1.13 billion individuals globally (World Obesity Federation, 2025). Furthermore, long-term forecasts from the Global Burden of Disease (GBD) BMI Collaborators (2025) suggest that by 2050, around 60 % of adults (nearly 3.8 billion people) and one-third of children and adolescents (≈ 746 million) will be overweight or obese worldwide ((IHME), 2025). These trends are particularly concerning in the Middle East and North Africa, where obesity rates are among the fastest rising. Obesity rates have been steadily rising in Gulf countries, with prevalence increasing from 6.5% in 1975 to 20% in 2016 (Ritchie, 2017; World Health Organization, 2016). According to the United Arab Emirates (UAE)'s 2017–2018 National Health Survey, obesity affected 27.8% of the UAE population, while in Lebanon, the prevalence was 30.8% as reported by the World Health Organization in 2016 (World Health Organization, 2016). Without effective prevention and policy interventions, obesity-related diseases—including type 2 diabetes, cardiovascular disease, and other metabolic complications—are expected to increase substantially, especially in low- and middle-income settings ((IHME), 2025; Ng et al., 2025; World Health Organization, 2024; World Obesity Federation, 2025).

Genetic predisposition, lifestyle and environmental factors contribute to obesity, whilst emerging evidence highlights the gut microbiota as a key regulator of in particular, environmental and diet-related effects on metabolism and energy homeostasis (Patrone et al., 2016; Turnbaugh et al., 2006). The gut microbiota comprises not only bacterial phyla but also fungi, archaea, viruses, and protozoa, with its bacterial component being primarily composed of *Bacillota, Bacteroidota, Actinomycetota, Pseudomonadota, Fusobacteriota*, and *Verrucomicrobiota* (Oren & Garrity, 2021), with *Bacillota* and *Bacteroidota* accounting for nearly 90% of microbial communities (Arumugam et al., 2011; Rinninella et al., 2019).

An imbalance in gut microbiota, commonly referred to as dysbiosis, remains a debated concept, as microbial composition varies considerably among individuals, making it difficult to establish universal criteria for distinguishing a dysbiotic state from a healthy profile. Nevertheless, variations in gut microbiota have been linked to several metabolic and inflammatory disorders, including obesity, diabetes, and allergies (Gupta et al., 2017). Early animal studies suggested a strong relationship between the *Bacillota/Bacteroidota* ratio and obesity (Turnbaugh et al., 2006), but subsequent findings in humans have been inconsistent (Magne et al., 2020; Sze Marc & Schloss Patrick, 2016). Beyond this ratio, specific bacterial phyla and genera have also been associated with obesity (OB) (Crovesy et al., 2020). Individuals with obesity, insulin resistance (IR), and type 2 diabetes mellitus (T2DM) frequently exhibit altered gut microbiota compared to lean counterparts, characterized by reduced microbial richness and diversity as well as lower levels of butyrate-producing bacteria, changes that have been linked to metabolic impairments (Jardon et al., 2022). Increasingly, researchers emphasize that definitions of dysbiosis should account not only for taxonomic shifts but also for functional consequences on host metabolism and health.

The composition of gut microbiota varies among individuals and across populations, influenced by lifestyle, genetics, age, diet, medication use, geography, ethnicity, and environment (Crovesy et al., 2020; Deschasaux et al., 2018; Gupta et al., 2017; Jardon et al., 2022; Pinart et al., 2021). Advances in high-throughput sequencing technology have enhanced our understanding of population-specific differences in gut microbiota (Yasir et al., 2015).

While the Lebanese and Emirati populations share some environmental and cultural similarities, variations in dietary patterns, lifestyle habits, and genetic backgrounds may shape distinct gut microbiota profiles. These differences raise concerns about the universality of microbiome-based therapies, emphasizing the need for geographically tailored, community-specific approaches to microbiome research (Gupta et al., 2017).

Although multiple studies have explored the relationship between gut microbiota and obesity in different populations (Escobar et al., 2014; Jain et al., 2018), comparative research in Middle Eastern populations remains scarce (Yasir et al., 2015). Furthermore,

data on ethnicity-driven gut microbiota differences in Arab populations remain inconsistent (Andrew W Brooks et al., 2018).

This study aims to bridge this research gap by analyzing gut microbiota composition and diversity in Lebanese and Emirati individuals with OB using high throughput 16S ribosomal RNA (rRNA) gene amplicon sequencing and assessing whether age or other factors contribute to these observed variations. Identifying population-specific microbiota signatures associated with OB will provide valuable insights for developing targeted interventions to manage and prevent obesity-related complications in the Middle East, where research remains limited.

2. Materials and Methods

Study design

We conducted a cross-sectional design among Lebanese and Emirati subjects with OB living in the UAE and Lebanon, respectively. The present study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Middle East Institute of Health University Hospital (MEIH-UH) in Lebanon (AL-04-02/2018-IRB/MEIH) and by the Research Ethics Committee of the Ministry of Health and Prevention of UAE (MOHP/DXB-REC-52/2018), the Dubai Health Care Regulatory Research Ethics Committee (DHCR-REC), and the Zayed University Ethical Committee Board (ZU19_51_F) in UAE.

Study population

Recruitment took place from October 2019 until April 2021. Sample size was determined by following standard calculations based on normal distributions. The current study utilized data from a mother study designed to investigate gut microbiota profiles in Lebanese and Emirati participants, both with OB and of normal weight, as well as changes following bariatric surgery. The present analysis was restricted to obese participants from a larger cross-sectional study that included both obese and normal-weight adults from Lebanon and the UAE. The current manuscript focuses solely on the obese subgroup to assess ethnicity-related microbial differences within obesity. In the mother study, the primary outcome was the change in the *Bacteriodota*/*Bacillota* ratio. Sample size calculation was based on

expected changes in this ratio before and after bariatric surgery, referencing findings from Damms-Machado et al. (Damms-Machado et al., 2015), which reported a significant increase in the fecal *Bacillota*/*Bacteriodota* ratio from 5.9 (SD 2.1) to 10.4 (SD 1.4) three months post-surgery. Based on these values, a minimum of two participants was required to achieve 80% power at a two-sided 5% significance level (Ali Ahmad et al., 2025). To account for protocol deviations and anticipated reductions in effect size (~30%), researchers conservatively multiplied the required sample by a factor of 15. This adjustment yielded a final target of 30 participants per group. Accordingly, we aimed to recruit 30 Emirati and 30 Lebanese adults with OB, including both sexes, residing in the UAE and Lebanon, respectively.

Inclusion and exclusion criteria

Participants were eligible if they were adults between 18 and 60 years of age, had a body mass index (BMI) ≥ 35 kg/m², and had maintained stable body weight (no fluctuation greater than 5%) during the three months prior to recruitment. Eligibility was restricted to Emirati and Lebanese nationals with both parents of the same nationality. Additional requirements included absence of antibiotic use in the previous three months and willingness to complete a 3-day 24-hour food diary (two weekdays and one weekend) to assess habitual energy and fiber intake. For Emirati participants, dietary intake was analyzed using the Middle Eastern food composition database available in the Nutritics software, whereas for Lebanese participants, food reference tables from *Understanding Nutrition* (Whitney & Rolfes, 2002) were used. All participants provided written informed consent before enrolment. Eligibility was assessed through a structured screening questionnaire (Supplementary Information), which captured information on general health, weight history, alcohol intake, physical activity, smoking, medication use, and supplement intake (including probiotics, fiber, and prebiotics).

Participants were excluded if they had experienced weight loss ≥ 5% within the last three months, were pregnant, reported excessive alcohol consumption (>½ ounce/day for females; >1 ounce/day for males) (Snetselaar et al., 2021), or were unwilling or unable to provide informed consent.

Recruitment

Lebanese participants with obesity were recruited from the weight-loss program at the Middle East Institute of Health University Hospital (MEIH-UH), while Emirati participants with obesity were recruited from hospitals in Dubai and Sharjah. Recruitment took place between October 2019 and April 2021.

Anthropometric measurements

Eligible subjects were asked to arrive at the testing site after a 12 h fast and were asked to avoid alcohol and caffeine consumption, as well as any unusual strenuous exercise 24 h prior to the screening. While subjects wore light clothes and no shoes, height and weight were measured using the "SECA 700" balance and BMI was calculated as the ratio of weight (kg) to height (m) squared (kg/m^2). Waist circumference (WC) (cm) was measured to the nearest 0.1 cm using a flexible, non-stretchable measuring tape at the umbilicus level midway between the lower ribs and the upper iliac crest while standing (Alberti et al., 2006). WC was defined as normal or 'at risk' according to the International Diabetes Federation (IDF) using Europids cutoff values for normal of <94 cm for males and <80 cm for females (Alberti et al., 2006). Waist-to-height ratio (WtHR) was calculated as the ratio of the waist circumference divided by height (both in cm). A cut-off of 0.5 was used to define abdominal obesity, whereby a WtHR < 0.5 was considered normal (Gibson & Ashwell, 2020).

The percentage of body fat (PBF) was measured using a bioelectrical impedance analyzer 200 (BC-420 MA, Tanita Corporation, Tokyo, Japan) for Emiratis and an InBody composition analyzer 720 (InBody Co., Ltd., Seoul, Korea) for Lebanese participants, according to the manufacturer's instructions. Cutoffs of ≥25% and ≥35% were used to define elevated PBF among males and females respectively (Nuttall, 2015).

Biochemical analyses

After a 12 h fast, on-site, venous blood samples were collected by a registered nurse for the analyses of fasting blood glucose (FBG, mg/dl), total cholesterol (TC, mg/dl), low-density lipoprotein cholesterol (LDL-C, mg/dl), high-density lipoprotein cholesterol (HDL-C, mg/dl), and triglyceride (TG, mg/dl) that were analyzed via an enzymatic colorimetric method using the Roche/Hitachi Cobas® 6000 analyzer (Roche, Basel, Switzerland) and

insulin via the Enzyme-Linked Immunosorbent Assay (ELISA) principle using the Elecsys insulin kit (Roche, Basel, Switzerland) for Lebanese participants. As for Emirates, the former were measured via the portable Lux Meter Blood Test (Biochemical Systems International, S.p.A; Arezzo, Italy) following the manufacturer's instructions. As for insulin: one 5 mL venous blood sample was drawn into an ethylenediaminetetraacetic acid (EDTA) tube and analyzed by using ELISA kits (Diametra Millipore, St. Louis, MO, USA).

Insulin resistance was calculated using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), defined as (fasting immunoreactive insulin (μU/mL) × fasting plasma glucose (mg/dL))/ 405 (Vaccaro et al., 2004). HOMA-IR values exceeding 1.8 for Emirati participants (Esteghamati et al., 2009) and 2.32 for Lebanese participants (Chedid et al., 2009) were considered indicative of insulin resistance.

Stool collection

Stool was collected using a fecal specimen collector along with the Zymo DNA/RNA Shield™ Fecal Collection tube which was pre-filled with DNA/RNA Shield™ (9 mL) (Zymo Research Corp., Irvine, USA). Subjects were asked to follow the instructions by the developer of the tubes, where two samples were collected from each participant, one of which was analyzed, and the second was held for accurate verification. Collection tubes (with a spoon attached to the cap for collecting 1 gram of faeces). The nucleic acids (DNA & RNA) in the samples were preserved at room temperature (DNA > 1 year, RNA up to 1 month).

DNA Isolation and Sequencing of the V3–V4 Region of the 16S rRNA Gene

DNA isolation and sequencing of the barcoded amplicons of the V3–V4 region of the 16S rRNA gene were performed according to the Illumina protocols (Illumina, Eindhoven, The Netherlands) which involved PCR amplification using region-specific primers, followed by indexing, purification, and paired-end library preparation (Surono et al., 2022). Sequencing was conducted on the Illumina MiSeq platform (San Diego, CA, USA) using barcodes and the paired end 2x 300 bp protocol. Raw reads were processed and demultiplexed using (QIIME 2, version 2023.2) (Bolyen et al., 2019), and taxonomic classification was performed using the SILVA 16S rRNA gene reference database, version 132 (Quast et al., 2013; Yilmaz et al., 2014).

Diversity analyses

The alpha diversity metrics used in this study included Faith's phylogenetic diversity (PD) (Faith, 1992), observed features (DeSantis et al., 2006), Chao1 index (Chao, 1984), Pielou's evenness (Pielou, 1966) and Shannon's indices (Shannon & Weaver, 1949). These indices were calculated using QIIME 2 and visualized in RStudio (version 4.2.1) using the phyloseq and ggplot2 packages (Wickham, 2016). Rarefaction curves were also generated using the ggplot2 package, with rarefication depth set at 3800. Beta diversity was assessed using Bray–Curtis dissimilarity (Bray & Curtis, 1957), Jaccard similarity (Jaccard, 1908), unweighted unique fraction metric (UniFrac), and weighted UniFrac (Lozupone & Knight, 2005) and visualized through Principal Coordinate Analysis (PCoA) plots using the vegan and phyloseq packages in R.

Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences software version 20 (SPSS Inc. Chicago, IL, USA) and software package R (4.2.1) (R Core Team, http://www.R-project.org) in RStudio. The normality of data using SPSS was tested using the skewness and kurtosis. Normally distributed data is expressed as mean (M) ± standard deviation (SD), and skewed data as median (Mdn) and interquartile range (IQR). Depending on the data distribution, differences between the groups were assessed using an independent samples t-test for normally distributed continuous variables or a nonparametric test using the Mann–Whitney U-test for skewed continuous variables and chi-square for categorical values. The value of $p < 0.05$ was used to denote statistical significance.

Comparisons of relative abundances at the phylum and genus levels were performed using the non-parametric Kruskal–Wallis H-test corrected with the Benjamini–Hochberg false discovery rate (FDR) for multiple comparisons by using the software package R in RStudio. Although only two groups were compared, the Kruskal–Wallis test was retained for the microbiota data to maintain consistency with the LEfSe workflow and because its distribution-free properties are well suited to highly skewed genus-level abundance data. In contrast, Mann–Whitney U-tests were used for clinical variables, as this is the standard non-parametric method for two-group comparisons. Spearman's rank correlation coefficient was used to evaluate associations between microbial taxa and

clinical variables. In addition, linear regression models were applied to examine the relationship between microbial composition and nationality, with age assessed as a potential confounder. Both unadjusted and age-adjusted models were reported. For each genus-level analysis, the relative abundance of the bacterial genus was used as the dependent variable in separate linear regression models, and nationality (Lebanese vs Emirati) served as the main independent variable (coded 1 and 0, respectively). Relative abundances were analyzed as proportional values without transformation (e.g., no log, arcsine-square-root, or CLR transformation), as exploratory inspection indicated that model residuals met acceptable assumptions for linear regression. Zero values were retained as observed without imputation. Age was added as a covariate in adjusted models to assess confounding, applying a ≥10% change-in-estimate criterion for identifying meaningful confounding effects. (Skelly et al., 2012). Model assumptions—including linearity, homoscedasticity, multicollinearity, and normality of residuals—were evaluated using residual plots and diagnostic checks. No additional covariates met criteria for inclusion. Taxonomic composition was summarized using stacked bar plots at the phylum and genus levels, illustrating the relative abundance of dominant microbial taxa across study groups.

The statistical significance of beta diversity between the two groups with OB (Emirati *vs*. Lebanese) was determined using Permutational Multivariate Analysis of Variance (PERMANOVA) using the software package R in RStudio.
Linear discriminant analysis (LDA) effect size (LEfSe) was done using the R software (4.2.1) in RStudio to explore microbial taxa with differential abundance between Lebanese and Emirati individuals with OB. Genera enriched in each group with an LDA score $>^{10}\log 2$ at a value of $p < 0.05$ were considered (Segata et al., 2011).

3. Results

Participant characteristics

The participant characteristics are presented in Table 1. A total of 43 Emirati and 30 Lebanese individuals with OB (BMI ≥35kg/m^2) were enrolled in the study. The difference in sample sizes between the two groups was mainly due to recruitment challenges in Lebanon. While the broader study initially aimed to enrol participants undergoing bariatric surgery from both countries, enrolment in Lebanon was hindered by financial constraints and disruptions caused by the COVID-19 pandemic.

Most of the subjects were females including 62.8% and 66.7% of the Emirati and Lebanese participants, respectively ($p = 0.807$). Anthropometric measurements showed that weight ($p=0.015$), BMI ($p= 0.002$), WtHR ($p<0.001$), fat mass (FM) ($p<0.011$), and probiotic food consumption ($p=0.001$) were significantly higher in the Emirati group. As for fiber intake ($p<0.001$), total cholesterol ($p=0.02$), dyslipidemia ($p=0.017$), and alcohol consumption ($p<0.001$), these were significantly higher in the Lebanese subjects. There was no significant difference between the two groups with respect to WC, PBF, energy intake (EI), FBG, insulin, HOMA-IR, HDL-C, LDL-C, TG, fiber supplement, smoking, diabetes, cardiovascular disease, irritable bowel syndrome, hypertension, and cancer. None of the participants were on probiotic or prebiotic supplements. Lebanese participants were significantly older than Emirati participants (41.0 ± 10.7 vs. 29.9 ± 9.1 years, $p < 0.001$; Table 1).

Table 1. Demographic, anthropometric, and biochemical parameters between Lebanese and Emirati Individuals with Obesity

	Emirati n=43	**Lebanese n=30**	**p-value**
Age (years)	29.9 ± 9.1	41.0 ± 10.7	**<0.001***
Female (n (%))	27 (62.8)	20 (66.7)	0.807
Weight (kg)	119.0 (98.8 -133.2)	101.1 (98.4-112.0)	**0.015***
Height (cm)	165.2 ± 9.8	164.06 ± 8.07	0.062
BMI (kg/m^2)	42.8 (37.8-46.1)	38.4 (35.7-41.7)	**0.002***
WC (cm)	123.3 ± 17.8	118.7 ± 8.5	0.149
WtHR	0.74 ± 0.08	0.63 ± 0.07	**<0.001***
PBF (%)	46.1 ± 5.3	45.4 ± 5.79	0.596
FM (Kg)	53.8 ± 13.3	47.4 ± 7.3	**<0.011***
EI (Kcal)	2075±738	1822±746	0.157
Fiber (g)	10 (7.8-13.8)	17.3 (13.1-21)	**<0.001***
Fiber (g)/1000 Kcal	4.9 (4.0-7.3)	10.5 (6.4-14.3)	**<0.001***
FBG (mg/dL)	97 (93.6-104.0)	98.5 (95.0-107.3)	0.346
Insulin	22.0±14.0	20.9±10.3	0.716
HOMA-IR	4.78 (3.08-7.59)	5.24 (3.47-6.73)	0.771
HDL-C (mg/dL)	41.7 ± 11.1	44.6 ± 11.3	0.279
LDL-C (mg/dl) (Mdn (IQR))	93 (75-118)	109 (81-136)	0.115
TG (mg/dL) (M ± SD)	128.5 ± 62.1	149.7 ± 50.2	0.127
TC (mg/dL) (Mdn (IQR))	161.6 (143.0-181.7)	177.0 (159.3-218.3)	**0.020***
Lifestyle factors	**Yes, n (%)**	**Yes, n (%)**	
Probiotic supplement	0 (0)	0 (0)	-
Fiber supplement	2 (4.7)	0 (0)	0.509

Probiotic food consumption	29 (67.4)	8 (26.7)	**0.001***
Probiotic Food Consumption Frequency			
None	15 (34.9)	22 (73.3)	**<0.001***
2 or less/week	13 (30.2)	7 (23.3)	
3 to 6/week	11 (25.6)	1(3.3)	
7 or more/week	4 (9.3)	1 (3.3)	
Prebiotic supplement	0 (0)	0 (0)	-
Smoker	12 (27.9)	14 (46.7)	0.137
Alcohol consumption	2 (4.7)	12 (40)	**<0.001***
Diabetes	0 (0)	2 (6.7)	0.166
Cardiovascular disease	0 (0)	3 (10)	0.065
Dyslipidemia	1 (2.3)	6 (20)	**0.017***
Hypertension	5 (11.6)	8 (26.7)	0.125
Irritable bowel syndrome	0 (0)	3 (10)	0.065
Cancer	0 (0)	0 (0)	-

*Denotes statistical significance ($p<0.05$) between groups. Independent-sample t-test was used for normally distributed continuous variables with mean, and SD (Age, Height, WC, WtHR, PBF, FM, EI, insulin, HDL-C, TG), Mann-Whitney U-test for skewed continuous variables (Weight, BMI, Fiber, Fiber/ 1000 kcal; FBG, HOMA-IR, LDL-C and TC) with medians and IQR, and Chi-square test was used for categorical variables. Abbreviations: BMI: Body mass index, WC: Waist circumference, PBF: Percentage body fat, FM: Fat mass, WtHR: Waist-to-height ratio, FBG: Fasting blood glucose, HOMA-IR: Homeostatic model assessment for insulin resistance, HDL-C: High- density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, TG: Triglycerides, TC: Total Cholesterol, M ± SD: Mean ± Standard deviation, Mdn (IQR): Median (Interquartile range).

Alpha and beta diversity

According to the alpha diversity metrics (Figure 1), Lebanese adults with OB showed significantly higher diversity (Shannon index, $p = 0.015$), coinciding with the higher observed operational taxonomic unit (OTU) ($p = 0.007$), higher Chao1 richness ($p = 0.006$), and higher Faith's phylogenetic diversity (PD) ($p = 0.007$) compared to the Emirati group, but no statistically significant difference in evenness (Pielou's evenness, $p = 0.964$). Microbial composition as reflected by the beta diversity measures including unweighted UniFrac, weighted UniFrac, Bray–Curtis dissimilarity, and Jaccard similarity showed a marked separation between the gut microbiota community of Lebanese and Emirati with OB Pairwise permanova analysis indicated a significant difference in beta diversity between the two groups (Figure 3); ($p = 0.001$; $p = 0.008$; $p = 0.001$; $p = 0.001$, respectively).

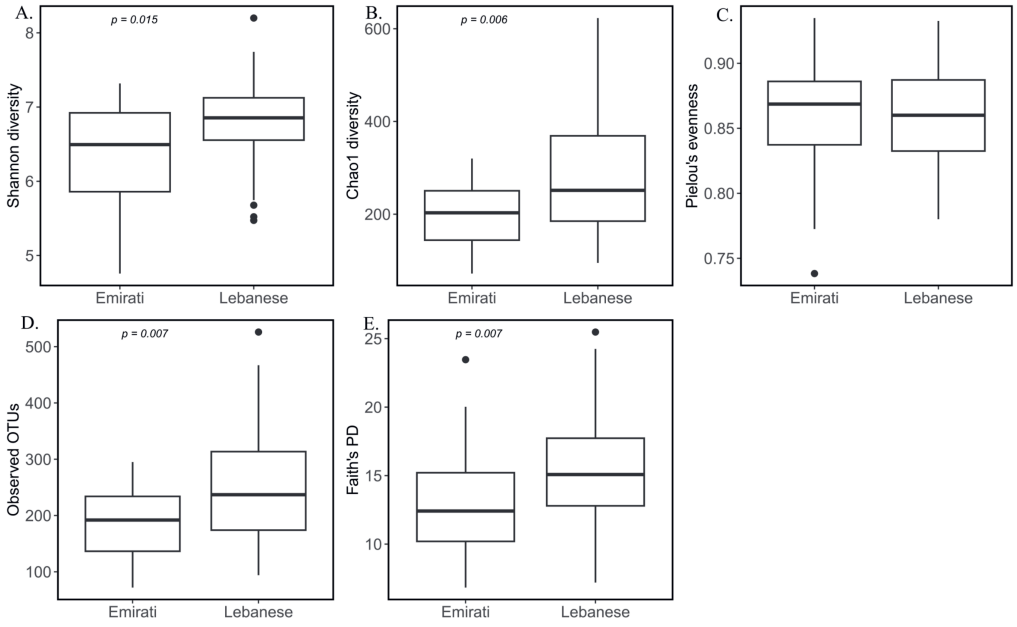


Figure 1. Alpha diversity of Lebanese and Emirati individuals with obesity

Alpha diversity boxplot showing Shannon diversity (A), Chao1 (B), Pielou's evenness (C), Observed operational taxonomic unit (OTU)(D), and Faith's Phylogenetic Diversity (PD) (E) Lebanese and Emirati individuals with obesity. For each sample, the alpha diversity indices are shown on the y-axis, and the groups (Emirati or Lebanese) are on the x-axis. Boxes denote the interquartile range (IQR) between the first and third quartiles, and the horizontal line defines the median. Whiskers represent the smallest (ymin) and largest (ymax). Outliers are indicated by black

circles. Kruskal Wallis test was performed. A *p*-value <0.05 denotes significant difference between Lebanese and Emirati individuals with obesity.

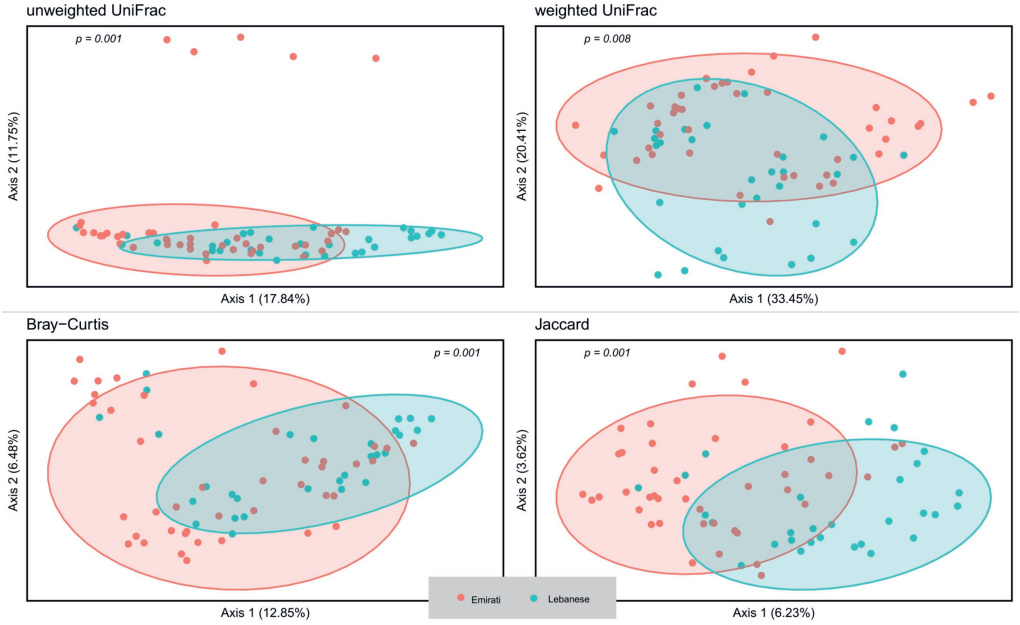


Figure 2. Beta diversity of Lebanese and Emirati individuals with obesity

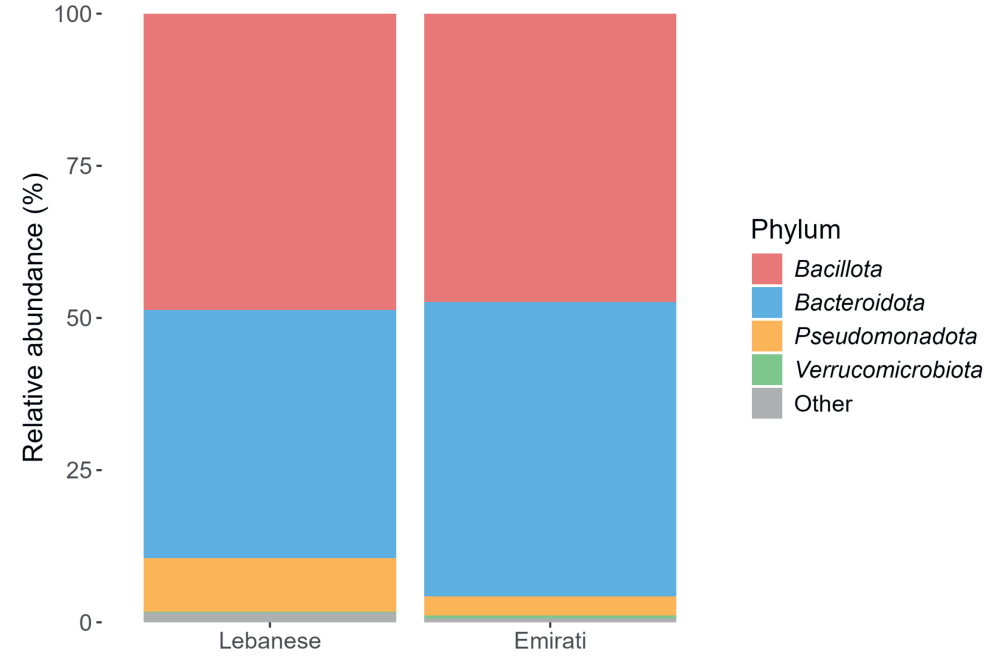
Principal coordinate analysis (PCoA) plot of unweighted UniFrac, weighted UniFrac, Bray-Curtis dissimilarity, and Jaccard similarity index between Lebanese and Emirati individuals with OB. Emirati is red and Lebanese is blue. A *p*-value <0.05 denotes a significant difference between the two groups.

Taxonomic analysis

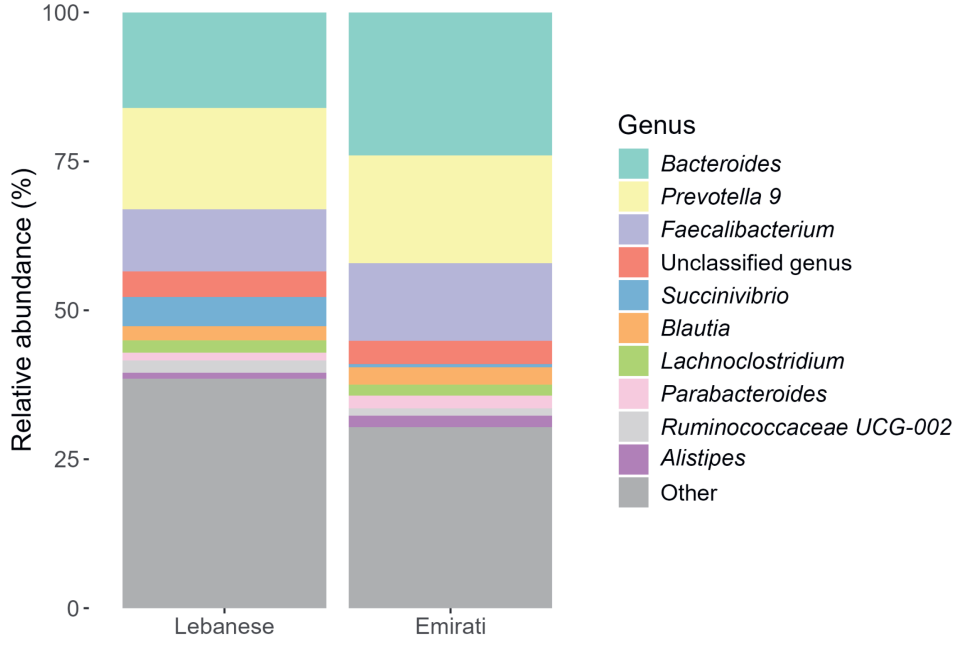
Taxonomic profiling of the gut microbiota was conducted and visualized using stacked bar plots. At the phylum level (Figure 3a), the microbial communities of both Lebanese and Emirati participants with obesity were dominated by *Bacillota* and *Bacteroidota*, which together accounted for nearly 90% of the total bacterial abundance. In the Lebanese group, *Bacillota* represented approximately 49% of the community, followed by *Bacteroidota* (41%), while *Pseudomonadota* (about 9%) and *Verrucomicrobiota* (<1%) were detected in lower proportions. Similarly, in the Emirati group, *Bacillota* (47%) and *Bacteroidota* (49%) were the most abundant phyla, with smaller contributions from *Pseudomonadota* (3%) and other phyla such as *Actinomycetota, Mycoplasmatota, Cyanobacteriota*, and *Lentisphaerota*, each present at very low levels.

At the genus level (Figure 3b), the gut microbiota composition included a range of taxa commonly reported in obese populations. The most represented genera included *Bacteroides, Prevotella, Faecalibacterium, Ruminococcus, Lachnospira, Blautia,* and *Alloprevotella,* reflecting their contribution to carbohydrate fermentation and short-chain fatty acid production. Additional taxa such as *Collinsella, Paraprevotella, Dialister, Eubacterium ruminantium* group, *Intestinimonas*, and *Caproiciproducens* were also present, alongside multiple low-abundance genera distributed across individuals.
It is noteworthy that several taxa were completely absent in one group but present in the other (figures 4 and 6). For example, *Allisonella, Howardella, Intestinimonas,* and *Caproiciproducens* were detected in the Lebanese cohort but absent in Emiratis, whereas other genera such as *Bacteroides* and *Parabacteroides* were enriched in Emiratis but nearly absent in Lebanese participants.

Taken together, these findings indicate that the gut microbiota in both Lebanese and Emirati individuals with obesity was shaped by a limited number of dominant phyla and genera, complemented by a broad range of low-abundance taxa that added to inter-individual variability within each population.

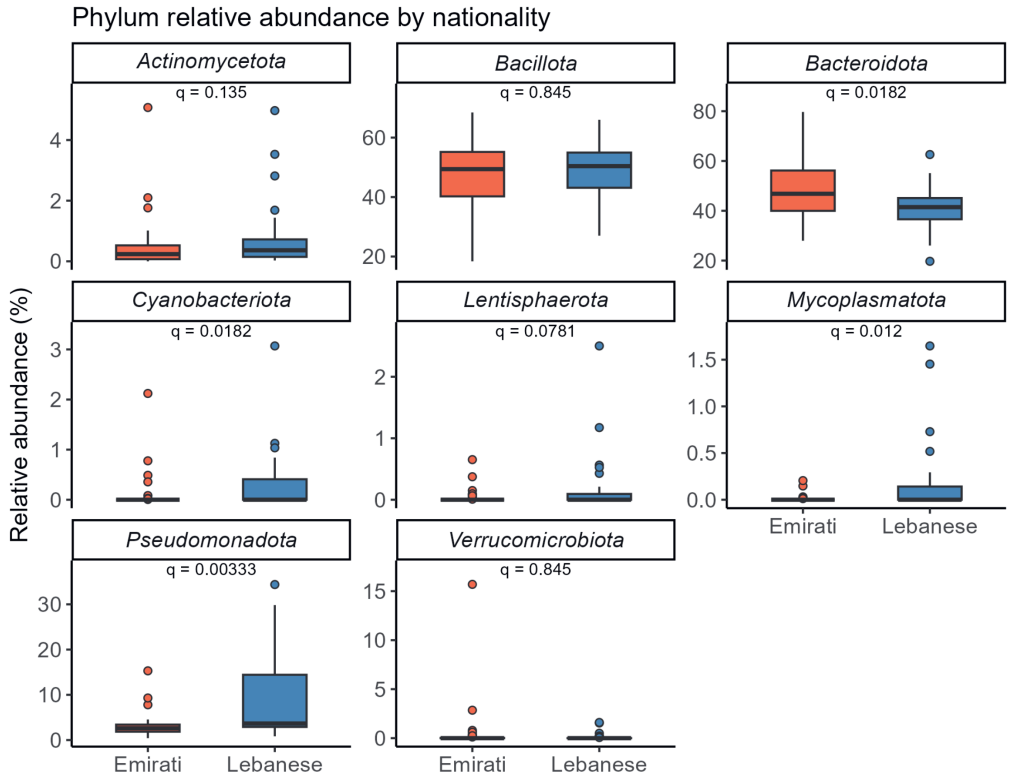


a.

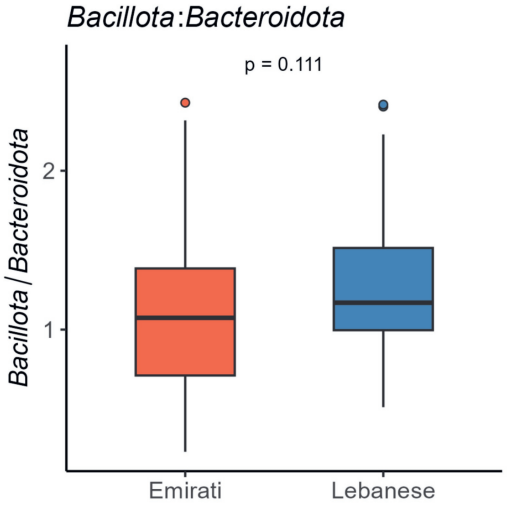


b.

Figure 3. Stacked bar plots a. Stacked bar plots of phylum- level and b. at genus level composition detected in datasets of Lebanese vs. Emirati individuals with obesity. Relative abundances (percentages) of the phyla are displayed.



a.



b.

Figure 4. Comparative relative abundance of selected bacterial phyla and Bacillota/ Bacteroidota ratio in Lebanese and Emirate participants with obesity. Figure 4 a: Boxplots showing relative abundance (%) of selected phyla, stratified by nationality with q-values. Figure 4 b: Boxplot of Bacillota/ Bacteroidota ratio stratified by nationality. Differences between groups were assessed using the Kruskal- Wallis test followed by false discovery rate (FDR). Adjusted q-values are indicated where applicable. *q*-value of $\leq$ 0.05 was considered statistically significant, as determined by the Kruskal-Wallis with FDR correction.

Differences in Gut Microbial Genera between Lebanese and Emirati individuals with obesity

Based on the relative abundances of the gut microbiota in the Lebanese *vs*. Emirati individuals with OB, LEfSe identified several genera that differed significantly between Lebanese and Emirati individuals, with only taxa exceeding the LDA threshold (>10 $\log_2$) and $p < 0.05$ reported.

In the Emirati group, *Bacteroides*, *Parabacteroides* (both of the *Bacteroidota* phylum), *Blautia, Acidaminococcus, Subdoligranulum, Phascolarctobacterium,* and *Lachnospiraceae UCG-004 (*all *Bacillota* phylum*)* were enriched. In contrast, Lebanese participants showed higher abundances of 22 bacteria: *Unchar.taxon Clostridiales vadinBB60* group 1, *Tyzzerella, Streptococcus, Anaerotruncus, Erysipelotrichaceae UCG-003, Ruminiclostridium 6, Ruminococcaceae NK4A214* group, *Ruminococcaceae UCG-010,* [*Eubacterium*] *ruminantium* group, *Ruminococcaceae UCG-005, Christensenellaceae R-7* group, *Dialister, Ruminococcaceae UCG-002, Lachnospiraceae NK4A* 136 group *(*all *Bacillota* phylum*); Rikenellaceae RC9* gut group, *Paraprrevotella, Prevotellaceae NK3B31* group, Unchar.taxon in *Prevotellaceae,* and *Alloprevotella (*all *Bacteroidota* phylum*); Desulfovibrio, Sutterella,* and *Succinivibrio (*all *Pseudomonadota* phylum*)* (Figure 5).

According to the Kruskal-Wallis analysis with Benjamini–Hochberg correction at the genus level, as seen in Figure 6, there is an overlap with some of the results from the LEfSe. For the Lebanese group with OB, in accordance with the LEfSe results, there is an increased relative abundance of 14 out of the 22 microbial taxa, which *are Alloprevotella (q<0.001), Lachnospiraceae NK4A136* group *(q<0.001), Ruminococcaceae UCG-005 (q*=0.002*), Eubacterium ruminantium* group *(q=0.003), Sutterella (q*=0.006*), Ruminococcaceae UCG-002 (q*=0.010*), Unchar.taxon Clostridiales vadinBB60* group 1 *(q*=0.013*), Paraprevotella (q*=0.016*), Dialister (q*=0.020*), Ruminiclostridium 6 (q*=0.021*), Rikenellaceae RC9* gut group *(q*=0.022*), Anaerotruncus (q*=0.029*), Erysipelotrichaceae UCG-003 (q*=0.035*), and Christensenellaceae R-7* group *(q*=0.037). None of the results overlapped with the Emirati subjects.

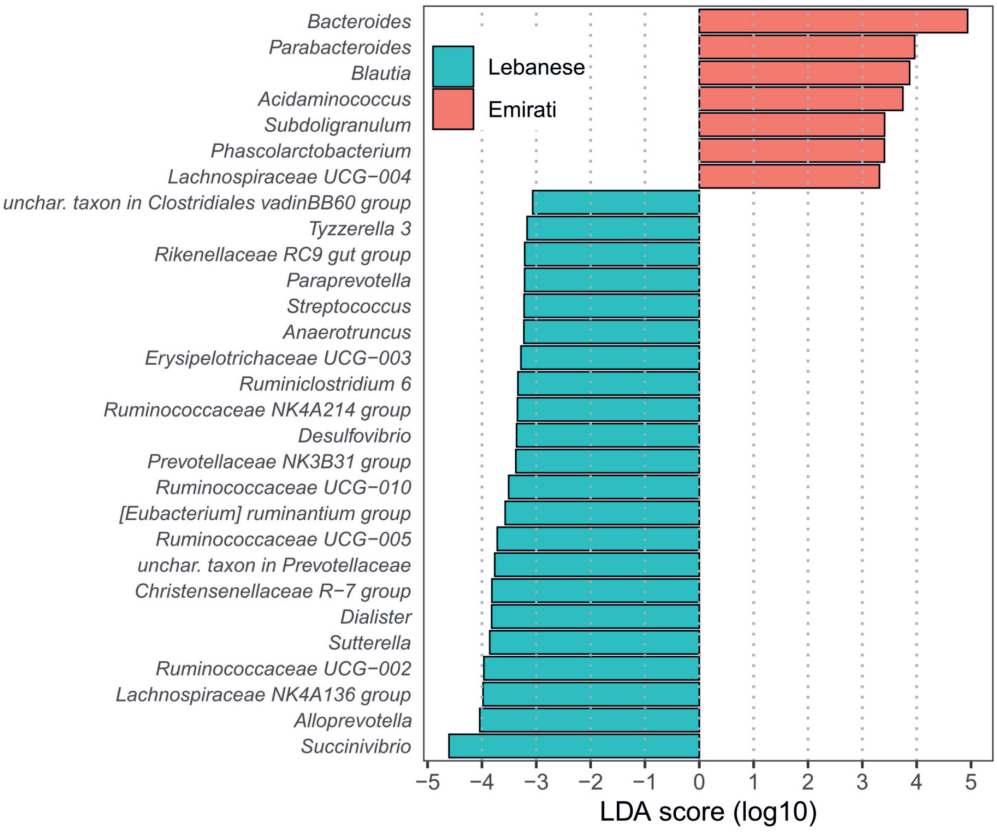


Figure 5. Linear discriminant analysis effect size (LEfSe) plot

The genera enriched in Lebanese and Emirati individuals with OB are colored by red and blue respectively. Histogram of the LDA scores computed for features differentially abundant among Lebanese and Emirati individuals with obesity. Only class meetings with an LDA significant threshold $>^{10}\log 2$ and $p < 0.05$ are shown.

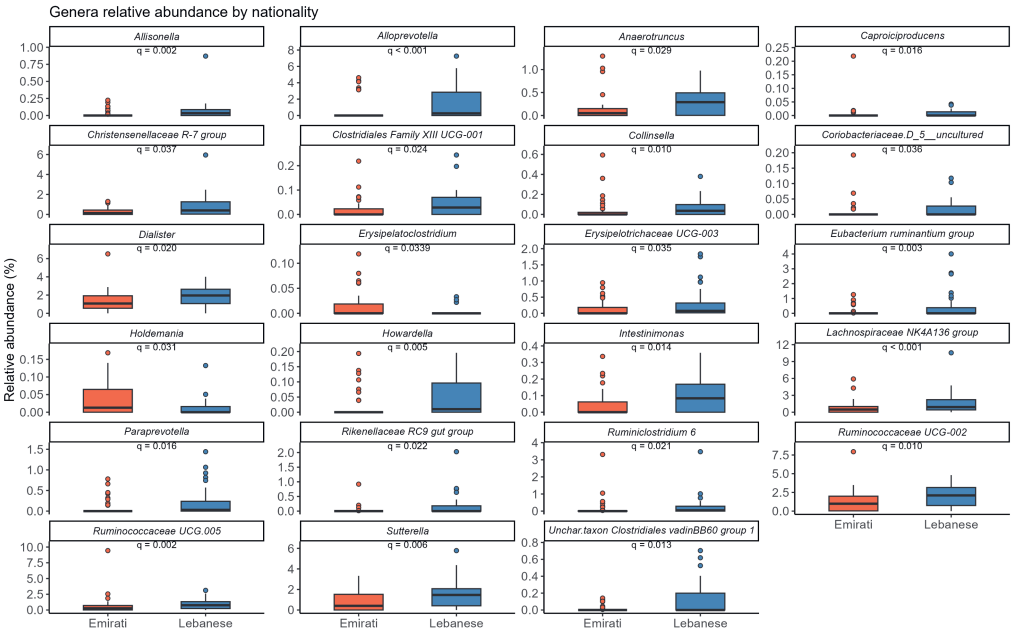


Figure 6. Relative abundance of selected gut bacterial genera by nationality.

Boxplots depict the relative abundance (%) of selected bacterial genera in Lebanese and Emirati participants with obesity. Group comparisons were performed using the Kruskal–Wallis test, with false discovery rate (FDR) correction applied to account for multiple testing. Adjusted q-values are displayed for each genus.

Spearman correlation between gut microbiota and age in Lebanese and Emirati individuals with obesity

There was on average more than 10-year difference between Lebanese and Emirati participants (Table 1). Spearman correlation analysis with FDR correction (Figure 7 and Table 2) identified several bacteria that were significantly positively associated with age ($q < 0.01$). The most pronounced correlation was observed for *Ruminococcaceae NK4A214* (rho = 0.397, $q = 0.0014$), followed by *Prevotellaceae D_5* uncultured (rho = 0.386, $q = 0.002$), *Ruminococcaceae UCG-005* (rho = 0.364, $q = 0.004$), and *Anaerotruncus* (rho = 0.359, $q = 0.005$). Additional significant associations were found for *Alloprevotella* (rho = 0.358, $q = 0.005$), *Ruminococcaceae UCG-002* (rho = 0.358, $q = 0.005$), *Clostridiales vadinBB60* group (rho = 0.354, $q = 0.005$), and *Ruminococcaceae UCG-010* (rho = 0.347, $q = 0.006$). Among all clinical and demographic variables assessed, age was the only factor significantly correlated with specific bacterial genera after FDR correction (Figure 7). Collectively, these findings indicate that age plays a substantial role in shaping gut

microbiota composition, particularly influencing members of the *Ruminococcaceae* family and other bacteria such as *Prevotellaceae D_5* uncultured and *Anaerotruncus*. The uniformity of positive associations suggests a gradual, age-related enrichment of these bacteria. None of the other clinical parameters were significantly correlated with any of the taxa.

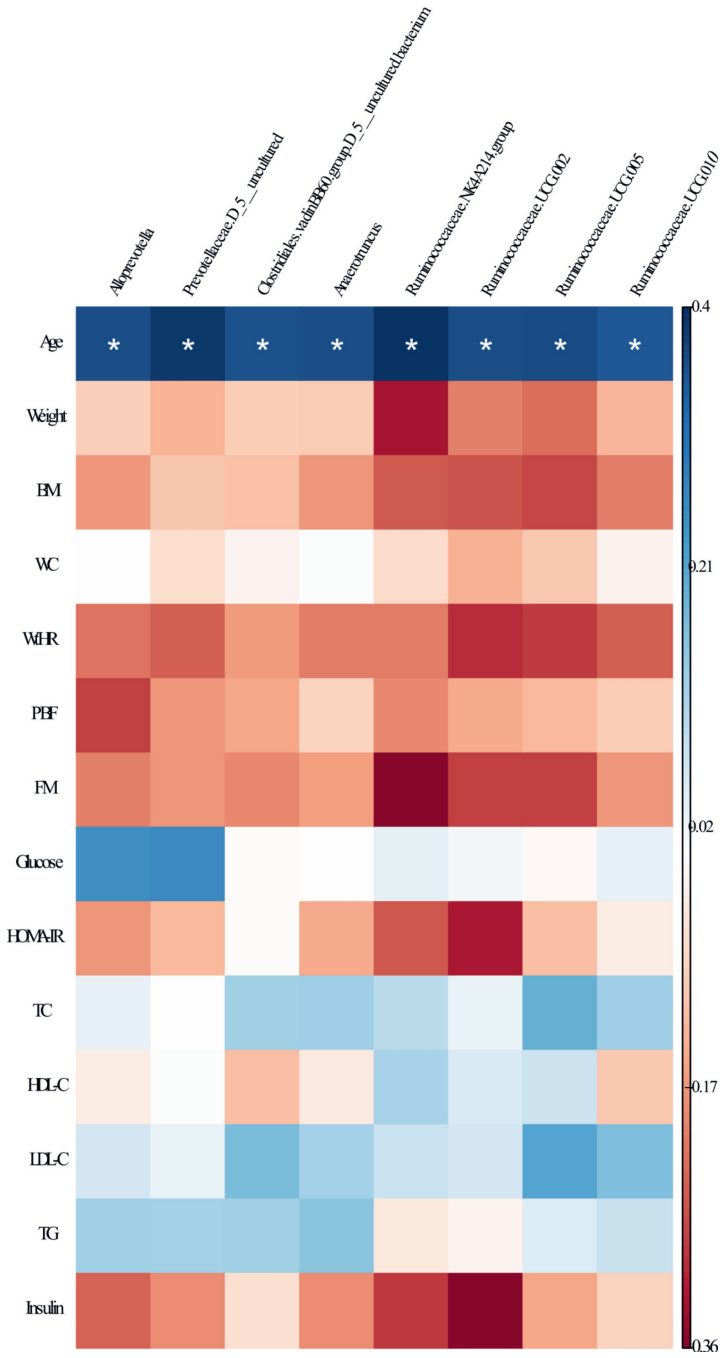


Figure 7. Spearman correlation analysis between gut microbiota and clinical variables in Lebanese and Emirati individuals with obesity. In the heatmap, blue squares indicate significant positive correlations, and red squares indicate significant negative correlations. * Significant correlation at $q<0.05$ (FDR-correction). Only age showed significant correlations with gut microbial taxa after FDR correction. Abbreviations: BMI: Body mass index; WC: waist circumference; WtHR: waist to height ratio, PBF: percent body fat; FM: fat mass; HOMA-IR: homeostatic model assessment for insulin resistance LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol; TG: triglyceride.

Table 2. Correlation analysis between gut microbiota and age in Lebanese and Emirati individuals with obesity

Bacteria	q-value	rho
Ruminococcaceae.NK4A214.group	0.0014*	0.397
Prevotellaceae.D_5__uncultured	0.0022*	0.386
Ruminococcaceae.UCG.005	0.0040*	0.364
Anaerotruncus	0.0046*	0.359
Alloprevotella	0.0046*	0.358
Ruminococcaceae.UCG.002	0.0046*	0.358
Clostridiales.vadinBB60. group. D_5__uncultured. bacterium	0.0052*	0.354
Ruminococcaceae.UCG.010	0.0064*	0.3467

* Indicates significant correlation $q < 0.05$ for Spearman correlation analysis with FDR correction

Table 3 Linear regression analysis between gut microbiota and age among Lebanese and Emirati individuals with obesity

Gut microbiota	Model	Intercept Estimate	Nationality (Lebanese) Estimate	Age Estimate	R^2 (Adj.)	p-value (Nationality)	p-value (Age)	Confounding (%)
Alloprevotella	Unadj.	5.00×10^{-3}	1.09×10^{-2}	-	0.085	0.007 **	-	-
	Adj.	-1.30×10^{-3}	8.40×10^{-3}	2.2×10^{-4}	0.089	0.067	0.263	22.9
Prevotellaceae D_5	Unadj.	8.00×10^{-4}	5.70×10^{-3}	-	0.074	0.012 *		-
	Adj.	-1.00×10^{-5}	5.50×10^{-3}	3×10^{-5}	0.061	0.037 *	0.821	5.0
Clostridiales vadinBB60	Unadj.	1.00×10^{-4}	1.20×10^{-3}	-	0.147	<0.001*	-	-
	Adj.	-1.10×10^{-3}	7.00×10^{-4}	4×10^{-5}	0.213	0.047 *	0.011*	38.9
Anaerotruncus	Unadj.	1.50×10^{-3}	1.50×10^{-3}	-	0.048	0.036 *	-	-
	Adj.	6.00×10^{-5}	9.7×10^{-4}	5.0×10^{-5}	0.059	0.229	0.181	35.3
Ruminococcaceae NK4A214	Unadj.	3.30×10^{-3}	1.90×10^{-3}	-	0.031	0.073	-	-
	Adj.	-3.00×10^{-4}	6.0×10^{-4}	1.2×10^{-4}	0.059	0.613	0.0247 *	-
Ruminococcaceae UCG-002	Unadj.	1.25×10^{-2}	7.90×10^{-3}	-	0.052	0.030 *	-	-
	Adj.	2.40×10^{-3}	4.20×10^{-3}	3.3×10^{-4}	0.085	0.301	0.0637	46.97
Ruminococcaceae UCG-005	Unadj.	6.40×10^{-3}	3.30×10^{-3}	-	0.002	0.284	-	-
	Adj.	2.50×10^{-3}	1.80×10^{-3}	1.3×10^{-4}	-0.0019	0.599	0.404	43.81
Ruminococcaceae UCG-010	Unadj.	5.00×10^{-4}	2.97×10^{-3}	-	0.140	<0.001*	-	-
	Adj.	-1.30×10^{-3}	2.3×10^{-3}	6.0×10^{-5}	0.153	0.018 *	0.1530	22.73

* Indicates significant correlation $p < 0.05$ for linear regression analysis

Linear regression analysis

The relationship between microbial composition and nationality was subsequently examined, comparing Lebanese and Emirati individuals with OB, while accounting for age as a potential confounder. Linear regression models were used (Table 3) to assess these associations, with both unadjusted and adjusted models reported. A change-in-estimate criterion with a cut-off of 10% was used to identify the confounder (Skelly et al., 2012).

In the unadjusted model, Lebanese individuals had a significantly higher relative abundance of *Alloprevotella* compared to Emiratis (β = 0.0109, $p = 0.007$). However, after adjusting for age, the effect size decreased (β = 0.0084) and was no longer statistically significant ($p = 0.067$), with a 22.9% reduction in effect size, suggesting that age may be a confounder.

For *Prevotellaceae D_5*, Lebanese nationality remained significantly associated with higher bacterial abundance in both unadjusted (β = 0.0057, $p = 0.012$) and adjusted models (β = 0.0055, $p = 0.037$). The 5% reduction in effect size suggests that age had minimal confounding influence.

In *Clostridiales vadinBB60*, a strong association was observed in the unadjusted model (β = 0.0012, $p < 0.001$). After adjusting for age, the effect size decreased by 38.9% (β = 0.0007, $p = 0.047$), indicating that age played a significant role as a confounder. Additionally, age was a significant predictor of bacterial abundance ($p = 0.011$).

For *Anaerotruncus*, Lebanese nationality was significantly associated with bacterial abundance in the unadjusted model (β = 0.0015, $p = 0.036$). However, after adjusting for age, the effect size declined by 35.3% (β = 0.00097) and was no longer significant ($p = 0.229$), further indicating confounding by age.

The unadjusted model for *Ruminococcaceae NK4A214* showed a weak association (β = 0.0019, $p = 0.073$), and in the adjusted model, the effect size further decreased (β = 0.0006, $p = 0.613$), indicating that age largely explained the observed relationship.
For *Ruminococcaceae UCG-002*, Lebanese individuals exhibited significantly higher levels in the unadjusted model (β = 0.0079, $p = 0.030$). However, after adjustment, the effect size decreased by 46.97% (β = 0.0042) and lost statistical significance ($p = 0.301$), suggesting that age was a strong confounder.

No significant association was found for *Ruminococcaceae UCG-005* in either the unadjusted ($p = 0.284$) or adjusted model ($p = 0.599$).
Finally, for *Ruminococcaceae UCG-010*, the unadjusted model showed a strong association (β = 0.00297, $p < 0.001$). After adjusting for age, the effect size decreased by 22.73% but remained statistically significant (β = 0.0023, $p = 0.018$), indicating that the association persisted but was partially influenced by age.

Overall, the regression analyses revealed a consistent pattern across taxa: several nationality–microbiota associations observed in the unadjusted models were attenuated

after controlling for age, indicating that age acted as a meaningful confounder for many genera. However, a subset of taxa—including *Prevotellaceae D_5*, *Clostridiales vadinBB60*, and *Ruminococcaceae UCG-010*—remained significantly associated with nationality even after adjustment, suggesting a robust nationality-linked microbial signature. In addition, age itself showed positive associations with several genera, reinforcing its influential role in shaping gut microbial composition within this cohort.

4. Discussion

This study contributes to the growing body of research exploring how geography, culture, and ethnicity shape gut microbiota and its relationship with obesity. By comparing obese individuals from two Arab nationalities—Lebanese and Emirati residing in their respective countries, we highlight distinct microbial patterns potentially driven by regional dietary and lifestyle habits. Existing studies, such as comparisons between French and Saudi individuals, have shown that dietary and cultural factors can significantly influence microbial composition (Yasir et al., 2015). Despite global interest in ethnic differences in microbiota composition, data from Middle Eastern populations remain limited. Our findings emphasize the need for more inclusive studies in this region to better understand how cultural and environmental factors influence metabolic health and to inform tailored intervention strategies.

The comparison of Lebanese and Emirati individuals with OB provides a meaningful framework to explore how cultural norms shape gut microbiota and potentially affect metabolic outcomes. The predominance of female participants 62.8% to 66.7% reflects a broader regional trend of higher obesity prevalence among cis-women in the Middle East and North Africa (MENA), a phenomenon attributed to sociocultural perceptions associating larger body size with beauty and affluence (Mallat et al., 2016; Naja et al., 2011; Sibai et al., 2003). These findings suggest that public health strategies targeting obesity in MENA countries should consider the cultural contexts influencing body image and dietary behaviours, alongside biological factors such as the gut microbiota.

Despite overall dietary similarities, Lebanese participants reported significantly higher fiber intake. While no direct correlations between fiber intake and microbial taxa were observed after FDR correction**,** fiber's known role in modulating gut microbiota may

partly explain the distinct microbial profiles observed. In contrast, Emirati individuals exhibited higher anthropometric measures—including weight, BMI, WtHR, and fat mass —although these were not significantly associated with specific bacterial genera in our dataset**.** These differences align with previous studies linking higher fiber intake to greater gut microbial diversity and improved metabolic health (Nasreddine et al., 2014; Nasreddine et al., 2012). Comparable patterns have been observed in other cross-cultural comparisons, such as between French and Saudi populations, where differences in fiber consumption and traditional versus Westernized diets influenced microbial composition (Yasir et al., 2015). Another important demographic difference in our cohort was age, with Lebanese participants being on average more than 10 years older than Emiratis (Table 1). Since age is a well-recognized determinant of gut microbiota composition, this imbalance needed to be considered when interpreting our findings.

Our findings revealed that Lebanese adults with OB exhibit significantly greater gut microbial diversity compared to their Emirati counterparts. This is supported by multiple alpha diversity indices, including the Shannon index, observed features, Chao1 richness, and Faith's PD. Greater microbial diversity may indicate a more complex and versatile ecosystem, potentially conferring resilience to metabolic diseases, as various studies have associated increased gut microbiota diversity with improved metabolic profiles in both humans and mice (Astbury et al., 2020; Cotillard et al., 2013; Le Chatelier et al., 2013; Meyer & Bennett, 2016). Additionally, although a meta-analysis found that obesity is linked to alterations in gut microbial composition, including changes in richness and diversity, significant discrepancies were observed across different cohorts (Chanda & De, 2024). For instance, high BMI has been associated with decreased gut microbial diversity, and certain taxa, such as *Christensenellaceae*, have been linked to BMI variations across ethnicities (Balakrishnan et al., 2021).

In terms of beta diversity, PCoA was used to visualize differences between the Lebanese and Emirati populations, and these were confirmed as statistically significant by pairwise PERMANOVA analyses, indicating marked differentiation in microbial community composition. This suggests that nationality—and by extension, environmental and dietary exposures—may play a critical role in shaping gut microbiota profiles (Parizadeh & Arrieta, 2023; Yasir et al., 2015).

Taxonomic analysis of gut microbiota revealed both shared and distinct features. While both groups exhibited comparable proportions of the dominant phyla *Bacillota* and *Bacteroidota* —consistent with established gut microbiota compositions (Chen et al., 2021; Zhang et al., 2023)—notable differences emerged in less abundant but potentially influential taxa. The Lebanese group displayed significantly higher levels of *Pseudomonadota* (9% vs. 3% in Emiratis), *Mycoplasmatota* ($q = 0.003$), *Cyanobacteriota* ($q = 0.009$), and *Lentisphaerota* ($q = 0.049$), whereas the Emirati group exhibited a greater abundance of *Bacteroidota* ($q = 0.009$). Elevated levels of *Mycoplasmatota* in the Lebanese group may reflect the influence of Western dietary components, such as high fat and sugar intake, which have been associated with increased obesity risk (Konstantinidis et al., 2020; Napoli et al., 2024). Although the *Bacillota/Bacteroidota* ratio has historically been linked to obesity-related microbiota changes (Boulange et al., 2016; Compare et al., 2016; John et al., 2018; Turnbaugh et al., 2008), our results underscore the limitations of using this ratio as a universal marker of obesity. In our study, Lebanese individuals with OB exhibited a higher, but not significant, *Bacillota/Bacteroidota* ratio than obese Emiratis, despite the latter presenting with higher overall obesity levels. This discrepancy aligns with recent research questioning the reliability of the *Bacillota/Bacteroidota* ratio across ethnically and geographically diverse populations (Magne et al., 2020). While some studies have observed elevated *Bacillota/Bacteroidota* ratios in obese individuals (Koliada et al., 2017), others report inconsistent or population-specific patterns (Kim et al., 2019). These findings highlight the need to move beyond broad phylum-level comparisons and instead investigate specific microbial genera and their functional contributions to host metabolism (Bauer et al., 2016) .

Geographic, ethnic, and cultural factors are well-documented determinants of gut microbiota composition (Yatsunenko et al., 2012). As previous studies show, Americans, Japanese, Koreans, and Chinese individuals each harbour distinct microbial profiles, with varying proportions of *Bacillota*, *Actinomycetota,* and *Bacteroidota,* respectively (A. W. Brooks et al., 2018). At the genus level, variations are similarly pronounced, with *Bacteroides* enriched in Chinese populations, *Prevotella* in Indians, and *Ruminococcus* in the Dutch (A. W. Brooks et al., 2018). Our findings reflect these ethnically stratified patterns. Emiratis—characterized by higher BMI—had increased levels of *Bacteroides* and *Parabacteroides*, which are associated with high-fat, high-sugar diets and enhanced energy

harvest. Conversely, Lebanese participants had higher abundances of *Alloprevotella* and *Paraprevotella*, linked to high-fiber intake and improved metabolic profiles (Christensen et al., 2018). These results bolster the existing evidence linking Bacteroides-dominant microbiota to increased adiposity, while leaner individuals often harbour Prevotella-rich communities (Lee et al., 2024). Supporting this, studies comparing French and Saudi populations (Yasir et al., 2015), as well as investigations among Emiratis (Ahmad et al., 2023) and Lebanese (Abou-Samra et al., 2025) have highlighted gut microbiota differences shaped by diet, lifestyle, and cultural practices.

LEfSe analysis further identified taxa that discriminate between the two populations. Emirati individuals showed enrichment in *Bacteroides*, while Lebanese participants had higher levels of *Lachnospiraceae*. Both genera have been previously implicated in metabolic functions related to energy harvest, lipid metabolism, and fat storage (Wu et al., 2024; Yang et al., 2022).However, interpretation of these differences must consider the significant age gap between the groups. Spearman correlation analysis confirmed that age significantly correlated with several taxa, and linear regression models showed that some between-group differences—particularly at the genus level—were attenuated or lost after adjusting for age. This underscores the role of age as a confounding factor in microbiota studies, as also reported in previous research (Chen et al., 2021; de la Cuesta-Zuluaga et al., 2019; Odamaki et al., 2016).

Age-related changes in the gut microbiome have been extensively documented across the human lifespan. Research spanning from infancy to old age has identified key transitions in microbial composition—particularly during early development and later life stages (Odamaki et al., 2016). Some studies report minimal age-related differences (Takagi et al., 2019), while others identify significant associations between age and microbial composition (de la Cuesta-Zuluaga et al., 2019). These inconsistencies indicate that age can act as a confounder in microbiome studies and must be carefully considered. In our cohort, although age explained part of the between-group variation, several taxa—including *Prevotellaceae D_5, Clostridiales vadinBB60,* and *Ruminococcaceae UCG-010*—remained significantly associated with Lebanese nationality after adjustment. This suggests that nationality-related dietary and environmental factors continue to influence gut microbiota composition independently of age, consistent with evidence that distinct microbial

signatures persist across populations even when controlling for demographic variables (Andrew W Brooks et al., 2018; Yatsunenko et al., 2012).

Each of these taxa has known links to health-promoting microbial functions. *Prevotellaceae* D_5 is associated with fiber-rich diets and improved glycaemic control (Kovatcheva-Datchary et al., 2015); *Clostridiales* vadinBB60 has been found in metabolically healthy individuals and centenarians and is implicated in lipid metabolism and anti-inflammatory effects (Zhang et al., 2019). *Ruminococcaceae* UCG-010 plays a role in fiber degradation and short-chain fatty acid production, which support gut health and are linked to GLP-1 release, satiety, and protection against type 2 diabetes (Gryaznova et al., 2022; Mancabelli et al., 2017; Tims et al., 2013; Tsai et al., 2020; Wei et al., 2018). The persistence of these associations despite age adjustment highlights the key role of cultural and environmental influences tied to nationality in shaping the gut microbiota.

Although ethnicity was not found to have a significant correlation with microbiota composition in a Middle Eastern population, lifestyle and environmental influences exhibited a greater degree of homogeneity compared to the ethnic, sociocultural, economic, and dietary diversity observed within the United States (A. W. Brooks et al., 2018). Importantly, our study controlled for methodological consistency—using identical DNA extraction and sequencing protocols—yet still observed significant differences between groups. This reinforces the notion that factors such as diet (Asnicar et al., 2021), genetics (Goodrich et al., 2014), physical activity, and medication use (Heiman & Greenway, 2016) contribute meaningfully to microbiota variation.

5. Strengths and Limitations

This study provides novel comparative insights into how regional and ethnic factors may shape gut microbiota composition in the context of obesity, focusing on two Arab populations living in the MENA region. The use of standardized microbiota sequencing protocols across sites minimized methodological variability, while comprehensive data collection—including anthropometric, biochemical, and selected dietary variables—facilitated robust analysis. The application of linear regression models adjusted for age, along with rigorous participant selection and harmonized methodologies, further strengthened the reliability and interpretability of the findings. Nonetheless, several limitations should be considered. The cross-sectional design limits the ability to infer

causality, suggesting that there must be longitudinal research to examine microbiota dynamics over time. The relatively small sample size may constrain the generalizability of the results. This study also focused exclusively on obese participants from a larger cross-sectional dataset that also included normal-weight adults. As such, comparisons with normal-weight controls were beyond the scope of the current analysis but will be explored in a separate study using the same parent cohort. Another notable limitation is the significant age difference between the Lebanese and Emirati participants, which could independently influence gut microbiota composition. While we addressed this through correlation and regression analyses, which confirmed age as a confounding factor, residual confounding cannot be fully excluded. Although age differences between the 2 samples were accounted for, other relevant factors—such as physical activity, medication use, psychosocial stress, socioeconomic status, and genetic background—were not assessed and may have influenced gut microbiota composition. Additionally, dietary assessment was limited to fiber and probiotic intake, omitting other potentially significant dietary components (e.g., fat and protein intake). Variability in laboratory assays between countries may have introduced inconsistencies in biochemical measurements, and the lack of functional microbiota profiling restricted insights into microbial metabolic potential. Additionally, the use of different bioelectric impedance analysis devices across study sites (Tanita in the UAE and InBody in Lebanon) may introduce inter-device variability and measurement bias, which could affect the comparability of body composition data between the two populations.

6. Conclusion

This study highlights significant gut microbiota differences between Lebanese and Emirati adults with OB, shaped by cultural, dietary, and geographic factors. Emiratis exhibited a higher abundance of *Bacteroides* and *Parabacteroides*, bacteria commonly linked to high-fat, low-fiber diets and greater energy harvest. In contrast, Lebanese individuals showed greater microbial diversity and elevated levels of *Alloprevotella*, *Paraprevotella*, and *Mycoplasmatota*, reflecting higher fiber intake. Furthermore, this study reveals that dietary patterns rooted in cultural and geographic context strongly influence the composition of gut microbiota. The study also identified age as key confounder, which significantly influenced

the associations between nationality and microbial profiles. These findings highlight the importance of incorporating both age - and ethnicity-specific microbiome signatures when designing targeted obesity interventions. Notably, microbial differences such as higher abundances of *Prevotellaceae D_5*, *Clostridiales vadinBB60*, and *Ruminococcaceae UCG-010* in Lebanese individuals remained significant after adjusting for age, emphasizing the potential for culturally driven, microbiota-informed strategies. Ultimately, the gut microbiota may serve as both a biomarker of lifestyle and a modifiable factor in the management of obesity and its metabolic consequences within Arab populations living in the MENA region. Future research should adopt longitudinal designs to elucidate the causal mechanisms linking gut microbiota to obesity and metabolic health, particularly in response to dietary and lifestyle interventions. Including non-obese control groups and diverse Arab populations will enhance generalizability and provide insights into ethnicity-specific microbial variations. Integrating functional metagenomics, metabolomic profiling, and detailed dietary, lifestyle, and genetic data will deepen our understanding of microbial activity and its metabolic impact. Additionally, culturally tailored dietary interventions and investigations into age-related microbial shifts across life stages are warranted to inform effective, personalized strategies for weight management in the MENA region.

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CHAPTER 6



Chapter 6 – General Discussion

1.Overview

Obesity represents a major public health concern in the Middle East, with rising prevalence driven by rapid lifestyle- and dietary transitions. The associated metabolic burden includes increased risk of insulin resistance, type 2 diabetes, and cardiovascular disease (Blüher, 2019; World Obesity Federation, 2022). In recent years, gut microbiota composition has been increasingly linked to metabolic health; however, its role in obesity appears heterogeneous and influenced by population-specific factors such as diet, environment, and lifestyle (Lynch & Pedersen, 2016; Rothschild et al., 2018; Turnbaugh et al., 2006).
In parallel, gut-derived peptide hormones play a central role in appetite regulation and energy homeostasis. Alterations in these hormonal pathways are commonly observed in obesity, yet their interaction with gut microbiota in human populations remains incompletely understood (Drucker, 2018; Gribble & Reimann, 2019). Investigating the relationship between microbial composition and peptide hormone concentrations may therefore provide further insight into the mechanisms underlying obesity related metabolic dysfunction.

The present thesis examines the relationship between gut microbiota composition, gut-derived peptide hormones, and metabolic characteristics in Arab populations, with a primary focus on Lebanese adults and an additional comparison to Emirati cohorts. The work evaluates associations between microbial, endocrine, and metabolic parameters and does not infer causal directionality.

Specifically, this thesis characterises differences in fasting gut-derived peptide hormone profiles between individuals with obesity and normal weight controls and evaluates their associations with markers of adiposity, glycemic control, and insulin resistance. In parallel, it examines gut microbiota composition at the genus level, identifying differences between individuals with obesity and normal weight individuals and relating these patterns to metabolic and endocrine profiles (Companys et al., 2021; Sze & Schloss, 2016).

In addition, the thesis investigates associations between specific bacterial taxa and circulating gut peptide concentrations, providing insight into potential microbiota–endocrine interactions. Furthermore, it explores population-specific variability by comparing gut microbiota composition between Lebanese and Emirati adults with obesity,

highlighting the influence of regional and environmental factors (Deschasaux et al., 2018; Gupta et al., 2017).

Across the studies included in this thesis, a consistent pattern emerges in which alterations in hormonal profiles, microbial community structure, and metabolic characteristics occur in parallel. However, their interpretation is complex and requires further mechanistic deepening. Furthermore, the interpretation is context dependent and influenced by population-specific factors, including dietary habits and environmental exposures.

2. Integrative Synthesis of Microbial, Endocrine, and Metabolic Findings

The findings of this thesis provide a perspective on the relationships between gut microbiota composition, gut-derived peptide hormones, and metabolic characteristics in obesity. When considered collectively, several key observations emerge that extend beyond the interpretation of individual results.

First, alterations in endocrine profiles and gut microbiota composition were consistently observed in individuals with obesity, as demonstrated in ***Chapters 2*** and ***3***; however, these changes did not always follow patterns commonly reported in Western cohorts. In particular, higher fasting concentrations of anorexigenic peptides such as GLP-1 and PYY were observed in individuals with obesity (***Chapter 2)***, despite their established roles in promoting satiety (Drucker, 2018; Gribble & Reimann, 2019). This is in contrast to most Western studies reporting reduced or unchanged fasting GLP-1 concentrations in obesity (Faerch et al., 2015; Steinert et al., 2017).

Similarly, microbial patterns traditionally associated with metabolic health, including the presence of fibre-associated taxa, such as members of the *Ruminococcaceae* family and other SCFA producing bacteria, were not uniformly linked to improved metabolic outcomes, as observed in ***Chapter 3***. These findings suggest that neither hormonal profiles nor microbial composition can be interpreted on their own, but rather reflect context-dependent adaptations influenced by metabolic state and population characteristics (Rothschild et al., 2018; Sze & Schloss, 2016).

Second, the integration of microbiota and endocrine data within the same individual (***Chapter* 4**) revealed associations between specific bacterial taxa including *Butyricimonas*, *Alistipes*, and members of the *Ruminococcaceae* family, and circulating

peptide hormones. Notably, inverse relationships were identified between selected bacterial genera, including *Butyricimonas*, *Alistipes*, and *Ruminococcaceae* related taxa, and anorexigenic peptides (***Chapter 4***). These taxa are known to be involved in SCFA production, which may initially appear counterintuitive given the established role of microbial metabolites, particularly SCFAs, in stimulating enteroendocrine secretion (Psichas et al., 2015; Tolhurst et al., 2012). However, similar inverse associations have been reported in other human studies and may reflect complex regulatory feedback mechanisms or altered signalling efficiency rather than direct stimulatory effects (Canfora et al., 2019; Chambers et al., 2015; Psichas et al., 2015). This highlights the importance of interpreting microbiota–endocrine relationships within the context of systemic metabolic dysregulation rather than as isolated interactions.

Third, population-specific variability emerged as a consistent theme across the studies, particularly in ***Chapter 5***. Differences in microbial diversity and taxonomic composition between Lebanese and Emirati cohorts indicate that microbial composition is shaped by environmental, dietary, and lifestyle factors (Deschasaux et al., 2018; Gupta et al., 2017). In ***Chapter 5***, Lebanese individuals exhibited higher microbial diversity and enrichment of fibre-associated taxa, whereas Emirati individuals showed lower diversity and enrichment of genera such as *Bacteroides* and *Blautia*. Importantly, microbial features often considered metabolically favourable, such as higher diversity or enrichment of carbohydrate fermenting taxa, did not uniformly correspond to improved metabolic profiles in all populations. This observation aligns with evidence that gut microbiota composition is strongly influenced by environmental exposures and dietary patterns rather than by host genetics alone (Rothschild et al., 2018). As such, microbial composition alone is insufficient to define metabolic health and must be interpreted alongside host and environmental context.

Taken together, these findings support the concept that obesity is characterised by context-dependent alterations across microbial, endocrine, and metabolic domains. Rather than identifying a single biomarker or uniform signature, this thesis demonstrates that biological patterns associated with obesity vary according to physiological state and population background. This integrative perspective underscores the need to move beyond reductionist interpretations and towards a more holistic understanding of obesity as a multi-system condition influenced by host–microbe interactions (Cani, 2018; Cryan et al., 2019).

3. Endocrine Alterations in Obesity

The endocrine findings of this thesis demonstrate that obesity in Lebanese adults is characterised by a distinct hormonal profile as compared to their normal weight counterparts, including elevated leptin and insulin concentrations and reduced circulating ghrelin levels, as demonstrated in ***Chapter 2***. These alterations are consistent with established patterns of endocrine dysregulation in obesity and are thought to reflect adaptive or compensatory responses rather than simple hormonal deficiencies (Friedman, 2019; Myers et al., 2010).

Elevated leptin concentrations observed in individuals with obesity and are proportional to the increased adipose tissue mass. Individuals with obesity develop leptin resistance, a state in which central appetite-regulating pathways become less responsive despite increased circulating leptin levels (Munzberg & Morrison, 2015; Munzberg et al., 2020). This phenomenon is widely recognized as a hallmark of obesity and may be one of the contributors to impaired satiety signaling and sustained positive energy balance. In parallel, elevated insulin concentrations, also observed in ***Chapter 2***, reflect underlying insulin resistance, which commonly accompanies adiposity related metabolic dysfunction (Saltiel & Olefsky, 2017).

In contrast, reduced ghrelin concentrations in individuals with obesity , as reported in ***Chapter 2***, are consistent with previous human studies demonstrating an inverse relationship between circulating ghrelin and body mass index (Tschop et al., 2001). Although ghrelin is classically described as an orexigenic hormone, its suppression in obesity may represent a compensatory response to chronic energy surplus rather than a primary driver of excessive food intake (Cummings et al., 2002; Muller et al., 2015; Tschop et al., 2001). Together, these observations highlight the complexity of endocrine regulation in obesity, where hormonal alterations often reflect adaptive responses to metabolic stress rather than isolated pathogenic mechanisms.

Importantly, these hormonal patterns should be interpreted within the context of obesity heterogeneity. As observed in ***Chapter 2***, not all individuals with similar body mass index exhibited comparable endocrine or metabolic profiles. Variability in insulin sensitivity, adipose tissue distribution, inflammatory status, and metabolic risk may influence circulating peptide concentrations (Blüher, 2019; Friedman, 2019; Myers et al.,

2010). The observed endocrine alterations therefore likely represent one dimension of a broader, multi-system metabolic phenotype.

While fasting peptide measurements provide insight into basal endocrine status, they do not capture dynamic postprandial responses, which may differ substantially between individuals with obesity and normal weight (Sumithran et al., 2011). This distinction is particularly relevant when interpreting fasting GLP-1 and PYY concentrations. In ***Chapter 2***, higher fasting concentrations of these anorexigenic peptides were detected in individuals with obesity, a finding that may initially appear counterintuitive given their role in promoting satiety. However, this pattern may reflect compensatory upregulation in response to reduced hormone sensitivity or impaired downstream signaling rather than enhanced physiological function. Evidence indicates that obesity may involve diminished responsiveness to gut-derived hormones, including incretins, thereby reducing their effectiveness despite elevated circulating levels (Drucker, 2018).

Taken together, the findings in this thesis support the concept that obesity is characterised by alterations in appetite-regulating and metabolic hormones (Stefan et al., 2018). However, these changes should not be viewed in isolation. Their potential interaction with gut microbial composition, explored in subsequent sections of this discussion (***Chapter 4***), may provide additional insight into mechanisms contributing to metabolic variability.

4. Gut Microbiota Alterations in Obesity

The genus-level analysis of gut microbiota composition revealed that obesity in Lebanese adults was associated with alterations in microbial diversity and taxonomic distribution, as demonstrated in ***Chapter 3***. Specifically, individuals with obesity exhibited reduced alpha diversity and shifts in the relative abundance of selected bacterial genera compared to normal weight controls. These findings are consistent with previous studies demonstrating that lower microbial richness is associated with metabolic dysfunction, insulin resistance, and increased adiposity (Le Chatelier et al., 2013; Turnbaugh et al., 2006).

Reduced microbial diversity has been proposed as a marker of impaired ecosystem resilience and metabolic flexibility, potentially limiting the capacity of the microbiota to respond to dietary and environmental challenges (Le Chatelier et al., 2013). In this context, the lower diversity observed in individuals with obesity may reflect a less adaptable

microbial ecosystem, which could contribute to impaired metabolic regulation. Individuals with lower microbial gene richness have been shown to exhibit greater systemic inflammation, insulin resistance, and dyslipidemia, suggesting that microbial ecosystem contraction may parallel metabolic impairment (Le Chatelier et al., 2013). However, the relationship between microbial diversity and obesity is not universally consistent across populations, and considerable heterogeneity has been reported in human studies (Falony et al., 2016; Sze & Schloss, 2016). This variability underscores the importance of examining microbial patterns within well-characterized regional cohorts rather than relying solely on findings derived from Western populations.

Historically, obesity associated microbiota signatures were often described in terms of alterations in the *Bacillota/ Bacteroidota* ratio (Ley et al., 2006; Turnbaugh et al., 2006). However, subsequent investigations have demonstrated that such phylum-level shifts are not consistently reproducible and may oversimplify the complexity of microbial community structure (Sze & Schloss, 2016). In line with this evolving perspective, the findings of this thesis (***Chapter 3***) highlight genus-level differences rather than uniform phylum-wide alterations, reinforcing the notion that obesity related microbial signatures are taxon specific and context-dependent.

Several bacterial taxa were differentially abundant between Lebanese individuals with obesity and normal weight controls, as shown in ***Chapter 3***. In particular, an increased abundance of *Lachnoclostridium* was observed in individuals with obesity, while taxa such as *Ruminococcaceae UCG-005*, *Ruminococcaceae UCG-010*, *Lachnospiraceae UCG-001*, and *Oxalobacter* were more abundant in normal weight individuals. These findings suggest that obesity is associated with shifts in bacterial groups that may differ in their metabolic roles and interactions with the host.

Members of the *Ruminococcaceae* and *Lachnospiraceae* families are commonly associated with the production of SCFAs, particularly butyrate, which plays an important role in maintaining gut barrier integrity and regulating host metabolism (Canfora et al., 2015). The reduced abundance of these taxa in individuals with obesity observed in this thesis (***Chapter 3***) may therefore indicate a reduction in microbial functions associated with metabolic homeostasis, including SCFA production. Such alterations could contribute to metabolic dysregulation through mechanisms involving inflammation, impaired gut barrier function, and altered energy metabolism (Canfora et al., 2015; Cani et al., 2008).

Conversely, the increased abundance of *Lachnoclostridium* observed in individuals with obesity has been reported in other studies of metabolic disorders and may be associated with insulin resistance and dyslipidemia (Vacca et al., 2020). Although the functional role of this genus remains incompletely characterized, its enrichment in obesity may reflect shifts toward microbial communities associated with altered metabolic activity. However, it is important to interpret these findings with caution, as microbial taxa can have context-dependent roles that vary across populations and dietary patterns (Rothschild et al., 2018; Zmora et al., 2019).

Importantly, the observed microbial shifts occurred within individuals who also exhibited endocrine and metabolic alterations (***Chapters 2*** and ***3***), suggesting that changes in community composition may reflect broader host–microbe interactions rather than isolated ecological variation. Nevertheless, 16S rRNA gene sequencing provides taxonomic resolution without direct insight into functional metabolic pathways. Consequently, while compositional differences were identified, the functional implications of these shifts cannot be determined. Integrative approaches incorporating metagenomics, metabolomics, or transcriptomics would be required to clarify whether these compositional shifts translate into functional metabolic changes (Falony et al., 2016).

Taken together, the findings of this thesis (***Chapter 3***) support the concept that obesity is associated with measurable alterations in gut microbial ecology. However, these alterations should be interpreted within a framework that recognizes inter-individual variability, population-specific influences, and methodological limitations. Rather than representing a single, uniform dysbiotic signature, obesity associated microbiota patterns likely reflect dynamic host–environment interactions shaped by dietary, metabolic, and regional factors.

5. Microbiota–Endocrine Interactions in Obesity

The integration of gut microbiota composition with circulating peptide hormone concentrations represents a central component of this thesis, as described in ***Chapter 4***. While several human studies have simultaneously assessed gut microbiota composition and circulating gut-derived peptide hormones, the interactions between these systems remain incompletely understood and are often interpreted independently rather than within an integrated metabolic framework (Gribble & Reimann, 2019; Lynch & Pedersen, 2016). The

present work specifically evaluated whether microbial taxa are associated with circulating gut hormones.

Significant associations were identified between specific bacterial taxa and circulating gut-derived peptide hormones in Lebanese adults. In particular, inverse associations were observed between several bacterial taxa, including *Butyricimonas*, *Alistipes*, and members of the *Ruminococcaceae* family, and circulating concentrations of anorexigenic peptides such as GLP-1 and PYY. These associations remained significant after adjustment for body mass index in several cases, suggesting that the observed relationships are not solely driven by differences in adiposity but may reflect more direct host–microbe interactions.

The observation of inverse relationships between GLP-1, PYY, and selected bacterial taxa involved in SCFA production —particularly members of the *Ruminococcaceae* family and related fibre fermenting taxa identified in ***Chapter 4***— may initially appear counterintuitive, particularly given the established role of SCFAs in stimulating L-cell secretion of GLP-1 and PYY (Psichas et al., 2015; Tolhurst et al., 2012), as well as the commonly described beneficial metabolic roles of some of these taxa. However, evidence from human intervention studies indicates that increasing SCFA availability does not consistently translate into improved hormonal or metabolic outcomes (Chambers et al., 2015; Psichas et al., 2015). These findings suggest that such associations may reflect complex regulatory feedback mechanisms or altered signalling efficiency rather than direct stimulatory effects. Importantly, fasting hormone concentrations do not necessarily reflect postprandial secretory capacity. Elevated circulating peptide levels in individuals with obesity may therefore represent compensatory endocrine responses to reduced receptor sensitivity or impaired downstream signaling rather than enhanced physiological function (Drucker, 2018). In this context, it can be speculated that the negative associations observed in this thesis (***Chapter 4***) may reflect altered regulatory feedback between microbial composition and enteroendocrine activity under metabolically compromised conditions.

Several mechanisms may underlie these observed relationships. Microbial fermentation of dietary fibers produces SCFAs, activate free fatty acid receptor 2 (FFAR2; GPR43) and free fatty acid receptor 3 (FFAR3; GPR41) expressed on enteroendocrine L-cells, thereby stimulating GLP-1 and PYY secretion (Tolhurst et al., 2012). In addition to

SCFAs, microbial metabolites such as secondary bile acids and indole derivatives may influence enteroendocrine signaling and host metabolic responses (Agus et al., 2018; Koh et al., 2016). Conversely, hormonal changes affecting gut motility and nutrient transit may alter the intestinal microbial environment, highlighting the bidirectional nature of microbiota–endocrine crosstalk (Gribble & Reimann, 2019).

Importantly, the associations identified in this thesis were modest in magnitude and derived from cross-sectional analyses. As such, they should not be interpreted as evidence of causation. It remains unclear whether microbial alterations precede hormonal changes, whether endocrine dysregulation modifies microbial ecology, or whether both processes are secondary to obesity-related metabolic disturbances. Addressing these questions will require longitudinal and interventional studies incorporating dietary modulation, microbial profiling, and dynamic hormone testing (Lynch & Pedersen, 2016).

Nevertheless, the identification of microbiota–peptide associations within a well characterized Middle Eastern cohort adds to emerging human evidence that microbial composition and enteroendocrine signaling may be functionally interconnected. Rather than viewing obesity solely as a disorder of excess adiposity or isolated hormonal imbalance, the findings of this thesis are consistent with the prevailing concept that microbial ecology and endocrine regulation interact within a broader metabolic framework.

6. Population-Specific Variability in Gut Microbiota

An important contribution of this thesis lies in the comparison of gut microbiota composition between Lebanese and Emirati adults with obesity, as described in ***Chapter 5***. Although both populations reside within the Middle Eastern region, distinct microbial patterns were observed, highlighting that obesity-associated microbiota signatures are context-dependent rather than uniform across populations (Gupta et al., 2017; Rothschild et al., 2018).

At the community level, clear differences in both alpha and beta diversity were identified. Lebanese individuals exhibited significantly higher microbial diversity, indicating a richer microbial ecosystem, while beta diversity analyses demonstrated clear separation between the two populations. This is relevant given that reduced microbial richness has been linked to adverse metabolic outcomes, including insulin resistance and low-grade inflammation (Falony et al., 2016; Le Chatelier et al., 2013). Similarly, global

comparisons of microbiome diversity have revealed substantial variability across populations and geographic regions (Nakayama et al., 2015; Yatsunenko, 2012).

At the phylum level, both populations were dominated by *Bacillota* and *Bacteroidota*, yet notable differences were observed beyond these dominant phyla. Lebanese individuals showed higher relative abundances of *Pseudomonadota*, *Mycoplasmatota*, *Cyanobacteriota*, and *Lentisphaerota*, whereas *Bacteroidota* was more predominant among Emirati individuals, suggesting subtle but meaningful compositional shifts. Similar population-level variability in phylum distribution has been reported across geographically distinct cohorts (Deschasaux et al., 2018; Gupta et al., 2017).

More pronounced differences emerged at the taxa and genus levels. Several genera were uniquely present or enriched in one population, indicating both quantitative and qualitative variability. Emirati individuals showed enrichment of genera such as *Bacteroides*, *Parabacteroides*, *Blautia*, *Acidaminococcus*, and *Subdoligranulum*, whereas Lebanese individuals exhibited enrichment of a broader range of taxa, including *Alloprevotella*, *Paraprevotella*, *Dialister*, *Anaerotruncus*, and multiple members of the *Ruminococcaceae* family, alongside taxa such as *Desulfovibrio*, *Sutterella*, and *Succinivibrio*, as demonstrated in ***Chapter 5***. These findings suggest that population-specific microbial signatures arise from coordinated shifts across multiple taxa rather than a single defining organism, consistent with previous microbiome studies (Falony et al., 2016; Sze & Schloss, 2016).

Some of these associations remained significant after adjustment for age, including *Prevotellaceae D_5*, *Clostridiales vadinBB60*, and *Ruminococcaceae UCG-010*, whereas others were attenuated, indicating partial confounding. This is consistent with evidence that age is an important determinant of gut microbiota composition, particularly influencing taxa within the *Ruminococcaceae* family (Odamaki et al., 2016; Xu et al., 2019).
The observed differences are likely driven by variations in diet, lifestyle, and environmental exposures. In ***Chapter 5***, dietary data indicated that fibre intake was higher among Lebanese participants compared to Emirati participants, which may partly explain the higher microbial diversity and enrichment of fibre associated taxa observed in this group. The Middle Eastern region is undergoing rapid nutritional transition; however, dietary patterns—particularly fibre intake—differ between countries and may influence microbial composition through substrate availability for fermentation (David et al., 2014; De Filippo

et al., 2010). In turn, these differences may contribute to variability in microbial metabolite production and host metabolic responses (Canfora et al., 2019; Koh et al., 2016).
These findings align with large-scale studies showing that environmental and lifestyle factors exert a stronger influence on gut microbiota composition than host genetics (Rothschild et al., 2018). They also reinforce that microbiota variability in obesity is better captured at the taxa and genus levels rather than at the phylum level, where important functional differences may be overlooked (Canfora et al., 2019; Magne et al., 2020; Sze & Schloss, 2016).

From a translational perspective, these results suggest that microbiota targeted interventions may require adaptation to population-specific dietary habits and baseline microbial profiles (Jardon et al., 2022). Extrapolation across populations without such context may lead to suboptimal outcomes (Zmora et al., 2019).
Taken together, these findings demonstrate that obesity associated microbial alterations are not uniform across Middle Eastern populations but are shaped by population-specific environmental and lifestyle factors. This underscores the importance of interpreting microbiota–metabolic relationships within a population-specific framework (Falony et al., 2016; Rothschild et al., 2018).

Finally, the cross-sectional design limits causal inference, and longitudinal studies are needed to determine whether these microbial differences contribute to metabolic outcomes or reflect underlying exposures (Sze & Schloss, 2016).

7. Methodological Considerations

The findings presented in this thesis should be interpreted in light of several methodological considerations. While the study design and analytical approach allowed for a detailed investigation of gut microbiota composition and its associations with metabolic characteristics, certain limitations need to be acknowledged alongside key strengths.
A primary limitation relates to the cross-sectional design. Although this approach enabled the identification of associations between gut microbiota composition, gut peptides, and metabolic outcomes, it does not allow conclusions regarding causality. It therefore remains unclear whether the observed microbial patterns contribute to the development of obesity or instead reflect underlying metabolic alterations. This challenge is well recognised in

microbiome research, where longitudinal and interventional studies are required to establish directionality (Sze & Schloss, 2016).

Another consideration is the relatively modest sample size. While sufficient to detect moderate associations, it may have limited the ability to identify smaller effect sizes, particularly at lower taxonomic levels. Given the high inter-individual variability in gut microbiota composition, larger cohorts are generally required to identify consistent microbial signatures across populations (Falony et al., 2016).

Residual confounding should also be considered. Although strict inclusion and exclusion criteria were applied and statistical adjustments were performed for key variables such as age, the influence of additional factors, including dietary habits and lifestyle behaviours, cannot be fully excluded. These factors are known to shape gut microbiota composition and may contribute to inter-individual variability observed in this study (David et al., 2014). Dietary intake was assessed using self-reported food records, which are subject to recall bias and reporting inaccuracies. Although multi-day records improve reliability, they may not fully capture habitual dietary patterns. This is particularly relevant given the strong influence of diet on gut microbiota composition (David et al., 2014).

From a methodological perspective, the use of partial 16S rRNA gene sequencing provided valuable information on microbial composition but does not allow direct assessment of microbial function. Taxonomic resolution is largely limited to the genus level, and functional interpretations are therefore indirect. More advanced approaches, such as metagenomics or metabolomics, would provide a more comprehensive understanding of microbiota–host interactions (Franzosa et al., 2018).

An additional methodological consideration relates to the use of relative abundance data. Microbiota analyses based on 16S rRNA gene sequencing typically report relative rather than absolute bacterial abundances, meaning that observed changes in one taxon may reflect proportional shifts rather than true increases or decreases in microbial load. As a result, relative abundance data can complicate the interpretation of ecological dynamics and may obscure biologically meaningful changes in total microbial biomass. Future studies incorporating quantitative approaches, such as absolute abundance measurements or microbial load estimation, would provide a more comprehensive understanding of microbiota–host interactions (Gloor et al., 2017).

In addition, the present analysis focused primarily on bacterial communities, whereas the gut microbiome also includes other components such as fungi, archaea, and bacteriophages (Marchesi & Ravel, 2015). These microbial groups may contribute to community structure, metabolic function, and host health, and their interactions with bacterial populations remain incompletely understood (Lynch & Pedersen, 2016; Marchesi & Ravel, 2015). The exclusion of these components therefore represents a limitation of current profiling approaches and highlights the need for more comprehensive multi-kingdom analyses in future research (Marchesi & Ravel, 2015).

The characteristics of the study population should also be considered. The study population was limited to Middle Eastern individuals, primarily Lebanese participants, with additional comparison to Emirati cohorts. While this represents a strength in terms of addressing an underrepresented population, it may limit the generalisability of the findings to other geographic or ethnic groups. At the same time, the identification of population-specific differences highlights the importance of studying microbiota–metabolic interactions within diverse populations and reinforces the need for region-specific research.

Despite these limitations, several strengths of this thesis should be acknowledged. The integration of microbiome and endocrine data within the same individuals allowed for the direct assessment of microbiota–peptide associations in a human population. In addition, the inclusion of clearly defined study groups with standardized clinical and biochemical measurements enhances the consistency and interpretability of the findings. Importantly, the focus on Middle Eastern populations provides valuable insight into microbiota–metabolic interactions in a region that remains underrepresented in the literature, addressing a critical gap related to ethnicity and geographic diversity in microbiome research.

Taken together, while the findings of this thesis should be interpreted within the context of these methodological considerations, they provide a valuable foundation for future research aimed at better understanding the complex interplay between gut microbiota, endocrine signalling, and metabolic health.

8. Implications for Future Research

Building on the findings and methodological considerations discussed in this chapter, several directions for future research can be identified. A key priority is the use of

longitudinal and interventional study designs with detailed mechanistic deepening to clarify the causal relationships between gut microbiota composition, gut-derived peptide hormones, and metabolic outcomes. While the present work demonstrates associations between these systems, the directionality and underlying mechanisms remain unresolved (Lynch & Pedersen, 2016).

In addition, future studies should also incorporate postprandial assessments to better capture the dynamic nature of gut-derived peptide hormone secretion. Fasting measurements provide insight into basal endocrine status but do not fully reflect physiological responses to nutrient intake, which are central to appetite regulation and metabolic control.

The integration of multi-omics approaches, including metagenomics, metabolomics, and transcriptomics, represents an important next step. Such approaches would allow direct assessment of microbial function and metabolic activity, moving beyond taxonomic descriptions toward a more comprehensive understanding of microbiota–host interactions (Franzosa et al., 2018). In particular, the measurement of microbial metabolites, such as short-chain fatty acids and bile acids, may help elucidate pathways linking gut microbiota to endocrine and metabolic regulation.

Finally, given the population specific differences observed in this thesis, future research should prioritise diverse cohorts and region-specific investigations. Understanding how dietary patterns, environmental exposures, and cultural factors shape gut microbiota composition will be essential for interpreting microbiota–metabolic interactions across populations and for developing targeted intervention strategies (Jardon et al., 2022; Rothschild et al., 2018).

9. Concluding Remarks

In conclusion, the findings of this thesis demonstrate that obesity in Middle Eastern populations is associated with alterations in endocrine, microbial, and metabolic domains. In addition, associations between gut microbiota composition and circulating peptide hormones were identified within individuals, highlighting the relevance of considering these systems together in the context of obesity.

Importantly, the present findings indicate that microbiota–metabolic relationships are context-dependent and influenced by population-specific environmental, dietary, and

lifestyle factors. This challenges the application of universal microbial or hormonal patterns and underscores the importance of interpreting metabolic phenotypes within their specific biological and epidemiological context.

By integrating microbial and endocrine analyses in Lebanese adults and examining population-specific microbiota differences between Lebanese and Emirati cohorts, this thesis contributes to addressing the underrepresentation of Middle Eastern populations in microbiome research and provides region-specific insight into microbiota–host relationships.

Overall, these findings support a shift toward more integrative and population aware approaches in metabolic research, with relevance for improving the interpretation of microbiota–host associations across diverse populations.

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CHAPTER 7



Chapter 7- Impact

Societal Relevance

Obesity as a regional health burden

Obesity represents a major public health challenge worldwide and is highly prevalent in the Middle East and North Africa (MENA) region, where rapid lifestyle and dietary transitions have contributed to increasing rates of metabolic disease (Ng et al., 2014; World Obesity Federation, 2022). Despite extensive research, current prevention and treatment strategies remain only partially effective, highlighting the need for improved understanding of the biological mechanisms underlying obesity.

The findings of this thesis show that obesity is associated not only with endocrine alterations but also with changes in gut microbiota composition. These results support the view that obesity is a complex and heterogeneous condition involving interactions between multiple physiological systems rather than a single underlying mechanism (Blüher, 2019). A key strength of this work is the focus on Middle Eastern populations, which remain underrepresented in microbiome research. The identification of population-specific microbial differences between Lebanese and Emirati individuals highlights the importance of regional, dietary, and environmental factors. This is particularly relevant for the MENA region, where culturally adapted prevention and management strategies are needed.

Cultural and dietary relevance

Diet is a major determinant of gut microbiota composition, particularly through the availability of fermentable substrates. While traditional dietary patterns in the region have historically included fibre rich foods, these have been partially replaced by diets lower in fibre and higher in processed foods (De Filippo et al., 2010).

The population-specific differences observed in this thesis, particularly the higher microbial diversity and enrichment of fibre associated taxa in Lebanese individuals compared to Emirati individuals, likely reflect differences in dietary intake, including fibre consumption. These findings support the development of culturally adapted nutritional strategies, including:

- Promoting fibre rich foods such as legumes, whole grains, and vegetables, consistent with the higher diversity observed in Lebanese participants
- Encouraging dietary patterns aligned with local food culture rather than adopting generalized dietary models

- Integrating microbiota-related concepts into nutrition education

Such approaches may improve both adherence and long-term effectiveness.

Public health implications

The variability in microbiota profiles observed across populations suggests that simplified or universal microbial markers should be interpreted with caution (Sze & Schloss, 2016). At present, microbiome data are not yet incorporated into public health strategies, and further research is required before such applications can be implemented. Nevertheless, these findings highlight the importance of developing context aware approaches in future microbiome related research and communication, particularly in regions where locally derived data remain limited.

Target Groups

The findings of this thesis are relevant to several key groups.

First, the scientific community benefits from the integrative approach combining gut microbiota profiling with circulating peptide hormone measurements. Therefore, the research in this thesis contributes to future mechanistic and longitudinal studies.

Second, healthcare professionals, including physicians, endocrinologists, and dietitians, may use these findings to better understand factors contributing to metabolic variability in obesity. The observed microbiota–peptide associations suggest that microbial and endocrine factors may contribute to variability in appetite regulation and metabolic responses and highlight the potential relevance of these systems in metabolic research.

Third, policymakers and public health authorities, particularly in the MENA region, represent an important target group. The findings indicate that prevention strategies should account for local dietary habits, environmental exposures, and cultural practices rather than relying solely on data derived from Western populations.

Finally, patients and the general population may benefit indirectly through the putative future translation of these findings into improved prevention strategies and more tailored approaches to metabolic health.

Innovation

This thesis provides several innovative contributions.

A key innovative aspect of this thesis is the focus on Middle Eastern populations, which remain underrepresented in microbiome research. The identification of differences in microbial composition between Lebanese and Emirati individuals reinforces the importance of considering environmental and dietary context in microbiome studies.

In addition, this thesis combines gut microbiota profiling with circulating gut-derived peptide hormone measurements within the same human cohort. While similar approaches have been applied in other populations, this work provides insight within a regional context that has been less extensively studied. Furthermore, the findings contribute to the field of precision nutrition by suggesting that microbiota composition may be linked to metabolic phenotypes and regulatory pathways (Fan & Pedersen, 2021; Jardon et al., 2022).

Translation to Practice and Application

The findings of this thesis provide several opportunities for future application.

From a clinical perspective, the observed microbiota–endocrine associations indicate that modulation of the gut microbiota could be explored as part of strategies aimed at improving metabolic health (Canfora et al., 2019). Potential approaches include:

- Dietary interventions targeting fibre intake
- Use of prebiotics and probiotics
- Integration of microbiota-informed principles into nutritional counselling

These approaches should be considered within the context of established lifestyle and clinical interventions rather than as stand-alone strategies.

From a public health perspective, the findings support the development of population-specific dietary and lifestyle strategies. The differences observed between Lebanese and Emirati individuals indicate that interventions should be adapted to regional contexts rather than directly extrapolated from Western populations.

In the longer term, microbiome profiling may contribute to identifying microbial patterns associated with metabolic risk or response to dietary interventions. However, further validation is required before such applications can be implemented.

In addition, the findings of this thesis have been disseminated through presentations at scientific conferences and academic discussions, contributing to knowledge exchange among researchers and healthcare professionals.

Future Research Directions

Further research is required to translate these findings into practical applications. Longitudinal and interventional studies are needed to determine whether the observed associations between gut microbiota and peptide hormones are causal.
In addition, the integration of multi-omics approaches, including metagenomics and metabolomics, may provide deeper insight into the mechanisms underlying microbiota–host interactions.

Expanding research to include diverse populations will also be essential to improve the generalisability of findings and to support the development of both globally relevant and regionally adaptable strategies for obesity prevention and management. Continued dissemination of findings through scientific conferences, collaborations, and engagement with healthcare professionals will be important to facilitate translation of research into practice.

Conclusion

This thesis demonstrates that microbiota–endocrine interactions in obesity are influenced by population-specific factors and should be interpreted within their broader context. By examining these relationships in Middle Eastern populations, it contributes to a more comprehensive understanding of metabolic regulation beyond traditionally studied settings. Overall, these findings support the need for more context-specific and integrative approaches in obesity research and provide a foundation for future studies aimed at translating microbiome science into clinically and publicly relevant strategies.

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SUMMARY

Summary

This thesis investigated the relationships between gut microbiota composition, gut-derived peptide hormones, and metabolic characteristics associated with obesity in Middle Eastern populations, with a primary focus on Lebanese adults and an additional comparison with Emirati adults with obesity with respect to gut microbiota composition.

In Lebanese adults, obesity was associated with distinct alterations in endocrine profiles and gut microbiota composition. Individuals with obesity exhibited higher circulating concentrations of leptin, insulin, GLP-1, and PYY, together with lower ghrelin concentrations compared with normal-weight individuals. Differences in gut microbiota composition were observed primarily at the genus level, with *Lachnoclostridium* enriched in individuals with obesity, whereas *Peptococcus, Ruminococcus 2, Lachnospiraceae UCG-001, Ruminiclostridium 6, Ruminococcaceae UCG-005, Ruminococcaceae UCG-010, Oxalobacter*, and an unclassified *Coriobacteriaceae* taxon were more abundant in normal weight individuals. These findings suggest that obesity-related microbial alterations are better captured at lower taxonomic levels than by broad phylum-level classifications. Importantly, within the Lebanese cohort, associations between specific bacterial taxa and circulating gut-derived peptide hormones were identified. GLP-1 concentrations were negatively associated with *Butyricimonas, Alistipes, Ruminococcaceae UCG-005, Ruminococcaceae UCG-002*, and unclassified taxa within the *Ruminococcaceae* and *Coriobacteriaceae* families. Negative associations were also observed between GLP-2 and *Butyricimonas* and an unclassified *Ruminococcaceae* taxon, while PYY was negatively associated with *Eubacterium ventriosum* group, *Parasutterella, Subdoligranulum, Alistipes*, and an unclassified *Ruminococcaceae* taxon. However, given the cross-sectional design of the study, these findings should be interpreted as associative, whilst causality cannot be determined.

In contrast, analyses involving Emirati participants were limited to individuals with obesity and focused on gut microbiota composition. Comparisons between Lebanese and Emirati adults with obesity revealed significant differences in gut microbiota diversity and composition. Lebanese individuals exhibited greater microbial alpha diversity and a distinct microbial profile characterised by higher abundances of taxa including *Prevotellaceae, Ruminococcaceae UCG-010, Clostridiales vadinBB60* group, *Alloprevotella*, and several other members of the *Ruminococcaceae* family. In contrast,

Summary

Emirati individuals showed higher abundances of *Bacteroides, Parabacteroides, Blautia, Subdoligranulum*, and *Phascolarctobacterium*. Several nationality-associated microbial signatures remained significant after adjustment for age, particularly *Prevotellaceae D_5, Clostridiales vadinBB60* group, and *Ruminococcaceae UCG-010*. These findings suggest that gut microbiota composition varies across Arab populations and may be shaped by differences in dietary habits, lifestyle, and environmental exposures.

Overall, this thesis demonstrates that obesity is associated with alterations in endocrine and microbial profiles, while microbiota–peptide associations and population level differences highlight the context dependent nature of microbiota–metabolic relationships. By providing data from underrepresented Middle Eastern populations, this work contributes to a more comprehensive understanding of host–microbe interactions and supports the need for population aware approaches in metabolic research.



SAMENVATTING



Samenvatting

Deze scriptie onderzocht de relaties tussen de samenstelling van de darm-microbiota, darm-gerelateerde peptide-hormonen en metabole kenmerken die samenhangen met obesitas in populaties in het Midden-Oosten. De focus lag primair op Libanese volwassenen, met een aanvullende vergelijking met Emirati volwassenen met obesitas wat betreft de samenstelling van de darm-microbiota.

Bij Libanese volwassenen bleek obesitas gepaard te gaan met duidelijke veranderingen in de endocriene profielen en de samenstelling van de darm-microbiota. Personen met obesitas vertoonden hogere circulerende concentraties van leptine, insuline, GLP-1 en PYY, en lagere ghreline concentraties in vergelijking met personen met een normaal gewicht. Verschillen in de samenstelling van de darm-microbiota werden voornamelijk op genus-niveau waargenomen, waarbij *Lachnoclostridium* in verhoogde mate voorkwam bij personen met obesitas, terwijl *Peptococcus*, *Ruminococcus 2*, *Lachnospiraceae UCG-001*, *Ruminiclostridium 6*, *Ruminococcaceae UCG-005*, *Ruminococcaceae UCG-010*, *Oxalobacter* en een niet-geclassificeerd taxon van *Coriobacteriaceae* vaker voorkwamen bij personen met een normaal gewicht. Deze bevindingen suggereren dat microbiële veranderingen gerelateerd aan obesitas beter worden weergegeven op lagere taxonomische niveaus dan door brede classificaties op fylum-niveau.

Belangrijk is dat binnen het Libanese cohort associaties werden geïdentificeerd tussen specifieke bacteriële taxa en circulerende, uit de darm afkomstige peptide-hormonen. GLP-1-concentraties waren negatief geassocieerd met *Butyricimonas*, *Alistipes*, *Ruminococcaceae UCG-005*, *Ruminococcaceae UCG-002* en ongeclassificeerde taxa binnen de families *Ruminococcaceae* en *Coriobacteriaceae*. Negatieve associaties werden ook waargenomen tussen GLP-2 en *Butyricimonas* en een ongeclassificeerd taxon van *Ruminococcaceae*, terwijl PYY negatief geassocieerd was met de *Eubacterium ventriosum*-groep, *Parasutterella*, *Subdoligranulum*, *Alistipes* en een ongeclassificeerd taxon van *Ruminococcaceae*. Gezien het cross-sectionele ontwerp van de studie moeten deze bevindingen echter als associatief worden geïnterpreteerd, terwijl causaliteit niet kan worden vastgesteld.

Daarentegen waren analyses met Emirati-deelnemers beperkt tot personen met obesitas en richtten ze zich op de samenstelling van de darm-microbiota. Vergelijkingen tussen Libanese en Emirati-volwassenen met obesitas lieten significante verschillen zien in de diversiteit en samenstelling van de darm-microbiota. Libanese individuen vertoonden een grotere microbiële alfa-diversiteit en een onderscheidend microbieel profiel, gekenmerkt door een hogere abundantie van taxa zoals *Prevotellaceae*, *Ruminococcaceae UCG-010*, de *Clostridiales vadinBB60*-groep, *Alloprevotella* en verschillende andere leden van de *Ruminococcaceae*-familie. Daarentegen vertoonden individuen uit de Verenigde Arabische Emiraten een hogere abundantie van *Bacteroides*, *Parabacteroides*, *Blautia*, *Subdoligranulum* en *Phascolarctobacterium*. Verschillende nationaliteitsgebonden microbiële kenmerken bleven significant na correctie voor leeftijd, met name *Prevotellaceae D_5*, de *Clostridiales vadinBB60*-groep en *Ruminococcaceae UCG-010*. Deze bevindingen suggereren dat de samenstelling van de darm-microbiota varieert tussen Arabische populaties en mogelijk wordt beïnvloed door verschillen in voedingsgewoonten, levensstijl en blootstelling aan de omgeving.

Samenvattend toont dit proefschrift aan dat obesitas gepaard gaat met veranderingen in endocriene en microbiële profielen, terwijl de associaties tussen microbiota en peptiden en de verschillen op populatie-niveau de context-afhankelijke aard van de relaties tussen microbiota en metabolisme benadrukken. Door gegevens te verstrekken van ondervertegenwoordigde populaties in het Midden-Oosten draagt dit werk bij aan een beter begrip van de interacties tussen gastheer en microben, en onderstreept het de noodzaak van populatiegerichte benaderingen in metabolisch onderzoek.

PUBLICATIONS

Publications

Published Articles

Abou-Samra M, Venema K, Ayoub Moubareck C, Karavetian M. The Association of Peptide Hormones with Glycemia, Dyslipidemia, and Obesity in Lebanese Individuals. Metabolites. 2022;12(11):1051. https://doi.org/10.3390/metabo12111051

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CURRICULUM VITAE

Curriculum Vitae



Murielle Abou-Samra was born on 8 May 1984 in Lebanon and holds Lebanese and Canadian citizenship. She obtained her Bachelor of Science in Nutrition and Dietetics from the American University of Beirut, Lebanon, in 2005 and completed her Master of Science in Nutrition and Dietetics at the same institution in 2014.

Mrs. Abou-Samra is a licensed dietitian accredited by the Lebanese Order of Dietitians and has more than 20 years of professional experience in clinical nutrition, obesity management, and nutrition education. She began her career at the Middle East Institute of Health University Hospital (MEIH-UH), where she served as Chief Clinical Dietitian and Head Dietitian, providing specialized nutritional care to individuals with obesity and coordinating dietetic internship training programs. Her clinical expertise includes bariatric nutrition, weight management, renal nutrition, pediatric nutrition, and medical nutrition therapy.

Alongside her clinical career, Mrs. Abou-Samra has been actively involved in higher education. Since 2012, she has taught nutrition courses at several Lebanese

universities, including the Lebanese University, the Lebanese American University, and the University of Balamand. Through her academic appointments, she has contributed to the education and training of future nutrition and dietetics professionals.

In 2019, Mrs. Abou-Samra enrolled as a PhD candidate at the School of Nutrition and Translational Research in Metabolism (NUTRIM), Maastricht University, the Netherlands. Her doctoral research focuses on the interactions between gut microbiota and gut-derived peptide hormones in obesity, with particular emphasis on Lebanese and Emirati populations. Her work aims to improve understanding of the microbiota–gut peptide axis and its potential implications for obesity and metabolic health in Middle Eastern populations.

Throughout her professional and academic career, Mrs. Abou-Samra has remained committed to advancing evidence-based nutrition practice through clinical care, teaching, and research. The work presented in this thesis reflects her longstanding interest in obesity, metabolic health, and the role of the gut microbiota in human physiology.