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The Role of the Lebanese Mediterranean Diet on Gut Health, Anxiety, and Depression

By

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Dedication Page

To my family

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The Role of the Lebanese Mediterranean Diet on Gut Health, Anxiety, and Depression

Dana Abdulghani

ABSTRACT

Objectives: A Mediterranean-style diet (MeDi) that is rich in high proportion of fibers, healthy fats, antioxidants, and polyphenols has been associated with the prevention of several metabolic diseases. Adherence to the MeDi has been also shown to lower the risk of mental disorders, including anxiety and depression. Several studies have shown that MeDi has been also associated with beneficial changes in gut microbiome composition. The gut microbiome plays an important role in host health and disease. A bacterial imbalance of the gut has been linked to several mental illnesses including anxiety and depression too. The role of gut health on anxiety and depression might be attributed to the effect of the MeDi on gut health. Previous studies have shown that the Lebanese population has low to moderate adherence to MeDi. Furthermore, the effect of this moderate adherence on gut microbiota and on mental health is still missing among Lebanese adults. Therefore, the aim of this study is to examine how adherence to the MeDi affects depression and anxiety via its effect on gut microbiota among Lebanese adults.

Methods: This is a pilot cross-sectional study conducted on 75 Lebanese adults aged between 45-65 years recruited from different Lebanese governorates, including Beirut, South Lebanon, and Mount Lebanon. Participants were sent an online invite to our study through different social media platforms (WhatsApp and Instagram), in which information on dietary intake, depression, anxiety, and gut health was collected. Dietary intake was assessed by a 61-item FFQ. Adherence to the Mediterranean diet was calculated by the (Lebanese Mediterranean Diet) LMD index. The gastrointestinal health of the Lebanese adults was evaluated using 9 items of the validated Structured Assessment of Gastrointestinal Symptom (SAGIS) related to the lower GI tract. Depression was assessed using the Patient Health Questionnaire (PHQ-9). Anxiety was assessed using the 7-item tool Generalized Anxiety Disorder (GAD-7). Data were analyzed using STATA version 13. Logistic regression models adjusted for

sociodemographic and clinical covariates were used to assess the association between LMD, gut health anxiety, and depression.

***Results*:** Among the 68 participants, 48 (70.59%) were females, and the mean age of the whole sample of 55.15 ± 5.5 years. At a logistic regression level, after adjusting for several confounders (age, gender, education, BMI, medications, and physical activity) it has been shown that having high adherence to LMD compared to low adherence were associated with depression (OR=8.16, 95%CI:1.16-57.28, P=0.035). However, high adherence to LMD was not associated with anxiety (OR=0.89,95%CI:0.23-3.4, P=0.866). nor gut health (OR=3.86, 95%CI: 0.78-18.96, p-value: 0.097). Also, scoring more than the mean (>4) for gut health appeared to be associated with depression (OR= 5.64,95%CI: 1.23-25.7, P=0.025) but not with anxiety ([OR= 2.29,95%CI: 0.66-7.93, P=0.188).

Conclusion: Our results have shown that a higher LMD adherence was associated with a higher prevalence of depression which is counterintuitive while no significant associations were observed between LMD and anxiety nor gut health. On the other hand, we observed a significant association between gut health and depression with worse gut health was associated with a higher prevalence of depression but not with anxiety. Furthermore, we were not able to demonstrate that LMD would influence depression and anxiety via its mediated effect on gut health ince LMD was not associated with mental health nor associated with gut health. Future longitudinal and randomized studies with a larger sample size are needed to clarify the relationship between LMD, gut health, depression, and anxiety.

Keywords: Lebanese Mediterranean diet, Gut health, Depression, Anxiety, Lebanese adults

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List of Abbreviations

MeDi: Mediterranean Diet

LMD: Lebanese Mediterranean Diet

SAGIS: Structured Assessment of Gastrointestinal Symptom

PHQ-9: Patient Health Questionnaire (9 items)

GAD-7: Generalized Anxiety Disorder (7 items)

CI: Confidence Interval

OR: Odds Ratio

Chapter 1

Literature Review

1.1: Overview of the Mediterranean Diet and Its Impact on Health

A Mediterranean-style diet (MeDi) is a nutritionally balanced diet that is based on the increased intake of fruits and vegetables, legumes, nuts, and fish. It is also characterized by the moderate consumption of alcohol and low consumption of red meat and poultry (Nagpal et al,2019). Many of the characteristic components of the MeDi have functional features and affect our health and well-being positively. Several studies indicated that higher adherence to the MeDi is associated with a lower risk of mortality (Canudas et al.,2020, Dinu et al, 2018, Estruch et al.,2018, Soltani et al., 2019). It has been also demonstrated that MeDi exerts a preventive effect on cardiovascular diseases (Khan et al., 2021). The PREDIMED study, a large Spanish cohort study including 744participants (55 to 80 years of age, 57% women) was done to assess the long-term effects of the MeDi on incident CVD in men and women at high cardiovascular risk. It showed that the incidence of major cardiovascular events was lower among those assigned to a MeDi supplemented with olive oil or nuts (Martinez-Lapiscina et al, 2013). There is also evidence that MeDi has a potential role in preventing certain types of cancers (Schwingshackl et al,2015). In fact, the European Prospective Investigation into Cancer and Nutrition (EPIC) study, which was conducted on 519,978 participants to investigate the relationships between diet the incidence of cancer and other chronic diseases. It was shown that MeDi represents the best food model in the prevention of cancer diseases.

Moreover, the intake of flavonoids would seem to reduce the risk of developing gastric cancer (Parke et al, 2012). A recent umbrella review of 13 meta-analysis of observational studies and 16 meta-analyses of RCTs that included a total population of over 12,800,000 persons aimed to evaluate the association between the adherence to the MeDi and multiple health outcomes. It was concluded that there is strong evidence from observational studies that greater adherence to MeDi is associated with lower risk of CVD, coronary heart diseases (CHD), myocardial infarction, diabetes, and other diseases (Dinu et al,2018). Furthermore, meta-analyses of RCTs demonstrated that participants following a MeDi had better metabolic parameters and lower risk of cardiovascular outcomes compared with participants following control diet (Dinu et al,2018).

Finally, recent studies have shown that higher adherence to the MeDi is associated with a lower risk of mental disorders, including cognitive decline and depression (Pagliai et al, 2018, Ventriglio et al,2020).

1.2: Effect of MeDi on Depression and Anxiety

The association between MeDi and mental health is well-established (Shafiei et al,2019, Fraga et al., 2021, Ventriglio et al, 2020). A large French prospective study investigated the association between adherence to the MeDi and incident depressive symptoms over a 13-year follow-up period in 3523 non-depressed participants with a mean age of 49.5 years at baseline (Adjibade et al,2018). MeDi adherence was measured by a relative Mediterranean diet score (rMed) and was computed as a continuous variable. Incident depressive symptoms were defined by the Center for Epidemiological Studies-Depression CES-D score. (Adjibade et

al,2018). Results have shown that adherence to MeDi was significantly associated with a 9% lower risk of incident depressive symptoms, particularly in men (OR=0.91; 95%CI:0.83-0.99; p=0.03)) However, the associations were non-significant among women (Adjibade et al,2018). Another cohort study was done on non-depressed 42,515 Swedish women with a mean age of 39.5 years (Yin et al,2021). Adherence to MeDi was calculated using a Mediterranean dietary pattern (MDP) and clinical depression was extracted from the National Patient Register (Yin et al,2021). After a follow-up of 20.4 years, it was shown that participants with medium and high adherence to MeDi had a lower risk of depression, and the risk of depression was reduced by a 5% per unit increase in adherence to MeDi (HR = 0.95, 95%CI = 0.92-0.98) (Yin et al,2021).

Additionally, a cross-sectional study aimed to examine the association between adherence to Mediterranean dietary patterns and the prevalence of depression and anxiety among a large population of 3172 Iranian adults aged with a mean age of 36.54 ± 4.97 years. They used a validated food frequency questionnaire for the assessment of dietary intakes, the Mediterranean Dietary Score (MDS) based on Trichoopoulo et al to examine the adherence to the MeDi, a validated version of the Hospital Anxiety and Depression Scale (HADS) for mental health. It has been observed that the higher adherence to the MeDi the lower odds for depression (OR=0.60, 95% CI: 0.46-0.78), anxiety (OR=0.61, 95% CI: 0.42-0.86) and anxiety (OR= 0.61, 95% CI: 0.42-0.86) compared with those with the lowest adherence (Sadeghi et al, 2021).

Another cross-sectional study examined the association between the adherence to Mediterranean Diet Score (MDS) with depression and anxiety in 1634 adults with a mean age of 52.0 ± 13.2 years in the Netherlands. Depression

symptoms were measured with the 30-item Inventory of Depressive Symptomatology and anxiety was measured using the 21-item Beck Anxiety Inventory. Results have shown that the higher the MDS score is protective against depression (OR= 0.77,95% CI: 0.66–0.90, P<0.01) and anxiety (β −0.11, 95% CI:−0.16, −0.06, p<0.01) (Gibson-Smith et al,2020).

In addition, a cross-sectional study examined whether adherence to MeDi was associated with depressive symptoms in a representative sample of 11,769 U.S. adults with a mean age of 46.2 years old. MeDi was determined by the Alternate Med Diet score (aMED) and depression was determined by the Patient Health Questionnaire-9 (PHQ-9) (Oddo et al, 2022). Compared to individuals with the lowest MeDi adherence (Q1), individuals in the third and fourth quartiles had 40p and 45% lower odds of moderate to severe depressive symptoms (odds ratio [OR] = 0.60, 95% confidence interval [CI]: 0.50- 0.74; OR = 0.55, 95% CI: 0.36-0.84, respectively) (Oddo et al, 2022). Lastly, a recent systematic review and meta-analysis of observational studies of 20 longitudinal and 21 cross-sectional studies aimed to study the link between diet quality using several indices, including MeDi, and depressive outcomes. It showed that higher adherence to MeDi decreased the incident risk of depression (Lassale et a,2019). Therefore, it is widely recognized that MeDi plays a positive role on depression and anxiety especially among the adult population (Lassale et a,2019).

1.3: Effect of MeDi on Gut Health

Gut microbiota is defined as the community of microorganisms residing in the intestine with the highest concentrations being found in the colon (Khan et al, 2021). It is colonized by different microbial species, some being protective as Bifidobacteria, Bacteriodetes, Lactobacillus, and others acting as harmful such as Enterobacter cloacae, Escherichia coli., Bacteroides, Clostridia…) (Del et al, 2014). Dysbiosis, defined as a bacterial imbalance, leads to a wide range of metabolic disorders and digestive disturbances including bloating, diarrhea, constipation, and stomach cramps (Hills et al,.2019).

The gut microbiota composition is generally formed in early childhood, depending on geographical factors, the type of delivery, breastfeeding, age of weaning, antibiotic exposure, and dietary regimens (Duranti et al, 2017). But one of the most important modulators that influence the composition of gut microbiota is the host's diet (Del et al, 2014). Several studies have shown that MeDi has been associated with beneficial changes in gut microbiome composition (Mitsou et al,2017). Adherence to MeDi appears to have significant beneficial effects on the gut microbiome composition (bacterial abundance and microbial metabolites) and function (Merra et al, 2020). A longitudinal prospective study examined the relationship between MeDi and the17-years risk of later-onset Crohn's disease (CD) that is characterized by significant gut dysbiosis. This study included 83 147 Swedish participants aged between 45–79 years. At baseline (in 1997), a validated FFQ was used to calculate an adherence score to a modified Mediterranean diet (mMED) and incident diagnoses of CD and UC were determined from the Swedish Patient Register. After a 17-years follow-up, the authors observed that a higher mMED score was associated with a lower risk of CD (p=0.03) (Khalili et al., 2020).

They speculated that the mechanism behind this relationship may be explained by the greater role of the gut microbiome in CD where CD is characterized by significant dysbiosis. Therefore, diet-induced changes in the gut microbiome may have a greater impact in preventing and treating CD (Khalili et al.,2020).
Another cross-sectional study was conducted on 27 volunteers (16 females and 11 males) whose mean age is 39.5 ± 7.3 years old and living in a Mediterranean area (Valencia, SPAIN) to study the effect of MeDi adherence on the gut microbiome (Garcia-Mantrana,2018). Results showed that low adherence to the diet (scoring less than 9) resulted in a higher Firmicutes-to-Bacteroidetes ratio, and when the participants had better adherence to the MeDi (scoring above 9), they had a greater presence of beneficial bacteria such as Bacteroidetes, indicating a better gut health (Garcia-Mantrana,2018). These results are in line with those of another cross-sectional study that aimed to explore the potential associations between MeDi adherence and gut microbiota characteristics and gastrointestinal health among 116 Greek participants (61% males with a mean age of 42±13.33 years) (Mitsou et al, 2017). Results have shown that high adherence to the MeDi as assessed via Mediterranean diet score (MDS) was correlated with lower Escherichia coli counts (harmful bacteria), increased level in beneficia bacteria such as Candida albicans, and a greater amount of SCFA acetate. This indicates that higher adherence to Medi is associated with a better gut microbial abundance which reflect a better gut health (Mitsou et al, 2017).

A connection between the MeDi and fecal metabolic phenolic profile has also been recently shown in a randomized, multicenter, single-blind, controlled trial that analyzed the gut microbiota in 612 non-frail participants across five European countries (UK, France, Netherlands, Italy, and Poland). Participants were profiled

before and after a 12-month intervention. The control group followed a habitual diet and received only a leaflet with national dietary guidelines. The intervention group followed the NU-AGE diet (described in Berendsen et al., 2014) which consisted of individually tailored MeDi and received education with dietitians. It was observed that adherence to the MeDi was associated with an increase in microbiome diversity and an increase in SCFA production which is an indication of a better gut health (Ghosh et al, 2020).

Lastly, a meta-analysis aimed to determine whether MeDi can be used as a preventive measure against gut related diseases. 16S RNA data from participants on diets such as MeDi or Western Diet, and patients with intestinal diseases. They observed that the microbiota associated with MeDi was enriched in beneficial bacteria that promote an anti-inflammatory environment (Oscar et al,2021). As a conclusion, dietary interactions especially MeDi play a vital role in modulating the gut microbiota and thus affecting the hosts gut health (Nagpal et al.,2019).

1.4: Impact of Gut microbiota on Health

Currently, the gut microbiota is considered one of the key elements contributing to the regulation of host health (de Vos et al,2022). By controlling numerous processes, such as nutrition absorption, inflammation, oxidative stress, immunological function, and anabolic balance, the human gut microbiota can have an impact on the host physiology (Valdes et al,2018). Two other ways how the gut microbiota influences the health of the host are the production of metabolic byproducts from dietary components like short-chain fatty acids (SCFAs) and the production of vitamins and vital amino acids (Fan et al, 2021). According to Valdes

et al. (2018), the primary metabolites produced in the colon by bacterial fermentation of dietary fibers and resistant starch are acetate, propionate, and butyrate (Silva et al, 2020). Intestinal epithelial cells rely heavily on SCFAs for their energy, which also helps to keep the gut's oxygen levels balanced and guard against gut microbiota dysbiosis (Valdes et al,2018). Recent studies have shown the association between gut microbiota dysbiosis and non-communicable diseases, such as diabetes, obesity, cancer, gastrointestinal diseases such as irritable bowel syndrome (IBS), and neurological disorders (Valdes et al, 2018). A cross-sectional study performed among 2166 participants from 2 Dutch population-based prospective cohorts to examine associations between gut microbiome composition and insulin resistance and type 2 diabetes (Chen et al, 2021). The study showed that higher microbiome richness as assessed by the 16S ribosomal RNA method was associated with less insulin resistance, and patients with type 2 diabetes had lower richness than participants without diabetes (Chen et al,2021). Additionally, many studies have examined the gut microbiome and its relevance in specific gastrointestinal disorders such as intestinal bowel diseases (IBD), coeliac disease, irritable bowel syndrome, and much more (Caruso et al., 2020, Menees et al., 2018, Pecora et al.,2020). In particular, one study followed 132 participants for one year in order to generate integrated longitudinal molecular profiles of their microbial activity during the flare period of IBD. The findings revealed that dysbiosis was present in the gut microbiome during inflammatory bowel disease activity (Lloyd-Price et al.,2019). In conclusion, it is becoming evident that the gut microbiome plays an important role in regulating host metabolism and physiology.

1.5: Effect of Gut Health on Anxiety and Depression

A bacterial imbalance of the gut has been also linked to several mental illnesses including anxiety and depression (Lach et al,2018). A cross-sectional study of 40 patients aimed to analyze the gut microbiome composition in patients with a generalized anxiety disorder (GAD) compared to healthy controls. The fecal microbiota of patients suffering from GAD showed a much lower prevalence of non-beneficial bacteria such as Shigella, Fusobacterium and Ruminococcus gnavus. (Jiang et al., 2018).

Another cross-sectional study recruited 116 Polish women with a mean age of 52.0 ± 4.7 years to analyze SCFAs in the stool of depressed and non-depressed women (Skonieczna-Żydecka et al.,2018). The Beck Depression Inventory (BDI) was used to analyze the severity of depressive symptoms and SCFAs was assessed by gas chromatography. Results have shown negative correlations between acetate, propionate and BDI score ($r = -0.2$, $p = 0.03$; $r = -0.21$, $p = 0.02$, respectively) indicating that metabolic by products of the gut are skewed among depressed people (Skonieczna-Żydecka et al.,2018). These results are consistent with those of another cross-sectional study where it was observed that beneficial bacteria (Taxa Dialister and Coprococcus) were reduced in participants diagnosed with depression. This study included 1054 participants and fecal microbiome was determined through genetic testing (16sRNA) (Valles-Colomer et al.,2019). Moreover, a double-blind clinical trial was done to compare the effect of supplementation with the probiotic and prebiotic on depression using BDI score as a primary outcome. Eighty-one participants with a mean age of 36.5 ± 8.03 completed the trial. After 8 weeks, probiotic supplementation resulted in a significant decrease in BDI score (17.39-9.1)

compared to the placebo (18.18-15.55) and prebiotic (19.72-14.14) supplementation ($p = 0.042$).This indicates that probiotic supplementation resulted in improvement in BDI scores (Kazemi et al.,2018)

Additionally, a recent systematic review that included 26 studies showed that participants with depression or anxiety had a higher abundance of proinflammatory species (e.g., Enterobacteriaceae), and lower SCFA producing-bacteria (e.g., Faecalibacterium) (Simpson et al,2021). Another systematic review and meta-analysis of 16 studies (10 observational and six interventional trials) examined gut microbiota in patients with major depressive disorder (MDD) (Sanada et al, 2020). It has been shown that in observational studies, significant reductions in beneficial bacteria were observed in patients with MDD compared to non-depressed controls (Sanada et al,2020). In the interventional studies with probiotics, a significant improvement in gut diversity was found in depressed participants compared to controls (SMD = -1.62, 95% CI = -2.73 to -0.51, $p< 0.01$) (Sanada et al,2020). Thus, this shows that there is a linkage of gut dysbiosis to anxiety and depression.

1.6: Gut health as a Modulator of the association between MeDi and depression and anxiety

The host's health and mental health (including depression and anxiety) has been shown to be influenced by the gut microbiota composition (Hills et al,2019). One of the most important factors influencing the gut microbial community and its metabolic processes is the host's diet (Del et al, 2014). For instance, low consumption of fibers can lead to changes in the gut microbiome that are associated with low production of beneficial microbial metabolites, such as SCFAs that are involved in the modulation of host immune and inflammatory health status (Merra et al,2020). Epidemiological studies have found that the 'Western-style' dietary habits that are characterized by low consumption of nutritious foods and high consumption of simple sugars, saturated fat, red meat, and processed foods attribute to several chronic diseases and gut microbiota dysbiosis (Merra et al,2020). On the other hand, it has been observed that MeDi has been associated with beneficial changes in gut microbiome composition (Mitsou et al,2017). This role of gut health on anxiety and depression might be attributed to the effect of the Mediterranean Diet (MeDi) on gut health (Ravinder et al.,2019). To the best of our knowledge, the relationship between the LMD, gut microbiota, and mental health, particularly depression and anxiety, remains to be examined among Lebanese adults.

Chapter 2

Aims and Hypothesis

Several studies have observed the beneficial effect of adherence to MeDi on depression and anxiety (Shafiei et al,2019, Fraga et al., 2021, Ventriglio et al, 2020). Adherence to MeDi also appeared to have significant beneficial effects on the gut microbiome composition and function (Merra et al, 2020). Furthermore, recent studies have shown that gut health also plays a protective role against anxiety and depression (Lach et al,2018). This role of gut health on anxiety and depression might be attributed to the effect of the Mediterranean Diet (MeDi) on gut health (Ravinder et al.,2019).

In Lebanon, it has been indicated that more than half of the Lebanese population had depressive symptoms (59.7%) and anxiety (β = .457) (Obeid et al,2020). Additionally, Nasreddine et al. (2019) reported that there is a shift towards a Westernized diet. Moreover, Lebanese adults are adopting a Westernized dietary pattern (Jomaa et al. (2016). Therefore, the effect of this shift on mental health is still missing among Lebanese adults.

Furthermore, MeDi is culturally sensitive, and its results cannot be translated to all countries since its characteristics are quite variable due to several factors (traditions, religion, etc.…) (Lăcătușu et al ,2019, Naja et al.,2015). Thus, researchers have created different scoring methods to assess the adherence of a population to the MeDi (Lăcătușu et al,2019). As for Lebanon, an index for assessing adherence to the MeDi was developed from the Traditional Lebanese dietary pattern by Naja et al (Naja et al, 2015). This index is called the Lebanese Mediterranean

Diet index (LMD). Based on the above data, adherence to the LMD among Lebanese adults is questionable.

To the best of our knowledge, the relationship between the LMD, gut microbiota, and mental health, particularly depression and anxiety, remains to be examined among Lebanese adults.

Hence, our aim in this study is to:

- Explore the potential associations of adherence to the LMD with gut microbiota characteristics and gastrointestinal symptomatology in an adult population.
- Explore the potential associations of adherence to the LMD with depression and anxiety among the adult population.
- Determine the association between gastrointestinal health, depression, and anxiety among Lebanese adults.

Thus, we hypothesize that:

Higher adherence to the LMD decreases the overall gastrointestinal disturbances and is associated with a lower prevalence of depression and anxiety through the beneficial effects of MeDi on gut health.

Chapter 3

Methodology

3.1: Study Design

This is a pilot cross-sectional study that was conducted among Lebanese men and women living in different Lebanese governorates, including Beirut, South Lebanon, and Mount Lebanon. Participants received an online invite to our study that was sent on social media platforms (WhatsApp and Instagram). The recruitment happened as well by word of mouth. We aimed to collect data through a survey that we put together aiming to evaluate its feasibility for future bigger studies of that kind. The study was approved by the Institutional Review Board at the Lebanese American University (IRB #: LAU.SAS.BR1.12/Sep/2021).

3.2: Study Participants

We initially anticipated a sample size of 196 participants. This number was calculated using the "A- priori Sample Size Calculator for Multiple regression" with a medium level anticipated effect size (f2) of 0.15, a statistical power level of 0.8, 13 predictors and a probability level of 0.05. This specification resulted in a total sample size of 131. The missing data is accounted for by inflating the sample size by 1.2 and the non-response is accounted for by inflating the sample size by 1.25, leaving a final sample size of 196 participants.

An invitation to participate in our study was shared on social media platforms allowing adults all over Lebanon to participate. Participants that were eligible to participate in this study were 45-65 years old, English or Arabic literate, residing in Lebanon, and digital Literate (to be able to fill the survey online). Participants were

excluded from the study if they had previous bariatric surgeries, chronic kidney disease (CKD), or were currently diagnosed cancer patients undergoing chemotherapy.

Participants who were sent an online invite to our study through different social media platforms (WhatsApp and Instagram), in which information on dietary intake, depression, anxiety, and gut health was collected. Initially, participants were provided with an informed consent that included the purpose and benefits of the study and ensures anonymity. Participants were asked to read the informed consent carefully and had access to the online survey after their agreement.

The sample reached was 75 Lebanese adults noting that due to the covid 19 pandemic we were hindered from having direct contact with our participants. The survey was challenging if filled online and might have been much more efficient if done during live interviews.

3.3: Dietary Assessment

Dietary intake was assessed by a 61-item semi-quantitative FFQ derived from previous studies conducted among the Lebanese population. The validity and clarity of this FFQ have been previously tested (Naja et al., 2015). The FFQ consists of 61 items for the commonly consumed foods in Lebanese households. The food items are divided into groups of breads and cereals, dairy products, fruits and juices, vegetables, meat and alternates, fats and oil, sweets and desserts, beverages and miscellaneous (such as manaeesh, falafel etc…). Subjects were asked to indicate the frequency of consumption (per day, per week, per month, per year, or never) with the standard portion size (1cup, 1 piece, 1 teaspoon…) over the past year. The subjects

were asked to report the portion size in terms of common household measurements (teaspoons, tablespoons, and cups), with the help of a validated two-dimensional (2D) food portion visual chart. This visual aid was validated on adults aged 20 to 70+years which suits our population (Posner et al., 1992). Participants were provided pictures of common kitchenware found in every Lebanese household to determine the exact portion size consumed. The FFQ was self-administered in an online format.

3.4: Lebanese Mediterranean Diet Score

To calculate the Lebanese MeDi score, nine characteristic foods of the Lebanese traditional pattern, constituted the index: "fruits, vegetables, legumes, olive oil, burghol (crushed whole wheat), milk and dairy products, starchy vegetables, dried fruits and eggs (Naja et al., 2014). Fruits group included citrus oranges/grapefruit, deep yellow or orange fruits, strawberry, grapes, banana, apple, and fresh fruit juice. Vegetables group included salads/greens (lettuce, celery, green pepper, cucumbers), dark green or deep yellow vegetables (spinach, hindbeh, carrots…), tomatoes, squash/ eggplant, and cauliflower/ cabbage/ broccoli. Starchy vegetables included corn/ green peas, and potato (baked, boiled, and mashed). Milk and dairy products group included low-fat milk, whole-fat milk, fat-free/ low-fat yogurt, whole-fat yogurt, cheese regular, cheese low-fat, labneh. All other food components of the LMD index were entered as individual food items. The calculation of the LMD score was based on the number of portions of these nine foods/food groups consumed weekly Specifically, consumption data of each of those food groups was divided into tertiles and a value of 1, 2, and 3 was assigned to the first, second and third tertiles of consumption, respectively. The Lebanese pattern

score was then calculated, for each subject, as the sum of points received on the consumption of the nine foods/food groups. The LMD score was categorized into two categories: low adherence if scoring less than 18 and high adherence if scoring above 18 based on the literature (Naja et al,2014).

3.5: Depression

Depression was assessed using the PHQ-9 that is a self-administered 9-item questionnaire developed by Kroenke et al. in 2001. It is a reliable and valid tool to measure depression severity and is part of the full Patient Health Questionnaire (PHQ) (McEwen et al., 2018). This diagnostic instrument also has a high sensitivity and specificity (Kroenke et al., 2001) and is validated in Arabic (Sawaya et al, 2016). It is composed of 9 items each presenting one possible disturbance or problem that a person might have faced over the last 2 weeks. The participants are asked on a scale from “not at all” to “nearly every day”, respectively scored as 0 and 3, how often they have been bothered by the 9 suggested problems. One of the items for example is “Little interest or pleasure in doing things”. The total scores range from 0 to 27, with scores less than 5 indicating the absence of a depressive disorder; scores of 5 through 9 a mild depression; scores of 10 through 14 a moderate depression; and scores of 15 or more reflect major depression (Kroenke et al, 2001). We regrouped our sample in two categories for the analysis, those scoring <5 have no or minimal depression and those scoring >5 have a mild to severe depression in order to differentiate between those with and those without depression.

3.6: Anxiety

Anxiety was assessed using the GAD-7, which is a 7 items tools with a strong criterion validity and reliability used to screen for Generalized Anxiety Disorder (GAD), and assess its severity (Spitzer et al., 2006). In this study, the validated Arabic version was used (Sawaya et al, 2016). The questionnaire, like the PHQ-9, asks the participants to report how frequently in the past 2 weeks they were bothered by the 7 anxiety symptoms suggested. The symptoms are chosen in accordance with the criteria mentioned in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). The participants are asked to answer either "not at all," "several days," "more than half the days," or "nearly every day," scored respectively as 0, 1, 2, and 3. The total score ranges between 0 and 21, and a score of 5 or higher would indicate a mild to severe GAD. Therefore, we classified our sample into two categories: minimal GAD if they scored lower than 5, and mild to severe GAD if they scored 5 or higher.

3.7 Assessment of Gut Health:

Gastrointestinal health of the Lebanese adults was evaluated using 9 questions from the validated Structured Assessment of Gastrointestinal Symptom (SAGIS) by Koloski. NA, et al. (2017). This questionnaire was used as a proxy for gut health since fecal testing was not available. A previous study had used this questionnaire as a proxy for gut dysbiosis (Sundaram et al,2022).

The SAGIS questionnaire supports the clinical assessment and symptom-based categorization of patients with different gastrointestinal symptoms. It includes 22 gastrointestinal symptoms scored on a five-point Likert scale from no problem, mild (can be ignored when you do not think about it), moderate (cannot be ignored but

does not influence daily activities), severe (influencing your concentration on daily activities), and very severe (markedly influences your daily activities &/or requires rest) problem. In addition, the SAGIS asks about the presence of selected non-gastrointestinal symptoms including depression and anxiety.

After that symptom domain score was calculated as the following (Koloski. et al, 2017):

Diarrhea and Discomfort scores: Q8 (pain defecation), Q11 (loose stool), Q12 (incontinence), Q13 (urgency), Q14 (diarrhea), and Q21 (gas) were summed together. The diarrhea score can range from 0 to 20. Higher scores will indicate higher severity of the disease.

Constipation score: Q9 (difficult defecation) and Q10 (constipation) are summed together. The constipation score can range from 0 to 8. Higher scores will indicate higher severity of the disease.

We added the bloating score where we extracted it using Q20 (feeling of swelling of the abdomen and excessive gas in the abdomen). The bloating score can range from 0 to 4. Higher scores will indicate higher severity of the disease.

Finally, we added the scores of the above domains and after that calculated the mean based on previous studies (Sameul et al, 2021, Koloski et al, 2017). Lastly, gut health was categorized into two domains: below mean ≤4, above mean >4. Scores above the mean indicated greater severity of symptoms.

3.8: Confounding Variables

Moreover, general questions were asked to collect additional needed information and due to the small sample size we regrouped the answers as follows:

gender (female/male), age (in years), marital status (single and windowed; married), level of education (intermediate school or below or 9th grade or high school graduate or with a diploma or the equivalent; trade/technical/vocational training, and bachelor's degree or a higher university degree), and average monthly income (Answered with a specific number; I don't have an income; I prefer not to say). The participants were also asked to report their weight and height; accordingly, we calculated the Body Mass Index (BMI) and used the recognized cut-off values to categorize the participants as underweight, normal weight, overweight or obese (WHO, 2010). Participants were also asked to report the presence of chronic diseases (Yes/No). We also asked for the intake of medications and or antibiotics (Yes/No), supplements (Never/Daily/Weekly/Monthly), and probiotics (Never, Yes, Yes after taking antibiotics). Physical activity was assessed using the validated International Physical Activity Questionnaire (IPAQ) (Lee et al., 2011).

Chapter 4

Statistical Analysis

Descriptive statistics were assigned by mean and standard deviations for continuous variables and percentage frequencies for categorical variables. The independent variable is the adherence to LMD, and the dependent variables are gut health, anxiety, and depression. Independent student t-test and Chi-square test were used to compare continuous and categorical variables respectively. Moreover, logistic regressions were performed to assess the relationship between adherence to LMD and gut health, depression, and anxiety while adjusting for the confounding variables. The level of significance was set as $p<0.05$. Model 1 was adjusted for gender, age, gender, and education, and model 2 for age, gender, education, BMI, medication, and physical activity. These variables were chosen based on clinical significance. Statistical analysis was carried out using STATA version 13.

Chapter 5

Results

5.1: Final Sample

Initially, we approached 75 participate in our study. 6 participants were excluded from the analyses since they had missing data. An additional participant was excluded because they did not fit the eligibility criteria as their age (25) was outside of our specified range. Therefore, the final sample included 68 participants.

Among the 68 participants, 48 (70.59%) were females and the mean age of the whole sample of 55.15 ± 5.5 years. Almost all participants were married (n= 56, 82.35%) and had reached a trade/ technical/ vocational training/ Bachelor's degree or a higher university degree (n=40, 60.29%). Half of the participants had mild to severe depression and mild to severe anxiety (n= 31, 50.82%; (n=39, 60.94%, respectively). Lastly, most of the participants had had low LMD adherence (n= 41, 75.93%) while one third only (n=21, 33.87%) scored above the mean of gut health.

5.2 Socio Demographic Characteristics stratified by gut health, depression, and anxiety

Table 1 shows the socio-demographic characteristics of the participants stratified by gut health status, depression, and anxiety. Although not significant, but in comparison to those scoring above the mean of gut health, those scoring below the mean were more likely to be females (63.41%), had reached a trade/ technical/ vocational training/ Bachelor's degree or a higher university degree (60.98%) and were married (80.49%). Additionally, most of the participants who had mild to severe depression and anxiety were also females (64.52% and 64.10% respectively) and

married (80.65% and 79.49% respectively). However, this difference was not significant between the groups. It has been also observed that almost half of depressed participants had reached intermediate school or below/9th grade/High school graduate, diploma or the equivalent (51.61%) but more than half of the participants with mild to severe anxiety had reached a trade/ technical/ vocational training/ Bachelor's degree or a higher university degree (58.97%). Still, this difference was also not significant between the groups.

5.3: Clinical and Dietary Characteristics

Table 2 shows the clinical and dietary characteristics of the participants stratified by gut health status, depression, and anxiety. The mean BMI of the participants is 25.72±4.38. Almost half of the participants (46.27%) have a normal BMI (18.5-24.9 kg/m2) followed by those who are overweight (34.33%). Approximately half of the participants (57.89%) reported having chronic diseases while more than half (65.67%) took medications and/or antibiotics in the last 6 months and almost all participants did not take probiotics (92.54%)On the contrary, 35.29% reported never taking supplements When it comes to smoking status and physical activity, more than half of the participants (64.18%) were nonsmokers ,and a little bit more than half reported moderate physical activity (51.47%). It has been shown that 80.65% of depressed participants were taking medications and/or antibiotics in the last 6 months ($p<0.01$). The results have also shown that 75.93 % of the participants in this sample had low adherence to the LMD. 91.67% of participants with low to minimal depression had lower adherence to LMD ($p<0.02$). Moreover, 73.68% of those scoring above the mean of gut health had mild-severe depression ($p=0.02$).

Table 1 Socio-Demographic characteristics of the study participants stratified by gut health, depression, and anxiety status.

Covariates	Whole Sample N=68	Males	Females	P-value [c]	Below mean of gut health N=41	Above mean of gut health N=21	p-value [c]	No-Minimal Depression N=31	Mild-Severe Depression N=30	p value [c]	Minimal Anxiety N=25	Mild-Severe Anxiety N=39	p value [c]
Gender N (%) [b]							0.16			0.46			0.32
Male	20 (29.41)				15 (36.59)	4 (19.05)		8 (26.67)	11 (35.48)		6 (24)	14 (35.90)	
Female	48 (70.59)				26 (63.41)	17 (80.95)		22 (73.33)	20 (64.52)		19 (76)	25 (64.10)	
Age (mean ±SD) [a]	55.15 ± 5.5	56.94 ± 6.02	54.43 ± 5.16	0.09	55.25 ± 5.57	54.53 ± 5.41	0.63	55.16 ± 5.31	55.16 ± 6.00	0.83	55.68 ± 6.14	54.63 ± 5.30	0.47
What is your highest achieved education? N (%) [b]				0.97			0.77			0.09			0.94
Intermediate school or below/9th grade/High school graduate, diploma or the equivalent	27 (39.71)	8(40)	19(39.58)		16 (39.02)	9 (42.86)		9 (30)	16 (51.61)		10 (40)	16 (41.03)	
Trade/ technical/ vocational training/ Bachelor's degree or a higher university degree	40 (60.29)	12(60.00)	29(60.42)		25 (60.98)	12 (57.14)		21 (70)	15 (48.39)		15 (60)	23 (58.97)	
What is your marital status? N (%) [b]				0.74			0.97			0.79			0.65

Single /Widowed	12 (17.65)	4(20.00)	8(16.67)		8 (19.51)	4 (19.05)		5 (16.67)	6 (19.35)		4 (16)	8 (20.51)	
Married	56 (82.35)	16(80.00)	40(83.33)		33 (80.49)	17 (80.95)		25 (83.33)	25 (80.65)		21 (84)	31 (79.49)	

* All data are presented as n (%) except for BMI where mean (±SD) was presented. Differences between the groups were tested by: [a] independent t test; [b] Chi-square, and [c] significance level at <0.05.

Table 2 Clinical and Dietary Characteristics of the study participants stratified by gut health, depression, and anxiety status.

Covariates	Whole Sample N=68	Males	Females	P value [c]	Below mean of gut health N=41	Above mean of gut health N=21	p-value [c]	No-Minimal Depression N=31	Mild-Severe Depression N=30	p value [c]	Minimal Anxiety N=25	Mild-Severe Anxiety N=39	p value [c]
Do you have any chronic diseases? N (%) [b]				0.45			0.1			0.73			0.75
No	24 (42.11)	8(50.00)	16(39.02)		15 (48.39)	5 (25)		9 (36)	11 (40.74)		7 (35)	13 (39.39)	
Yes	33 (57.89)	8(50.00)	25(60.98)		16 (51.61)	15 (75)		16 (64)	16 (59.26)		13 (65)	20 (60.61)	
BMI (mean ±SD) [a]	25.72 ± 4.38	26.35 ±3.5	25.45 ±4.71	0.44	25.33 ± 3.86	26.49 ± 4.88	0.31	24.75 ± 4.18	26.75 ± 4.34	0.07	25.06 ± 4.38	26.18 ± 4.32	0.32
BMI N (%) [b]				0.68			0.71			0.44			0.61
Normal	31 (46.27)	8 (40)	23(48.94)		20(48.78)	8(38.10)		16(53.33)	12(38.71)		12(48)	17(43.59)	
Underweight	1 (1.49)	0 (0)	1(2.13)		1(2.44)	0(0)		1(3.33)	0(0)		1(4)	0(0)	
Overweight	23 (34.33)	7(35)	16(34.04)		13(31.71)	9(42.86)		9(30)	13(41.94)		8(32)	15(38.46)	

Obese	12 (17.91)	5(25)	7(14.89)		7(17.07)	4(19.05)		4(13.33)	6(19.35)		4(16)	7(17.95)	
Do you take any medication(s)?/ Have you taken antibiotics, in the last 6 months? N (%) [b]				0.94			0.75			**0.01**			0.13
No	23 (34.33)	7(35.00)	16(34.04)		15 (37.50)	7 (33.33)		15 (50)	6 (19.35)		11 (44)	10 (25.64)	
Yes	44 (65.67)	13(65.00)	31(65.96)		25 (62.50)	14 (66.67)		15 (50)	25 (80.65)		14 (56)	29 (74.36)	
How often do you take supplements (Fish oil, Iron, Vitamin D, Vitamin C…)? N (%) [b]				0.37			0.49			0.69			0.99
Never	24 (35.29)	10(50.00)	14(29.17)		14 (34.15)	10 (47.62)		10 (33.33)	14 (45.16)		10 (40)	14 (35.90)	
Daily	23 (33.82)	6(30.00)	17(35.42)		12 (29.27)	7 (33.33)		10 (33.33)	10 (32.26)		8 (32)	13 (33.33)	
Weekly	13 (19.12)	210.00)	11(22.92)		10 (24.39)	2 (9.52)		7 (23.22)	4 (12.9)		4 (16)	7 (17.95)	

Monthly	8 (11.76)	2(10.00)	6(12.50)		5 (12.20)	2 (9.52)		3 (10)	3 (9.68)		3 (12)	5 (12.82)	
How often do you take probiotics (ProbioLife...)? N (%) [b]				0.65			0.58			0.51			0.69
Never	62 (92.54)	18(94.74)	44(91.67)		36 (90)	20 (95.24)		27 (93.10)	29 (93.55)		24 (96)	35 (92.11)	
Yes	3 (4.48)	1(5.26)	2(4.17)		2(5)	1 (4.76)		1 (3.45)	2 (6.45)		1 (4)	2 (5.26)	
Yes, after taking antibiotics	2 (2.99)	0(0)	2(4.17)		2(5)	0 (0)		1 (3.45)	0 (0)		0 (0)	1 (2.63)	
Do you smoke cigarettes/Narghileh? N (%) [b]				0.31			0.9			0.53			0.34
Never Smoked	43 (64.18)	11(55.00)	32(68.09)		26 (65)	14 (66.67)		20 (68.97)	19 (61.29)		17 (70.83)	23 (58.97)	
Smoked either one or both	24 (35.82)	9(45.00)	15(31.91)		14 (35)	7 (33.33)		9 (31.03)	12 (38.71)		7 (29.17)	16 (41.03)	
Physical Activity N (%) [b]				0.91			0.59			0.35			0.95
Low	30 (44.12)	8(40.00)	22(45.83)		16 (39.02)	11 (52.38)		11 (36.67)	17 (54.84)		11 (44)	17 (43.59)	

Moderate	35 (51.47)	11(55.00)	24(50.00)		23 (56.10)	9 (42.86)		18 (60)	13 (41.94)		13 (52)	21 (53.85)	
High	3 (4.41)	1(5.00)	2(4.17)		2 (4.88)	1 (4.76)		1 (3.33)	1 (3.23)		1 (4)	1 (2.56)	
LMD Adherence (mean ±SD) [a]	16.19 ± 3.70	14.82±3. 67	16.81±3. 55	0.07	15.85±3. 92	16.93±3. 64	0.35	15.16±3. 47	16.92±3. 49	0.08	16.90±4. 01	15.71±3. 57	0.36
LMD Adherence N (%) [b]				0.15 2			0.05			**0.02**			0.62
Low Adherence	41 (75.93)	15(88.24)	26(70.27)		28 (82.35)	9 (56.25)		22 (91.67)	16 (64)		15 (71.43)	24 (77.42)	
High Adherence	13 (24.07)	2(11.76)	11(29.73)		6 (17.65)	7 (43.75)		2 (8.33)	9 (36)		6 (28.57)	7 (22.58)	
Depression N (%) [b]				0.46			**0.02**						0.05
No-Minimal Depression	30 (49.18)	8(26.67)	22(73.33)		23 (60.53)	5 (26.32)					17 (73.91)	12 (32.43)	
Mild-Severe Depression	31 (50.82)	11(35.48)	20(64.52)		15 (39.47)	14 (73.68)					6 (29.09)	25 (67.57)	
Anxiety N (%) [b]				0.32			0.11			**0.000 1**			
Minimal Anxiety	25 (39.06)	6(24.00)	19(76)		18 (46.15)	6 (28.57)		17 (58.62)	6 (19.35)				
Mild-Severe Anxiety	39 (60.94)	14(35.90)	25(64.10)		21 (53.85)	15 (71.43)		12 (41.38)	25 (80.65)				
Gut Health N (%) [b]				0.15						**0.02**			0.19

Below Mean	41 (66.13)	15(78.95)	26(60.47)					23 (82.14)	15 (51.72)		18 (75)	21 (58.33)	
Above Mean	21 (33.87)	4(21.05)	17(39.53)					5 (17.86)	14 (48.28)		6 (25)	15 (41.67)	

* All data are presented as n (%) except for BMI where mean (±SD) was presented. Differences between the groups were tested by: [a] independent t test; [b] Chi-square, and [c] significance level at <0.05.

5.4: Intake for each LMD component

Intake for each LMD component is presented in Table 3. To start with, the intake of bulgur in our sample is very low (0.5 servings per week) compared to the proposed recommendations of the Lebanese Cedar Food Guide (½ cup cooked burghul). Our participants had also low intake of fruits (1 serving per day) but good intake of vegetables (2.5 servings per day) where recommendations for fruits and vegetables are 2 and 2-3 servings per day respectively. As for the dairy group it is recommend having 3 servings per day and our participants were taking almost 2 servings per day. Additionally, for olive it is recommended by Food and Drug Administration (FDA) to have 1½ - 2 tablespoons per day and our participants were having lower than this (< 1 serving per day).

Table 3 Intake for each component of LMD of our study participants

Food group	Median	Mean ±SD	Minimum-Maximum
Fruits	9.37	15.54±14.45	1.625 – 73.5
Vegetables	18.3	22.69±19.02	2.5-74
Starchy Vegetables	3.5	4.7±6.36	0.25-49
Dried Fruits	0.5	2.45±3.68	0.125-17.5
Legumes	1	1.98±1.69	0.125-7
Milk and Dairy	14.125	19.01±18.50	0.875-54.75
Eggs	1	2.49±3.25	0.125-17.5
Bulgur	0.5	1.49±2.59	0.125-17.5
Olive Oil	5.5	8.21±8.28	0.125-31.5

All intakes are represented as number of servings/weeks

5.5: Correlation between LMD gut health, depression, and anxiety

The association between adherence to the LMD and the prevalence of depression, anxiety and gut health problems was assessed using logistic regressions, The results are presented in **Table 4.** Compared to those with the lowest adherence, participants with high adherence to LMD were shown to have a higher prevalence of depression when adjusted for age, gender, and education (OR=8.67, 95% CI: 1.44-53.21, P=0.018). When additionally adjusted for BMI, medications, and physical activity in model 2, the association between LMD adherence and depression remained significant with those in the high adherence having 8 times more prevalent depression (OR=8.16, 95%CI:1.16-57.28, P=0.035). Furthermore, adherence to LMD was not associated with prevalence of anxiety and gut health problems after adjustment for age, gender and education (model 1: OR=0.89, 95%CI:0.23-3.4, p=0.86 and OR=3.84, 95 CI%: 0.89-16.68, p= 0.072 respectively) and further adjustment for BMI, number of medication and physical activity level (model 2: OR=0.79, 95%CI:0.19-3.40, p=0.757 and OR=3.86, 96CI% 0.78-18.96, p=0.097 respectively).

Table 4 - Correlation between LMD adherence and depression, anxiety and gut health among Lebanese Adults.

Depression							
LMD adherence		**Crude Model OR (95%CI)**	**P**	**MODEL 1 OR (95% CI)**	**p**	**MODEL 2 OR (95% CI)**	**p**
	n depressed/ Total						
LOW ADHERENCE	16/38	*ref*		*ref*		*ref*	
HIGH ADHERANCE	9/11	6.19(1.17-32.61)	**0.032**	8.67(1.44-53.21)	**0.018**	8.16(1.16-57.28)	**0.035**
Anxiety							
	n anxiety /Total	**Crude Model OR (95%CI)**	**P**	**MODEL 1 OR (95% CI)**	**p**	**MODEL 2 OR (95% CI)**	**p**
LOW ADHERENCE	24/39	*ref*		*ref*		*ref*	
HIGH ADHERENCE	7/13	0.73(0.21-2.59)	0.625	0.89(0.23-3.4)	0.866	0.79(0.19-3.40)	0.757
Gut health							
	n above mean of gut health/Total	**Crude Model OR (95%CI)**	**P**	**MODEL 1 OR (95% CI)**	**p**	**MODEL 2 OR (95% CI)**	**p**
LOW ADHERENCE	9/37	*ref*		*ref*		*ref*	
HIGH ADHERENCE	7/13	3.63(0.96-13.64)	0.06	3.84(0.89-16.68)	0.072	3.86(0.78-18.96)	0.097

OR: odds ratio, CI confidence intervals; Ref: Reference; n depression: number of participants with mild-severe depression; n anxiety: number of participants with mild-severe anxiety; n above mean of gut health: number of participants with gut health problems Model 1: Adjusted for age, gender, and education Model 2: Adjusted for Model 1+ further adjustments for BMI, medication, and physical activity

5.6: Correlation between gut health depression and anxiety

Table 5 shows the results of the logistic regressions evaluating the association between gut health and depression. When adjusted for age, gender, and education (model 1), scoring more than the mean (>4) was positively associated with depression. These results indicate that those having a higher probability of gut health problems were 4 more times likely to be depressed (OR=4.58, 95%CI: 1.29-16.28, p= 0.018). After further adjusting for BMI, medication, and physical activity in model 2, such as the association between scoring more than the mean (>4) for gut health and prevalence of depression remained significant, with those scoring more than the mean of gut health have 5.64 times more prevalent depression (OR= 5.64,95%CI: 1.23-25.7, p=0.025). However, scoring higher than the mean (>4) of gut health was not associated with anxiety in model 1 (model 1: OR=2.54, 95%CI: 0.78-8.27, p= 0.121) and model 2 (OR= 2.29,95%CI: 0.66-7.93, P=0.188).

Table 5 - Correlation between gut health status with depression and anxiety.

Depression							
Gut Health		**Crude Model OR(95%CI)**	**P**	**MODEL 1 OR (95% CI)**	**P**	**MODEL 2 OR (95% CI)**	**p**
	n depression /Total	**Crude Model OR(95%CI)**	**P**	**MODEL 1 OR (95% CI)**	**P**	**MODEL 2 OR (95% CI)**	**p**
Less than mean ≤4	**15/38**	*ref*		*ref*		*ref*	
Higher than mean>4	**14/19**	4.29(1.28-14.4)	**0.018**	4.58 (1.29-16.28)	**0.018**	5.64(1.23-25.7)	**0.025**
Anxiety							
	n anxiety /Total	**Crude Model OR(95%CI)**	**P**	**MODEL 1 OR (95% CI)**	**P**	**MODEL 2 OR (95% CI)**	**p**
Less than mean ≤4	**21/39**	*ref*		*ref*		*ref*	
Higher than mean>4	**15/21**	2.14(0.68-6.68)	0.189	2.54(0.78-8.27)	0.121	2.29 (0.66-7.93)	0.188

OR: odds ratio, CI confidence intervals; Ref: Reference; n depression: number of participants with mild-severe depression; n anxiety: number of participants with mild-severe anxiety

Model 1: Adjusted for age, gender, and education

Model 2: Adjusted for Model 1+ further adjustments for BMI, medication, and physical activity

Chapter 6

Discussion

In this pilot cross-sectional study, we evaluated the correlation between adherence to LMD, gut health, anxiety, and depression among 68 community dwelling Lebanese participants aged between 45-65 years old. The current study showed that there is a high prevalence of depression and anxiety in our sample. Our results have shown that LMD is positively associated with depression which is against our hypothesis. Furthermore, no significant associations were observed between LMD with anxiety nor gut health. On the other hand, we observed a significant association between gut health and depression indicating that those with higher probability of having gut health problems had a higher prevalence of depression, while no significant association was observed between gut health and anxiety.

The observed high prevalence of depression and anxiety is in line with a previous study that was done among the Lebanese population and has indicated that more than half of the Lebanese population had depressive symptoms (59.7%) and anxiety ($\beta = .457$) (Obeid et al,2020). This high prevalence of depression could be explained with the several tragedies that has been ongoing from the Lebanese civil war that had many negative consequences on mental health, to the constant political and socioeconomical issues (Obeid et al,2020).

Surprisingly, our study observed a result opposing our hypothesis regarding LMD adherence and depression prevalence. We initially hypothesized that higher adherence to LMD will be associated with a lower prevalence of depression. Nevertheless, our results showed that high adherence to LMD was associated with a

higher depression prevalence. Contrary to our results, almost all studies in the literature show that MeDi plays a protective role against depression. A systematic review of a total of 20 longitudinal and 21 cross-sectional studies showed that MeDi confers protection against depression (Lassale et al,2018). These results can be explained by the several tragedies Lebanon has been going through including the economic crisis, the currency devaluation and people losing their life savings, COVID-19 pandemic, and the explosion of the fourth of August in Beirut port. Given the current difficulties in Lebanon, recent data in the country, and the literature on adversity and mental health outcomes of man-made disasters, we speculate the Lebanese population is most likely facing an epidemic of poor mental health regardless of their intake. Diet at this stage might not be protective anymore especially that we have seen that there is a low adherence to MeDi according to Cordahi et al. 2015 and that there is a shift to a western diet according to Nasreddine et al, 2019. This shift also increases the prevalence of depression (Shafiei et al,2019).

Moreover, in the present study, we did not observe a significant association between LMD and anxiety prevalence, contrary to what is shown in other studies. In fact, a recent cross-sectional study aimed to examine the association between adherence to MeDi patterns and the prevalence of psychological disorders among a large population of 3172 Iranian adults aged with a mean age of 36.54 ± 4.97 years. It showed that adherence to the MeDi had lower odds of anxiety (OR: 0.61, 95% CI: 0.42-0.86) compared with those with the lowest adherence (Sadeghi et al, 2021). Another significant relationship was shown in a cross-sectional study that aimed to examine the effects of the adherence levels of adults to MeDi on depression, anxiety, and sleep quality in 1053 Turkish participants. It was found that a 1-point increase in MeDi adherence decreased anxiety scores 3.4 units ($p<0.001$) (Burcu et al, 2021).

Several reasons can explain this absence of significant association in our study. It is possible that no association was found since we had a small sample size, and we were not able to reach the sample size that allows detecting significance. In the literature, the sample size of studies assessing the relationship between anxiety and MeDi had around 1000 - 3000 participants, which is a sample size larger than ours (Burcu et al, 2021, Sadeghi et al,2021). In addition, similar to depression, we speculate that the Lebanese population has a high prevalence of anxiety because of all what is going in the country where they reached a point that diet might not be able to offset the consequences of stress leading to anxiety.

Furthermore, our study has also shown no associations between LMD adherence and gut health. These results do not align with the current evidence. One main reason for the absence of association can be attributed to the small sample size we had compared to that of the studies that observed a relationship having a minimum of 100-600 participants (Ghosh et al,2020, Mitsou et al,2017). This small sample size might not allow us to detect any significance.

Other than the small sample size, several factors might explain the absence of association between LMD and gut health. First, we speculate that there is a low consumption of some food groups of the LMD which are known to be important in improving gut health, such fruits and bulgur. In our sample, it has been shown that there are low consumptions of fruits (9 servings per week) and bulgur (0.5 servings per week) compared to the recommendations proposed by the Lebanese Cedar Food guide of 2 and 0.5 servings per day, respectively. This low consumption might be due to the decreased purchasing power that is exacerbated by the current economic situation in Lebanon. Moreover, food insecurity has been associated with lower adherence to a MeDi among Lebanese population (Naja et al., 2020). Furthermore,

there is a shift towards a Westernized diet, especially among Lebanese older adults (Jomaa et al, 2016). Furthermore, our study has shown that gut health is associated with higher prevalence of depression which is in line with our hypothesis. A cross-sectional study was done in Belgium on 1054 participants to examine the relationship between fecal microbiome via 16S rRNA and general practitioner-reported depression (Valles-Colomer et al.,2019). It showed that Taxa Dialister and Coprococcus (beneficial bacteria) were reduced in subjects with depression (Valles-Colomer et al.,2019). Physiologically, there are several plausible mechanisms on how gut microbiota affects depression (Nagpal et al, 2019). A good microbial diversity helps in the production of beneficial metabolites, neurometabolites, vitamins and SCFAs (Clara et al, 2016). SCFAs can also induce the secretion of γ-aminobutyric acid (GABA), and serotonin (Silva et al, 2020). Many of these molecules have neuro-active functions due to their capacity to modulate neural signaling within the enteric nervous system and consequently influence brain function and host behavior (Appleton, 2018). Furthermore, a good bacterial diversity can reduce metabolic endotoxemia by suppressing the growth of gram-negative bacteria and improving the gut barrier integrity by modulating tight junctions and mucus secretion (Nagpal et al, 2019). These mechanisms can help in reducing systemic inflammation, which is the hallmark of many conditions including depression and anxiety in the human body (Clara et al, 2016).

Additionally, no association was found between gut health and anxiety. This lack of association was also found in a cross-sectional study where no association was found between anxiety and microbiota diversity. However, they mentioned that the lack of association might be related to their small sample size (91 participants) where other studies have a minimum of 1000 participants (Kleiman et al,2017). Unfortunately,

due to this small sample size, we dichotomized our continuous data which reduces the statistical power to detect a relation between the variable and outcome. Additionally, our sample did not have a broader variability in gut health problems which might also affected those results.

Since previous studies have shown that MeDi plays a role on gut health and gut health impacts mental health independently, we speculated that LMD would influence mental health via its effect on gut health. Nevertheless, we were not able to prove this hypothesis since LMD was not associated with mental health nor associated with gut health. Therefore, the effects of gut health on depression might be independent of the effect of MeDi on depression. The discrepancy happening between LMD and depression and gut health and depression can be explained by the bidirectional relationship between the psychological stressors (depression) and gut (Carabotti et al.,2015). Not only the gut affects depression, as previously mentioned, but also being depressed can affect our gut health (Appleton et al.,2018). Psychological stressors can also modulate the gut microbiota (Appleton et al.,2018). These effects may be mediated by the central nervous system and in particular hypothalamic pituitary adrenal (HPA) axis that can be activated in response to stressors. HPA is finalized to cortisol release that is a major stress hormone that affects many human organs (Carabotti et al.,2015). Thus, cortisol targets the muscle layers and gut mucosa, modulating its motility, immunity, and secretion of mucus (Carabotti et al.,2015). This might also affect microbiota composition and function by alteration of intestinal permeability, allowing bacterial antigens to penetrate the epithelium and stimulate an immune response in the mucosa (Appleton et al.,2018).

Future studies with larger sample sizes and fecal genetic testing to assess microbiota are needed to confirm the association between gut health and depression and to evaluate if these effects are due to the role of MeDi on mental health.

Despite our counterintuitive results, our study has several strengths worth mentioning. First, it is the first study done to examine the relationship between the MeDi with gut health, depression, and anxiety among the Lebanese population. Second, we used valid and reliable tools to assess adherence to LMD, depression, and anxiety. Third, the sample was recruited from different regions in Lebanon allowing to take into consideration the different dietary and lifestyle characteristics of each region, therefore making our sample representative of the Lebanese population.

Nonetheless, several limitations of the study should be considered, especially its cross-sectional design, which limits any causal conclusions. Another limitation is due to the political instability and COVID-19 pandemic, we were not able to collect enough data in person, to reach a sample size that allows us to detect significance, thus our small sample size. Additionally, this survey was collected online which has several limitations. People have low internet literacy and we only targeted people who had access to social media, a smart phone and access to internet on their phone. This might have led to reaching a specific population with a certain education level and from certain socioeconomic status, thus limiting the generalizability of our results. Several participants had missing data on the FFQ therefore we speculate that it was a bit long for them to fill online in its current format especially that it was filled on a smart phone on a small screen, without any explanations on how to fill it.

Moreover, we cannot eliminate the social desirability bias leading to a tendency to underreport unhealthy behaviors and dietary intake and the presence of depression or anxiety and over-report healthy dietary habits, thus influencing our findings. Nevertheless, if overreporting of healthy lifestyles was the case, we would have observed a much higher adherence to LMD and a better intake in general. Therefore, two scenarios are present here: either the diet quality of our participants is much worse than what was actually reported, or underreporting was minimal due to the fact that the questionnaire was anonymous therefore minimizing the social desirability bias. In both scenarios, the results indicate that the diet quality of our sample is not healthy and lots of work should be done at the national level in order to increase the awareness about how to eat healthy and its general beneficial effects on preventing chronic diseases. Another limitation is that mental health is still a taboo in Lebanon and remains underreported because Lebanese do not often seek the help of a specialist to diagnose and treat mental symptoms due to religious and cultural norms (Karam et al,2008). This might not only have led to underestimating the depression and anxiety prevalence but also to being unable to reach out to a larger portion of people. People might have discarded our invitation to participate in the study when they knew that we are discussing mental health. Apart from this, we did not have access to fecal sampling and sequencing which is one of the most used tools to assess gut microbiota composition. Thus, we used SAGIS to measure gut health, which still is a good tool that reflects gastrointestinal symptoms but remains a proxy (Koloski et al.,2017). Thus, this might also have affected our results. Since we had a small sample size, we dichotomized our data which might affected the variability in our sample and therefore the significance of our results. Finally, our study is not generalizable since we conducted our study through tough times, where stress levels

were high, lots of people lost their life savings, their jobs, their purchasing power, their children who are leaving the country, and much more. All these factors might have affected our results and skewed our observations. Therefore, our results should be interpreted with caution, and future studies should be designed in different countries with more stable situation to be able to mirror the participants lifestyles.

Chapter 7

Conclusion

To conclude, this pilot cross-sectional study was conducted on Lebanese adults aged between 45-65 years recruited from different regions in Lebanon allowing us to take into consideration the different dietary and lifestyle characteristics of each region and making our sample representative of the Lebanese population. Our results have shown that LMD is positively associated with depression which was counterintuitive while no significant associations were observed between LMD with anxiety nor gut health. These results are also not aligned with the established literature. On the other hand, we observed a significant association between gut health and depression but not with anxiety with poor gut health being associated with a higher prevalence of depression. Furthermore, we speculated that LMD would have an effect on depression and anxiety and this effect would be mediated via the effect of MeDi on gut health. Nevertheless, we were not able to prove this hypothesis since LMD was not associated with mental health nor associated with gut health. These results can be explained by the several tragedies Lebanon has been going through which has negative consequences on mental health. Not to also mention that the Lebanese diet is shifting toward the westernized diet that has several negative effects on health and mental health too.

In fact, future studies should be designed in different countries with more stable situation to be able to mirror the participants lifestyles. Future longitudinal and randomized studies with larger sample sizes and fecal genetic testing are needed to assess microbiota and to confirm the association between gut health and mental health and to evaluate if these effects are due to the role of MeDi on mental health.

Potential preventative strategies and awareness are warranted to improve mental health and wellbeing and to reduce the taboo associated with mental illness. Despite everything, policies should also be targeted toward supporting healthy eating in general and the MeDi in particular, since it is essential to promote this lifestyle again among the Lebanese population.

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