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## Modelling A Low-Fat, Low-Energy, Nutrient-Dense Diet with A Low Dietary Inflammatory Index to Be Used as An Intervention Tool in A Clinical Trial on University Students of Pakistan – A Quasi Experimental Study

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### ABSTRACT

**Objective:** The main objective of the present study was to model a diet based on the fundamental characteristics of a Mediterranean dietary (MD) pattern that encourages a number of healthy foods choices (e.g. olive oil, fruits and vegetables, omega-3 and 6 rich foods, lean meat, low saturated foods etc). the model MD pattern will be used as an intervention in a larger study on university students. **Methods:** The modelling process of the MD pattern and the subsequent nutrient analysis was completed in the following steps: 1) dietary goals were set-up; 2) different menus were designed to achieve these dietary goals; 3) nutritional profiling of the proposed meal menus was done using food composition database of Pakistan as well as of the USD. **Results:** We developed a list of dietary goals. Amounts of all target nutrients were determined including macronutrients (n=17), vitamins (n=14) and minerals (n=14) separately for male and female university students according to their age and general anthropometric characteristics. These nutrients were then translated into different foods in a way to considering MD patterns and menus were developed. The resulting menus have an energy content between 1800-2400 Kcal. The mean DII score is -5.32 showing an anti-inflammatory dietary pattern. **Conclusion:** In conclusion, a diet was modelled based on MD patterns with an overall anti-inflammatory DII score that is expected to positively impact overall physical health as well as stress in the university students.

**Keywords:** university students, nutrition, stress, Mediterranean diet

### INTRODUCTION

Diet is one of the fundamental factors and determinants of a good health (Mialon, 2020). The link between balanced diet and health has been established all over the world. This link is bidirectional, i.e. diet affects health and the physical health also affects the dietary choice of an individual. In addition, there is now substantial evidence supporting the notion that diet is also related to the mental health, particularly, the stress, which has become a public health concern especially in the young individuals with particular note of the university-going students (Merlo et al., 2024).

The typical university student's diet is generally low in foods that are likely to contribute to optimal dietary patterns and perceived as healthier choices, such as vegetables, fruits, and dairy products. It is also higher in fat, sugar, and salt, and high in alcohol, which is perceived as less healthy (Kent et al., 2024). Diet that is high in energy and snacks, is linked to an elevated risk of obesity and a variety of non-communicable diseases, including type 2 diabetes, cardiovascular disease, and certain cancers. Therefore, it may be proposed that the importance of making healthy food choices as opposed to solely satisfying the need to consume sufficient energy increases at higher levels of the hierarchy and is prioritized only after other needs have been met. The fundamental requirement of obtaining an adequate amount of energy is satisfied; however, when confronted with an abundance of less nutritious foods, the act of making healthy choices may be perceived as a necessity for achieving self-fulfillment. Consequently, it becomes a need that is not satisfied until all lower-level needs are met, and it is not a priority. Consequently, university life is a period of vulnerability that is linked to negative health and food behaviors that are associated with weight gain. So, it is crucial to comprehend the factors that influence the eating behaviors of students while they are living away from home, as this knowledge may aid in the development of the most effective health promotion strategies.

The overarching objective of this study was to tailor a diet based on the MD patterns from the local available food ingredients. This diet is expected to play a vital role in reducing the stress level and the related physical health determined by some laboratory indicators (e.g. blood lipid profile, IL-6, sCD14, hsCRP etc). The model diet will be used as an intervention in a larger study on university students (Effects of Nutritional Counselling on the Academic Stress, Inflammatory Biomarkers And Quality Of Life In University Students: A Single Blinded Open Randomized Trial) (Alam et al., 2023).

**Materials & Methods**

A research panel including the PhD student’s Major Supervisor, Co-Supervisor, expert in computer programming, two dietitians and the PhD student herself was constituted. There were continuous meetings held and thorough discussion followed.

The following main activities were accomplished:

- 1) Setting dietary goals for intervention
  - 2) Designing meal menus according to the targeted dietary goals
  - 3) Nutritional profiling and Analysis of the resulting menu and comparison with a typical traditional Pakistani menu
- Setting Dietary Goals for Intervention

We conducted the baseline survey to establish two purposes:

- 1) The current nutritional status and nutrients intake of the subjects.
- 2) To assess the desirable nutritional status and calculate nutrients intake of the subjects.

The baseline survey revealed that majority of the subjects were overweight (BMI range 26-28). It was also noted that the energy intake of majority of the subjects were well above the RDA (> RDA in a range of 20-35%), while the intake of most of the vitamins and minerals was below their respective RDA. Therefore, our primary goal was to reduce the additional weight of all of the individuals in the intervention group by reducing their energy intake. This goal was expected to be accomplished by dietary modification.

We designed a dietary pattern based on the Mediterranean dietary (MD) pattern that would help in reducing the energy intake while also normalizing the intake of other essential micronutrients (vitamins and minerals).

Figure 1 shows nutrients intake target for male and female students.

(A)					(B)				
Nutrient	Unit	Target	Minimum	Maximum	Nutrient	Unit	Target	Minimum	Maximum
<b>Macronutrients</b>					<b>Macronutrients</b>				
Energy	kcal	2411	-	-	Energy	kcal	2100	-	-
Carbohydrates (Total)	g	323.4	130	382.2	Carbohydrates (Total)	g	281.7	130	332.9
Dietary Fiber	g	29.4	-	-	Dietary Fiber	g	25.61	-	-
Fat (Total)	g	68.49	54.8	95.89	Fat (Total)	g	59.66	47.73	83.52
N-6, Linoleic Acid	g	5.48	5.48	27.4	N-6, Linoleic Acid	g	4.773	4.773	23.86
N-3, Total (incl. ALA)	g	2.382	1.096	5.48	N-3, Total (incl. ALA)	g	2.075	0.955	4.773
N-3, (EPA & DHA)	g	2.382	0.301	5.48	N-3, (EPA & DHA)	g	2.075	0.263	4.773
Protein	g	117.6	58.8	205.8	Protein	g	102.4	51.22	179.3
Histidine	g	2.117	1.058	-	Histidine	g	1.844	0.922	-
Isoleucine	g	2.94	1.47	-	Isoleucine	g	2.561	1.28	-
Leucine	g	6.469	3.234	-	Leucine	g	5.634	2.817	-
Lysine	g	5.998	2.999	-	Lysine	g	5.224	2.612	-
Methionine & Cysteine	g	2.94	1.47	-	Methionine & Cysteine	g	2.561	1.28	-
Phenylalanine & Tyrosine	g	5.528	2.764	-	Phenylalanine & Tyrosine	g	4.815	2.407	-
Threonine	g	3.175	1.588	-	Threonine	g	2.766	1.383	-
Tryptophan	g	0.823	0.412	-	Tryptophan	g	0.717	0.359	-
Valine	g	3.764	1.882	-	Valine	g	3.278	1.639	-
<b>Vitamins &amp; Nutrients</b>					<b>Vitamins &amp; Nutrients</b>				
Vitamin A	µg	-	900	3000	Vitamin A	µg	-	700	3000
Thiamin (B1)	mg	-	1.2	-	Thiamin (B1)	mg	-	1.1	-
Riboflavin (B2)	mg	-	1.3	-	Riboflavin (B2)	mg	-	1.1	-
Niacin (B3)	mg	-	16	35	Niacin (B3)	mg	-	14	35
Pantothenic acid (B5)	mg	-	5	-	Pantothenic acid (B5)	mg	-	5	-
Vitamin B6	mg	-	1.3	100	Vitamin B6	mg	-	1.3	100
Biotin (B7)	µg	-	30	-	Biotin (B7)	µg	-	30	-
Folate (B9)	µg	-	400	1000	Folate (B9)	µg	-	400	1000
Cyanocobalamin (B12)	µg	-	2.4	-	Cyanocobalamin (B12)	µg	-	2.4	-
Choline	mg	-	550	3500	Choline	mg	-	425	3500
Vitamin C	mg	-	90	2000	Vitamin C	mg	-	75	2000
Vitamin D	IU	-	600	4000	Vitamin D	IU	-	600	4000
Vitamin E	mg	-	15	1000	Vitamin E	mg	-	15	1000
Vitamin K	µg	-	120	-	Vitamin K	µg	-	90	-
<b>Elements &amp; Electrolytes</b>					<b>Elements &amp; Electrolytes</b>				
Calcium	mg	-	1000	2500	Calcium	mg	-	1000	2500
Chromium	mg	-	35	-	Chromium	mg	-	25	-
Copper	mg	-	0.9	10	Copper	mg	-	0.9	10
Fluoride	µg	-	4000	10000	Fluoride	µg	-	3000	10000
Iodine	µg	-	150	1100	Iodine	µg	-	150	1100
Iron	mg	-	8	45	Iron	mg	-	18	45
Magnesium	mg	-	400	-	Magnesium	mg	-	310	-
Manganese	mg	-	2.3	11	Manganese	mg	-	1.8	11
Molybdenum	µg	-	45	2000	Molybdenum	µg	-	45	2000
Phosphorus	mg	-	700	4000	Phosphorus	mg	-	700	4000
Selenium	µg	-	55	400	Selenium	µg	-	55	400
Zinc	mg	-	11	40	Zinc	mg	-	8	40
Potassium	mg	-	4700	-	Potassium	mg	-	4700	-
Sodium	mg	-	1500	2300	Sodium	mg	-	1500	2300

**Figure 1: Target Nutrients for Male (A) and Female (B) University Students.** Amounts of all target nutrients were determined including macronutrients (n=17), vitamins (n=14) and minerals (n=14) separately for male and female university students according to their age and general anthropometric characteristics. An excel-based software, designed especially for this study, was used for these calculations.

**Model of MD Pattern**

After the target nutrient intakes were determined, the subsequent stage was to determine how to obtain these nutrients from the diet. To achieve this objective, it was necessary to develop an intervention diet that closely resembled the MD patterns. The primary objective of the intervention was to facilitate the adaptation of the Mediterranean dietary pattern to the subjects in the intervention group. It is always difficult to implement abrupt and significant changes in subjects who are already adhering to a predetermined dietary regimen. Consequently, we were of the opinion that it would be impossible to completely alter the dietary patterns of our subject. Nevertheless, it was deemed challenging to implement specific dietary modifications that would bring the diet closer to the Mediterranean diet, but it was not entirely unfeasible. Consequently, in collaboration with dietitians and experts, we established specific objectives that were determined to enhance the nutritional profile.

The diet objectives were determined by the varieties of food commodities available in the local market, Pakistani intakes, and local dining behaviors. The fruit and vegetable objective were 7 to 9 servings per day, contingent upon energy intake. The fat intake target was a PUFA: SFA:MUFA ratio of 1:2:5, which was both feasible and reasonably comparable to the Pakistani intakes. The objective was to achieve this fat aim by halving the typical fat intake through the consumption of low-fat foods, and subsequently incorporating olive oil or other high-MUFA foods into the diet to maintain baseline levels of energy and total fat intake. In particular, it was crucial to restrict the consumption of polyunsaturated fatty acids (PUFAs) due to their high levels in eastern cuisines, which significantly influence the desired fat ratio. In order to alter the type of fat ingested, participants were instructed to select "very lean" and "lean" meats from the exchange list and to restrict their overall meat intake. Margarine and other high-PUFA foods were largely eliminated, and the use of commercially prepared foods with polyunsaturated fats was restricted to a minimum. The diet was supplemented with fats from olive oil and high-MUFA plant sources (Table 1).

Foods were classified as "high-MUFA foods" on the exchange list if their polyunsaturated content was 10% of energy or less and their MUFA content was 54% of energy or more (e.g., PUFA: SFA:MUFA ratio of 1:1.6:8.7 for olive oil). The moderate MUFA diets contained up to 23% of energy from PUFA and 18% to 53% of energy from MUFA (a PUFA: SFA:MUFA ratio of 1:0.5:3 for almonds). Lastly, the "MUFA fats with high PUFA" were foods that contained 24% to 30% of energy from PUFA and 31% to 60% of energy from MUFA (a PUFA: SFA: MUFA ratio of 1:0.4:1.6 for peanuts). Participants were instructed to incorporate olive oil into salad dressings, breads, vegetables, and food preparation. At both the baseline and three-month visits, the subjects were administered three liters of extra-virgin olive oil.

By substituting fruits and vegetables for other carbohydrates, the target energy intake was maintained while an increase in fruits and vegetables was achieved. The objective was to consume 7 to 9 servings of fruits and vegetables per day, with the number of servings varying based on energy intake: 7 servings for a daily energy intake of less than 1,700 kcal, 8 servings for a daily energy intake of 1,700 to 2,000 kcal, and 9 servings for a daily energy intake of more than 2,000 kcal (note: 7 servings can weigh approximately 600 g). This was in order to maintain a fruit and vegetable intake that was comparable to the traditional Pakistani diet. Variety was accomplished by employing exchange lists that categorized fruits and vegetables into eight groups, a modification of the approach that we and others had previously employed to increase carotenoid intake. Figure 1, which illustrates serving proportions and an abbreviated exchange list, is shown. Initially, subjects kept food records and enumerated all exchanges daily for about 1 month. After subjects became adept at enumerating exchanges, they were given a fast-track checklist to use daily that only enumerated the high-MUFA fats and fruit/vegetable categories.

### Traditional Pakistani Diet

A menu representative of a typical traditional Pakistani diet was also constituted based on the previous literature. The nutritional profiling of the menu was done using the same procedure as mentioned above for the Model MD pattern. This traditional Pakistani diet was used for the purpose of comparisons as it is expected to be in practice by the participants in the control group in the large proposed study.

**Table 1: Content of Monounsaturated fats in some foods**

Oils	
Olive oil	73.9
Sunflower oil	3.18
High-oleic -acid sunflower oil	83.6
Safflower oil	14.4
High-oleic-acid-safflower oil	74.6
Corn oil	29.3
Soyabean oil	24.3
Canola oil	58.9
Foods	
Peanuts	23.4
Almonds	42.2
Hazelnuts	36.6
Butter	26.8

### Results

A one-day menu of the modelled intervention MD and the typical traditional Pakistani diet (TPD) is illustrated in Table 2. The nutrient composition and DII score of these regimens are presented in Table 4. The anti-inflammatory (-) DII scores of both meal plans were similar; however, the mMD meal plan was significantly more anti-inflammatory than the TPD (-5.23 vs. -0.01, respectively). In terms of the ingestion of DII parameters, the meal arrangements exhibited several significant differences. The MD was higher in the pro-inflammatory nutrient parameter of total fat content, but also higher in numerous anti-inflammatory parameters of the index (MUFA, PUFA, omega-3 and -6, fiber, vitamin A, D and E, flavonoids, garlic, onion, rosemary and thyme/oregano).

A cocktail in the form of coarse powder with added vanilla flavor and containing a number of functional foods was prepared in the Laboratory of Human Nutrition and Dietetics, Bacha Khan University Charsadda. The cocktail contained foods of high nutritional values, e.g. garlic powder, Ginger, black-pepper, Rosemary, Saffron, thyme/Oregano, turmeric, eugenol, mint leaves etc. The cocktail was oven-dried and packed in green plastic bags of 25 gram each. Each student in the intervention group was

instructed to use the cocktail in a variety of ways e.g., sprinkle it on the meals, eat as such with spoon, boil in water or milk etc. The details of the cocktail are available in (Table 3 and Table 4)

**Table 2: Meals of a one-day menu of the modelled intervention MD and the typical traditional diet Pakistani**

	Breakfast	Lunch	Dinner
Traditional Pakistani Diet	1 parata OR naan, egg OR Choley; black tea	1 naan, 1 plate aloo-gosht, 2 pieces of achaar, 2 spoons of chitin,	1 plate of rice with chicken, 1 medium plate of daal-aloo, ½ naan
Mediterranean Diet	1 whole-grain naan, 1.5 cup yogurt, 0.5 cup shelled walnuts 3 tsp honey, 1 cup green seedless grapes, 1 cup herbal tea	1 whole-wheat naan, 1 plate of chicken curry, 150-gram Greek salad (with spinach, lettuce, feta cheese, tomatoes, Onion, cucumber, olives). 1 tbsp olive oil, 1 whole wheat pita, 0.25 cup hummus, 1 small coffee 2 tbsp milk, 1 tbsp vinegar, 20 gram of donated cock-tail* (Garlic powder, Ginger, black-pepper, Rosemary, Saffron, thyme/Oregano, turmeric, eugenol, mint leaves,);1 fruit cup green tea	1 plate rice cooked with green lentils, 1 medium plate curry of mixed vegetables with 2 spoons of olive oil, 150-gram Greek salad (with spinach, lettuce, feta cheese, tomatoes, Onion, cucumber, olives), 20 gram of donated cock-tail* (Garlic powder, Ginger, black-pepper, Rosemary, Saffron, thyme/Oregano, turmeric, eugenol, mint leaves,) 1 cup green tea

\*The donated cock-tail had a composition with foods that are recommended for the anti-inflammatory diet. The cock-tail was donated as an incentive to the students in the test group.

**Table 3: Ingredients and Nutritional Values of Cocktail**

Ingredient	Quantity/Portion	Nutritional Value
Garlic Powder	1-2 grams	High in vitamins C, B6, manganese, and selenium
Ginger	1-2 grams	Contains gingerol, high in vitamins and minerals
Black Pepper	0.5-1 gram	Rich in piperine, enhances nutrient absorption
Rosemary	0.5-1 gram	Contains carnosic acid and rosmarinic acid
Saffron	0.1-0.2 grams	Contains crocin and safranal, rich in antioxidants
Thyme/Oregano	0.5-1 gram	High in vitamins C, A, and K, rich in antioxidants
Turmeric	1-2 grams	Contains curcumin, high in antioxidants
Eugenol	0.1-0.2 grams	Found in cloves, rich in antioxidants
Mint Leaves	1-2 grams	High in vitamin A and antioxidants

**Table 4: Packaging and Usage Instructions**

Packaging	Details	Method
Drying Method	Oven-dried	Sprinkle on meals
Packaging Material	Green plastic bags	Eat as is (1-2 tsp)
Package Size	25 grams per bag	Boil in water (1-2 tsp)
		Boil in milk (1-2 tsp)

**Table 5: Nutrient composition and DII score of a one-day menu of the modelled intervention MD and the typical traditional Pakistani diet**

	Traditional Pakistani Diet	Mediterranean Diet
DII Score*	-0.01	-5.23
Food parameters (units):		
Energy intake (kcal/day)	2013	2021
Total fat (g/d)	102.5	109.24
Total carbohydrate (g/d)	188.0	193.57
Total protein (g/d)	85.0	61.25
Cholesterol (mg/d)	365	105.85
Saturated fat (g/d)	33.2	20.84
Monounsaturated fat (g/d)	35.6	45.74
Polyunsaturated fat (g/d)	25.5	36.75
Trans fatty acid (g/d)	3.70	0.53

Omega 3 fatty acids (g/d)	2.81	6.42
Omega 6 fatty acids (g/d)	25.11	30.29
Total dietary fiber (g/d)	13.01	26.81
Vitamin A (RE/d)	246.1	1097.68
Beta Carotene (mcg/d)	146.9	5780.23
Vitamin D (mcg/d)	2.67	5.45
Vitamin E (AT EQ/d)	11.2	14.92
Vitamin C (mg/d)	35.21	181.64
Thiamin (mg/d)	1.13	1.18
Riboflavin (mg/d)	1.65	1.54
Niacin (mg/d)	23.23	21.39
Vitamin B6 (mg/d)	1.45	1.74
Total folate (mcg/d)	213.87	423.66
Vitamin B12 (mcg/d)	3.90	2.75
Magnesium (mg/d)	191.23	371.62
Iron (mg/d)	8.21	12.4
Zinc (mg/d)	11.23	8.67
Selenium (mcg/d)	103.27	75.56
Caffeine (mg/d)	0.1	0.09
Flavonols (g/d)	3.44	48.75
Flavones (g/d)	0.32	6.37
Isoflavones (g/d)	0.19	0.25
Anthocyanidins (g/d)	0	204.36
Flavonones (g/d)	0.34	9.02
Flavan-3-ol (g/d)	0.19	736.66
Garlic (g/d)	2	5
Ginger (g/d)	0	5
Onion (g/d)	50	50
Pepper (g/d)	0	50
Rosemary (mg/d)	0	20
Saffron (g/d)	0	0.5
Thyme/Oregano (mg/d)	0	5
Turmeric (g/d)	0	10
Eugenol (g/d)	0	10
Green/Black Tea (g/d)	0	25

## Discussion

Pakistanis are suffering from malnutrition (Alam et al., 2019), and university students are no exception. This research study was designed to plan a meal with the characteristics of the famous Mediterranean pattern that is known for its positive health effects (Alam et al., 2023).

The main purpose of this research study was to design a diet based on ingredients from a typical MD pattern. This tailored diet was deemed to be balanced in terms of nutrients. The tailored diet was expected to be feasible in preparation as well as affordability. Energy intake was kept reduced in order to have a room of its increase if needed so. The fruit and vegetable's objective was 7-9 servings per day (Alexander et al., 2010). The fat intake target was a PUFA: SFA:MUFA ratio of 1:2:5. consumption of polyunsaturated fatty acids (PUFAs) was kept minimum (Hussein, 2003). Meat intake was encouraged to be furnished by taking "very lean" and "lean" meats from the exchange list and to restrict their overall meat intake (Li et al., 2005). The diet was supplemented with fats from olive oil and high-MUFA plant sources (Yubero-Serrano et al., 2019). In the actual survey of the study, the RDs would determine exchange goals for each subject at the baseline visit. The objectives are expected to be satisfied the study's criteria while simultaneously respecting the reported caloric and total fat intakes of each participant. A typical diet will contain 30% of energy from fat, 20% of energy from protein, and 50% of energy from carbohydrates (Austin et al., 2011). Participants will also receive 3-7 days of example menus for their own particular caloric and fat intake levels. Based on these targets, we calculated the desirable target nutrients intake of our subjects. These target nutrients intakes were expected to normalize the weight of the subjects as great majority of the subjects were overweight at baseline. In addition, these intakes would ensure the optimum intake of certain nutrients which were found lacking (<45% of RDA) in the diet of

majority of the subjects. One of the main characteristics of the tailored MD pattern is a low level of DII (-5.23), which is particularly expected to help in reducing stress (Abid et al., 2022), and the stress-related inflammation (Alam et al., 2018).

### Conclusion

A diet was modelled based on MD patterns with an overall anti-inflammatory DII score that is expected to positively impact overall physical health as well as stress in the university students.

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### Conflict of interest

All authors declare 'no conflict of interest'. The present work is part of PhD dissertation of Ms. Juweria Abid (the first author) at Bacha Khan University Charsadda, KP, Pakistan

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