Micronutrients and Amino Acids Food Intakes and Their Associations with Obesity, Type 2 Diabetes and Apo B/Apo A1 Ratio in Middle-Aged Algerian Adults

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Objective: The present work investigates the interrelationship of food intakes of micronutrients and amino acids with the apolipoprotein (apo) B / apo A1 ratio as a potential indicator of atherosclerosis risk in patients with obesity and/or type 2 diabetes (T2D). Methods: A cross-sectional comparative study was performed on 285 participants divided into three groups; normal-weight type 2 diabetics, overweight/obese type 2 diabetics and overweight/obese non-diabetics. Anthropometric and blood pressure were measured. Biochemical parameters were evaluated and the apo B/apo A1 ratio was calculated. Food dietary intakes were assessed using a validated 3-days food record. The Association between intakes of micronutrients (vitamins and minerals) and amino acids was studied using Principal Component Analysis (ACP). Results: Significant differences were revealed with respect to age, fasting and postprandial blood glucose, triglycerides and glycated hemoglobin (Hba1c) (p<0.005). In normal-weight diabetics, ACP showed that the highest apo B/apo A1 ratios were positively associated with branched-chain amino acids intake, vitamins E and B6, and calcium, zinc and phosphorus levels. In overweight/obese T2D patients, amino acid intakes were associated with vitamins B3 and B12 and minerals; phosphorus and zinc. However, results in overweight/obese patients without diabetes revealed that amino acid intakes were specifically correlated with vitamin D. Conclusion: Micronutrients and amino acids play a pivotal role in the development of cardiovascular risk in obese and/or diabetics. Branched-chain amino acids, B and D vitamins, as well as calcium and zinc, show considerable associations with higher apo B/apo A1 ratio and therefore with atherosclerosis.

INTRODUCTION

Statistics reveal an alarming worldwide increase in the number of diabetes cases, and a large proportion of patients with type 2 diabetes (T2D) are simultaneously affected, currently, we are talking about a new concept of “diabetes” (Guarisco & Leonetti, 2021). In parallel, recent data have shown that cardiovascular disease is still the leading global cause of death in Western countries despite a steady decrease recorded over the past 10 years (Karupaiah et al., 2019; Casas et al., 2018).

A range of disorders is described by cardiovascular diseases affecting the heart and blood vessels, such as peripheral arterial disease, venous disease, hypertension, stroke and atherosclerosis (Benjamin et al., 2018).
Pathologically, atherosclerosis is closely related to dyslipidemia, this last is a metabolic disorder characterized by a high plasma concentration of total cholesterol (TC), triglycerides (TG) and low-density lipoproteins (LDL) which is the most atherogenic lipoprotein in both fasting and postprandial blood (Ama Moor et al., 2017; Yaseen et al., 2021).

Previous research has firmly established atherosclerosis as a cardiovascular complication induced by lipoproteins. The apolipoprotein B (apo B) represents the major protein constituent of LDL particles. However, apolipoprotein A1 (apo A1) is linked to high-density lipoproteins (HDL) cholesterol. This makes apo B a better predictor of LDL particle number than the LDL cholesterol molecule itself (Talmud et al., 2002).

According to several studies, the apo B/apo A1 ratio is the helpful marker of metabolic risk, especially atherosclerosis risk, in patients with dyslipidemia caused by overall obesity, abdominal obesity, impaired glycaemia and T2D (Yaseen et al., 2021; Diaf (a) et al., 2015; Walldius et al., 2001).

Various atherosclerosis risk factors are to consider, including age, smoking, physical inactivity and co-morbidities. However, nutrition is one of the key factors in the aetiology of the cardiovascular disease.

MATERIALS AND METHODS

1. Population Samples:

The experimental part of the present work was carried out during the year 2018 at the level of three health facilities in the northwestern region of Algeria; the Public Establishment of Local Health Centre (“Maison du Diabétique” of Ex Gambetta and Mostefa Ben Brahim Polyclinic) in Sidi-Bel-Abbes city and Meslem Tayeb Hospital in Mascara city.

After establishing inclusion and exclusion criteria, the simple random sampling technique without replacement was used. Thus, and through a cautious analysis of their medical records, diabetic patients recruited for the study were selected during several investigations (van der et al., 2005; Diaf (b) et al., 2015; Sunkara & Raizner, 2019), although not all (Vergnaud et al., 2007; de Oliveira et al., 2011), have revealed a significant association between dietary micronutrients (vitamins and minerals) and cardiovascular events. Currently, a range of micronutrient supplements is widely used in the prevention of some chronic diseases including atherosclerosis thus proving the role that these micronutrients play in the treatment of cardiovascular complications (de Oliveira et al., 2011).

In addition, and for some years, the role of dietary amino acids intakes such as L-arginine (Arg), L-homoarginine (hArg) and L-tryptophan (Trp) has been widely discussed in the context of atherosclerosis risk, and their roles in vascular homeostasis and immune regulation are generally accepted (Nitz et al., 2019).

Very few nested studies evoking associations of dietary micronutrients and amino acids with markers of inflammation and atherosclerosis are scarce. So, the present work describes the associations between food intakes of micronutrients, in particular vitamins and minerals, and amino acids intake with the apo B / apo A1 ratio as a potential marker of atherosclerosis risk by comparison between three groups of patients according to their corpulence and their diabetic profile.

Periodic consultation sessions with their treating physicians in the three health facilities. Adult subjects meeting the following criteria were solicited: aged 19 to 75 years, with confirmed T2D for less than 15 years, exclusively on oral antidiabetics and not suffering from any complications related to diabetes. However, the excluding criteria were: pregnancy, insulin therapy, patients with hypothyroidism, patients with primary hyperlipidemia and those with renal failure and hepatic dysfunction.

Therefore, and in order to determine the suitable sample size, the following equation was used:

\[ n_0 = \left[ \frac{Z^2 \times P \times (1 - P)}{e^2} \right] \]

(Z) is the Z-score or confidence level (set at
1.96 which represents a risk $\alpha$ of 5% and a confidence interval of 95%).

$P$ is the true percentage value of patients meeting the inclusion and exclusion criteria; $P=0.5$.

(e) is the error margin ($e=0.03$).

Then, the value of $n$ found was employed to determine the sample size as follows:

$$n = n_0 / \left[1 + \left(\frac{n_0}{N}\right)\right].$$

($n_0$) is the sample size (before correction)

(N) is the population size.

(n) is the corrected sample size (final sample).

The approximate number of T2D patients in the study area (Sidi-Bel-Abbes and Mascara cities) was 25,000. About 18% (nearly 4500 patients) were exclusively under oral anti-diabetic agents and met our inclusion and exclusion criteria.

The estimated sample of patients who were supportive to be part of our study was about (n0=240) (type 2 diabetes patients). After correction, via the formula:

$$n = n_0 / \left[1 + \left(\frac{n_0}{N}\right)\right].$$

($n_0$) is the sample size (without correction)

(N) is the population size.

(n) is the sample size corrected (final sample).

The final sample was n=238 T2D patients.

Regarding the group of overweight/obese patients without diabetes, 50 patients with the same including (except T2D) and excluding criteria, with a body mass index (BMI)$\geq25$ kg/m$^2$, were randomly selected. However, 47 patients only completed the study till the end.

2. Anthropometric Measurements:

All measurements of anthropometric dimensions were performed in the morning with strict respect to the World Health Organization (WHO) recommendations (WHO, 2005). Bodyweight (in kilograms) was measured using an electronic balance (TS-2003A: 360 lb, Capacity: 180 Kg, Graduations 0.1Kg) and height (in meters) was measured with a body meter (Seca 206, Germany; Measuring range: 0 - 220 cm, Graduation Length: 1 mm) which measured to the top of the head of a shoeless patient. The BMI was then calculated as follow: BMI (kg/m$^2$) = weight (kg)/height$^2$ (m$^2$). Patients should be lightly dressed and must respect the appropriate position for height measurement (gathered feet, straight body, heels touching the wall and staring out the horizon). Waist circumference was measured respecting every single cm with a measuring tape (Maximum: 150 cm, Graduation Length: 1 mm), the tape is gently tightened around the patient’s abdomen roughly in line with the navel without depressing the skin. Thus, three groups of patients were constructed namely, normal weight T2D patients, overweight/obese T2D subjects and overweight/obese non-diabetic subjects.

3. Blood Pressure Measurement:

The blood pressures (diastolic and systolic) were measured during the morning time for all participants (Usually at nine o’clock AM). Three evaluations were carried out with a time interval of 5 minutes between every two measurements in order to calculate the mean blood pressure value. As an instrument, the Blood Pressure Monitor OMRON M3 (Omron Healthcare, Ltd. Kyoto, Japan) was employed. The machine can determine systolic blood pressures between 30-240 mmHg, diastolic of 10-210 mmHg and heart rate of 40-200 beats per minute.

4. Collection of Nutrients Intake Information:

A three days (2 days per week and a weekend day) food record was used in order to evaluate both quantitative and qualitative food and beverages intakes (including the use of dietary supplements) for each patient. In order to fill in these food diaries correctly, explanatory sessions were organized with patients in sub-groups of 5 to 6 patients. The first instruction was to refrain from the use of vitamin and mineral supplements at least two weeks before the start of the dietary assessment. Then, the instructions were to record and mention all the details concerning food, beverages (including voluntary drinking water) consumed during each day meal or even outside traditional meals (snacking) with the exact quantity, quality (if possible), or commercial brand name, time and circumstance of eating a meal, cooking and processing method, additions while cooking and/or at the table and other details. Standard
units were used to quantify the amount units of consumed food such as tea or coffee spoon, slice (thin or thick), cup, glass (juice glass, water glass), a corner for cheese, bag, bottle, can, packet, middle or normal piece, meatball and standard portion for fruits and cakes.

Regarding patients who are illiterate and/or unable to remember their food consumption, we previously instructed a family member to complete the food file for them. After filling out the three-day food diaries, a verification step via face-to-face interview was carried out with each patient to correct and rectify any oversights or recording errors. All food information was directly computerized and entered on the NutriSurvey software for windows 2007, SEAMEO-TROPMED RCCN-University of Indonesia (NutriSurvey, 2007). This program is based on the German food database (BLS) with English names in addition to other databases (USDA food table, FAO-Minilist worldwide and Egypt food database) and provides information on energy, fat, and carbohydrate, protein, vitamins and minerals.

The main characteristics of the NutriSurvey program are as follows:
- The German food database (BLS) includes 11000 foods with more than 130 nutrients;
- It contains all useful functions which are typical for this kind of software (nutrient analysis and calculation of energy requirements, planning of diets, diet history, food frequency, searching of nutrients in foods, handling of recipes…);
- The possibility to analyze several food records together with a variety of options;
- The nutrient values of the foods in the database can easily be changed and complemented with additional information;
- Recipes can be modified and the nutrient content corrected depending on the preparation of the recipe.

5. Biochemical Blood Parameters:

Blood samples were drawn from each patient 12 h after an overnight fast (for fasting glucose and lipids parameters), 3 to 4 hours after breakfast meal (for postprandial biochemical parameters) and during the day for the non-fasting parameters (glycated haemoglobin ‘HbA1c’, apo A1 and apo B). The evaluation of fasting and postprandial glucose, TC, HDL, TG and direct LDL were performed through direct enzymatic colorimetric methods using “Spinreact-Spain” reagents (Bergmeyer, 1974). HbA1c levels were determined using an ion exchange resin separation method. However, for apo A1 and B, turbidimetric tests (Spinreact-Spain) (Tietz, 1987) were used.

6. Ethical Considerations:

All participants gave their written consent to take part in the study. The study protocol was approved by the scientific council of the faculty of natural and life sciences of Djillali LIABES University and the head of health and Population Department of the Wilaya of Sidi-Bel-Abbes, Algeria (agreement No. 142 dated 13 February 2013).

7. Statistical Methods:

Statistical analysis was processed using SPSS 24.0 (Statistical Package for the Social Sciences, IBM Corporation; Chicago, IL, 2015). Results are expressed as means ± standard deviations, percentages and odds ratios. Dependant and independent Student’s t-tests were used to comparing continuous variables. The non-parametric ANOVA Kruskal-Wallis test was used for comparing (median and mean values) between the three patients groups. A p-value lower than 0.05 was considered statistically significant with a confidence interval (CI) of 95% for all statistical used tests.

The Principal Component Analysis (ACP) was used as a method for projecting quantitative observations; intake of micronutrients (vitamins and minerals) and amino acids according to the levels of the apo B/apo A1 ratio (as the main index of atherogenicity), along with the independent variables, diabetic status and BMI (adjusted for patients’ gender).

RESULTS

Two hundred eighty-five patients (238 T2D and 47 Non-diabetics) participated in this study. The mean age of all participants was 55.41±12.77 years and the diabetes duration was 5.69±4.25 years. All diabetic
Role of Micronutrients and Amino Acids in Atherosclerosis Development

patients were exclusively treated using metformin (biguanide), however, about 41.21% of them were under biotherapy of metformin and sulfonylurea. 17.32% of women were menopausal (for 3±1.1 years approximately). Patients using oral contraceptives constitute 65.45% of all women.

Table 1 displays a comparison of basic characteristics between the three participants’ groups using the Kruskal-Wallis test. No significant dissimilarities were observed for blood pressures (either systolic or diastolic), fasting and postprandial HDL cholesterol, postprandial TC, Apolipoproteins levels (A1 and B), carbohydrates intakes, proteins, fibres and daily meals contributions to total energy intake. However, significant differences between the three groups were disclosed for age, diabetes duration (between the two groups of diabetic patients), fasting and postprandial glucose, triglycerides and Hba1c levels (p<0.005). Moreover, the total energy and the lipid food intakes were considerably higher in overweight/obese T2D patients compared to the others.

### Table 1. Comparison of basic characteristics of studied patients’ groups.

<table>
<thead>
<tr>
<th></th>
<th>Total sample (n=210)</th>
<th>Overweight/Obese Patients Without Diabetes (n=97)</th>
<th>Overweight/Obese T2D Patients (n=64)</th>
<th>Normal Weight T2D Patients (n=47)</th>
<th>ANOVA Kruskal-Wallis test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>285 (100)</td>
<td>167 (58.9)</td>
<td>57 (14.4)</td>
<td>5 (9.5)</td>
<td>57.24 (p&lt;0.001)</td>
</tr>
<tr>
<td>Sex ratio, male:female</td>
<td>87:190</td>
<td>57:118</td>
<td>17:34</td>
<td>5:2</td>
<td></td>
</tr>
<tr>
<td>Age (years, mean±SD)</td>
<td>49±13.77</td>
<td>47±13.28</td>
<td>57±8.80</td>
<td>56±14.60</td>
<td>57.24 (p&lt;0.001)</td>
</tr>
<tr>
<td>Diabetes duration (years, mean±SD)</td>
<td>4.76±3.71</td>
<td>--</td>
<td>6.3±5.74</td>
<td>6.5±3.57</td>
<td>118.52 (p&lt;0.001)</td>
</tr>
</tbody>
</table>

#### Anthropometric characteristics, mean±SD

| Body weight (kg)        | 71.5±4.23            | 69.4±4.23                                     | 71.5±4.23                         | 69.4±4.23                         | 69.4±4.23                |
| Height (cm)             | 165.5±4.16           | 165.5±4.16                                    | 165.5±4.16                        | 165.5±4.16                        | 165.5±4.16              |
| Waist (cm)              | 91.1±5.57            | 91.1±5.57                                     | 91.1±5.57                         | 91.1±5.57                         | 91.1±5.57               |
| BMI (kg/m²)             | 30.7±4.53            | 30.7±4.53                                     | 30.7±4.53                         | 30.7±4.53                         | 30.7±4.53               |
| Ideal body weight (kg)  | 59.9±6.50            | 59.9±6.50                                     | 59.9±6.50                         | 59.9±6.50                         | 59.9±6.50               |
| Blood pressure, mmHg    | 125±9.51             | 125±9.51                                      | 125±9.51                          | 125±9.51                          | 125±9.51                |
| SBP (mmHg)              | 70±10.97             | 70±10.97                                      | 70±10.97                          | 70±10.97                          | 70±10.97                |

#### Fasting biochemical parameters

| Glucose (g/L)           | 5.2±0.61             | 5.2±0.61                                      | 5.2±0.61                          | 5.2±0.61                          | 5.2±0.61                |
| Total Cholesterol (g/L) | 5.4±0.56             | 5.4±0.56                                      | 5.4±0.56                          | 5.4±0.56                          | 5.4±0.56                |
| HDL-Cholesterol (g/L)   | 1.2±0.11             | 1.2±0.11                                      | 1.2±0.11                          | 1.2±0.11                          | 1.2±0.11                |
| LDL-Cholesterol (g/L)   | 3.0±0.21             | 3.0±0.21                                      | 3.0±0.21                          | 3.0±0.21                          | 3.0±0.21                |
| Triglycerides (g/L)     | 1.1±0.68             | 1.1±0.68                                      | 1.1±0.68                          | 1.1±0.68                          | 1.1±0.68                |

#### Fasting biochemical parameters

| Glucose (g/L)           | 5.2±0.98             | 5.2±0.98                                      | 5.2±0.98                          | 5.2±0.98                          | 5.2±0.98                |
| Total Cholesterol (g/L) | 5.4±0.47             | 5.4±0.47                                      | 5.4±0.47                          | 5.4±0.47                          | 5.4±0.47                |
| HDL-Cholesterol (g/L)   | 1.2±0.11             | 1.2±0.11                                      | 1.2±0.11                          | 1.2±0.11                          | 1.2±0.11                |
| LDL-Cholesterol (g/L)   | 3.0±0.20             | 3.0±0.20                                      | 3.0±0.20                          | 3.0±0.20                          | 3.0±0.20                |
| Triglycerides (g/L)     | 1.1±0.68             | 1.1±0.68                                      | 1.1±0.68                          | 1.1±0.68                          | 1.1±0.68                |

#### Fasting biochemical parameters

| HbA1c (%)               | 7.4±0.91             | 7.4±0.91                                      | 7.4±0.91                          | 7.4±0.91                          | 7.4±0.91                |
| apo A (g/L)             | 1.2±0.57             | 1.2±0.57                                      | 1.2±0.57                          | 1.2±0.57                          | 1.2±0.57                |
| apo B (g/L)             | 0.9±0.58             | 0.9±0.58                                      | 0.9±0.58                          | 0.9±0.58                          | 0.9±0.58                |

#### Total energy intake

| Total energy intake (kcal) | 2037±682.70          | 2037±682.70                                   | 2037±682.70                       | 2037±682.70                       | 2037±682.70             |

#### Vitamin dietary intakes

Results analysis of vitamin dietary intakes in our patients (Table 2) revealed several significant differences between the two genders within the overweight/obese non-diabetic group. These differences were observed in vitamin E (p=0.010), vitamin C (p=0.009) and vitamins of the B group, notably B3 “pantothenic acid” (p=0.035), B6 (p=0.034) and B9 “folic acid “(p=0.031). One single difference between the three groups was noticed in vitamin B12 (p=0.006).
Table 2. Assessment and comparison of dietary vitamins intakes within/between the three patients’ groups.

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Overweight/Obese Patients: Without Diabetes (n=28)</th>
<th>Overweight/Obese Diabetic Patients (n=31)</th>
<th>Normal Weight Diabetic Patients (n=31)</th>
<th>Requirements</th>
<th>p-value of ANOVA Kruskal-Wallis test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin</td>
<td>Male 19 Means±S.D</td>
<td>Female 28 Means±S.D</td>
<td>Male 49 Means±S.D</td>
<td>Female 31 Means±S.D</td>
<td>Male 37 Means±S.D</td>
</tr>
<tr>
<td>A (ug)</td>
<td>3792±512</td>
<td>1577±496.8</td>
<td>0.284</td>
<td>1585±670</td>
<td>1535±472</td>
</tr>
<tr>
<td>D (ug)</td>
<td>4.1±8.09</td>
<td>5.12±0.97</td>
<td>0.888</td>
<td>2.7±6.63</td>
<td>2.7±3.01</td>
</tr>
<tr>
<td>E (mg)</td>
<td>23.65±5.45</td>
<td>14.78±6.22</td>
<td>0.010</td>
<td>10.18±17.4</td>
<td>18.28±6.25</td>
</tr>
<tr>
<td>K (ug)</td>
<td>502.2±234</td>
<td>474±212</td>
<td>0.278</td>
<td>470±312</td>
<td>528±318</td>
</tr>
</tbody>
</table>

Recommended Dietary Allowances (RDA) in **bold type**: Adequate Intakes (AI) **underlined** according to the Institute of Medicine, 2011 (Institute of Medicine, 2011).

1. The Student t test "independent samples" was used to compare means value between men and women within each group of patients. (Results are given as p value for Student t-test).
2. For comparing results of men and women overweight/obese diabetics on the one hand, with patients of the same gender from the two other groups, on the other hand, the Student t test "paired samples" was employed (Results are indicated by cross brace *p*<0.05).
3. ANOVA Kruskal-Wallis test is used for comparing between the results of the three groups of patients; results are indicated by p value for ANOVA Kruskal-Wallis test, in the last column.

Table 3 displays mineral status in our three groups of patients. No difference between the two genders was observed with respect to macro- and trace elements except for the non-diabetic overweight/obese individuals. In this last, we noticed a significant difference between men and women regarding potassium (p=0.007), calcium (p=0.016), magnesium (p=0.022) and phosphorus (p=0.022) as macro-elements and also in copper (p=0.012) and manganese (p=0.016) as micro-elements. No differences were found between all groups of patients neither in macro-elements nor in trace elements.

As summarised in Table 4, the comparison between the two genders’ results regarding the essential amino acids in each group did not show any significant difference. Likewise, the comparison between the three groups using the Kruskal-Wallis test showed no significant differences regarding all the evaluated dietary amino acids.

Within the three groups of patients, the statistic treatment of correlations between micronutrients (vitamins and minerals) and amino acids, on one hand, and apo B/apo A1 ratio levels (≤0.7 as 1st component, or >0.7 as 2nd component), on the other hand, was studied through principal component analysis (PCA). Our findings disclosed that amino acids intakes are generally correlated to the higher level (>0.7) of apo B/apo A1 ratio (2nd component), so to higher atherogenic risk, in the three groups (Figs. 1, 2 and 3).

In the normal weight T2D patients (Fig. 1), the PCA showed that amino acids food intakes (so the higher apo B/apo A1 ratio and atherogenic risk) were positively correlated to vitamins E and B6 and to calcium, zinc and phosphorus levels. In the overweight/obese T2D patients (Fig. 2), the intake of the amino acid was associated with vitamins B2 and B12 and minerals; phosphorus and zinc. However, results in overweight/obese patients without diabetes revealed that amino acid intakes were specifically correlated to vitamin D (Fig. 3).
Table 3. Assessment and comparison of dietary minerals intakes within/between the three patients’ groups.

<table>
<thead>
<tr>
<th>Minerals</th>
<th>Overweight/Obese Patients Without Diabetes (n=7)</th>
<th>Overweight/Obese Diabetic Patients (n=18)</th>
<th>Normal Weight Diabetic Patients (n=71)</th>
<th>Requirements</th>
<th>p-value for ANOVA Kruskal-Wallis test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male 19 Means±S.D</td>
<td>Female 28 Means±S.D</td>
<td>p-value for Student</td>
<td>Male 19 Means±S.D</td>
<td>Female 118 Means±S.D</td>
</tr>
<tr>
<td>Na (mg)</td>
<td>5022±825</td>
<td>4504±1876</td>
<td>0.120</td>
<td>7016±3045</td>
<td>4566±1493</td>
</tr>
<tr>
<td>K (mg)</td>
<td>3636±607</td>
<td>2708±715</td>
<td>0.007</td>
<td>3386±78</td>
<td>3281±690</td>
</tr>
<tr>
<td>Ca (mg)</td>
<td>777±361</td>
<td>488±152</td>
<td>0.016</td>
<td>604±301</td>
<td>701±258</td>
</tr>
<tr>
<td>Mg (mg)</td>
<td>351±17</td>
<td>259±72</td>
<td>0.022</td>
<td>319±60.9</td>
<td>305±61.6</td>
</tr>
<tr>
<td>P (mg)</td>
<td>1489±240</td>
<td>1323±244</td>
<td>0.022</td>
<td>1377±68</td>
<td>1241±32</td>
</tr>
<tr>
<td>Cr (mg)</td>
<td>886±1354</td>
<td>769±380</td>
<td>0.123</td>
<td>1074±680</td>
<td>885±211</td>
</tr>
<tr>
<td>NaCl (g)</td>
<td>13.6±2.3</td>
<td>11.1±4.6</td>
<td>0.167</td>
<td>17.6±12.6</td>
<td>10.6±5.5</td>
</tr>
</tbody>
</table>

Recommended Dietary Allowances (RDA) in bold type; Adequate Intakes (AI) underlined according to the Institute of Medicine 2011 (Institute of Medicine, 2011). NaCl requirements intake according to the Department of Health and Human Services (DHHS, 2005) & WHO/FAO, 2003 (DHHS, 2005).

- The Student t test “independent samples” was used to compare means value between men and women within each group of patients (Results are given as p value for Student t-test).
- For comparing results of men and women overweight/obese diabetics on the one hand, with patients of the same gender from the two other groups, on the other hand, the Student t test “paired samples” was employed (Results are indicated by cross brace *p<0.05).
- ANOVA Kruskal-Wallis test is used for comparing between the results of the three groups of patients; results are indicated by p value for ANOVA Kruskal-Wallis test, in the last column.

Table 4. Assessment and comparison of dietary amino acids intakes within/between the three patients’ groups.

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Overweight/Obese Patients Without Diabetes (n=7)</th>
<th>Overweight/Obese Diabetic Patients (n=18)</th>
<th>Normal Weight Diabetic Patients (n=71)</th>
<th>Highest Median Intake (g/day)</th>
<th>p-value for ANOVA Kruskal-Wallis test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male 19 Means±S.D</td>
<td>Female 28 Means±S.D</td>
<td>p-value for Student</td>
<td>Male 19 Means±S.D</td>
<td>Female 118 Means±S.D</td>
</tr>
<tr>
<td>His (g)</td>
<td>2.57±0.75</td>
<td>3.26±0.91</td>
<td>0.527</td>
<td>2.71±0.64</td>
<td>2.02±0.88</td>
</tr>
<tr>
<td>Ile (g)</td>
<td>4.85±1.10</td>
<td>4.22±1.40</td>
<td>0.331</td>
<td>4.83±1.28</td>
<td>3.85±1.18</td>
</tr>
<tr>
<td>Leu (g)</td>
<td>5.74±1.49</td>
<td>6.06±1.52</td>
<td>0.354</td>
<td>7.61±1.80</td>
<td>9.04±1.81</td>
</tr>
<tr>
<td>Lys (g)</td>
<td>6.58±2.09</td>
<td>5.89±2.11</td>
<td>0.567</td>
<td>7.01±1.68</td>
<td>5.18±1.83</td>
</tr>
<tr>
<td>Met (g)</td>
<td>2.18±0.59</td>
<td>1.92±0.90</td>
<td>0.472</td>
<td>2.12±0.49</td>
<td>1.79±0.60</td>
</tr>
<tr>
<td>Phe (g)</td>
<td>4.44±0.78</td>
<td>3.82±0.35</td>
<td>0.250</td>
<td>4.23±1.34</td>
<td>3.47±1.07</td>
</tr>
<tr>
<td>Thr (g)</td>
<td>3.95±0.87</td>
<td>3.48±1.56</td>
<td>0.401</td>
<td>4.11±0.94</td>
<td>3.27±0.99</td>
</tr>
<tr>
<td>Trp (g)</td>
<td>1.15±0.20</td>
<td>0.96±0.33</td>
<td>0.166</td>
<td>1.11±0.24</td>
<td>0.92±0.27</td>
</tr>
<tr>
<td>Val (g)</td>
<td>5.22±1.09</td>
<td>4.62±1.88</td>
<td>0.420</td>
<td>5.41±1.36</td>
<td>4.86±1.30</td>
</tr>
</tbody>
</table>

**Miscellaneous Notes:**
- Highest Median Intake according to NRC, 2005 (NRC, 2005).
- The Student t test “independent samples” was used to compare means value between men and women within each group of patients (Results are given as p value for Student t-test).
- For comparing results of men and women overweight/obese diabetics on the one hand, with patients of the same gender from the two other groups, on the other hand, the Student t test “paired samples” was employed (Results are indicated by cross brace *p<0.05).
- ANOVA Kruskal-Wallis test is used for comparing between the results of the three groups of patients; results are indicated by p value for ANOVA Kruskal-Wallis test, in the last column.
Fig. 1. Correlations between vitamins, minerals and amino acids food intakes and apo B/apo A1 ratio in normal weight T2D.

Fig. 2. Correlations between vitamins, minerals and amino acids food intakes and apo B/apo A1 ratio in overweight/obese T2D.
DISCUSSION

To our knowledge, this is the first work studying the atherogenic risk (using the apo B / apo A1 ratio) in correlation with dietary intakes of micronutrients and amino acids in patients with/without T2D and according to different corpulence classes.

Our study was conducted on 285 subjects divided into three groups (non-diabetic individuals with BMI ≥ 25, T2D patients with BMI ≥ 25 and normal weight T2D patients). First, we compared anthropometrics, blood pressure, and fasting, postprandial and non-fasting biochemical parameters between the three groups of patients. Likewise, food intakes were evaluated through a “three days” food record and then compared between the patients’ groups. The obtained results suggest that blood pressure averages were at the limit of the recommended values (140/80-85-90 mmHg). Systolic pressure in overweight/obese T2D patients was higher than systolic pressure in the two other groups. The same findings were reported by Min et al. (2021).

Physiologically, the accumulation and the enlargement of adipose tissue are associated with the increase in the number of adipose tissue macrophages, which are responsible for the increased plasma concentration of pro-inflammatory cytokines and more precisely IL-6 and TNF-α expression (Omran & Christian, 2020). In their work, Oishi et al. (2018) have confirmed that the increased levels of these two cytokines (IL-6 and TNF-α), in patients with T2D who suffer from an excess weight (overweight or obese), lead to an increase in systolic blood pressure.

As an important purpose of the present study, we evaluated, during both fasting and postprandial states, glucose level and lipids blood parameters. Likewise, HbA1c and apolipoproteins levels were evaluated during a non-fasting state, in all patients. Our findings revealed significant differences when comparing fasting and postprandial glucose and triglycerides levels between the three groups of patients. As well
as, a significant difference between the groups of patients was disclosed for HbA1c concentrations. Chakraborty et al. (2020) have reported the same observations when comparing fasting and postprandial lipid parameters in the evaluation of cardiovascular risk assessment between prediabetes and diabetes patients.

Strong evidence suggests that dietary factors can influence the development of atherosclerosis directly or through their effects on traditional risk factors, such as diabetes, obesity and blood pressure. Our results showed that overweight/obese T2D patients had significantly the highest total energy and lipids intakes, but lower dietary fibres consumptions, compared to the two other groups. These findings agree with those of Bounihi et al. (2021). The relationship between diet and atherosclerosis has been addressed in a tremendous number of observational epidemiological studies and even in controlled trials (Spector, 2016). Several methodological deficiencies and ambiguities are reported in the majority of these studies, and this affects their results.

As nutrients, vitamins and minerals play diverse roles in our bodies. Micronutrients can regulate metabolism and gene expression and influence the development and progression of many chronic diseases. Vitamins are vital to cardiovascular health (i.e. vitamin B1), nerve function (i.e., vitamins B6 and B12), the production of red blood cells (i.e. vitamins B9 and B12), and coagulation (i.e., vitamin K), among many other functions (Christie-David et al., 2015).

Results of the present study indicated a very low dietary intake of vitamins D and B9 and some minerals (calcium, potassium, magnesium and iodine) in all patients groups. However, we noticed also a low dietary intake of B group vitamins such as B12, B5 and B12, in normal-weight diabetic patients. In some studies, robust correlations have been perceived between food intake of vitamin D, on the one hand, and obesity and insulin resistance, on the other hand. These results remain suggestive and no causal relationship has been mentioned. The role of vitamin D in weight reduction is supported by plausible mechanisms in vitro. However, the close relationship between vitamin D itself and calcium makes the explanation of these mechanisms difficult and inconclusive (Vanlint, 2013).

Vitamin D is involved in insulin sensitivity and pancreatic insulin secretion, through the modification of intracellular calcium signals. As a summary of their systematic and meta-analysis study based on transversal investigations, Zhu et al. (2019) have reported that dietary calcium intakes are inversely associated with body weight and adiposity. Therefore, these studies have suggested a potential role of calcium in the development of T2D. Likewise, dietary supplementation with cholecalciferol and calcium improves the lipid profile in diabetic and dyslipidemic subjects and plays a preventive role against overweight, cardiovascular diseases and atherosclerosis risk (Hiemstra et al., 2019).

The usual diet of diabetic and/or obese patients in all three groups is unable to meet the needs for minerals and vitamins. It is clear that low-calorie diets generally involve vitamin and mineral deficits, especially in obese subjects for whom vitamin supplementation is still under discussion (Damms-Machado et al., 2012). Considering that anti-diabetic (especially metformin) treatment is associated with a decrease in serum vitamins B12, B9 and D (Kim et al., 2019; Wakeman & Archer, 2020), people with diabetes should be encouraged to meet their daily needs by eating in a balanced way. The use of vitamin or mineral supplements is suitable only when the food consumption becomes very inadequate.

Zinc (Zn) is another vital micronutrient and antioxidant that prevents the reactive response of free radicals that damage cells and cause degenerative diseases (Narayanam et al., 2021). The daily dietary zinc intake for men and women is 11 and 8 mg, respectively, and should be below 40 mg/day (Ruz et al., 2019). The dietary zinc intakes in our groups were slightly above the
recommended values. Zn deficiency contributes to the development of atherosclerosis; thus, Zn manipulations, including Zn drug supplementation, have beneficial and also detrimental effects on many cardiovascular and metabolic disorders (Knez & Glibetic, 2021).

Regarding the assessment of amino acids intakes, our results did not show any significant differences either between the three groups of patients or between males and females within each group. The role of amino acids is not only in protein synthesis, but they have other metabolic functions including improving or restoring metabolic imbalance and muscle catabolism.

Our findings showed that amino acids intake, in the three groups of patients, was in accordance with the highest median intake of essential amino acids recommended by the Institute of Medicine (2011). Beneficial effects on BMI, lean mass, insulin sensitivity and cardiovascular disease have been suggested through clinical studies based on supplementations with essential amino acids and in particular branched-chain amino acids (leucine, isoleucine and valine) (Almeida et al., 2020). These amino acids help prevent and reverse obesity in a model animal through several mechanisms (Ruocco et al., 2020). Furthermore, the population-based International Study of Macro-/Micronutrients and Blood Pressure (INTERMAP) recommended evaluating the effects of dietary branched-chain amino acids (BCAAs) (Almeida et al., 2020).

Dietary intake of essential amino acids in the three patients’ groups does not seem to show any deficiencies, which is in favor of our patients. However, supplementation in branched-chain amino acids may provide further positive improvements for obese and/or T2D patients.

Several epidemiological studies have consistently and reliably reported that LDL-c is the major lipoprotein in the process of atherosclerosis progression. The LDL is the main target of lipid-lowering therapies aiming to prevent cardiovascular disease and atherosclerosis. Surprisingly, even subjects with atherosclerosis can have normal or even low LDL-c concentrations (Bodde et al., 2019). For this reason, it is preferable to determine the apolipoprotein B level rather than the LDL level. Thus, the apo B/apo A1 ratio has in recent times been highly recommended as an evocative of cardiovascular risk and in particular myocardial infarction and atherosclerosis (Yaseen et al., 2021).

As another major objective of the present study, we have grouped micronutrients and amino acids according to the level of the apo B/apo A1 ratio (either less/or equal to 0.7 or greater than 0.7), in each group of patients. Our findings revealed that amino acids intakes are generally associated with the higher levels (>0.7) of apo B/apo A1 ratio, so to higher atherogenic risk, in the three groups of patients. Moreover, along with amino acids which are linked to the atherosclerotic risk, other minerals such as zinc, phosphorus and vitamin B12 are added in T2D subjects and vitamin D in particular in obese non-diabetics.

In their review study published in 2020, Zarik et al. summarized the current knowledge on essential amino acids’ role in the atherogenic risk and cardiovascular disease associated with atherosclerosis. They have stated that catabolic defects in branched-chain amino acids BCAAs metabolism increase their plasma concentrations, representing a significant risk factor for cardiometabolic disease. These intriguing discoveries open up future approaches for new anti-inflammatory therapies which integrate the peculiarities of the inflammatory system according to the patient’s condition and according to other associated risk factors, in particular diabetes and obesity.

Our study isn’t without limitations, the micronutrient requirements determination, according to different ages and health situations, has always been difficult since assessment methods are based on patients’ self-declaration as well as databases are not perfectly accurate. These methods are subject to a number of reporting biases, which may lead to misrepresentation of food intake.
For these reasons, micronutrient assessment methodologies are constantly being improved and revisited towards more comprehensive and precise approaches. Moreover, the Nutriservey software employed in our food estimates does not allow evaluating the selenium food intake. The selenium constitutes a key element among others to estimate the cardiovascular risk in diabetic and/or obese subjects; this can be another limitation of our work.

Conclusion

Taken together, our results have led us to conclude that T2D and high weight (overweight or obesity) both constitute an increased cardiovascular risk including atherosclerotic risk. The role of micronutrients and amino acids in the development of this risk is pivotal. The branched-chain amino acids such as leucine, isoleucine and valine, and vitamins; $\text{B}_1$, $\text{B}_3$, $\text{B}_9$ $\text{B}_{12}$ and D as well as the mineral salts as calcium and zinc constitute the key elements of the atherosclerosis phenomenon through their significant correlations with higher levels of the apo B/apo A1 ratio as an indicator of major cardiovascular risk and in particular atherosclerosis. Further trials and clinical studies are required to establish the existing relationships between micronutrients, dietary amino acids and cardiovascular risk in vulnerable patients.

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Role of Micronutrients and Amino Acids in Atherosclerosis Development


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Zaric, B.L.; Radovanovic, J.N.; Gluvic, Z.; Stewart, A.J.; Essack, M.; Motwalli, O. et al. (2020) Atherosclerosis Linked to Aberrant Amino Acid Metabolism and
المغذيات الدقيقة والمدخل الغذائي من الأحماض الأمينية وارتباطاتها مع السمنة ومرض السكري من النوع الثاني وكذا نسبة صميم البروتين الشحمي ب/أ عند الجزائريين البالغين

ضياف مصطفى. وخالد مغيث بومدين
قسم البيولوجيا، مختبر التغذية، علم الأمراض، البيوتكنولوجيا الزراعية والصحة، كلية علوم الطبيعة والحياة، جامعة جيلالي لباس، سيدي بلعباس، الجزائر ...

الهدف: يبحث العمل الحالي في العلاقة المتبادلة بين ماهج الطعام من المغذيات الدقيقة والأحماض الأمينية مع نسبة صميم البروتين الشحمي ب/أ كمؤشر محتمل لخطر مرض تصلب الشرايين لدى المرضى الذين يعانون من السمنة والسكري من النوع 2.

طرق الدراسة: تم إجراء دراسة مقارنة مقطعة على 285 مشاركًا مقسمين إلى ثلاث مجموعات. مرضى السكري من النوع 2 ذوي الوزن الطبيعي، المرضى الذين يعانون من زيادة الوزن/السمنة والسكري من النوع 2، الذين يعانون من زيادة الوزن/السمنة غير المصابين بالسكري. تم قياس متغيرات البيوكيميا وحساب نسبة الشحم الشحمي ب/أ مع (أقل أو مساوي ل 0.7 وأعلى من 0.7) وقياس المدخل الغذائي باستخدام سجل غذائي لمدة ثلاثة أيام. قمنا بردءат الارتباط بين نسبة الشحم الشحمي، نسبة المغذيات الدقيقة (الفيتامينات والمعادن) والأحماض الأمينية باستخدام تحليل المكونات الرئيسية وفقًا لمستويات جنبًا إلى جنب مع المتغيرات المستقلة وحالة السكري ومؤشر كتلة الجسم (معدل حسب جنس المريض) باستخدام طريقة تحليل العنصر الرئيسي.

 النتائج: تم الكشف عن فروق ذات دلالة إحصائية فيما يتعلق بالعمر ونسبة الجلوكوز في مرحلة الصيام وبعد الأكل والدهون الثلاثية والهيموجلوبين السكري.

فيما يخص مرضي السكري ذوي الوزن الطبيعي، أظهرت النتائج أن أعلى نسبة كانت مرتبطة بشكل إيجابي مع تناول الأحماض الأمينية متفرعة السلسلة، وفيتامينات ه وبي-6، ومستويات الكالسيوم والزنك والفوسفور. أما مرضى السكري من النوع الثاني الذين يعانون من زيادة الوزن/السمنة، ارتبط تناول الأحماض الأمينية (الفيتامينات والمغذيات) مع نسبة الشحم الشحمي ب/أ ومستوى الكالسيوم والكربوهيدرات الدائمة.

خلاصة: تلعب المغذيات الدقيقة والأحماض الأمينية دورًا مهماً في تطوير مخاطر القلب والأوعية الدموية لدى مرضى السكري. تظهر الأبحاث الأخيرة أن هناك علاقة تحليلية بين الأحماض الأمينية المتفرعة والفيتامينات د وبي-6، وذلك يرتبط بالفرق في دقائق ضغط الدم ونسبة الشحم الشحمي، وبالتالي، ترتبط هذه المتغيرات بال})(اندلع الصيام والدورة المغذية.

الكلمات المفتاحية: المغذيات الدقيقة، الأحماض الأمينية، السمنة، السكري من النوع الثاني، تصلب الشرايين.