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CORRELATION BETWEEN DIETARY FAT INTAKE AND ATHEROGENIC INDICES IN NORMAL, OVERWEIGHT AND OBESE ADULTS WITH OR WITHOUT TYPE 2 DIABETES

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Abstract

Background and aims: *We investigated the association of dietary intake, particularly fat and its constituent fatty acids, with atherogenic indices in adult patients with overweight, obesity and/or type 2 diabetes (T2D).* ***Material and Methods:*** *Two hundred eighty-five outpatients were selected in two cities located in the Northwestern region of Algeria. Anthropometric measurements for body weight, height, body mass index (BMI) and waist circumference were performed. Relationships between dietary intakes, estimated by a 3-days food record, and fasting blood atherogenic indices - total cholesterol-to-high-density lipoprotein cholesterol ratio (TC/HDL-c) and apolipoprotein (apo) B-to-apo A1 ratio, were analysed.* ***Results:*** *Study group included 58.59% overweight/obese T2D patients, 24.91% normal weight T2D patients and 16.49 % overweight/obese patients without diabetes. Higher dietary consumption (p= 0.003) of total fat, saturated fatty acids (SFAs) and polyunsaturated fatty acids (PUFAs), was recorded in the group of overweight/obese T2D patients. Significant positive correlations were observed between apo B/apo A1 and total fat (p= 0.035), total SFAs (p= 0.042) and palmitic acid (p= 0.042) in the group of overweight/obese T2D patients and with ω6 fatty acid (p= 0.030) in the group of overweight/obese patients without diabetes. In the two groups of T2D patients, whether normal weight, overweight/obese, numerous positive correlations with TC/HDL-c were disclosed for PUFAs, ω6 and fatty acids ratios, namely, ω6/ω3, monounsaturated fatty acids (MUFA)/SFAs and (MUFAs+PUFAs)/SFAs.* ***Conclusion:*** *Most adults, whom are either affected by an excess weight or T2D or both together, are prone to cardiovascular risk. Dietary intakes, particularly in fat and its constituent fatty acids, have an important effect on blood lipid atherogenic indices (TC/HDL-c and apo B/apo A1 ratios).*

key words: *Dietary intake, atherogenic indices, obesity, type 2 diabetes, cardiovascular risk*

Background and aims

The prevalence of chronic noncommunicable diseases is increasing dramatically. The mortality rate due to cardiovascular disease (CVD) goes beyond 18 million cases annually, for which diabetes and hypertension are major predisposing factors. However, the growing

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prevalence of overweight and obesity contributes to an upsurge in cases of type 2 diabetes (T2D) and hypertension [1]. Excess weight is a primary risk factor in the emergence of more than 90% of cases of T2D. Furthermore, the number of people with impaired glucose tolerance, often due to obesity and associated metabolic syndrome, is expected to increase from 197 million in 2007 to 420 million by 2025 [2].

The inclusion of the effect of body weight and waist circumference on diabetes risk, blood pressure, and blood lipid concentrations would provide the opportunity to develop strong cardiometabolic risk equations which gather all the these risk factors at once [3-6]. Furthermore, cardiovascular risk events may vary according to some conditions such as age, gender, family history, smoking, physical activity and dietary patterns [7].

Cumulative evidence supporting a pivotal role of apolipoprotein (apo) B to apo A1 ratio as a convenient risk predictor for cardiovascular events, especially myocardial infarction, which can represent 66% of the global risk of heart attack [8-11]. Moreover, the total cholesterol/high-density lipoprotein (TC/HDL-c) ratio is the most frequently used index that have higher association with CVD than individual lipid parameters [12-14].

For several decades, the causality of the association between diet and plasma lipid levels and thereafter coronary artery disease is receiving a close attention. This link is heavily influenced by the saturated or unsaturated nature, as well as by the number of carbon atoms in the chain, of the fatty acids consumed [7].

In the present study, we investigated whether dietary intake, particularly in fat and its constituent fatty acids, correlates with serum TC, lipoprotein cholesterol, apolipoproteins and atherogenic indices (TC/HDL-c and apo B/apo A1 ratios), and whether these correlations are influenced by obesity and/or diabetes. The main study population was composed of overweight/obese type 2 diabetic patients who are compared with two other groups as control; overweight/obese individuals without diabetes and normal weight ones with T2D.

Materials and methods

Study population

Our prospective observational study took place in two cities located in the Northwestern region of Algeria: Sidi-Bel-Abbes city (the Public Establishment of Local Health Centre and Mostefa Ben Brahim policlinic) and Mascara city (Meslem Tayeb Hospital). The study duration was 18 months, from March 2013 to August 2014.

At the level of the three health facilities, and using the simple random sampling method without replacement, T2D patients were randomly solicited considering the inclusion criteria, that is, aged between 20 and 75 years, diabetes duration of less than 15 years and exclusively under oral anti-diabetic treatments (for diabetic patients). However, for overweight/obese non-diabetic patients, the inclusion criteria were the same with a body mass index (BMI) $\geq$ 25 Kg/m^2 and the absence of diabetes. On the other hand, exclusion criteria were used to reduce confounding effects of other factors that might have an impact on blood profiles; hypothyroidism, primary hyper-lipidaemia, pregnancy, renal impairment, liver dysfunction and all other confirmed health complications were absent in our studied population. Patients using lipid-lowering drugs or diabetic patients treated with insulin were excluded from our study.

Anthropometric measurements

Measurement of body weight (in kilograms) was performed using an electronic balance (TS-

2003A: 360 Ib, Capacity: 180 Kg, Graduations 0.1Kg) and height (in meters) was measured using 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 individual. Each patient 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).

The BMI was calculated as follows: BMI (kg/m^2) = weight (kg)/height^2 (m^2). Waist circumference was measured with a tape (Maximum: 150 cm, Graduation Length: 1 mm), the tape is gently tightened around the patient's abdomen roughly above the iliac crest (passing through the navel in men) and a bit above the navel in women, without depressing the skin.

Blood pressure measurement

OMRON *M3* Digital Automatic Blood Pressure Monitor (Omron Healthcare., Ltd. Kyoto, Japan) was used for calculating the morning blood pressure. The machine can determine systolic blood pressures between 3-24 cmHg, diastolic 1-21 cmHg and heart rate 40-200 beats per minute. Patients were in a supine position for blood pressure readings, with three readings taken over a 5-minute period. The average of the three readings is taken as the blood pressure.

General habits and nutrient intake assessment

A structured questionnaire to get necessary information about personal data, socioeconomic data, lifestyle information and food and hygiene behaviour was used. Likewise, the evaluation of nutrient intake was carried out by means of a three-day food record. Patients were given verbal and written instructions on filling out their questionnaire and recording their food and drinks, which included the recording of the type of food, time of the meal, serving size and method of cooking and other details. For patients who were unable to fill out their food diaries alone, we asked from a family member to fill out the diaries for them.

For the amount units of consumed food, we asked the surveyed patients to use some standard units such as: tea or coffee spoon, slice (thin or thick), cup, glass (juice glass, water glass), corner for cheese, bag, bottle, can, packet, middle or normal piece, meatball and standard portion of fruits and cakes. However, for food products packaged in small unit such as dairy products, cakes and small tin cans, we asked patients to indicate the commercial brand of the product.

A thorough verification step following the filling of both questionnaires and food records was organized with every patient individually to correct inaccurate data and oversights.

Energy intake and diet composition, nutrients were calculated using the software program NutriSurvey for windows 2007, SEAMEO-TROPMED RCCN-University of Indonesia (NutriSurvey, 2007) [15]. This program is based on the German food database (BLS) with English names.

Blood sampling and assay methods

Between 8:00 and 9:00 am, venous blood samples were drawn 12 hours after an overnight fast from the ante-cubital fossa of the forearm. The enzymatic colorimetric methods (Spinreact Reagents, Spain) [16] were used to determine the serum concentrations of glucose, total cholesterol, high-density lipoprotein cholesterol, triglycerides (TG) and direct low-density lipoprotein cholesterol (LDL-c). Apo A1 and apo B were determined quantitatively using turbidimetric tests (Spinreact Reagents, Spain) [17], then the TC/HDL-c and the apo B/apoA1 ratios were calculated. The glycosylated

haemoglobin (HbA1c) levels were quantified by an ion exchange resin separation method.

Statistical Analysis

All data were processed and analysed through SPSS 20.0 (*Statistical Package for the Social Sciences*, IBM Corporation; Chicago, IL August 2011) for Windows. For all analyses, a *p*-value of 0.05 or less was considered to be significant with a confidence interval of 95%. The student *t*-test was used to compare means of anthropometric measurement and blood parameters by gender and the Chi-square test was employed for comparing percentage values.

The Kruskal Wallis ANOVA test was used to compare total energy intake and results of food rations between the three groups of patients. A multiple linear regression analysis, adjusted for the gender and age, was performed to evaluate the relationship between dietary intake and blood lipid TC/HDL-c and apo B/apo A1 ratios.

Ethical Considerations

By referring to Article 25 of Decree N° 387 of 31 July 2006 about ethical trials, we obtained the agreement N° 142 dated 13 February 2013 from the Director of Health and Population of the *Wilaya* of Sidi-Bel-Abbes (Algeria) in order to accomplish our study protocol. Furthermore, we obtained informed written consent from all participants and their physicians after the study protocol had been explained to them.

Results

Two hundred eighty-five patients (105 males and 180 females) participated in this study. The mean ages of men and women were 55.19±13.42 and 55.54±12.40 years, respectively. Amongst all patients, of both genders, 58.59% were overweight/obese with T2D, 24.91% were normal weight diabetic patients and 16.49 % were overweight/obese patients without diabetes.

In diabetic patients, whether normal weight, overweight or obese, the average diabetes duration was 6.80±3.70 years (6.30±3.40 years for males *vs.* 7.10±3.90 years for females). The commonest prescribed individual drug among anti-diabetic agents was metformin (biguanide) (38.70%). However, fixed dose combination of biguanide and sulfonylurea was prescribed commonly for the majority of patients (61.29%).

Table 1 displays the characteristics of the participants. No differences were observed for age, waist circumference, systolic and diastolic pressures between males and females. However, comparison of anthropometric parameters for weight, height and BMI revealed significant differences between the two genders ($p<0.05$). The prevalence of overweight and/or obesity was more pronounced in females, whereas the prevalence of normal weight was rather encountered in male patients.

Our results about biochemical parameters showed a significant effect of gender differences on fasting glycaemia ($p= 0.014$), triglycerides ($p= 0.043$), apo B ($p= 0.048$) and TC/HDL-c ratio ($p= 0.037$). However, no gender influence was observed for HbA1c, TC, HDL-c, LDL-c, apo A1 and apo B/apo A1 levels.

Comparing the three groups of patients (Table 2), high significant differences were observed for total energy intake (TEI) ($p=0.011$) and meal frequency ($p<0.001$). The group of overweight/obese diabetic patients is characterised by the highest TEI (2212.89±233.64 Kcal), but by the lowest meal frequency (3.18±0.385 meal/day). The lunch was the most important daily meal providing the highest amount of calorie intake, in all patients, exceeding by far the daily requirements [18]. However, the breakfast calorie intakes were lower than the dietary recommendations within the three groups.

Table 1. Characteristics of the patients, n=285.

Variables	**All patients**	**Men**	**Women**	***p*-value for statistical test***
N	285	105	180	–
Age (years), mean±S.D.	55.41±11.77	55.19±13.42	55.54±12.40	0.821
Anthropometric characteristics, mean±S.D.				
Weight (Kg)	78.26±13.75	81.43±14.77	76.41±12.81	**0.003**
Height (cm)	165.98±8.85	172.60±7.34	162.12±7.23	**<0.001**
Waist circumference (cm)	97.68±12.59	96.80±11.36	98.19±13.26	0.369
BMI (kg m-^{2})	28.37±4.50	27.29±4.57	28.99±4.35	**0.002**
Blood pressure, mean±S.D.				
Systolic pressure (cmHg)	12.86±1.45	12.83±1.47	12.88±1.44	0.804
Diastolic pressure (cmHg)	7.62±0.95	7.68±0.93	7.59±0.59	0.458
Type 2 diabetes, n (%)	238 (83.50)	86 (30.17)	152 (53.33)	**0.001**
Category, n (%)				
Overweight/Obese patients without diabetes	47 (16.49)	19 (40.42)	28 (59.57)	0.104
Overweight/Obese diabetic patients	167 (58.59)	49 (29.34)	118 (70.65)	**0.045**
Normal weight diabetic patients	71 (24.91)	37 (52.11)	34 (47.88)	0.053
Biochemical parameters, mean±S.D.				
HbA1c (mmol mol^{-1})	57.3±10.1	56.7±10.6	57.6±9.7	0.597
Fasting glycaemia (g L^{-1})	1.51±0.60	1.62±0.63	1.44±0.57	**0.014**
Total cholesterol (g L^{-1})	1.65±0.36	1.67±0.36	1.64±0.36	0.630
HDL-c (g L^{-1})	0.38±0.11	0.37±0.11	0.39±0.10	0.091
LDL-c (g L^{-1})	1.08±0.31	1.08±0.31	1.08±0.31	0.975
TG (g L^{-1})	1.47±0.68	1.36±0.61	1.53±0.72	**0.043**
Apo A1 (g L^{-1})	1.28±0.37	1.23±0.40	1.32±0.34	0.052
Apo B (g L^{-1})	0.93±0.38	0.87±0.26	0.97±0.43	**0.048**
Apo B/ Apo A1	0.76±0.31	0.76±0.29	0.76±0.33	0.952
Total cholesterol/HDL-c	4.56±1.41	4.78±1.37	4.42±1.42	**0.037**

*Comparison between men and women; means are compared using Student *t* test. Percentages are compared using Chi-square. $p<0.05$ was considered to be significant.

Table 3 summarizes the comparison of dietary intake characteristics between the three groups of patients. There were highly significant intakes ($p= 0.003$) of total fat, saturated fatty acids (SFAs), particularly palmitic and stearic acids, and polyunsaturated fatty acids (PUFAs), namely linolenic acid, in the group of overweight/obese T2D patients, comparing to the two other groups. The consumption of proteins was important in the three groups and

exceeded the dietary recommendations (10-15% of TEI) [19]. However, the dietary fibre intakes and ω3 fatty acid was lower than requirements (27-40 g/day) [19]. Higher levels of cholesterol consumption, above the requirement of 300mg/day, were noticed in overweight/obese patients (either with or without diabetes). With regard to the diet quality for all patients, ω6/ω3 and MUFA/SFAs ratios indicated that they consumed a healthy diet, nonetheless the (MUFAs + PUFAs)/SFAs ratio was below the requirements [20].

Table 2. Daily meals contribution in total energy intake between the three groups of patients.

	Normal weight type 2 diabetic patients		**Overweight/obese type 2 diabetic patients**		**Overweight/obese patients without diabetes**		**R**	***p*–value***
	Mean±S.D. (Kcal)	%	Mean±S.D. (Kcal)	%	Mean±S.D. (Kcal)	%	%	
TEI	1839.50±310.98[#*]	100	2212.89±233.64	100	2027.68±376.12	100	100	**0.011**
Breakfast	236.24±102.06[#*]	12.84	350.44±111.07	15.83	287.95±155.33	14.20	20	0.051
Morning snack	–	–	37.30±92.46	1.68	–	–	10	–
Lunch	821.44±331.84	44.65	1005.73±268.61	45.44	786.98±208.23[#*]	38.81	30	0.054
Afternoon snack	152.90±139.01	8.31	179.06±199.18	8.09	172.40±122.87	8.50	10	0.979
Dinner	628.91±146.13	34.18	640.34±252.25	28.93	780.33±182.77	38.48	30	0.086
Daily meal frequency	3.42±0.604		3.18±0.385		3.62±0.419		–	**<0.001**

TEI: total energy intake, **R:** Required frequency of meals contributions in daily intake (18), [#] significantly different from the group of overweight/obese type 2 diabetic patients (each two groups separately), * significantly different at $p<0.05$. ANOVA Kruskal-Wallis test is used for all comparisons.

Table 3. Comparison of dietary intake characteristics between the three groups of patients.

	Normal Weight Diabetic Patients (n=71) Median Mean±S.D	***p*-value[a]**	**Overweight/Obese Diabetic Patients (n=167) Median Mean±S.D**	***p*-value[b]**	**Overweight/Obese Patients Without Diabetes (n=47) Median Mean±S.D**	**Recommendations**	***p*-value for statistical test[c]**
TEI (Kcal)	1868.20 1839.50±310.98	0.001	2195.40 2212.84±233.59	0.255	2149.70 2027.68±376.12		**0.011**
Carbohydrates (g)	198.00 203.55±32.64 (57.29%)	0.901	204.60 209.06±44.33 (50.07%)	0.426	222.95 204.72±84.38 (49.95%)	55-75%[d]	0.491
Proteins (g)	76.80 78.03±21.95 (21.66%)	0.101	88.00 89.48±23.31 (22.22%)	0.492	90.25 96.32±26.06 (25.69%)	10-15% [d]	0.094
Dietary fibres (g)	25.30 25.32±6.89	0.836	23.80 24.30±4.95	0.871	27.50 24.56±9.52	27-40 g/day [d]	0.977
Fat (g)	71.10 76.88±28.94 (21.03%)	0.001	103.00 111.43±20.70 (27.68%)	0.019	90.65 89.42±26.99 (24.35%)	15-30% [d]	**0.003**
SFAs (g)	25.00 23.83±8.12 (6.51%)	0.079	38.50 40.03±10.03 (9.94%)	<0.001	34.80 33.21±12.20 (9.04%)	< 10% [d]	**<0.001**
Myristic acid (g)	1.60 2.04±1.33	0.758	3.20 3.70±1.99	0.014	3.50 3.50±1.79	-	**0.019**
Palmitic acid (g)	13.30 13.73±4.73	0.031	21.20 21.08±4.41	<0.001	17.80 17.06±5.84	-	**0.001**
Stearic acid (g)	5.40 5.36±1.68	0.049	8.80 8.60±1.86	<0.001	7.15 7.25±2.56	-	**0.001**

Table 3. *Continued.*

	Normal Weight Diabetic Patients (n=71) Median Mean±S.D	***p*-value[a]**	**Overweight/Obese Diabetic Patients (n=167) Median Mean±S.D**	***p*-value[b]**	**Overweight/Obese Patients Without Diabetes (n=47) Median Mean±S.D**	**Recommendations**	***p*-value for statistical test[c]**
MUFAs (g)	21.00 26.80±15.04 (7.32%)	0.270	33.60 34.44±11.29 (8.55%)	0.042	31.55 30.44±9.85 (8.28%)	By difference*	0.082
Oleic acid (g)	18.30 23.97±14.22	0.481	29.50 30.40±12.26	0.065	26.80 26.78±8.40	-	0.144
PUFAs (g)	17.90 21.00±11.29 (5.74%)	0.100	21.80 27.26±13.93 (6.77%)	0.165	180.80 19.76±8.40 (5.37%)	6-10% [d]	0.210
Linoleic acid ω6 (g)	15.80 19.01±10.31 (5.19%)	0.158	19.40 22.60±11.90 (5.61%)	0.419	16.50 17.23±7.41 (4.68%)	5-8% [d]	0.407
Linolenic acid ω3 (g)	1.10 1.18±0.76 (0.32%)	0.014	1.70 3.24±2.66 (0.80%)	0.002	1.30 1.31±0.43 (0.35%)	1-2% [d]	**0.002**
Cholesterol (mg)	236.70 292.36±171.06	0.613	341.20 490.87±353.41	0.130	296.80 435.83±284.87	< 300 mg/day [d]	0.189
ω6/ω3	18.00 19.16±1.28	0.024	8.80 10.22±0.72	0.065	13.41 13.89±0.61	5-10/1[e]	**0.041**
MUFA/SFAs	1.16 1.12±0.42	0.101	0.90 0.87±0.02	0.329	0.97 0.95±0.01	≥0.5[e]	0.207
(MUFAs + PUFAs)/SFAs	1.98 2.12±0.95	0.206	1.49 1.64±0.60	0.914	1.45 1.62±0.50	≥2.0[e]	0.302

TEI: total energy intake, **SFAs:** saturated fatty acids, **MUFAs:** monounsaturated fatty acids, **PUFAs:** polyunsaturated fatty acids, **ω6:** omega 6 fatty acid (Linoleic acid), **ω3:** omega 3 fatty acid (Linolenic acid), ANOVA Kruskal-Wallis test is used for all comparisons. [a] *p* values of asymptotic significance between the group of normal weight diabetic patients and the group of overweight/obese diabetic patients. [b] *p* values of asymptotic significance between the groups of overweight/obese diabetic patients and the group of overweight/obese patients without diabetes. [c] *p* values of asymptotic significance between the three groups. [d] Recommendations of diet, nutrition in the prevention of chronic diseases [19]. [e] Recommended healthy diet [20].
* This is calculated as total fat-(Sat. FA + PUFA + *trans* fatty acids)

As illustrated in Table 4, there were some positive relationship between dietary fat consumption and apo B/apo A1 ratio. Significant positive correlations were observed regarding total fat (r= -0.481, *p*= 0.035), total SFAs (r= -0.460, *p*= 0.042) and palmitic acid (r= -0.461, *p*= 0.042) in the group of overweight/obese T2D patients and regarding ω6 fatty acid (r= 0.453, *p*= 0.030) in the group of overweight/obese patients without diabetes.

Table 4. Multivariate linear regression of dietary fat intake and Apo B/Apo A1 ratio in the three groups of patients.

	Normal weight type 2 diabetic patients			**Overweight/obese type 2 diabetic patients**			**Overweight/obese patients without diabetes**		
	β (CI)*	r	*p*[#]	β (CI)*	R	*p*[#]	β (CI)*	r	*p*[#]
Total Fat	3.546	-0.125	0.329	-3.210	-0.481	**0.035**	4.439	0.390	0.055
SFAs	-7.572	-0.158	0.287	-0.317	-0.460	**0.042**	4.213	0.264	0.145
Myristic acid	7.205	-0.325	0.119	1.077	-0.263	0.172	-7.186	0.111	0.330
Palmitic acid	1.569	-0.031	0.456	1.162	-0.461	**0.042**	-6.617	0.252	0.156
Stearic acid	2.387	-0.070	0.402	-0.495	-0.334	0.112	-1.593	0.333	0.088
MUFAs	2.658	-0.068	0.405	-5.734	-0.228	0.207	3.042	0.374	0.063
Oleic acid	-5.361	-0.065	0.408	1.649	-0.063	0.412	-3.235	0.395	0.052

Table 4. *Continued*

	Normal weight type 2 diabetic patients			**Overweight/obese type 2 diabetic patients**			**Overweight/obese patients without diabetes**		
	β (CI)*	r	$p^{\#}$	β (CI)*	R	$p^{\#}$	β (CI)*	r	$p^{\#}$
PUFAs	-0.583	-0.143	0.306	4.096	-0.138	0.312	-0.090	0.385	0.057
Linoleic acid	2.314	-0.150	0.297	2.712	-0.088	0.378	-1.124	0.453	**0.030**
Linolenic acid	2.315	0.264	0.171	-0.203	-0.144	0.304	-0.803	0.138	0.293
Cholesterol	1.810	0.182	0.258	-0.734	-0.326	0.118	0.112	0.007	0.490
ω6/ω3	4.720	-0.239	0.196	-1.090	0.023	0.468	0.501	0.249	0.160
MUFA/SFAs	7.059	0.017	0.476	0.714	-0.011	0.485	-0.348	0.015	0.477
(MUFAs + PUFAs)/SFAs	-9.251	-0.103	0.358	0.372	0.234	0.201	-0.257	0.054	0.415
Regression model: Apo B/Apo A1 as dependent variable	r=0.940, r^2=0.883, r^2adjusted=0.184, F=1.263, p=0.525			r=0.933, r^2=0.871, r^2adjusted=0.398, F=1.843, p=0.337			r=0.814, r^2=0.663, r^2adjusted=-0.434, F=0.604, p=0.780		

SFAs: saturated fatty acids, **MUFAs:** monounsaturated fatty acids, **PUFAs:** polyunsaturated fatty acids, **ω6:** omega 6 fatty acid (Linoleic acid), **ω3:** omega 3 fatty acid (Linolenic acid), **β:** standardized coefficient of linear multiple regression, **Apo:** apolipoprotein, **r:** Pearson regression coefficient, **F:** fisher test, $^{\#}$significantly different at $p<0.05$, *adjusted by age and gender.

Correlations between dietary fat consumption and TC/HDL-c ratio are depicted in Table 5. In the two groups of T2D patients, whether normal weight, overweight or obese, numerous positive relationships with TC/HDL-c were disclosed for total PUFAs, ω6 and for fatty acids ratios, namely, ω6/ω3, monounsaturated (MUFA)/SFAs and (MUFAs + PUFAs)/SFAs.

Table 5. Multivariate linear regression of dietary fat intake and total cholesterol/HDL-c ratio in the three groups of patients.

	Normal weight type 2 diabetic patients			**Overweight/obese type 2 diabetic patients**			**Overweight/obese patients without diabetes**		
	β (CI)*	r	$p^{\#}$	β (CI)*	r	$p^{\#}$	β (CI)*	r	$p^{\#}$
Total Fat	3.740	0.491	**0.032**	-2.089	0.195	0.244	-35.809	-0.093	0.356
SFAs	8.698	-0.049	0.431	2.578	-0.108	0.305	1.037	0.072	0.388
Myristic acid	-5.294	-0.462	**0.042**	2.059	-0.041	0.443	8.396	0.165	0.256
Palmitic acid	-4.333	0.197	0.241	0.973	-0.153	0.293	8.674	0.049	0.424
Stearic acid	-0.679	0.143	0.305	0.319	-0.285	0.176	2.971	-0.072	0.388
MUFAs	-1.903	0.417	0.061	-7.189	0.053	0.426	1.371	-0.089	0.363
Oleic acid	3.685	0.423	0.058	-0.009	0.154	0.291	12.019	-0.056	0.412
PUFAs	4.646	0.667	**0.003**	-0.910	0.315	**0.012**	8.261	-0.301	0.113
Linoleic acid	-7.471	0.648	**0.004**	-0.169	0.449	**0.004**	5.937	-0.301	0.112

Table 5. *Continued.*

	Normal weight type 2 diabetic patients			**Overweight/obese type 2 diabetic patients**			**Overweight/obese patients without diabetes**		
	β (CI)*	r	$p^{\#}$	β (CI)*	r	$p^{\#}$	β (CI)*	r	$p^{\#}$
Linolenic acid	-1.439	0.098	0.364	0.950	-0.158	0.287	-1.184	0.205	0.207
Cholesterol	-0.640	0.185	0.254	0.425	0.367	0.089	0.818	0.175	0.244
ω6/ω3	-2.310	0.529	**0.021**	1.270	0.518	**0.024**	-6.356	-0.333	0.088
MUFA/SFAs	-6.697	0.561	**0.015**	-0.341	0.141	**0.038**	0.229	-0.192	0.222
(MUFAs + PUFAs)/SFAs	7.919	0.665	**0.003**	2.335	0.470	**0.039**	5.113	-0.226	0.184
Regression model: TC/HDL-c as dependent variable	r=0.953, r^2=0.907, r^2adjusted=0.352, F=1.634, p=0.442			r=0.956, r^2=0.914, r^2adjusted=0.600, F=2.913, p=0.205			r=0.938, r^2=0.879, r^2adjusted=0.487, F=2.240, p=0.227		

Sat. FAs: saturated fatty acids, **MUFAs:** monounsaturated fatty acids, **PUFAs:** polyunsaturated fatty acids, **ω6:** omega 6 fatty acid (Linoleic acid), **ω3:** omega 3 fatty acid (Linolenic acid), **β:** standardized coefficient of linear multiple regression, **TC:** total cholesterol, **HDL-c:** high-density lipoprotein cholesterol, **r:** Pearson regression coefficient, **F:** fisher test, #significantly different at $p<0.05$, *adjusted by age and gender.

Discussion

The increasing prevalence of overweight and obesity is becoming a major public health crisis owing to its upsurge association with risk of major chronic diseases, such as T2D and coronary heart disease [21]. Moreover, CVD, are now emerging as public health challenges in developing countries. The worldwide mortality due to CVD is expected to reach 20 million per year by 2020 [22].

Research is now focusing on nutritional approaches in order to detect and understand dietary factors interacting with lipid profiles and markers of cardiovascular risk. The purpose of the present study was to investigate the relationship between dietary fat intake and plasma lipid atherogenic indices represented by TC/HDL-c and apo B/apo A1 as markers of CVD risk.

Results about anthropometric characteristics from this study revealed that males were taller and heavier as compared to females. However, females have higher BMI values. These results are in consistency with data of many studies [23,24].

Systolic blood pressure was moderately high in females comparing to males (12.88±1.44 *vs.* 12.83±1.47 cmHg). The increase in systolic blood pressure in female gender is partly explained by a decrease in arterial compliance with age, which is significantly correlated with modest weight gain and the impact of insulin resistance [25].

After 50 years of age, hypertension is more common in menopausal women. Our findings indicate a limited effect of gender on major biochemical parameters. However, elevated levels of triglycerides and apo B were observed in women. In females, and during the menopausal transition, levels of triglycerides show a noticeable rise generally estimated at 16% [26]. Most often, a reduced gap in triglyceride levels is observed between men and women aged 50 to 59 years, while, from 60 years and above, females are characterized by higher levels than men [27].

The TC/HDL-c ratio is the most frequently used index in the assessment of CVD risk. In men, it has been established that each unit increase in the ratio was found to be associated with a 53% higher cardiovascular risk [12-14,28]. In parallel, the use of apo B/apo A1 ratio as marker of risk of CVD is increasingly promising [8-11]. Our findings indicate that TC/HDL-c ratio levels were beyond the therapeutic target of 4.0 [29,30]. Likewise, after adapting our data with risk ranges from AMORIS and INTERHEART studies [8,9], apo B/apo A1 ratio showed that all patients of both genders are prone to a moderate cardiovascular risk.

Evaluation of habitual food intake is now representing a cornerstone in the dietary management of obesity and T2D. Results of this study disclosed that overweight/obese diabetic patients had the highest energy intake comparing to the two other groups. No gender effect on the level of energy intake was reported. Similar results have been presented in other studies [31,32].

In all our patients (three groups), the lunch is the most important daily meal that brings the highest amount of caloric intake, exceeding by far the daily requirements [18]. Our result is in agreement with Tounian findings in 2006 [33]. Meal frequency is becoming more influenced by work schedules and children's activities after school. People tend to have their lunch outside and therefore take more fast-food, packaged food and soft drinks that bring much more calories. However, dinner remains the most respected meal (as frequency), the reason is the attachment to tradition and family especially in the Mediterranean region [33,34]. Therefore, encouraging the frequency of more family meals is becoming a target to the diabetic population as well as their respective families [35].

Lunch and dinner should each account for about 30% of the daily caloric intake, and the rest must be distributed as snacks throughout the day [18,36]. On the total, the diet should consist of three meals a day and some snacks. Daily meal frequency was significantly different between our three groups, with the lowest frequency recorded in the groups of overweight/obese T2D patients.

Regarding the comparison between main energy nutrients within the three groups, carbohydrates and proteins did not reveal any significant differences. However, we noticed a difference of total fat consumption. This result matches Visockienė et al. conclusions [32].

In our results, assessment of SFAs intake revealed significant differences between the three groups for both medium- and long chain SFA ($p<0.001$). Overweight/obese diabetic patients (both genders) had the highest levels of dietary SFAs intake followed by overweight/obese non-diabetic patients. It is suggested that dietary SFAs intake participates in modulating the genetic predisposition to obesity. This may constitute a relevant explanation of the relationship between diet and metabolic disease in individuals with an increased cardiometabolic risk [37]. Equally, dietary SFAs consumption modulated the relationship between “fat mass and obesity-associated protein” gene *(FTO) rs9939609* and waist circumference [38]. Overweight and obesity are accentuated among high-SFAs consumers. These results may give a satisfactory explanation for our findings. However, the potential role of SFAs intake in the development of diabetes is also in line with data from the ULSAM cohort study [11].

The outcomes of the current investigation about MUFAs and PUFAs indicated, for both genders, that the overweight/obese diabetic patient group had the highest dietary intake of

MUFAs and PUFAs followed by overweight/obese non-diabetic patients, and then the normal weight diabetic ones. The beneficial effect of diets rich in MUFAs and/or PUFAs has been shown in several studies. In KANWU study, in which a diet with a content of 38% total fat was used, impaired insulin sensitivity was reported in those who consumed a SFA-enriched diet compared with those who consumed a MUFA-rich diet [39].

In the same context, the negative feedback of cholesterol metabolism is well known, if large amounts of cholesterol are brought through the dietary food intake, the body`s cholesterol production will decrease. However, with a high SFAs consumption, the metabolism of cholesterol become activated and the total cholesterol level increases [40]. Our findings indicated a higher dietary cholesterol intakes in overweight/obese individuals, whether diabetic or not, comparing to non-diabetic ones. Likewise, these two groups have already been characterized by a high consumption of SFAs and total fat as well.

Among the fatty acid ratios measured in the present study, the ω6/ω3 ratio exhibited a significant difference between the three groups of patients and between each two groups separately. Nevertheless, no differences were observed for the other fatty acid ratios (MUFAs/SFAs and (MUFAs + PUFAs)/SFAs).

The correlation analysis illustrated a complex association between the dietary fat intake components for both apo B/apo A1 and TC/HDL-c ratios. There was a significant negative moderate correlation between apo B/apo A1 ratio and total fat intake (r= -0.481, *p*= 0.035), and between apo B/apo A1 ratio and total SFAs (r= -0.460, *p*= 0.042) in the overweight/obese T2D patients. When analysed separately, the only SFA that showed this pattern was the palmitic acid. So, as the total fat intake decreases, the apo B/apo A1 increases. Given the fact that there was a statistically significant difference between the three groups regarding the fat intake, but not protein and carbohydrate. So, should we modify the proportions of nutrients in the patients` diet for this group in order to lower the cardiovascular risk?

For the overweight/obese patients without diabetes, there was a positive significant moderate correlation (r= 0.453, *p*= 0.030) between the linoleic acid, but not total PUFAs, and apo B/apo A1 ratio. So, should we recommend diets with less linoleic acid for this group?

Regarding the TC/HDL-c ratio, there was a moderate positive correlation with PUFAs (r= 0.667, *p*= 0.003) and linoleic acid (r= 0.648, *p*= 0.004) in normal weight patients with T2D. So, should we decrease total PUFAs and linoleic acid content?

Several studies suggested these differences between apo B/apo A1 and TC/HDL-c ratios in CVD risk prediction. If the TC/HDL-c can be used as a simple, cost-effective and cumulative marker of cardiovascular risk in patients with T2D [41,42], the apo B/apo A1 seems to be an effective predictor of coronary heart disease risk in overweight and obese individuals [43,44].

In the end, fat dietary intake might be one of the many factors of an important causal relationship between food habits and cardiovascular risk prediction. However, T2D, overweight and obesity have relevant contributing effects on TC/HDL-c and/or apo B/apo A1 levels as independent and advantageous risk predictors for CVD.

One limitation of this study is that the information on the relationship between fat dietary intake and serum risk markers is derived from intervention studies, while our investigation is purely observational. However, we argue that an important causal correlation of

serum cholesterol, lipoproteins and apolipoproteins is influenced by various confounding genetic factors and stimuli such as lack of exercise, chronic stress, anxiety, depression and insufficient sleep. Moreover, the evaluation of dietary intakes is obtained through self-reported records and declaration, this method is subject to a number of reporting biases. A final worth mentioning limitation is the absence of reliable information on the CVD mortality in the studied population.

Conclusions

In conclusion, most patients with overweight, obesity and/or T2D, whether males or females, are prone to cardiovascular risk. Dietary intake, particularly fat and its constituent fatty acids, have a main effect on blood lipid atherogenic indices (TC/HDL-c and apo B/apo A1 ratios). However, it is more accurate to use fatty acid ratios in order to make interpretations more helpful and understandable. We contend that many lifestyle and pathological factors, not fat intake alone, are determining the CVD risk prediction.

In individuals at risk, whom are affected either by obesity or diabetes or both together, the strategy of health risk management should consider the entire diet and its contents, together with non-dietary lifestyle conditions, as opposed to the reductionist approach of studying each parameter separately.

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