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SHORT COMMUNICATION

Trans fatty acids consumption in type 1 diabetic patients: evaluation by dietary records and measurement in serum phospholipids

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Abstract The consumption of foods containing trans fatty acids (TFA), especially those produced by food industries, induces pleiotropic negative effects on health. Therefore, it is important to assess the amount of TFA consumed, especially in age groups more exposed to the consumption of TFA-containing foods. The present pilot study evaluates TFA intake in 54 young people with and without type 1 diabetes (29 young subjects with type 1 diabetes and 25 healthy subjects) through both dietary records (7-day food record) and the measurement of TFA levels in serum phospholipids, a possibly more objective marker of TFA intake. The comparison between the two groups was made by the student *t* test for independent samples. The intake of synthetic TFA was low in both groups (type 1 diabetic patients: 0.25 ± 0.25 g/day; healthy subjects 0.48 ± 0.37 g/day), but significantly lower in diabetic patients vs controls ($P < 0.05$); TFA levels in serum phospholipids also confirmed a low intake of these fatty acids. These data indicate that the intake of trans fatty acids is relatively low in our population, i.e.,<1% of total calories in the diet, in line with what recommended by the World Health Organization.

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Introduction

Trans fatty acids (TFA) are produced by the partial hydrogenation of vegetable oils during industrial processes (synthetic TFA) and by the partial hydrogenation of unsaturated fatty acids due the bacteria present in the stomach of ruminants (natural TFA) [1].

The most important dietary sources of trans fatty acids are: commercially produced bakery products, snacks, crackers, margarine (trans fatty acids content over 60% of total fat), meat and dairy products (trans fatty acids content does not exceed 6% of total fat) [2, 3].

Over the last few years, a growing body of scientific evidence has shown that the consumption of trans fatty acids, especially the synthetic ones, induces pleiotropic negative effects on our body, by increasing LDL cholesterol, decreasing HDL cholesterol [4, 5], inducing systemic inflammation [6], altering endothelial function [6–8], thus increasing the risk of cardiovascular disease [9–17].

Given the negative effects of trans fatty acids on health, as evidenced by numerous epidemiological and experimental studies [4–17], several expert committees have recognized the importance of implementing strategies that reduce the consumption of these fats. In 2003, the World Health Organization (WHO) [18] and, in 2006, the American Heart Association (AHA) [19] stated that an intake of trans fatty acids <1% of total calories in the diet can help minimize its unhealthful effects. In addition to these recommendations, some governmental agencies have issued specific regulations aiming to reduce the consumption of trans

fatty acids [20–24]. Nonetheless, the data on the intake of TFA in Italy are quite scanty.

Moreover, since the data derived from dietary records are always based on the subjects' compliance, it would be useful to have more reliable information coming from the measurement of TFA in serum or other body compartments.

Therefore, we aimed to conduct a pilot study to evaluate the consumption of trans fatty acids, through dietary records or by their measurement in serum phospholipids, in a group of individuals likely to be especially exposed to consumption of TFA-containing foods, i.e., young people with and without type 1 diabetes.

Materials and methods

Study design and participants

Twenty-nine young subjects with type 1 diabetes and 25 healthy subjects, matched for age, sex, and body mass index (BMI), were recruited for this study after giving their informed consent; the inclusion criteria were age between 18 and 30 years, and BMI < 30 kg/m^2, and the exclusion criteria were anemia (Hb < 12 g/dl), renal or liver failure, or other chronic disease, treatment with lipid-lowering agents. Blood samples were taken in the morning after a 12-h fast together with anthropometric measurements.

The protocol was approved by the Federico II University Ethics Committee.

Dietary evaluation

Eating habits were assessed by asking participants to report in a diary all the foods and beverages consumed on 7 consecutive days (7-day food record), with the corresponding weight measured on their own scales. The diary was first checked by the dietitian who collected it and was then analyzed by a single dietitian, utilizing the Italian food composition tables [25, 26] for energy, protein, fat, carbohydrate, fiber, and cholesterol, and the UK Nutrient Databank [27] for trans fatty acids.

Trans fatty acids measurement in serum phospholipids

Serum samples were kept at -80°C until analyzed. 500 ml of serum samples were added with 1 ml of chloroform ($CHCL_3$) and 0.5 ml of methanol (CH_3OH). Proteins were removed by centrifugation at 12,000 rpm at room temperature for 3 min. Solid supernatant containing the proteins was removed, and polar lipid fraction containing the phospholipids was recovered by methanol using SPE silica gel column after eluting apolar lipid fraction with ethyl ether [28]. Fatty acid methyl esters from phospholipids were prepared by transesterification with 10% boron trifluoride (BF_3) in methanol. The trans fatty acids methyl esters (TFAs) were determined by gas chromatography using capillary column Restek 2330 (60 m $\times$ 0.32 mm id; ft: 0.25 μ) [29]. TFAs were identified by means of standard mix, and the results were expressed in % in weight (w/w). Each sample was extracted and identified twice to correct the variability of the method. Although gas chromatographic conditions separated all the trans isomers of 16 to 20 carbons (C16:1 *trans* 9, C18:1 *trans 9*, C18:1 *trans* 11, C18:2 *trans* 9-*trans* 12, C18:2 *cis* 9-*trans* 11, C20:1 *trans* 11), in general, 18:1 *trans* isomers were the most prominent component in all samples.

Statistical analysis

Data are expressed as mean $\pm$ standard deviation (M $\pm$ SD). The comparison between the diabetic patients and controls was made by the *t* test for independent samples. A *P*-value <0.05 was considered statistically significant. All statistical evaluations were performed with SPSS (Statistical Program for Social Science) 15.0 for Windows.

Results

As many as 54 young subjects, 29 type 1 diabetic and 25 healthy subjects were studied.

The two groups are very similar for age, anthropometric characteristics, and fasting plasma lipids (Table 1).The blood glucose control in people with type 1 diabetes was not completely satisfactory, as shown by their glycated hemoglobin values (HbA1c: 7.7 $\pm$ 1.2%).

The diet followed by the two groups was not different except for protein and fiber intake, which was slightly higher in type 1 diabetic patients compared to the control group (protein: 18.5 $\pm$ 3.0 vs 16.2 $\pm$ 2.9% of total caloric intake, with $P < 0.05$; fiber: 12.8 $\pm$ 4.3 vs 9.4 $\pm$ 3.5 g/1,000 kcal, with $P < 0.05$).

Table 1 reports the intake of trans fatty acids evaluated on the basis of the 7-days food records. The consumption of synthetic TFA was low in both groups of subjects and well below the recommended intake (<1% of calories). However, the intake of synthetic TFA was significantly higher in the control group compared to type 1 diabetic patients (0.48 $\pm$ 0.37 vs 0.25 $\pm$ 0.25 g/day, 0.22 $\pm$ 0.14 vs 0.13 $\pm$ 0.13% of calories, with $P < 0.05$ for both). No differences were found between the two groups for intake of natural TFA (Table 1).

The amount of synthetic trans fatty acids in serum phospholipids, expressed both as single trans fatty acid (C16:1 *trans* 9, C18:1 *trans* 9, C18:1 *trans* 11, C18:2 *trans* 9-*trans* 12, C18:2 *cis* 9-*trans* 11, C20:1 *trans* 11) and as the

Table 1 Characteristics of the participants and their intake of trans fatty acids by 7-day food record

	Type 1 diabetic patients	Control group
N	29	25
Men/Women	16/13	11/14
Age (years)	24 ± 3	23 ± 3
BMI (kg/m^2)	24 ± 3	24 ± 4
Waist circumference (cm)	83 ± 8	86 ± 12
HbA1c (%)	7.7 ± 1.2	–
Plasma triglycerides (mg/dl)	67 ± 27	75 ± 44
Plasma cholesterol (mg/dl)	157 ± 24	162 ± 28
Plasma HDL cholesterol (mg/dl)	51 ± 12	54 ± 12
Synthetic		
TFA (g/day)	0.25 ± 0.25	0.48 ± 0.37*
% of total energy	0.13 ± 0.13	0.22 ± 0.14*
Natural		
TFA (g/day)	0.58 ± 0.28	0.54 ± 0.37
% of total energy	0.31 ± 0.15	0.27 ± 0.18

Mean ± SD

* Significance controls vs diabetics $P < 0.05$

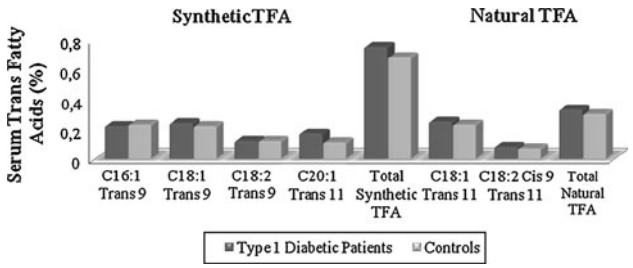

Fig. 1 Synthetic and natural trans fatty acids in serum phospholipids

sum of the single TFA, was quite low, and there were no differences between type 1 diabetic patients and the control group (Fig. 1). The same applies for natural TFA (Fig. 1).

Discussion

The results of this pilot study show that the consumption of trans fatty acids is less than 1% of the total calories of the diet, in line with what recommended by the World Health Organization (WHO) [18], in both type 1 diabetic patients and control subjects; this finding is of relevance because it was obtained in a group of young subjects who, for their eating habits (eating out, use of pre-packaged products), may be potentially more exposed to a higher consumption of foods containing trans fatty acids—at least for those industrially produced.

In addition, the data based on the dietary evaluation have been confirmed by the serum phospholipid levels, which are a possibly more objective marker of trans fatty acids intake.

This result shows that Italian youngsters have a low intake of trans fatty acids.

To this respect, the data on the Italian population are rather scanty. In fact, only two studies assessed trans fatty acids intake by dietary surveys: one conducted in Italian teenagers [30] and another one conducted in various European cohorts including one from Italy [31].

The first study showed that the intake of trans fatty acids was 1.23 ± 0.44% of total energy in the diet, which is slightly higher than our results. This study was conducted in Italy a few years ago among students attending a High School in the city of Arezzo, (central Italy), and, therefore, both age and different food habits, possibly related to regional differences, may account for the slight difference in the intake of trans fatty acids observed between the two study populations. The other study, published in 1999 and conducted in 14 European States, shows that the intake of trans fatty acids in Italy was quite low (0.5% of total energy), similarly to our results. These data were obtained with a market analysis and show some differences between populations: lower levels in Southern Europe (France and Italy, 0.5% of total calorie intake) and higher levels in Northern Europe (Iceland, 2.1% of total calorie intake).

In our study, young type 1 diabetic patients are characterized by a lower TFA intake, compared to controls. This is in contrast with the results of another study reported in the literature, where young type 1 diabetic patients living in the United States were characterized by a higher TFA intake compared to both our patients (2.36 ± 2.41 g/day vs 0.48 ± 0.37 g/day) and their control group [32]. The differences with our patients are likely ascribed to the different dietary habits but also to the different periods in which the two studies were conducted, as some governmental regulations aimed to reduce TFA in foods have been applied in recent years. Furthermore, our diabetic patients consumed significantly less TFA compared to their control group, at odds with what emerged from the US study, showing that our group of type 1 diabetic patients are well educated in terms of healthy eating pattern.

Data on TFA serum phospholipids are in agreement with those deriving from the analysis of food records, which supports the evidence of a low TFA intake in our participants. In only other two studies, TFA was measured in serum [33] and adipose tissue [34] and both studies show slightly higher levels compared to ours; however, in both cases, the populations studied were much older and this, together with different dietary habits, may explain the differences.

In conclusion, these data indicate that the intake of trans fatty acids is relatively low in our study population despite the fact that youngsters are, in general, particularly exposed to the use of packaged food rich in trans fatty acids. The low intake of TFA is confirmed also by the TFA levels in serum phospholipids.

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