PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 11 (5): 423-433, 2012
ISSN 1680-5194

Association of Dietary Intake of Trans Fatty Acids and Coronary Heart Disease Risk in Jordanian Subjects

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Abstract: The underlying role of dietary Trans Fatty Acid (TFA) intake in the aetiology of Coronary Heart Diseases (CHD) and its influence on serum lipid levels is very well established. The present study was conducted to estimate the dietary intake of TFA among selected cases of CHD and healthy participants and to investigate the strength of the association between TFA intake and CHD risk in Jordan. Using a case-control design, 100 patients known to have CHD of either sex and 91 healthy controls of age ≤60 y were examined. Daily intake of TFA was estimated using a semi-quantitative Food Frequency Questionnaire (FFQ). Mean daily dietary intake of %TFA was significantly higher in cases (0.78±0.55) as compared to controls (0.62±0.28, p = 0.01). Daily TFA intake was significantly positively associated with CHD risk in cases as compared to controls [RR: 2.4 (1.1-4.9), p = 0.01]. The RR of CHD for TFA intake within the highest quartile as compared to the lowest was associated with increased risk of CHD by 4.9 fold (95% CI: 1.3-17.4, p = 0.01) in cases as compared to controls. Finally, the major food sources of TFA intake was contributed by fast food, meats and dairy products. Therefore, proper food labeling of TFA, especially on local foods would help to minimize TFA intake and therefore reduce the risk of CHD incidence in Jordan.

Key words: Hydrogenation, trans fatty acid Intake (TFA), cardiovascular diseases, lipid profile

INTRODUCTION

Dietary Trans Fatty Acids (TFA) originate naturally from dairy and meat fats and commercially during hydrogenation of plant oils (Craig-Schmidt, 2006). Trans fatty acids are unsaturated fatty acids with at least one double bond in the *trans* configuration (Lichtenstein, 2000). Several studies have reported that TFA are similar in conformation and behavior to saturated fatty acids (Fernandez-San Juan, 2009; Lichtenstein, 2000). Moreover, the isomerization process occurs also in the rumen of cattle resulting in low levels of TFA in beef and dairy products (Ratnayake *et al*., 2009).

Cardiovascular diseases have been the leading cause for about 40% of all deaths in Europe and the United States (Ferdinand, 2006). It has been reported that partially hydrogenated oils might contain 30-50% TFAs, resulting in significant TFA intake in many populations (Mozaffarian *et al*., 2007). Many studies have shown that TFA intake is associated with increased risk of CHD (Fernandez-San Juan, 2009; Lichtenstein, 2000; Mozaffarian *et al*., 2007). Moreover, several studies have recently shown that dietary TFA intake is associated with other health problems such as breast cancer, poor growth and development (Innis *et al*., 1999; Stender and Dyerberg, 2004).

Although many studies have suggested an association between TFA intake and CHD incidence, they are limited in some respects. Several studies estimates of TFA intake were based on old or incomplete databases. Further, studies that provide evidence of correlation between TFA intake and CHD have used Food Frequency Questionnaire (FFQ) for TFA estimates. This method may provide estimates of questionable validity as compared to food records or recalls (Allison *et al*., 1999). Innis *et al*. (1999) stated that the validity of TFA content of foods could result in misclassification of TFA intake, hence, weaken the Relative Risk (RR) estimates of CHD. However, the Nurses' Health Study Cohort, a large prospective study, started in 1976 and involved 121,700 female nurses in 11 US states. Two-Food Frequency Questionnaire (FFQ) approximately two years apart and two diet recordings approximately six months apart were completed by 140 participants in the study. The authors compared fatty acid amounts in tissue with amounts that calculated from two FFQ and two food records. A moderate correlation (r = 0.40) has been reported between adipose TFA content and TFA intake. Many researchers highlighted the significance and the need for stating the amount of TFA content on food labels (Klurfeld, 1999; Ascherio *et al*., 1999).

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Nonetheless, data regarding TFAs intake and its association with CHD in developing countries are relatively scarce (Mozaffarian *et al*., 2006). As any developing country, Jordan experienced trends toward urbanization that is accompanied by changes in the lifestyle of individuals. In addition, there has been a shift in the lifestyle from active to sedentary and in the eating patterns from Mediterranean diet to fast food. Fast food pattern is high in fats, added sugars and salt, as well as it displaces healthy foods from the diet including milk, cereals, fruits and vegetables (Schmidt *et al*., 2005). The combination of these factors has posed dietary changes among Jordanians, increasing obesity probabilities and non-communicable diseases such as coronary heart diseases and diabetes mellitus (Alwan and Kharabsheh, 2006). It has been estimated that about 35% of all deaths in Jordan during the year 2005 are accounted for heart diseases (Brown *et al*., 2009).

Information on nutrition and health status of the Jordanian population is relatively scanty. The prices of fruits, vegetables and fortified cereals are relatively high in Jordan. Middle and lower-middle class families have no other alternative but to depend on carbohydrate and fat as the major sources of energy. Trans fatty acid content of foods is variable in processed foods, in hydrogenated oils and in smaller amounts, in meats and milk products (Fernandez-San Juan, 2009; Innis *et al*., 1999; Ratnayake *et al*., 2009). The major sources of TFA in the US are baked foods (37% TFA), deep-fried foods (36%) and margarines (11-49%) (Feldman *et al*., 1996). Data from our previous work involving the analysis of TFA content of local foods have illustrated for the first time that the average TFA levels in food groups in Jordan varied from 2.46±0.97 g/100 g fat to high up to 5.6±4.9 g/100 g fat (Mashal *et al*., 2011). Further, the highest level of TFA was contributed by fast food, fat and oils and baked sweets. Among milk and dairy products, TFA content of the traditional foods in this food group such as Jameed and Arabic cheese were up to ~5% (Mashal *et al*., 2011). Hence, the present study was conducted to estimate the dietary intake of TFA among selected cases of CHD and healthy participants and to investigate the strength of the association between TFA intake and CHD risk in Jordan.

MATERIALS AND METHODS

Research design: A case-control design was used to investigate the association between dietary intake of TFA and CHD risk in Jordanian subjects. Matching of cases and controls by age and gender was not feasible. Therefore, to satisfy the objectives of the present study, subjects were divided into 4 groups according to their gender and groups: 1) Male cases of CHD, 2) Female cases, 3) Male controls and 4) Female controls.

Human subjects: In the present case-control study, 100 patients known to have CHD of either sex and 91 healthy controls of age ≤60 y were examined. The protocol approval was obtained from the University of Jordan and the Protection of Human Subjects Ethics Committee in King Hussein Medical Center in Amman, Jordan. Only cases who were diagnosed during the previous year and confirmed to have clinical evidence of CHD were considered eligible to participate in the study. The inclusion of only recently diagnosed cases is performed to exclude any confounding factors that may be caused by treatment or lifestyle modifications. The clinical and investigational evidence of CHD was determined by an experienced cardiologist. Furthermore, patients with a history of chronic diseases such as: renal disease and diabetes mellitus that are known to increase the risk of CHD were excluded from the study. Controls who were apparently healthy and take no medication were considered eligible to participate in this study. All subjects were recruited from King Hussein Medical Center in Amman, Jordan. Healthy controls were selected from the personnel of the same center and their relatives or friends. Matching the cases with subjects from the personnel was relatively difficult due to demographic variations among both groups. Subjects from the personnel or their relatives were younger and mostly males, which made the matching more complicated.

Overall, signed informed consent was obtained from all subjects who were eligible to participate in the study.

Procedures

Dietary intake data collection: A semi-quantitative Food Frequency Questionnaire (FFQ) was developed and adapted to the Jordanian setting and was structured to follow a typical Jordanian daily meal pattern using a sample questionnaire provided by Gibson (1990). The FFQ was designed to make it more sensitive to fat intake. In addition, additional information on preparation method in cooking, sauces or dressings added and the type and amount of fat added in both cooking and at the table were also recorded. A comprehensive list of foods that accounted for the total fat intake was prepared. The final questionnaire included more than 85 food items or 9 food groups after it was pilot-tested in a group of 10 participants (5 cases, 5 controls), then minor modifications were made for precision. The test retest reliability value was 0.75 which falls within the conventionally accepted range from 0.4 to 0.7 (Frances, 1994). The reference period included the four weeks preceding the interview. The frequency of consumption of each food item was recorded as frequency per day and per week. All subjects were asked to respond to questions on usual frequency of intake of food items and portion size. Visual aids and food models were used to minimize errors in estimation of portion size. Calculations for TFA intake was estimated using a food processor software program (esha), which was updated with a food database assessed in a previous work of

122 local and imported foods analyzed specifically for this study (Mashal *et al*., 2011). The total intake of TFA was estimated by multiplying the reported frequency of each food by the amount of TFA in a serving of that food.

Demographic data: The questionnaire provided information on demographic data including age, sex, height, weight, family history of CHD and education.

Biochemical analyses: Blood samples for analyses of lipid profile were obtained from all subjects after an overnight fast. Lipid profile values including Total Cholesterol (TC), Low-Density Lipoprotein (LDL-C) and High-Density Lipoprotein (HDL-C) for cases were obtained from the medical records. Additional fasting blood samples were obtained from controls for lipid profile analyses at the laboratories of the King Hussein Medical Center.

Statistical analysis: The statistical analyses were performed using the SPSS Graduate Pack 17.0 for Windows (2007). The case-control differences in TFA intake and CHD predictors were examined using ANOVA for continuous variables and chi square test for categorical variables. Data was presented as means± SE and frequency distributions.

To satisfy the objectives of the present study, logistic regression analyses were performed to examine the association between TFA intake and other determinants in relation to CHD risk. In these analyses, all subjects were stratified into four strata according to their gender and groups. The RR was reported as odds ratios with 95% Confidence Interval (CI) for these analyses. All two-tailed p values of $\leq$0.05 were considered significant.

To determine the association between TFA intake and other suspected predictors of CHD risk, linear regression analyses were performed. In these models, TFA intake was the independent variable and all other predictors were entered as the dependent variables. Overall, TFA intake was divided into quartiles and then differences in CHD predictors were examined in each quartile by Analysis of Variance (ANOVA).

RESULTS

General characteristics of the full cohort: Mean age for cases was significantly higher than that of controls (51±8.8 vs. 33.1±5.3, p = 0.00) (Table 1). There were non-significantly more males than females in cases group. However, cases of both gender were significantly older than controls (p = 0.00). Family history of CHD was significantly higher in cases as compared to controls. Approximately 41.9% of the subjects were with a family history of CHD (Case Subjects: 51%; Control subjects: 31.9%). Among males, approximately 49% of the cases had a family history of CHD as compared to controls (14.3%, p = 0.002). Similarly, approximately 65% of the cases of CHD had hypertension as compared to controls (1.1%, p = 0.000). There were significantly more hypertensive males and females in cases group as compared to controls, respectively (64.4% and 65.4%, p = 0.000). Cases of CHD had significantly higher mean BMI and were overweight as compared to controls, respectively (29.2±5.4 and 24.8±4.0, p = 0.000). Within groups, there were non-significantly different mean BMI between males and females. However, cases of CHD of both gender had significantly higher mean BMI as compared to controls (p<0.05). Overall, mean systolic blood pressure was significantly higher in cases

Table 1: General characteristics of the full cohort*

	Total (n = 191)	Males	Females	P†
Age				0.00
Case	50.8±8.8	50.2±8.4[a]	52.6±9.7[a]	
Control	33.1±5.3	31.9±4.3[b]	33.6±5.5[b]	
Gender				0.1
Case		74 (74.0)	26 (26.0)	
Control		28 (30.8)	63 (69.2)	
Family history of CHD				0.005
Case	51(51.0)	36 (48.6)[a]	15 (57.7)[a]	
Control	29 (31.9)	4 (14.3)[b]	25(39.7)[a]	
Hypertension				0.000
Case	64 (64.6)	47 (64.4)[a]	17(65.4)[a]	
Control	1 (1.1)	-[b]	1 (1.6)[b]	
Body Mass Index (BMI)†				0.000
Case	29.2±5.4	28.8±4.9[a]	30.1±6.5[a]	
Control	24.8±4.0	24.4±3.4[b]	25.0±4.3[b]	
Systolic blood pressure (mm Hg)				0.00
Case	125.1±1.5	124.9±1.6[a]	125.7±4.0[a]	
Control	116.4±0.7	118.2±0.7[a]	115.6±1.0[b]	
Diastolic blood pressure (mm Hg)				NS
Case	77.5±1.0	78.4±1.2	75.2±1.9	
Control	77.4±0.5	79.1±0.5	76.7±0.7	

*Values with different letters in columns and with same letters in rows are significantly different from each other.
†Values represents p for the difference between groups of the full cohort

Table 2: Biochemical variables for cases and controls by gender*

	Full cohort	Males	Female	Sig.§
Total cholesterol levels (mg/dl)				NS
Case	149.4±05.1	194.1±04.4	195.1±12.3	
Control	206.2±05.2	213.0±12.1	203.2±05.3	
Triglycerides levels (mg/dl)				0.00
Case	215.0±11.5	225.6±13.7[a]	184.8±20.0[b]	
Control	155.4±14.5	225.7±38.3[a]	124.1±10.2[a]	
HDL-cholesterol (mg/dl)				0.00
Case	47.4±02.1	45.8±02.1[a]	54.9±05.3[b]	
Control	57.4±01.7	47.2±02.2[a]	61.8±01.9[a]	
LDL-cholesterol (mg/dl)				NS
Case	125.2±16.4	108.7±06.1[a]	205.9±91.8[a]	
Control	117.4±04.4	118.0±09.9[a]	116.7±04.6[b]	

*Mean±SE. §Values represents p for the difference between groups of the full cohort; values in rows with the same letter are significantly different from each other (p<0.05)

Table 3: Percent daily dietary intake of TFA in cases and controls by gender

	TFA Intake*		
	Full cohort	Males	Females
TFA intake/day (%)†			
Case	0.78±0.55[a]	0.79±0.53[a]	0.75±0.61[a]
Control	0.62±0.28[b]	0.54±0.17[a]	0.64±0.31[a]
Total	0.70±0.03	0.73±0.04	0.68±0.04
% TFA of fat calories/day			
Cases	0.09±0.006	0.087±0.006	0.089±0.015
Controls	0.07±0.004	0.074±0.012	0.072±0.004
Total	0.08±0.004	0.08±0.006	0.07±0.005
Total TFA intake/day (g)			
Cases	4.9±0.45[a]	5.5±0.58[a]	3.3±0.45[b]
Controls	4.1±0.31[a]	4.5±0.64	3.9±0.35
Total	4.6±0.28	5.2±0.46[a]	3.7±0.28[b]

*Mean±SE. †Values in columns and rows with different letters are significantly different from each other

(125.1±1.5) as compared to controls (116.4±0.7, p = 0.00). Among females, cases had significantly higher mean systolic blood pressure as compared to controls (125.7±4.0 and 115.6±1.0, respectively; p = 0.003). Diastolic blood pressure did not differ significantly between groups or by gender in both groups (p = 0.07).

Differences in lipid profile among groups: Case-control differences in lipid profile by gender are shown in Table 2. Mean cholesterol levels were non-significantly higher in controls than in cases (p = 0.1). Triglyceride levels were significantly higher in cases than in controls (p = 0.001). In cases as a group, males had non-significantly higher levels of Triglyceride than females. Among controls, males had significantly higher levels of Triglyceride than that of females (p<0.05). However, females cases had significantly higher levels of Triglycerides than female controls (p = 0.001). High-density lipoprotein levels were significantly lower in cases as compared to controls (p = 0.00). Moreover, males had significantly lower HDL-C levels than females in controls group (p = 0.00). Low-density lipoprotein levels did not differ in both groups. Among cases, females had significantly higher LDL-C levels than males. Interestingly, among females, LDL-C levels were significantly higher in cases than that in controls (p = 0.00).

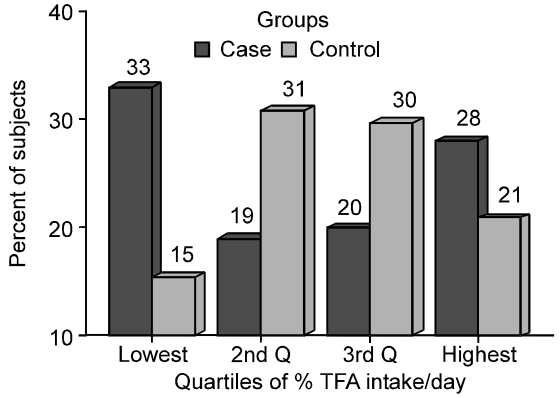

Fig. 1: Distribution of participants by quartiles of TFA intake

Dietary intake pattern of TFA: Mean daily dietary intake of %TFA was significantly higher in cases (0.78±0.55) as compared to controls (0.62±0.28, p = 0.01). Similarly, mean dietary intake of %TFA consumed per week was also higher in cases as compared to controls (p = 0.01). Daily and weekly dietary intake of %TFA did not differ significantly by gender in both groups (Table 3). Figure 1 illustrates the percent of subjects in each group at each quartile of total TFA intake of participants. The percent of subjects in the case group who were within

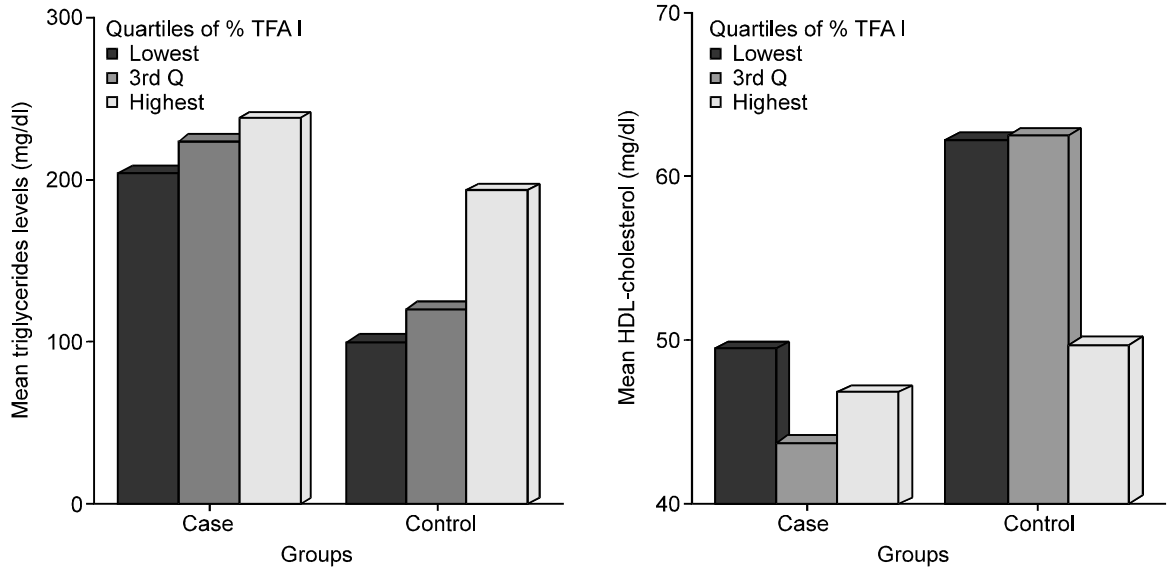

Fig. 2: Differences in triglyceride and HDL-C levels by quartiles of TFA intake in cases and controls

Table 4: The frequency of daily TFA intake by groups and gender*

	Quartiles of TFA intake/day			
Groups**	1st Q	2nd Q	3rd Q	4th Q
Cases				
Males	35.1%	18.9%	21.6%	24.3%
Females	26.9%	19.2%	15.9%	38.5%
Controls				
Males	-	52.0%	24.0%	24.0%
Females	22.2%	23.8%	33.3%	20.6%

**Differences among groups were significant at p = 0.005

the 4th quartile of TFA intake (28%) was significantly higher ($p<0.05$) from those who were in the control group (21%). Within the control group, the proportion of participants with the highest quartile of TFA intake (21%) was higher as compared to participants who fall within the lowest quartile of the percent TFA intake (15%).

Among males, the proportion of cases who fall in the highest quartile of TFA intake was similar to that of controls (24.3% and 24%, $p<0.05$; respectively). Conversely, the proportion of the female cases who were within the 4th quartile of TFA intake was significantly higher than those in the control group (38.5% and 20.6%, respectively; $p>0.05$) (Table 4).

Differences in selected predictors of CHD by the quartiles of daily TFA intakes are presented in Table 5. For all participants, only BMI and Triglyceride levels showed a significant difference across the quartiles of TFA intake. Surprisingly, participants who were within the 1st quartile of TFA intake had significantly higher BMI than those who fall within the 3rd quartile of TFA intake (28.2±5.3 and 25.4±4.3, respectively; $p<0.05$). Participants who fall within the 4th quartile of TFA had significantly higher Triglyceride levels than those who fall within the 1st quartile of TFA intake (219.5±123 and 172.7±97.1, respectively). In a separate bivariate model in which BMI was the dependent variable, daily dietary intake of total TFA (%) was highly positively associated with BMI ($\beta = 1.8$, $p = 0.02$) (Fig. 3). Similarly, DBP was significantly inversely associated daily intake of TFA as a percent of fat ($\beta = 2.4$, $p = 0.04$).

The percent contribution of food items to total TFA per day of the full cohort, cases and controls are illustrated in Table 6. In our study, the major source of dietary TFA intake was contributed by meat (Kofta) (7.4%) followed by chocolates (5.4%), Nabulsi white cheese (4.8%), falafel (3.5%), jameed (3.2%), French fries (3.1%) hamburger (3.0%) and shawerma (1.6-1.7%) come after. Kofta is a Middle Eastern meatballs or meatloaf, which consists of ground beef or lamb mixed with spices and onion. Jameed is an ingredient found in the Jordanian dish called "mansaf.". Mansaf is the national dish in Jordan that is made on special occasions. It is composed of meat, Jameed, thin wheat bread and rice. Jameed is a hard dry rocklike form of yogurt made from sheep's milk and is diluted when used in cooking. Nabulsi white cheese is the principle cheese consumed in Jordan and made up mainly from sheep or goat milk. Shawerma is a fast food staple across the Arab world and Europe. It is made in small pita breads which are sliced open and filled in with stoved grilled meat or chicken, garlic sauce and pickles.

Cases had significantly ($p<0.05$) higher mean TFA levels from Jameed, dried ready meals (soup) and crackers food items as compared to controls. Controls had significantly higher mean daily dietary intake of TFA from French fries and cakes as compared to cases ($p < 0.05$). Mean daily dietary intake of TFA from Nabulsi white cheese, pizza, Hamburger and Arabic sweets was non-significantly higher in cases as compared to controls.

Table 5: Differences in selected predictors by quartiles of TFA Intake in all participants

	Quartiles of TFA intake/day			
Predictors	1st Q	2nd Q	3rd Q	4th Q
Age (y)	53.1±2.0	49.8±1.6	50.5±1.6	49.9±1.737
Gender				
Males	26.3%	27.3%	22.2%	24.2%
Females	23.6%	22.5%	28.1%	25.8%
Body mass index (kg/m^2)	25.4±4.3[a]	27.1±6.6	28.2±5.3b	27.7±4.2
Systolic blood pressure (mm Hg)	122.5±14.6	118.7±11.2	119.8±12.9	122.8±13.8
Diastolic blood pressure (mm Hg)	76.6±11.0	76.7±6.2	78.1±7.2	78.3±8.8
Total cholesterol levels (mg/dl)	192.7±44.7	209.2±49.8	202.9±48.0	191.5±49.6
Triglycerides levels (mg/dl)	172.7±97.1[a]	172.8±88.3	163.4±123.0	219.5±123.0[b]
HDL-cholesterol (mg/dl)	54.9±19.5	54.4±14.0	56.1±17.5	48.4±14.8
LDL-cholesterol (mg/dl)	101.6±38.5	125.7±53.3	140.8±145.4	108.0±36.5

Table 6: Trans fatty acid contribution of individual food items for the full sample and differences between groups

	Contribution of TFA/day*	TFA (g)/day†		
Food items	Full Cohort	Cases	Controls	P
Dairy products				
Jameed	3.2 (3.9)	4.40±0.71	1.60±0.17	0.00
Nabulsi white cheese	4.8 (42.0)	2.00±0.34	1.30±0.20	0.07
Snacks				
Soup	0.07 (0.62)	3.40±0.75	1.40±0.20	0.00
Cakes	1.6 (14.1)	0.75±0.12	1.60±0.32	0.02
Chips	1.2 (10.2)	0.10±0.02	0.14±0.02	NS
Crackers	0.03 (1.2)	0.30±0.14	0.05±0.02	0.02
Arabic sweet (Kinafeh)	1.3 (11.2)	0.87±0.13	0.60±0.08	0.09
Chocolates	5.4 (46.5)	0.27±0.01	0.24±0.04	NS
Fast food				
Pizza	0.6 (5.1)	1.30±0.44	0.65±0.09	0.06
Hamburger sandwich	3.0 (26.0)	1.30±0.34	0.82±0.14	0.08
French fries	3.1 (27.3)	0.72±0.12	1.20±0.22	0.05
Meat kofta/kabab	7.4 (448.6)	0.45±0.09	0.27±0.03	NS
Meat shawerma	1.7 (15.2)	0.87±0.35	0.71±0.11	NS
Chicken shawerma	1.6 (14.5)	0.22±0.03	0.18±0.02	NS
Falafel sandwich	3.5 (30.6)	0.32±0.04	0.25±0.02	NS

*TFA presented as% (g) from each food item consumed daily by the full sample.
†Mean±SE of TFA (g) consumed daily from each food item by cases and controls

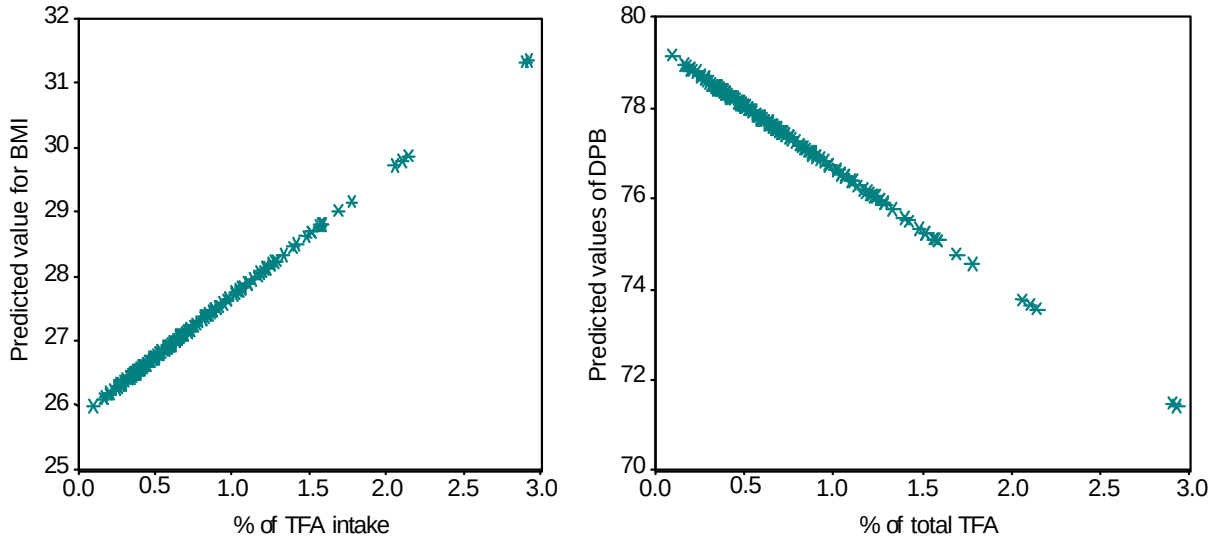

Fig. 3: Association between dietary intake of TFA, body mass index and diastolic blood pressure in both groups

Table 7: Relative risks for CHD in cases as compared to controls

	Relative risk (95% CI)	Sig.
Age	1.4 (1.2-1.7)	0.00
Gender†	15.6 (4.6-52.1)	0.03
BMI†	1.2 (1.0-1.3)	0.00
Cholesterol levels (mg/dl)	0.97 (0.91-1.05)	NS
Triglycerides (mg/dl)‡	1.0 (1.000-1.009)	0.04
HDL-cholesterol (mg/dl)§	0.96 (0.93-0.98)	0.00
LDL-cholesterol (mg/dl)	1.0 (0.99-1.00)	NS
Systolic BP	1.1 (1.0-1.2)	0.00
Diastolic BP	0.87 (0.78-0.97)	0.01
%TFA intake (/100 g fat/day)	5.2 (1.0-26.9)	0.04

†Controlling for age.
‡Controlling for age, sex and cholesterol.
§Controlling for age, sex, cholesterol, TG, SBP and DBP

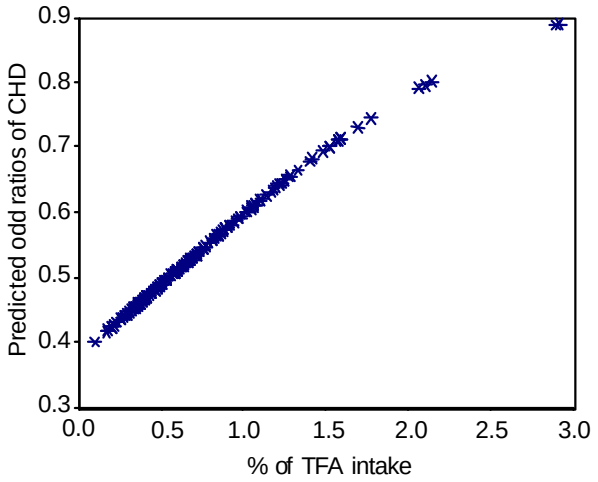

Fig. 4: Association between dietary intake of TFA and CHD risk in cases as compared to controls

Trans fatty acid intake, risk factors and coronary heart disease (CHD): The Relative Risks (RR) for cases of CHD as compared to controls in the presence of selected predictors are presented in Table 7. When all predictors were entered into the model, only age [RR: 1.4 (1.2-1.7), p = 0.00] and daily intake of TFA were significantly related to CHD risk [RR: % TFA: 16.5 (0.95-284.3), p = 0.05] (data not shown). Controlling for age in this model, the RR of CHD for daily TFA intake was decreased but remained a significant predictor of CHD risk in cases as compared to controls as shown in Table 7. However, the RR for CHD was also examined in a single logistic regression analyses (Fig. 2). In the previous model, daily TFA intake was significantly positively associated with CHD risk in cases as compared to controls [RR: 2.4 (1.1-4.9), p = 0.01]. Table 7 shows that gender, BMI, triglyceride levels and systolic blood pressure were significantly associated with increased risk of CHD [15.6(4.6 - 52.1), p = 0.03; 1.2 (1.0-1.3, p = 0.00; 1.0 (1.0-1.009), p = 0.04 and 1.1 (1.0-1.2), p = 0.01; respectively]. Body mass index, gender and systolic blood pressure were significant predictors of CHD when adjusted for age ($p<0.05$). Only after additional adjustment for gender and cholesterol levels, triglyceride level was found to be a significant predictor of CHD ($p<0.05$). Both cholesterol levels and LDL-C were not significant predictors of CHD. Controlling for age, diastolic blood pressure was inversely related to CHD risk in cases as compared to controls [RR: 0.87 (0.78-0.97), p = 0.01]. Similarly, HDL-C level was negatively associated with CHD risk when proper adjustments were made (Table 7).

DISCUSSION

Cardiovascular Disease (CVD) has been a public health problem of widespread occurrence in many parts of the world, which accounts for most of the mortality and morbidity. However, age, gender, hyperlipidemia, hypertension and obesity are all known CHD risk factors (Smith, 2006). Many studies have addressed the possible adverse effects of TFA on health and its association with CHD risk in particular. The underlying role of dietary TFA intake in the aetiology of cardiovascular diseases and Its influence on serum lipid levels is very well established (Katan, 2000; Lichtenstein, 1997; Lichtenstein, 2000; Lopez-Garcia *et al*., 2005). Concern has arisen from a number of metabolic and epidemiologic investigations. Generally, many metabolic studies have found that diet high in TFA increases LDL cholesterol levels and also decreases HDL cholesterol levels (Mozaffarian *et al*., 2009).

Ascherio *et al*. (1999) summarized the results of several randomized studies that investigated the effects of high TFA diet or saturated fatty acids diet on the ratio of LDL/HDL cholesterol. It has been found that the effects of TFA on the LDL/HDL cholesterol ratio was significantly greater than that of saturated fatty acid in each of the six studies. Moreover, the best-fit regression line indicates that the ratio of LDL/HDL cholesterol would increase by 0.1 unit with an absolute increase of 2% in TFA intake. A 1-unit increase is associated with 53% increase in the risk of CHD. Moreover, several case-control studies have been conducted to investigate the association between TFA intake and the risk of CHD. Ascherio *et al*. (1994) investigated the association between TFA intake and the risk of Myocardial Infarction (MI) using a case-control design. The study involved 239 patients with MI and 282 healthy controls in Boston area. The authors found a strong positive association between TFA intake and the risk of MI. the RR for MI was 2.44 (95% CI: 1.42, 4.19; $p<0.0001$) in subjects who fall in the highest quintile as compared to the lowest quintile of TFA intake. This relation remained highly significant after adjustment for conventional coronary risk factors, multivitamin use and the intake of other dietary components such as saturated fat, monounsaturated fat, linoleic acid, cholesterol, vitamins E and C, carotene and fiber.

Results from the present study were consistent with the previous findings. Trans fatty acid intake was highly significantly independently associated with increased risk of CHD. The risk of CHD for daily intake of TFA was increased by 2.4 (95% CI: 1.1-4.9, p = 0.01) fold in cases as compared to controls. However, controlling for age, the multiplicative effect of TFA intake in the presence of selected conventional predictors on CHD risk was increased by 5.2 (95% CI: 1.0-26.9, p = 0.4) in cases as compared to controls. In our study, the RR of CHD for TFA intake within the highest quartile as compared to the lowest was associated with increased risk of CHD by 4.9 fold (95% CI: 1.3-17.4, p = 0.01) in cases as compared to controls.

Louheranta *et al.* (1999) investigated the effect of TFA on serum lipids and lipoproteins among 14 young healthy women. The mean age was 23±3 years (mean±SD). All subjects were assigned to a high trans fatty acid diet [(TFA diet)] and a [high-oleic acid diet (MUFA diet)] for 4 weeks in a randomized crossover design. The subjects were asked to keep 7-day food records during the experimental diet periods. The food composition tables were based on estimates attained from Finnish food analyses and from the international composition tables. It has been found that total cholesterol/HDL ratio was significantly ($p>0.05$) increased after the TFA diet compared to the MUFA diet and so does HDL, LDL triglycerides and apo B. The authors concluded that moderate increase in TFA intake has changed the lipid profile to a higher atherogenic direction (Louheranta *et al.*, 1999). In our sample, only triglycerides and HDL-C levels were significantly associated with TFA intake. Compared to subjects at the lowest quartile of TFA intake, triglyceride levels were significantly higher ($p<0.05$) and HDL-C levels were non-significantly lower (p for trend = 0.06) in subjects at the 4th quartile of TFA intake. Similarly, among groups, cases with CHD at the highest quartile of TFA intake had significantly higher triglyceride and lower HDL-C levels as compared to that for controls ($p<0.05$).

Generally, most of these assertions were based on the following: the changes induced in plasma lipid profile (Mozaffarian *et al.*, 2007; Ratnayake *et al.*, 2009); the relation between adipose tissue composition and TFA dietary intake (Garland *et al.*, 1998) and the effects of TFA on calcium influx human arterial cells (Kummerow *et al.*, 1999). Kummerow *et al.* (1999) study, using arterial endothelial cells as a model, investigated TFA and Magnesium (Mg) influence on cell membrane composition and Calcium (Ca)influx into arterial cells. The results showed that a diet high in TFA and low in Mg increased the risk of atherosclerosis ($p<0.05$) by increasing the calcification of endothelial cells. The correlation between Mg and the atherogenic effects of TFA can be explained by the pronounced effect of Mg on the physical state of cell membrane bilayer lipids. Mg acts as a cofactor in the activity of desaturase enzymes that are involved in decreasing the levels of Unsaturated Fatty Acids (USFA) in cell membrane.

Although the associated increased risk of CHD with TFA intake would be contributed to its effects on serum lipids, the available evidence from prospective studies on the relationship between TFA intake and CHD risk has been stronger than that was based on the changes induced in serum lipid levels alone. This may indicate that TFA intake may also influence other risk factors for CHD (Mozaffarian *et al.*, 2006). In line with this, the present data illustrated that among the examined classical CHD risk factors; only BMI and DBP were significantly associated with TFA intake. The best-fit regression line indicated that the predicted increase in BMI was 1.8 for each unit change in TFA intake ($p<0.05$). Conversely, DBP was significantly inversely associated with TFA intake. For each unit change in TFA, DBP was decreased significantly by 0.15 ($p = 0.04$).

Although many epidemiologic studies have supported the association between TFA intake and the disease incidence, they are limited in some respects. It is believed that the assessment of TFA intake in populations is determined by two major factors. First, the usual food intake using dietary recalls, records or FFQs and the availability of updated food composition tables that include TFA composition of food items (Skeaff, 2009). I fact, the availability of these updated food composition databases is limited to only few countries including the United States, Germany and the United Kingdom (Skeaff, 2009). Several studies estimates of TFA intake were based on old or incomplete databases. Moreover, studies that provide evidence of a correlation between TFA intake and CHD have used FFQ for TFA estimates. Food frequency questionnare may provide estimates of questionable validity in terms of portion size, food categories and recall of past events as compared to food recordings. Lemaitre *et al.* (1998) assessed the validity of using a self-administered FFQ to estimate TFA intake by comparing dietary intake of TFA with trans-fatty acid concentrations in adipose tissue. The authors concluded that the FFQ method can provide accurate estimates of dietary TFA intake. Conversely, Cantwell *et al.* (2004) results were inconsistent with the previous findings. The authors conducted a study to assess the validity of Fat Intake Questionnaire (FIQ) in determination of usual dietary fat intake. The study compared the dietary estimates of linoleic acid and TFA obtained by FIQ and Dietary history (DH) with adipose tissue concentrations of these fatty acids. Although estimates of fatty acid intake obtained by both methods were significantly correlated, the estimates of TFA intake provided by the FIQ were poorly correlated with the concentrations in adipose tissue as compared to estimates of DH.

Fortunately, dietary intake of TFA in the present study was assessed using the database provided by the U.S. Department of Agriculture and an updated food database that included TFA content of 122 Arabic foods including imported and locally produced foods that are available in the Jordanian market (Mashal *et al*., 2011). For instance, Allison *et al*. (1999) estimated the mean level of TFA intakes among a representative sample of the US population, using dietary recall or records and the most updated food composition databases. The results suggested that the US population intake of TFA was 5.3g per day and 2.6% of their total energy and 7.4% of their fat energy. Moreover, results have shown that about 20-25% of TFA intake was from naturally occurring sources, therefore, dieticians would be advised to take it into consideration when planning diet for individuals. However, based on extensive body of epidemiological and experimental evidence relating the health implications of TFA intake, the Joint WHO/FAO Expert Consultation on Diet, Nutrition and the Prevention of Chronic Diseases recommended that the mean dietary intake of TFA from hydrogenated oils and fats should be limited to less than 1% of energy intake (Skeaff, 2009). Recently, a national study conducted in Canada has reported that the estimated average intake of TFA in 2008 has been 3.4 g/day (1.4% energy) (Ratnayake *et al*., 2009). Researchers have provided that mean daily intakes of TFA in different European countries has been 1.4 g/day (0.6% energy) in Greece, 2.3 g/day (1.2% energy) in France, 2.8 g/day (1.3% energy) in United Kingdom and 5.4 g/day (2% energy) in Iceland (Fernandez-San Juan, 2009; Craig-Schmidt, 2006). Overall, it has been reported that Asian countries such as Iran consumes high amounts of hydrogenated oils which constitute about 12.5% of their total energy intake (Mozaffarian *et al*., 2007).

In this context, data from the present study showed that total TFA intake for the full cohort was 4.6 g/day, 0.7% of their fat intake and 0.8% of their fat energy intake. TFA percentage of fats was significantly higher in cases (0.78 %) than that of controls (0.62%). However, total TFA intake was non-significantly higher in cases with CHD as compared to controls (4.9 g and 4.1 g, respectively) corresponding to 0.09% and 0.07% of their fat energy (p for trend = 0.06). Generally, total TFA intake was significantly higher in males than in females (5.2 g and 3.7 g, respectively; p = 0.00). Among cases but not controls, males had significantly higher intake of total TFA as compared to females. Moreover, the percent of females whose TFA intake was at the highest quartile for daily TFA intake was 38.5% in the case group as compared to 20.6% in the control group. Conversely, the percent of males who were at the highest quartile for TFA intake was similar in both the case and control groups (24% and 24.3%, respectively).These gender differences between cases and controls in relation to TFA intake could be due to the fact that the majority of cases in our sample were males as compared to controls in which the majority of them were females. In addition, it is believed that the validity of FFQ method in assessing dietary intake could be influenced by a number of factors. Marks *et al*. (2006) investigated the validity of FFQ in obtaining food intake estimates using a community-based sample of 96 Australian adults who completed a self-administered semi-quantitative FFQ. The authors concluded that there is a gender difference in the relative validity of food intake estimates, which indicates the importance of appropriate adjustment for factors affecting the validity of food intake estimates when assessing diet-disease associations.

Trans fatty acids are commercially produced when unsaturated fatty acids undergo hydrogenation. Moreover, the isomerization process occurs also in the rumen of cattle resulting in low levels of TFA in beef (6%) and dairy products (2%) (Ratnayake *et al*., 2009). Trans fatty acid content of foods is variable in processed foods, in hydrogenated oils and in smaller amounts, in meats and milk products (Fernandez-San Juan, 2009). The major sources of TFA in the US are baked foods (37% TFA), deep-fried foods (36%) and margarines (11-49%) (Feldman *et al*., 1996). In our sample, the majority of TFA intake was contributed by meats, chocolates and dairy products and to a lesser extent by snacks and fast food. Generally, cases of CHD tended to consume higher amounts of TFA from dairy products, snacks and fast food. However, whether the associated changes in lipid profile with TFA intake is influenced by the source of TFA (naturally occurring vs. industrially produced) is not yet clear. Further studies are needed to address these associations (Food Safety, 2007).

However, many researchers highlighted the significance and the need for stating the amount of TFA content on food labels. Neuhouser *et al*. (1999) examined the relation between label use and diet. The association between reading nutrition labels and eating lower fat diet was significantly high. The results provide evidence that the use of labels helps individuals who are concerned to select lower fat foods. Further, it has been suggested that by providing the proper motivations for the food industry, partially hydrogenated oils could be replaced with unhydrogenated oils in which it would reduce the CHD risk at a moderate cost (Ascherio *et al*., 1999; Mozaffarian *et al*., 2007) In addition, labeling regulations should also include fast foods due to the fact that 1 doughnut contains 3.2 g of TFA and large French fries contain up to 6.8 g TFA. However, improving the hydrogenation process through modification of temperature, pressure, catalyst and starting oils can decrease the formation of TFA. Moreover, new food technologies such as genetic engineering of oil seed plants may result in lowering the TFA content of the US diet (Feldman *et al*., 1996).

In Europe, food producers responded and TFA-free margarine is available in the market; these products are becoming also available in the US markets (Ascherio *et al*., 1999). Recently, the FDA proposed new regulations to require that the amount of TFA be included in the nutrition fact panel. The new regulations were based on the consistent results provided by a large number of metabolic studies and recent reports of other government bodies relating TFA intake and its adverse effect on LDL-C levels, which contribute to increased risk of CHD. The FDA is convinced that the new regulations will provide beneficial nutrition information so that consumers will not be misled about the negative impact of a specific product on CHD risk and it would help consumers to maintain healthy dietary habits as well (FDA, 1999).

Conclusion: TFA are formed when unsaturated fatty acids undergo hydrogenation. This process may occur naturally in beef, butter and milk fats. Many metabolic and epidemiologic studies provided strong evidence that TFA intake is associated with increased risk of CHD. Trans fatty acid intake was highly significantly independently associated with increased risk of CHD in a selected Arab Jordanian sample. The risk of CHD for daily intake of TFA was increased by 2.4 (95% CI: 1.1-4.9, p = 0.01) fold in cases as compared to controls. However, controlling for age, the multiplicative effect of TFA intake in the presence of selected conventional predictors on CHD risk was increased by 5.2 (95% CI: 1.0-26.9, p = 0.4) in cases as compared to controls. In our study, the RR of CHD for TFA intake within the highest quartile as compared to the lowest was associated with increased risk of CHD by 4.9 fold (95% CI: 1.3-17.4, p = 0.01) in cases as compared to controls.

The major source of TFA was contributed primarily by meats, deep fried foods and dairy products. Therefore, we fully support the suggestion that consumers have a fundamental right to be informed of what they are eating and that it is safe and has no adverse effects on their health. Moreover, proper food labeling of TFA, especially on foods that have misleading claims such as "cholesterol free" or "low fat", would help to minimize TFA intake and therefore reduce the risk of CHD incidence in Jordan. Recently, the FDA proposed new regulations to require that the amounts of TFA be included in the nutrition fact panel. Further investigations are needed to eliminate the overall limitations in favor of providing better understanding toward motivation of the general health and well being.

ACKNOWLEDGEMENTS

Sponsored by: Research Support Fund of the University of Jordan.

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