Circulating concentrations of trans fatty acids between 2004 and 2010: A cross-sectional study of young healthy Canadian adults

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Abstract

Trans fatty acids (TFA) produced from industrial partial hydrogenation of vegetable oils (PHVO) have been the subject of much debate regarding their negative effect on the development of chronic diseases and the need for improved regulatory guidelines to limit or eradicate their use in industrially-produced foods. TFA are produced both naturally and industrially; however, only PHVO TFA have been linked to inflammation and increased risk of cardiovascular disease. In Canada, recommendations to limit PHVO levels in foods were introduced in 2007. Voluntary adherence to guidelines by Canadian food suppliers resulted in the elimination of the majority of PHVO in foods as well as a decrease in estimated intake of PHVO. However, direct measurement of circulating TFA concentrations in Canadians as an accurate and confirmatory measure of exposure, post-introduction of guidelines, has not been performed. In this study we determined circulating concentrations of natural and PHVO derived TFA over time (2004-2010) in a cross-sectional cohort of young healthy Canadians. Our results demonstrated that over the study period minimal changes in total TFA concentrations occurred (R = -0.02, p = 0.0043); however, these changes reflected a statistically small increase in PHVO TFA (R = 0.19, p <0.0001), but significant declines in circulating natural TFA (R = -0.32, p = 0.0009). Our findings provide direct insight into the exposure level of TFA in young Canadians, which suggests that while levels of TFA have changed in many foods, levels of PHVO TFA in this segment of the population has not declined.

Introduction

A large body of research has demonstrated the negative impact of partially hydrogenated vegetable oils (PHVO) trans fatty acids (TFA) on health in contrast with naturally occurring TFA (1-3). Intake of PHVO TFA has been shown to be pro-inflammatory, increase LDL-cholesterol, decrease HDL-cholesterol and increases the risk for coronary heart disease (4, 5). These major findings have lead to the establishment of initiatives around the world regulating TFA in the food supply. In 2003, the Danish government limited the accepted amount of PHVO TFA to 2% of total fat in foods (6). In 2007, the Canadian government adopted recommendations from the 2006 Health Canada's Trans Fat Task Force (TFTF) with respect to limits on trans fats in vegetable oils and soft margarines to 2% of total fats and limits on trans fats in all other foods to 5% of total fats (7). Health Canada called for these limits to be achieved within two years and according to Health Canada's Trans Monitoring Program (from 2007 to 2009) PHVO TFA were eliminated from the majority of foods sold in Canada (7, 8). The United States has considered TFA as "generally regarded as safe" (GRAS) for decades; however, in 2013 the US Food and Drug Administration proposed to remove the GRAS status of PHVO (9).

TFA are products of natural bio-hydrogenation of unsaturated fats by micro-organisms of ruminants or industrial partial hydrogenation of vegetable oils (10). PHVO are desirable to the food industry because they improve the texture and stability of food products and extend their shelf life (4, 8, 11). TFA isomers present in food products include C16:1t, C18:1t, C18:2t and other long chain polyunsaturated trans fatty acids (4, 12, 13). The C18:1t isomers account for 80-90% of total TFA in foods with C18:1t9 and t10 being the major industrially produced isomers (4, 12, 13). Similar isomers of TFA are also found in ruminant products, but the overall profile of different types and proportions of TFA isomers is markedly different (14, 15). TFA in

ruminant meats and milk account for about 2-6% of total fat content (8). The predominant and unique TFA found in ruminant products are C16:1t9, C18:1t11 and conjugated linoleic acid (CLA) isomers C18:2c9t11 and C18:2t10c12 (4). It is challenging to determine where specific TFA are obtained in the diet due to the similarities in the profile in natural and industrial sources of TFA. Nevertheless, it is possible to identify trends in natural and PHVO TFA intake by examining the major TFA isomers found in these types of foods.

Studies by Health Canada reported a significant decrease in estimated average intake of TFA in Canada, in children and adults, from 8.4 g/d (~3.7% of Energy) in 1990s to 3.4 g/d (~1.4% of Energy) in 2008 (8). Although dietary intake of PHVO TFA has decreased, confirmatory measurement in blood has not yet been comprehensively examined. Such data would compliment dietary intakes findings in Canada. While indirect measures strongly suggest positive changes have occurred, direct measures provide objective evidence to assess such changes in the food supply. A recent analysis of TFA from archived plasma from the US NHANES study from 2000 to 2009 demonstrated a significant reduction in total TFA from 93.1 to 39.0 µmol/L (16). Only one small study of Canadian childbearing women have shown a decrease in TFA over time (17); therefore, the aim of the present study was to examine changes in circulating TFA concentrations over time between 2004-2010 in a larger population of young healthy Canadians.

Subjects and Methods

Study population

Participants, age 20-29 (total population, n = 968; males, n = 366; females, n = 602), were part of the cross-sectional Toronto Nutrigenomics and Health (TNH) Study (18) recruited between September 2004 and Nov 2010. Written informed consent was obtained from all

participants. Anthropometric measurements, health, lifestyle, food frequency questionnaires and levels of health biomarkers were obtained and described elsewhere (19). Women who were pregnant or breastfeeding were not included in the study. Women who were using hormonal contraceptives were excluded from analyses. Research Ethics Boards at the University of Toronto and University of Guelph approved the study protocol.

Gas chromatography (GC) analysis

Subjects were required to fast overnight for a minimum of 12 h prior to blood collection, separation of plasma and subsequent freezing of samples at -80ºC. Sample preparation and fatty acid analysis were carried out as described previously (19, 20). The internal standard C17:0 was used to calculate fatty acid concentrations (µmol/L). C17:0 standard was prepared as a single batch and aliquoted to be used throughout the study. Total TFA concentrations were calculated by adding the concentrations of the following TFA: 16:1t9, 18:1t4, 18:1t5, 18:1t6-8, 18:1t9, 18:1t10, 18:1t11, 18:1t12, 18:2t9t12, 18:2t9c12, 18:2c9t12, 18:2c9t13, 18:2tt, 18:2c9t11-CLA, 18:2c11t13-CLA, 18:2t10c12-CLA, and 18:2tt-CLA. Total CLA included concentrations of the following CLA isomers: 18:2c9t11-CLA, 18:2c11t13-CLA, 18:2t10c12-CLA, two 18:2c/c isomers, and 18:2tt-CLA.

Statistical analysis of Data

Results are expressed as mean ± standard deviation (SD). All data was analyzed using JMP genomics software V5 (SAS Institute, Cary, NC). P values were adjusted for age, BMI, sex, ethnicity, % energy from dietary fat, physical activity, and date of GC processing in linear regression models. A p-value of < 0.05 was considered statistically significant.

Results

Study population

Participant levels of various biomarkers are shown in Table 1.

Concentrations of trans fatty acids and change over time

Average concentrations of circulating TFA are shown in Table 2. Correlation analyses [time (2004-2010) and TFA concentrations] showed that total TFA was negatively correlated and significant (R = -0.02, p=0.0043). Total TFA is comprised of both natural and PHVO TFA. Therefore, analyses were further stratified by source. Total concentrations of natural TFA were inversely and significantly correlated with time (R = -0.32, p = 0.0009). Circulating concentrations of individual natural TFA 18:2c9t11-CLA, 18:2t10c12-CLA, and 18:1t11 (Table 2, Figures 1 and 2) were inversely and significantly correlated with time. Overall, circulating concentrations of PHVO TFA were positively correlated with time (R = 0.19, p < 0.0001) (Table 2). There was a significant statistical increase over time in circulating concentrations of PHVO TFA 18:1t9, 18:2t9t12, 18:2t9c12, and 18:2c9t12 (Table 2, Figure 1). Concentrations of PHVO TFA 18:1t10 were inversely and significantly correlated with time. While statistically significant, the magnitude change over time is relatively small and not necessarily indicative of a biologically meaningful change.

Discussion

Our study determined changes in circulating TFA concentrations between 2004 and 2010 in a cohort of young healthy Canadians. The correlation between time and change in total TFA was weak (R = -0.02), but further examination of natural and PHVO TFA showed a decline in natural TFA, concurrent with a slight rise in PHVO TFA. These changes were similar in males and females (data not shown).

Total circulating TFA constitute about 2% of total circulating fatty acids; thus, the strength of the correlations and absolute concentrations need to be carefully interpreted. Strong

positive correlations for the PHVO TFA 18:2t9t12 were observed ($R > 0.3$). However, the absolute plasma concentrations of this specific TFA are low (2.1±3.1 µmol/L). The biological significance of these small changes of polyunsaturated TFA has been suggested to be highly potent as trans-18:2 have been shown to have stronger coronary heart disease effects than the predominant monounsaturated forms of TFA (21). However, direct experimental evidence for the adverse effects of these polyunsaturated TFA has not yet been examined. It is worth noting that the young age and healthy status of our cohort resulted in weak correlations between average circulating concentrations of TFA and different biomarkers of health such as LDL- and HDL-cholesterol, insulin, glucose, TG and FFA (19). Therefore, the healthy status of this cohort makes it challenging to definitively assess disease outcomes.

The decline in circulating concentrations of naturally-produced TFA demonstrated in this study is consistent with data from the Canadian Dairy Information Centre on Canadian consumption of Dairy from 2004 to 2010 which showed a decrease in consumption of total dairy products per capita in Ontario (22). A recent study of 229 individuals, aged 20-80, from the United States NHANES reported a decline in plasma PHVO and natural TFA levels of 16:1t9, 18:1t9, 18:1t11 and 18:2t9t12 from 2000-2009 (16). Although their findings agree with findings from the present study regarding a decrease in natural TFA, our findings of higher PHVO TFA are inconsistent. Average total TFA in this study (156.1±67.1 µmol/L) was higher than average total TFA reported by the NHANES study 93.1 to 39.0 µmol/L. Furthermore, the mean age of subjects in the present study was ~23 years. In contrast, the mean age of the subset of NHANES subjects was ~45 years. Another study reported declines in erythrocyte trans fatty acids levels in 299 older Americans (62±9 years old) between 1999-2006 (23). However, the study reported fatty acids levels as percent composition which makes comparison of the results to

concentrations of fatty acids presented in the present study infeasible (23). It is also possible that these different age groups consume and prepare their foods differently. Our study cohort consists mainly of young Canadians that are less likely to prepare their own meals. While significant progress has been made to reduce TFA in Canadian products, TFA in some food categories such as bakery products remain high, which may be a source of TFA in the diet of our young cohort of Canadians (8). Thus, we have potentially identified a sensitive segment of the population having high TFA to monitor future changes.

Our study provides a direct measurement of TFA exposure during the early period when marked reductions in TFA started to occur in the Canadian food supply. It was originally anticipated that plasma TFA levels would have decreased over time complementing changes reported in the food supply by Health Canada's Trans Monitoring Program (7, 8). However, we observed little change in PHVO TFA in a young cohort of Canadians from 2004-2010. In contrast, circulating concentrations of PHVO TFA are lower in other population groups, such as pregnant Canadian women, that are more likely to adhere to a healthy diet that is low in industrial TFA (17, 24). Our data suggests that this young segment of the population remains at risk for TFA exposure from PHVO. Young Canadians are a unique age group that likely has a higher consumption of convenience foods containing trans fats. The TFTF recommendations were clearly implemented by 2009, however fatty acids have long 1-2 year half-lives in adipose tissue (25, 26), which may have also contributed to the lack of change in plasma TFA. Nevertheless, these data suggest that TFA consumption by young Canadians warrants further investigation to identify sources and levels of intake.

In summary, our data does not show a decrease in TFA from PHVO in young Canadians but a significant decline in natural TFAs from 2004-2010. These results, while not reflective of

the entire Canadian population in terms of age and health status, demonstrate the importance of directly measuring circulating levels of TFA as an accurate reflection of exposure and highlight possible differences in circulating TFA levels in different segments of the population. While changes in PHVO TFA were not observed in this cohort, these data potentially provide useful baseline measures that can be used in the future to assess, in a sensitive manner, changes in PHVO TFA intake in young Canadians.

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Competing interests

AE holds shares in Nutrigenomix Inc., a genetic testing company for personalized nutrition. No other authors declare a conflict of interest.

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Table 1

General characteristics of study population.

	Total Population
Population (#)	968
Age (yrs)	22.7 ± 2.5
BMI (kg/m^2)	22.8 ± 3.5
HOMA-IR	1.4 ± 1.3
Glucose (mmol/L)	4.8 ± 0.5
Insulin (pmol/L)	46.6 ± 37.9
Total cholesterol (mmol/L)	4.2 ± 0.7
HDL-cholesterol (mmol/L)	1.5 ± 0.4
LDL-cholesterol (mmol/L)	2.3 ± 0.6
Triglycerides (mmol/L)	0.9 ± 0.4
Free fatty acids (µmol/L)	472.0± 249.8
% Energy from dietary fat	27.1 ± 6.0

Data represented as Mean±SD.

Table 2

Concentrations (µmol/L) of select trans fatty acids and correlation over time from 2004-2010.

Trans fatty Acid	Mean±SD	Minimum	Maximum	*R*	p-value
18:2c9t11-CLA	14.5±6.4	*t*	52.48	-0.26	0.0008
18:2t10c12-CLA	4.3±2.5	*t*	12.25	-0.03	<0.0001
Total CLA	29.1±13.0	*t*	102.18	0.13	<0.0001
16:1t9	17.1±9.2	*t*	65.16	-0.13	0.0533
18:1t11	14.2±8.3	*t*	74.19	-0.46	0.0166
Total natural TFAs	50.02±21.15	2.85	148.41	-0.32	0.0009
18:1t9	16.8±11.5	*t*	87.96	0.05	<0.0001
18:1t10	17.3±11.4	*t*	71.14	-0.03	<0.0001
18:2t9t12	2.1±3.1	*t*	20.87	0.41	0.0007
18:2t9c12	9.9±4.4	*t*	61.48	0.27	0.0027
18:2c9t12	15.4±6.4	*t*	45.15	0.21	0.0081
18:2tt	3.5±4.3	*t*	38.74	0.49	0.2082
Total PHVO	64.92±33.81	12.26	244.45	0.19	<0.0001
Total TFAs	156.1±67.1	35.54	446.80	-0.02	0.0043

Total population: n = 968. *A p-value < 0.05 was considered statistically significant. Multiple linear regression models were adjusted for age, BMI, physical activity, % Energy from dietary fat, and ethnicity. SD, standard deviation; *t*, trace.

Figure 1.

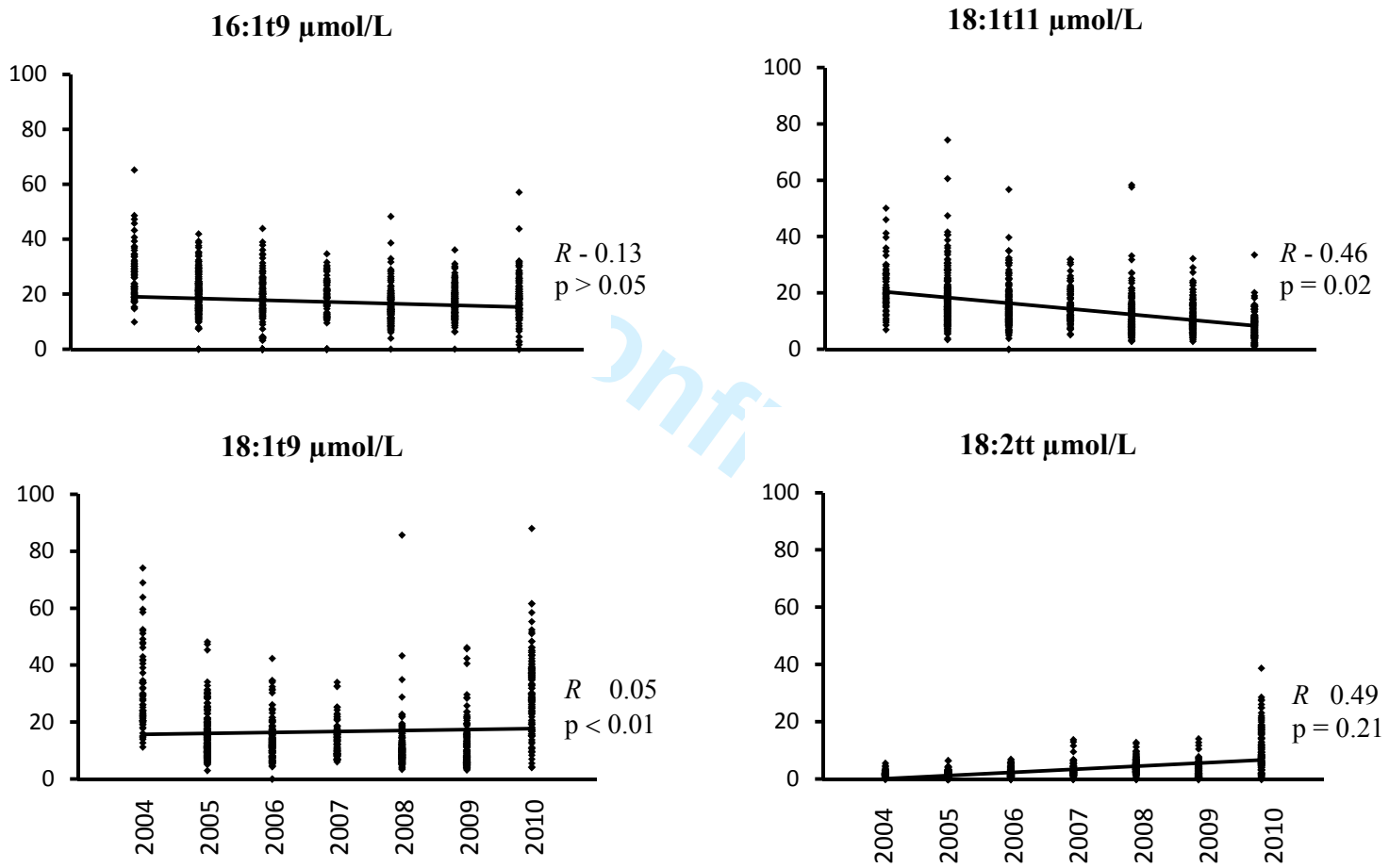

Figure1. Changes in circulating concentrations of natural TFA,16:1t9 and 18:1t11 and PHVO TFA 18:1t9 and 18:2tt from 2004-2010. Multiple linear regression models were adjusted for sex, ethnicity, age, BMI, physical activity score and % energy from dietary fat. n= 968.

Figure 2

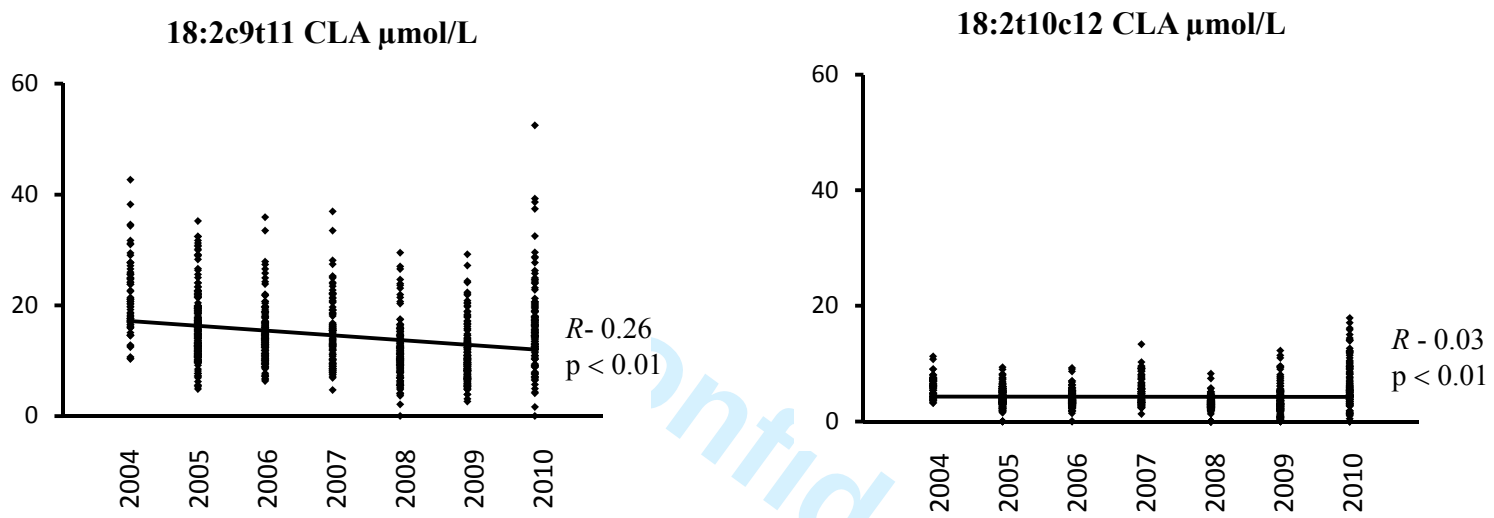

Figure 2. Changes in circulating concentrations of CLA isomers 18:2c9t11 and 18:2t10c12 from 2004-2010. Multiple linear regression models were adjusted for sex, ethnicity, age, BMI, physical activity score and % energy from dietary fat. n= 968.