Gender differences in the myocardial response to dietary CLA isomers during diabetes

Paramjit S. Tappia, Seyla Bun[1], Mark Bernardino,[1] Nina Aroutiounova,[1] Lori Tessler,[1] Naranjan S. Dhalla[2]

Asper Clinical Research Institute, St Boniface Hospital Research and [1]Institute of Cardiovascular Sciences, Department of Physiology and Pathophysiology, College of Medicine, Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada

Abstract

This study was undertaken to examine if conjugated linoleic acid (CLA) isomers c9,t11 and t10,c12, provide protection against cardiac dysfunction in diabetic male and female rats. Diabetes was induced by an intravenous injection of streptozotocin (65 mg/kg body weight) in male and female rats. Four wks after the induction of diabetes, the animals were treated daily for 4 wks with or without 0.5% of each CLA isomer by oral gavage. Eight wks diabetes was associated with elevated levels of plasma total cholesterol, HDL-cholesterol, triglycerides and glucose as well as depressed rate of cardiac relaxation (-dP/dt) in both male and female animals. However, unlike females, male diabetic rats showed an increase in heart weight: body weight ratio and decreased rates of cardiac contraction (+dP/dt) and fractional shortening (FS). Both CLA isomers improved the lipid profile in male diabetic rats, whereas only CLA c9,t11 isomer improved the lipid profile in females. CLA intervention did not affect blood glucose levels in male or female diabetic animals. While CLA c9,t11 isomer improved ± dP/dt in the male rats, a deterioration of +dP/dt and -dP/dt in the female diabetic rats was observed. Although FS was normalized by both CLA isomers in males, CLA t10,c12 isomer depressed FS in the female diabetic rats. CLA treatment normalized GLUT 4 gene expression levels, but markedly increased insulin receptor gene expression in the heart of male diabetic rats. These data suggest that, unlike females, improved myocardial function in male diabetic rats in response to CLA may be related to a favorable shift in myocardial energetics.

Key words: *Conjugated linoleic acid isomers, diabetes, cardiac function, gender differences, gene expression, metabolism*

Introduction

Conjugated linoleic acid (CLA) is a group of positional and geometric isomers of dienoic derivatives of linoleic acid.[1] The major dietary source of CLA for humans is ruminant meats, such as beef and lamb, and dairy products, such as milk and cheese.[2,3] The major isomer of CLA found naturally in food is cis-9, trans-11 (c9,t11) and is largely in the triglyceride form, whereas commercial CLA preparations contain approximately equal amounts of c9,t11 and trans-10, cis-12 (t10,c12) isomers as free fatty acids or triglycerides. CLA has attracted considerable attention as a weight loss supplement because CLA can reduce fat stores and increase muscle mass.[4] Several studies have shown increases in the amounts of CLA isomers which are incorporated into myocardial membrane phospholipids of experimental animals fed different CLA isomers.[5-7]

Received on: 20 January, 2015
Accepted on: 18 March 2015

Correspondence to: Paramjit S. Tappia, Ph.D.
Asper Clinical Research Institute, CR3129-369 Tache Avenue, Winnipeg, Manitoba, Canada R2H 2A6, E-mail: ptappia@sbrc.ca

Although some studies have shown that CLA could exert cardiovascular benefits through its hypolipidemic and anti-atherosclerotic effects,[8-16] a blood pressure lowering effect of CLA t10,c12 isomer in obese rats[17] and spontaneously hypertensive rats[18] has been reported.

Earlier, we have reported that dietary CLA can modulate cardiac function and myocardial gene expression for Ca^{2+}-cycling proteins in both male and female rats [19,20] demonstrating that dietary CLA has a direct impact on heart function under normal physiological conditions. However, virtually nothing is known about the possible gender-related benefits of specific CLA isomers on cardiovascular function during diabetes. In view of the clinical prevalence of diabetes and mortality linked to cardiovascular disease (CVD),[21-28] this study was undertaken to examine if dietary c9,t11 or t10,c12 CLA isomers protect against cardiac dysfunction during diabetes in a well-established model of diabetes that resembles human type 1 diabetes,[29,30] in male and female rats. In addition, myocardial gene expression of some of the key regulators of glucose utilization and fatty acid metabolism were also examined. This study reports the importance of gender in the cardiac response to diabetes as well as to two major CLA isomers present in the human diet.

Materials and Methods

Diabetes model

The use of animals and protocols in this study was approved by the University of Manitoba Animal Care Committee in accordance with the guidelines of the Canadian Council on Animal Care and the Guide for the Care and Use of Laboratory Animals, NIH, Bethesda. Male (225-250 g) and female (125-150 g) Sprague-Dawley rats were made diabetic with a single tail vein injection of streptozotocin (STZ) (65 mg/kg body weight, dissolved in 0.1 M citrate buffer, pH 4.5) as described previously.[31,32] Age-matched normal animals received citrate buffer and served as controls; all rats fed ad libitum. Diabetes was confirmed by measurement of glucose in the urine > 55 mM (Diastix reagent test strips for glucose, Bayer Inc., Toronto, ON, Canada). Blood samples from diabetic animals treated with and without CLA isomers were taken at the time of sacrifice (8 wks post-STZ) and analyzed for plasma glucose levels, total cholesterol, triglycerides, and high density lipoprotein (HDL) cholesterol levels using standard methods by Laboratory Services at St. Boniface Hospital operated by Diagnostic Services of Manitoba.

Dietary interventions

The CLA isomers were a kind gift from Loders Croklaan (Lipid Nutrition, Channahon, IL, USA). These products are 84% c9, t11 CLA plus 16% t10, c12 CLA or 84% t10, c12 plus 16% c9, t11 CLA. Single isomer products (i.e. 100% pure) in amounts necessary to conduct this study were not available. The selection of these isomers is in line with the 80% of CLA in butter fat being c9, t11 CLA with the remainder being predominantly t10, c12 CLA. The 4 wk male and female diabetic rats were treated with CLA (200 µl) by oral gavage, whereas control rats were treated with equivalent corn oil, daily for 4 wks to provide the following amount of CLA: 0.5 % corn oil (for control and untreated diabetes groups); 0.5 % c9,t11 CLA and 0.5% t10c12 CLA ; for CLA treated diabetic rats. The amounts of CLA isomers are as used in our previous studies.[19, 20]

Echocardiography of the heart

An ultrasound imaging system (SONOS 5500 ultrasonograph, Agilent Technologies, Mississauga, ON, Canada) was used for the measurement of heart rate (HR), systolic and diastolic pressures, left ventricle (LV) wall thickness and internal diameters during systole and diastole in control and diabetic animals as described previously.[32] Cardiac output and fractional shortening (FS) were calculated. Echocardiographic measurements were made in rats anaesthetized with 2.5 % isoflurane in 2L/min oxygen. Briefly, the transthoracic short axis measurements were performed using a 12-MHz annular array ultrasound transducer. The heart was first imaged in the two-dimensional mode in the parasternal long-axis view. From this view, an M-mode cursor was positioned perpendicular to the interventricular septum and LV posterior wall at the level of the papillary muscle. M-mode images were obtained for wall thickness and chamber dimensions. Images were stored in digital format for review and analysis.

Assessment of LV function by in vivo catheterization

The LV function was assessed in rats by in vivo catheterization as described previously.[27] At the end of the feeding period, rats were anesthetized with 5% isofluorane in a flow rate of oxygen of 2L/min. The right carotid artery was then exposed and a micromanometer-tipped catheter (2-0; model SPR-249, Millar Instruments, Houston, TX, USA) inserted and advanced into the LV. The catheter was secured with a silk ligature around the artery and, after a 15 min stabilization of the heart function, measurements for heart rate (HR), systolic pressure (SP), diastolic pressure (DP), mean arterial blood pressure (MAP), LV systolic pressure (LVSP), LV end diastolic pressure (LVEDP), rate of pressure development (+dP/dt) and rate of pressure decay (-dP/dt) were recorded. Hemodynamic data were computed and displayed using AcqKnowledge Software version 3.7.1 (MP System "Quick Start", Biopac System, Inc., Goleta, CA, USA).

RNA isolation and quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from LV tissue using RNA isolation kit (Life Technologies, Burlington, ON, Canada) according to the manufacturer's procedures. The iScript One-Step RT-PCR kit with SYBR Green was used for real-time qPCR. cDNA synthesis and PCR amplifications were carried out in the same tube. 500 ng of total RNA was used for reverse transcription. 2X SYBR Green RT-PCR. The Superscript First-Strand Synthesis System for RT-PCR (Bio-Rad, Hercules, CA, USA) was used according to the instructions of the manufacturer. Primers used for amplification were synthesized as follows: insulin receptor (IR), TTCGAGGAGAGACCTTGGAA (forward) and TCGTGAGGTTGTGCTTGTTC (reverse); insulin receptor substrate-1 (IRS-1), TGGATGC AAGTGGATGACTC (forward) and CGGAGGATTGTTGAGATGGT; phosphatidylinositol 3-kinase (PI 3-Kinase), TGGAGAGCAAACACCACAAG (forward) and TATGTGAGCTGCTTGGTTGG (reverse); glucose trans-porter-1 (GLUT 1), TCTCT GTGGGCCTCTTTGTT (forward) and AGGCCAACA GGTTCATCATC (reverse); glucose transporter-4 (GLUT 4), ACAATGTCTTGGCT GTGCTG (forward) and TCCCACATACATAGGCACCA (reverse); phosphotyrosyl protein phosphatase-1B (PTP 1B), ATGGAGAAGGAATTCGAGCA (forward) and TTCTGCATGGGAAGTCACTG (reverse); acyl-CoA oxidase 1, GCCAATTTTGTGGAACCTGT (forward) and TCCAAGCCTCGAAGATGAGT (reverse); carnitine palmitoyl transferase-1B (CPT 1B), GGTCCATTG CATCCAGAGAT (forward) and TCCACCCATG ATAGGAAAGC (reverse). qRT-PCR was performed using the Bio-Rad iCycler detection System. For analysis, cycle threshold (Ct) values were calculated for each sample; gene expression was further analyzed by the 2-ΔΔCt method.[33]

Statistical analysis

Each observation was made with 4-8 different experiments and results are presented as mean ± S.E. The statistical difference between mean values for two groups was evaluated by the Student's t-test. For the comparison of more than two groups, multiple analysis of variance was carried out with post-hoc testing by the Scheffe's procedure.

Table 1: General characteristics of male and female diabetic rats treated with CLA isomers.

	BW (g)	HW (g)	LVW (mg)	HW/BW ratio (mg/g)
A.MALE				
Control	541 ± 15.1	1.47 ± 0.13	974 ± 11.7	2.72 ± 0.08
Diabetes	375 ± 26.2*	1.34 ± 0.05	883 ± 52.6*	3.61 ± 0.10*
Diabetes + CLA:				
c9, t11 isomer	389 ± 14.3	1.28 ± 0.04	836 ± 28.2*	3.31 ± 0.10*
t10, c12 isomer	360 ± 13.0	1.24 ± 0.05	767 ± 20.6#	3.45 ± 0.03
B.FEMALE				
Control	300 ± 8.7¶	1.00 ± 0.04¶	657 ± 31.7¶	3.33 ± 0.09¶
Diabetes	289 ± 11.2	0.99 ± 0.04	646 ± 30.9	3.42 ± 0.03
Diabetes + CLA:				
c9, t11 isomer	263 ± 5.9#	0.94 ± 0.02	611 ± 17.0	3.57 ± 0.1
t10, c12 isomer	254 ± 7.8#	0.91 ± 0.04	577 ± 26.9	3.58 ± 0.04

Values are means ± S.E. of 6-8 animals for each group. CLA, conjugated linoleic acid; BW, body weight; HW, heart weight; LVW; left ventricle weight; * P<0.05 vs. age- matched control, # P<0.05 vs. non-treated diabetic; ¶ P<0.05 vs. corresponding male value.

Table 2: Effect of dietary CLA isomer intervention on plasma glucose levels and lipid profile of male and female diabetic rats.

	Total Cholesterol	Triglycerides (mM)	HDL- Cholesterol	Glucose (mM)
A. MALE				
Control	2.30 ± 0.37	1.51 ± 0.21	1.53 ± 0.25	23.8 ± 1.1
Diabetes	3.94 ± 1.18*	15.22 ± 5.73*	0.75 ± 0.18*	34.9 ± 2.2*
Diabetes + CLA: c9, t11 isomer	2.51 ± 0.32#	6.22 ± 1.37#	1.14 ± 0.07#	34.4 ± 1.4*
t10, c12 isomer	2.48 ± 0.54#	5.67 ± 2.38#	0.84 ± 0.16	34.89 ± 2.2
B.FEMALE				
Control	1.91 ± 0.27	0.34 ± 0.43¶	1.12 ± 0.14	17.8 ± 1.5¶
Diabetes	4.44 ± 1.28*	15.64 ± 7.23*	0.71 ± 0.13*	37.0 ± 2.0*
Diabetes + CLA: c9, t11 isomer	1.94 ± 0.38#	4.04 ± 2.50#	1.10 ± 0.19#	35.1 ± 3.4
t10, c12 isomer	4.05 ± 1.46	16.45 ± 9.23	0.73 ± 0.18	31.7 ± 4.7*

Values are means ± S.E. of 6-8 animals for each group. CLA, conjugated linoleic acid; HDL, high density lipoprotein; * P<0.05 vs. age-matched control, # P<0.05 vs. non- treated diabetic, ¶ P<0.05 vs. corresponding male value.

Results

General characteristics of male and female diabetic rats treated with or without CLA isomers

The body wt (BW) of male diabetic rats was lower than control males; treatment of male diabetic rats for 4 wks with CLA (c9, t11 or t10, c12) did not affect the BW (Table 1). While diabetes did not change the BW of female rats, treatment with CLA resulted in a decrease in the BW relative to control and un-treated diabetic female rats. Although no significant changes were observed in heart weight (HW), the LV weight (LVW) was decreased in male diabetic rats only. The LVW was further reduced in the male diabetic rats upon treatment with CLA. Unlike the male rats, no changes in LVW were observed in the female diabetic rats treated with or without CLA isomers. The HW/BW ratio (as an indicator of cardiac hypertrophy) was increased in the male diabetic rats only; this did not revert to control values with CLA treatment. Unlike the males, no differences in the HW/BW ratio were observed in the female diabetic rats treated with or without CLA. It is pointed out that the values for BW, HW and LVW in control female rats were lower whereas that for HW/BW ratio was higher than those in control male rats (Table 1).

Effect of dietary CLA isomers on plasma glucose and lipid levels of diabetic rats

Plasma levels for both TG and glucose in control female rats were significantly lower than those in control male rats. An increase in the circulating level of total cholesterol (TC) was observed in both male and female diabetic rats (Table 2); however, the increase in females was greater than that in males. Significant increases in plasma triglycerides (TG) were also seen in both male and female diabetic rats, while HDL-cholesterol levels were reduced. The increase in blood glucose was greater in female diabetic rats as compared to that in males. Dietary intervention with c9, t11 or t10, c12 CLA normalized plasma TC levels in male diabetic rats. While c9, t11 CLA normalized plasma TC levels in the female diabetic rats, treatment with t10, c12 CLA did not affect the TC levels (Table 2). Both CLA isomers partially corrected the high TG levels of male diabetic rats. c9, t11

CLA was able to partially normalize TG levels in the femalediabetic rats, treatment with t10,c12 CLA did not change the

Table 3: Cardiac function of male and female diabetic rats treated with and without CLA isomers.

	+dP/dt (mm Hg/s)	-dP/dt (mm Hg/s)	LVSP (mm Hg)	LVEDP (mm Hg)
A.MALE				
Control	6691 ± 199	5723 ± 240	127 ± 8.0	6.5 ± 0.7
Diabetes	5329 ± 507*	3730 ± 358*	97 ± 3.7*	8.8 ± 1.1
Diabetes + CLA:				
c9, t11 isomer	6321 ± 295#	4843 ± 287#	119 ± 7.9#	8.1 ± 1.1
t10, c12 isomer	4799 ± 600	3493 ± 345	109 ± 9.2	7.9 ± 1.7
B.FEMALE				
Control	6528 ± 204	5899 ± 251	130 ± 5.0	8.0 ± 0.5
Diabetes	6320 ± 463	4591 ± 224*	125 ± 7.2	8.0 ± 1.1
Diabetes + CLA:				
c9, t11 isomer	4784 ± 468#	3694 ± 248#	102 ± 9.2#	7.5 ± 0.8
t10, c12 isomer	5788 ± 526	4086 ± 541	115 ± 10.9	9.5 ± 1.9

Values are means ± S.E. of 6-8 animals for each group. CLA, conjugated linoleic acid; +dP/dt, rate of pressure development; -dP/dt, rate of pressure decay; LVSP, left ventricular systolic pressure; LVEDP, left ventricle end-diastolic pressure; * P<0.05 vs. age-matched control, # P<0.05 vs. non-treated diabetic.

Table 4: Heart rate and blood pressure of male and female diabetic rats treated with CLA isomers.

	HR (beats/min)	MAP (mmHg)	Systolic Pressure (mm Hg)	Diastolic Pressure (mm Hg)
A.MALE				
Control	236 ± 6	93 ± 7	123 ± 8	78 ± 7
Diabetes	206 ± 4*	69 ± 6*	91 ± 5*	57 ± 6*
Diabetes + CLA:				
c9, t11 isomer	242 ± 5#	95 ± 7#	118 ± 9#	84 ± 6#
t10, c12 isomer	256 ± 8#	80 ± 10#	101 ± 11#	70 ± 10#
B.FEMALE				
Control	261 ± 8¶	102 ± 4	127 ± 6	90 ± 4
Diabetes	239 ± 11*	100 ± 8	125 ± 9	87 ± 8
Diabetes + CLA:				
c9, t11 isomer	247 ± 11	73 ± 9#	95 ± 11#	63 ± 8#
t10, c12 isomer	260 ± 6	90 ± 10	113 ± 11	78 ± 9

Values are means ± S.E. of 6-8 animals for each group. CLA, conjugated linoleic acid; HR, heart rate; MAP, mean arterial pressure; * P<0.05 vs. age-matched control, # P<0.05 vs. non-treated diabetic; ¶ P<0.05 vs. corresponding male value.

elevated levels of TG (Table 2). Although the mean values for the HDL-cholesterol levels in both the male and female diabetic rats were higher in the CLA treated as compared to non-treated diabetic rats, they however; did not reach significance. No changes in blood glucose levels were seen with CLA in the diabetic rats (Table 2).

Hemodynamic function of male and female diabetic rats treated with or without CLA isomers

Hemodynamic assessment of male and female diabetic animals revealed depressed rates of pressure development (+dP/dt) and pressure decay (-dP/dt) in male diabetic rats, whereas only -dP/dt was depressed in the female diabetic rats (Table 3). While an improved cardiac function was seen with c9, t11 CLA treatment of the male diabetic rats, treatment with t10, c12 CLA did not improve hemodynamic function. On the other hand, c9, t11 CLA actually worsened + dP/dt and -dP/dt of female diabetic animals, whereas t10, c12 CLA had no significant effect on these parameters. A depressed LVSP was seen only in the male diabetic rats and only treatment with c9, t11 CLA normalized LVSP to control value. In contrast, while diabetes itself did not affect LVSP in the female rats, treatment of the female diabetic rats with c9, t11 CLA reduced LVSP. No significant changes in LVDP due to diabetes or treatment with CLA were observed. The heart rate (HR) in control female rats was significantly higher than that in control male rats (Table 4). While the HR was lower in both male and female diabetic rats as compared to control animals, treatment with CLA improved heart rate to control values in both sexes. Depressions in MAP, systolic pressure and diastolic pressure were only seen in the male diabetic rats (Table 4); treatment of the male diabetic rats with both CLA isomers reversed these changes. In contrast, while diabetes in the

female rats did not induce changes in the above parameters, treatment of female diabetic rats with c9, t11 CLA resulted in a depression of MAP, systolic and diastolic pressures as compared to diabetic values (Table 4).

Echocardiography of male and female diabetic rats treated with and without CLA isomers Cardiac output, both systolic and diastolic PWT and diastolic LViD, unlike FS and

Table 5: Cardiac performance and cardiac remodeling parameters upon echocardiographic assessment of male and female diabetic rats treated with CLA isomers.

			Systolic		Diastolic	
	Fractional shortening (%)	**Cardiac output (L/min)**	**LViD (cm)**	**PWT (cm)**	**LViD (cm)**	**PWT (cm**
A.MALE						
Control	43 ± 4	0.146 ± 0.016	0.336 ± 0.026	0.224 ± 0.010	0.577 ± 0.014	0.142 ± 0.006
Diabetes	31 ± 4*	0.149 ± 0.010	0.412 ± 0.029*	0.185 ± 0.013*	0.656 ± 0.013*	0.129 ± 0.011
Diabetes + CLA:						
c9, t11 isomer	44 ±2#	0.137 ± 0.023	0.348 ± 0.021#	0.220 ± 0.014#	0.606 ± 0.039	0.144 ± 0.006
t10, c12 isomer	42 ±4#	0.155 ± 0.012	0.386 ± 0.033	0.213 ± 0.020#	0.661 ± 0.028	0.132 ± 0.008
B.FEMALE						
Control	42 ±3	0.097 ± 0.006¶	0.308 ± 0.015	0.188 ± 0.007¶	0.520 ± 0.014¶	0.119 ±0.008¶
Diabetes	41 ±2	0.116 ± 0.018	0.350 ± 0.025	0.186 ± 0.010	0.574 ± 0.026*	0.127 ± 0.006
Diabetes + CLA:						
c9, t11 isomer	42 ±3	0.097 ± 0.007	0.307 ± 0.022	0.195 ± 0.013	0.534 ± 0.005	0.117 ± 0.009
t10, c12 isomer	36 ±2	0.075 ± 0.004#	0.343 ± 0.016	0.168 ± 0.005	0.534 ± 0.013#	0.127 ± 0.006

Values are means ± S.E. of 6-8 animals for each group. CLA, conjugated linoleic acid; LViD, left ventricle internal diameter; PWT, posterior wall thickness; * P<0.05 vs. age-matched control, # P<0.05 vs. non-treated diabetic, ¶ P<0.05 vs. corresponding male value.

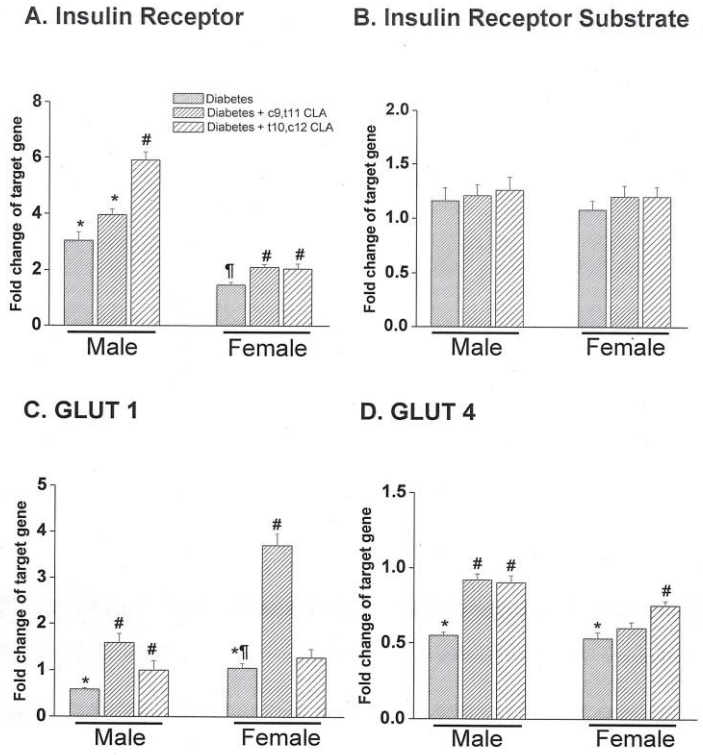

Figure 1: Myocardial gene expression for glucose regulatory and handling proteins in male and female diabetic rats treated with or without CLA isomers. Quantitative real time RT-PCR showing mRNA levels for insulin receptor, insulin receptor substrate-1; GLUT 1 and GLUT 4 expressed as fold change of target gene. Values are means ± S.E.M. of 4 independent measures in each group. Control value is 1.0. *Significantly different (P<0.05) from control value; # significantly different (P<0.05) non-treated diabetes value; ¶ significantly different (P<0.05) from corresponding male value. GLUT 1, glucose transporter 1; GLUT 4, glucose transporter 4; CLA, conjugated linoleic acid.

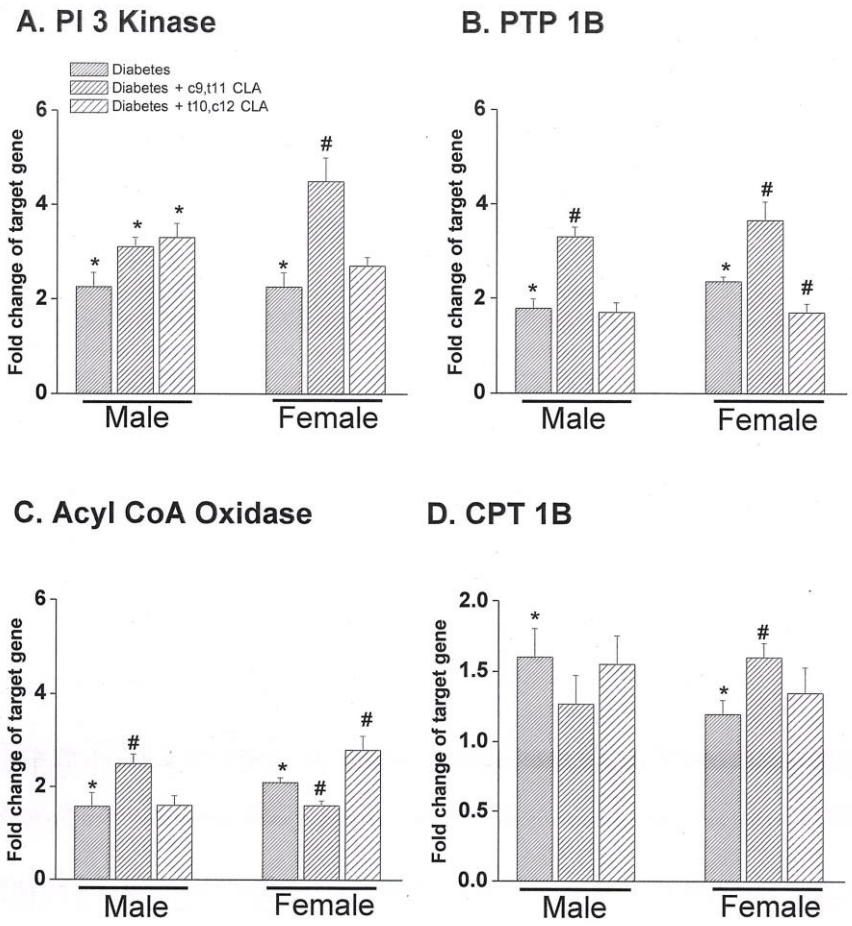

Figure 2: Myocardial gene expression for glucose handling and fatty acid metabolism proteins in male and female diabetic rats treated with or without CLA isomers. Quantitative real time RT-PCR showing mRNA levels for PI3-kinase, PTP-1B, acyl CoA oxidase and CPT1B expressed as fold change of target gene. Values are means ± S.E.M. of 4 independent measures in each group. Control value is 1.0. *Significantly different ($P<0.05$) from control value; # significantly different ($P<0.05$) non-treated diabetes value. PI3- kinase, phosphatidylinositol 3-kinase PTP1B, phosphotyrosyl protein phosphatase 1B; CPT 1B, carnitine palmitoyl transferase 1B ; CLA, conjugated linoleic acid.

systolic LViD, in female control rats were lower than those in male control animals (Table 5). A significant depression in the FS was observed in male diabetic rats only; treatment of male diabetics with CLA isomers normalized the FS. Significant increases in LViD during systole and diastole due to diabetes were observed, which were corrected by treatment with CLA. Likewise decreases in the PWT were seen in the male diabetic rats only, which were corrected by CLA treatment. On the other hand, an increase in LViD during systole and diastole were seen in the female diabetic rats as compared to control animals. However, treatment of the female diabetic rats with t10, c12 CLA resulted in a decrease in the FS as well as cardiac output. While treatment with c9, t11 CLA corrected the LViD, t10, c12 CLA was only able to correct the diastolic LViD (Table 5).

Expression of genes involved in myocardial glucose metabolism in diabetic rats treated with and without CLA isomers

Diabetes induced a 3-fold increase in IR gene expression in the male diabetic rats (Figure 1A). Treatment of the male diabetic rats with CLA further increased the myocardial expression levels of IR gene, although the extent of the increase was much greater with t10, c12 CLA than with c9, t11 CLA. In female diabetic rats, no increase in IR gene expression was seen; however, treatment with CLA isomers induced a small and comparable increase in its gene expression (Figure 1A). No significant changes were observed in the mRNA levels for IRS-1 in both male and female diabetic rats with or without CLA treatment (Figure 1B). Gender differences were also observed in the expression levels of GLUT 1 in the hearts of diabetic animals (Figure 1C). In this regard, a decrease in GLUT 1 gene expression was seen in the heart of male diabetic rats only. However, a significant increase in GLUT 1 gene expression was seen in male diabetic rats treated with c9, t11 CLA (Figure 1C). Likewise, an increase in GLUT 1 expression was seen in the female diabetic hearts with c9, female vs. male (Figure 1C). Although diabetes depressed GLUT 4 gene expression in both male and female diabetic

rats, normalization of the gene level was only seen in the male diabetic rats treated with either CLA isomer (Figure 1D). Diabetes in both male and female rats induced an increase in PI 3-kinase gene expression (Figure 2A). Treatment of male diabetic rats with CLA resulted in a further small increase in PI 3-kinase gene expression, whereas a further marked increase in myocardial PI 3-kinase gene expression in the female diabetic rat in response to c9, t11 CLA was observed. Diabetes induced an increase in PTP1B gene expression in the hearts of male and female diabetic rats, but further increases were seen upon c9, t11 CLA only in both genders (Figure 2B).

Expression of genes involved in myocardial fatty acid metabolism in diabetic rats treated with and without CLA isomers

A significant increase in acyl-CoA oxidase 1 gene expression was seen in diabetic male rats (Figure 2C), which was further increased in response to c9, t11 CLA only. Acyl-CoA oxidase 1 gene expression was also increased in diabetic female rats; treatment of these animals with t10, c12 CLA resulted in a further increase in acyl-CoA oxidase 1 gene expression (Figure 2C). Although diabetes induced an increase in CPT 1B gene expression in both male and female diabetic animals, the degree of increase was greater in male diabetic animals (Figure 2D). A further increase in CPT 1B expression was seen in female diabetic rats treated with c9,t11 CLA only.

Discussion

In this study, gender differences were seen in the normal healthy male and female animals. In this regard, the BW of the age-matched female rats was lower than the males. While the female HW and LVW was less than in the male rats, the HW/BW ratio was higher in the control female rats as compared to males. In addition, plasma triglyceride and glucose levels as well as HR and cardiac output were lower in control female rats than in male counterparts. Furthermore, echocardiography revealed thinner PWT and smaller LV internal diameters of female hearts as compared to male control hearts.

Similarly some differences in male and female diabetic rats were observed. A weight reduction was only seen in the female diabetic rats. While no changes in HW, LVW as well as HW/BW ratio were seen in the female diabetic rats, a reduction in LVW was observed in the male diabetic rats. However, cardiac hypertrophy as evidenced by an increase in the HW/BW ratio was evident in only the male diabetic rats. In addition, while +dP/dt and -dP/dt were depressed in male diabetic rats, only a depression in -dP/dt was seen in the females. Furthermore, decreases in HR, MAP, systolic and diastolic pressures were only seen in the male diabetic animals. These observations are consistent with earlier reports.[19, 20]

Although gender differences were seen in some of the parameters measured in the untreated diabetic animals, gender differences in the response to dietary CLA isomers were observed. Both CLA isomers improved the lipid profile in male diabetic rats, whereas only the c9,t11 CLA improved lipid profile in females. CLA intervention did not affect blood glucose levels in male and female diabetic animals. While c9,t11 improved ± dP/dt in the male rats, a deterioration of ± dP/dt in the female diabetic rats was observed. Although FS was normalized by CLA isomers in males, the t10,c12 CLA depressed FS in the female diabetic rats. Both CLA isomers were able to normalize the HR, MAP as well as systolic and diastolic pressures in male diabetic animals. Interestingly, in a study involving incident cases of a first nonfatal acute myocardial infarction (MI), adipose tissue c9, t11 CLA was found to be associated with a lower risk of MI in basic and multivariate models.[34]

In terms of mechanisms for the beneficial effects of c9, t11 CLA on improving heart function in the male diabetic rats, it is difficult to propose clear cut sites of action; however, normalization of GLUT 4 gene expression was only seen in the male diabetic rats treated with c9, t11 CLA and t10, c12 CLA. Although CLA has been reported to improve insulin resistance in obese rats possibly due to increased expression of GLUT 4 in skeletal muscle,[35] c9,t11 CLA has been shown to inhibit the TNFα-induced downregulation of GLUT 4 mRNA and promote insulin- stimulated glucose transport in 3T3-L1 adipocytes.[36] Whether the normalization of GLUT 4 expression in the male diabetic heart is related to improved cardiac function remains to be determined, but it is possible that c9,t11 CLA may have improved metabolic flexibility through co-ordination between carbohydrate and energy metabolism in the heart.[37] It should also be noted that IR gene expression in male diabetic hearts was increased, but IR mRNA levels were further increased in response to the CLA isomers. While increases were also seen in the females the extent was much lower as compared to males. Taken together, the increases in GLUT 4 and IR gene expression could point to a shift in myocardial energetics (increased glucose utilization); however, if this is related to improved function of the heart in male diabetics remains to be determined. While the comparable increases in myocardial acyl-CoA oxidase and CPT 1B gene expression observed in the male and female diabetic rats would seem to imply increased capacity for fatty acid oxidation, the relationship to cardiac contractile function needs to be further investigated.

Since we have shown that dietary CLA isomers can also modify gene and protein expression of elements involved in regulating myocardial Ca^{2+}-cycling,[20] these interventions could be useful candidates to study for their contribution to improved function of the male diabetic heart. Similarly, the impact of dietary CLA isomers on Ca^{2+}-homeostasis in the female diabetic heart should be examined. In this regard, it is pointed out that CLA has been reported to depress intracellular Ca^{2+}-concentration in neonatal cardio-myocytes although the type and proportion of the CLA isomer was not identified and contractile activity not measured;[38] the female diabetic heart may adversely respond to CLA through this mechanism. It is pointed out that blood pressures were only reduced in male diabetic rats. Since we[19] and others[17,18,39] have shown blood pressure lowering effects of CLA, this was not the case in the male diabetic rats as MAP, SP and DP were increased but, in female diabetic rats these

parameters were depressed to below control values, which can be considered as an adverse response to CLA.

While the regulation of genes of lipid and glucose metabolism have been reported to be more effective with t10,c12 than with c9,t11 CLA,[40] long-term feeding of CLA isomers in C57B1/6J mice revealed contrasting effects on glucose and insulin metabolism. t10,c12 CLA increased insulin resistance and liver steatosis, whereas c9,t11 CLA prevented the insulin resistance.[41] In the diabetic apoE deficient mouse, supplemental 0.9% CLA (c9, t11) failed to reduce the severity of aortic atherosclerosis, although plasma TG concentration was substantially lowered and HDL cholesterol raised.[42] On the other hand, t10,c12 CLA has been reported to reduce atherosclerotic lesion development in apoE and LDL receptor deficient mice, despite exerting an adverse effect on cardiovascular risk parameters.[43] CLA has been reported to deteriorate insulin resistance in obese/diabetic mice. [44] In contrast, c9,t11 adipose tissue content has been observed to be inversely correlated to diabetes risk in adults.[45] In vitro studies have found that both c9,t11 and t10, c12 CLA activate the cell surface receptor, FFA1, which results in an increase in insulin secretion.[8] Despite these observations a daily supplementation of 8 g CLA for 16 wks in post-menopausal, obese diabetic women had no effect on HbA1c, C- reactive protein, blood lipid and glucose levels.[46] Synthetic isomers, particularly t10,c12 CLA has been suspected of having pro-diabetic effects in individuals who are already at risk of developing diabetes;[47] in fact, detrimental effects of CLA appear to be largely in mice and are due mainly due to t10,c12 isomer.[48] Thus some caution must be exercised in the interpretation of animal data to human applicability. Indeed, the efficacy of dietary CLA supplementation remains inconsistent in randomized clinical trials. Furthermore, evidence has emerged that supplemental CLA is associated with subclinical inflammation and oxidative stress.[49] It is pointed out that CLA derived from ruminant foods differ from commercial CLA supplements in their isomer composition/distribution, consumption level and bioactivity.[49] Therefore further substantiation of the scientific evidence relating to CLA and human health benefits is still required.

A major limitation of our study is the lack of information on the amounts of CLA isomers incorporated into myocardial membrane phospholipids. In addition, we did not determined protein levels of the genes that were examined and subsequent measurements of glucose utilization and fatty acid oxidation were not undertaken. Nonetheless, our data have revealed that while CLA may be of benefit to male diabetic rats, it would appear that CLA exerts an adverse effect on heart function in diabetic female rats, despite the improvements in the lipid profile at least by c9, t11 CLA. These data are suggestive that metabolic differences owing to gender may account for the nature of the cardiac response to CLA during diabetes. The findings of the present study could be of value to male diabetic populations as a natural and affordable adjunct for prevention/treatment of associated cardiovascular complications. This study has also revealed the importance of gender in relation to understanding the mechanisms of diabetes induced cardiovascular complications and raises the possibility of gender-specific therapies.

Acknowledgements

The work reported here was supported by a grant from the Dairy Farmers of Canada (PST). The infrastructure support for this project was provided by the St. Boniface Hospital Research Foundation.

References

1. Griinari JM, Corl BA, Lacy SH, Chouinard PY, Nurmela KV, Bauman DE. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Delta(9)-desaturase. J Nutr 2000; 130: 2285-2291.
2. Chin SF, Liu W, Storkson JM, Ha YL, Pariza MW. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J Food Compos Anal 1992; 5: 185-197.
3. Lin H, Boylston TD, Chang MJ, Luedecke LO, Shultz TD. Survey of the conjugated linoleic acid contents of dairy products. J Dairy Sci 1995; 78: 2358-2365.
4. Schmidt J1, Liebscher K, Merten N, Grundmann M, Mielenz M, Sauerwein H, et al. Conjugated linoleic acids mediate insulin release through islet G protein-coupled receptor FFA1/GPR40. J Biol Chem 2011; 286:11890-11894.
5. Li Y, Watkins BA. Conjugated linoleic acids alter bone fatty acid composition and reduce ex vivo prostaglandin E2 biosynthesis in rats fed n-6 or n-3 fatty acids. Lipids 1998; 33:417-425.
6. Kramer JK, Sehat N, Dugan ME, Mossoba MM, Yurawecz MP, Roach JA, et al. Distributions of conjugated linoleic acid (CLA) isomers in tissue lipid classes of pigs fed a commercial CLA mixture determined by gaschromatography and silver ion-high-performance liquid chromatography. Lipids 1998;33: 549-558.
7. Alasnier C, Berdeaux O, Chardigny JM, Sébédio JL. Fatty acid composition and conjugated linoleic acid content of different tissues in rats fed individual conjugated linoleic acid isomers given as triacylglycerols small star, filled. J Nutr Biochem 2002; 13:337-345.
8. Rudel LL. Atherosclerosis and conjugated linoleic acid. Br J Nutr 1999; 81: 177-179.
9. Khosla P, Fungwe TV. Conjugated linoleic acid: effects on plasma lipids and cardiovascular function. Curr Opin Lipidol 2001; 12: 31-34.
10. Kritchevsky D, Tepper SA, Wright S, Tso P, Czarnecki SK. Influence of conjugated linoleic acid (CLA) on establishment and progression of atherosclerosis in rabbits. J Am Coll Nutr 2000; 19: 472S-477S.
11. Nicolosi RJ, Rogers EJ, Kritchevsky D, Scimeca JA, Huth PJ. Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters. Artery 1997; 22: 266-277.
12. Toomey S, Roche H, Fitzgerald D, Belton O. Regression of pre-established atherosclerosis in the apo-

E (-/-) mouse by conjugated linoleic acid. Biochem Soc Trans 2003; 31: 1075-1079.
13. Lee JH, Cho KH, Lee KT, Kin MR. Antiatherogenic effects of structured lipid containing conjugated linoleic acid in C57BL/6J mice. J Agric Food Chem 2005; 53: 7295-7301.
14. Mitchell PL, Langille MA, Currie DL, McLeod RS. Effect of conjugated linoleic acid isomers on lipoproteins and atherosclerosis in the Syrian Golden Hamster. Biochim Biophys Acta 2005; 1734: 269-276.
15. Pal S, Takechi R, Ho SS. Conjugated linoleic acid suppresses the secretion of atherogenic lipoproteins from human HepG2 liver cells. Clin Chem Lab Med 2005; 43:269-274.
16. Valeille K, Ferezou J, Amsler G, Quignard-Boulangé A, Parquet M, Gripois D, et al. cis-9,trans-11-conjugated linoleic acid-rich oil reduces the outcome of atherogenic process in hyperlipidemic hamster. Am J Physiol 2005; 289: H652-H659.
17. Nagao K, Inoue N, Wang YM, Hirata J, Shimada Y, Nagao T, et al.The 10 trans,12 cis isomer of conjugated linoleic acid suppresses the development of hypertension in Otsuka Long-Evans Tokushima fatty rats. Biochem Biophys Res Commun 2003; 306: 134-138.
18. Inoue N, Nagao K, Hirata J, Wang YM, Yanagita T. Conjugated linoleic acid prevents the development of essential hypertension in spontaneously hypertensive rats. Biochem Biophys Res Commun 2004 ; 323: 679-684.
19. Tappia PS, Mangat R, Gabriel C, Dent MR, Aroutiounova N, Weiler H. Gender differences in the cardiac response to dietary conjugated linoleic acid isomers. Can J Physiol Pharmacol 2006; 84: 257-264.
20. Tappia PS, Dent MR, Aroutiounova N, Babick AP, Weiler H. Gender differences in the modulation of cardiac gene expression by dietary conjugated linoleic acid isomers. Can J Physiol Pharmacol 2007; 85: 465-475.
21. An D, Rodrigues B. Role of changes in cardiac metabolism in development of diabetic cardiomyopathy. Am J Physiol Heart Circ Physiol 2006; 291: H1489-H14506.
22. Marwick TH. Diabetic heart disease. Heart 2006; 92: 296-300.
23. Hartel U. Gender issues in the epidemiology of cardiovascular diseases. Ther Umsch. 2007; 64: 297-304.
24. Gu K, Cowie CC, Harris MI. Diabetes and decline in heart disease mortality in US adults. J Am Med Assoc 1999; 281: 1291-1297.
25. Scirica BM. Prevalence, incidence, and implications of silent myocardial infractions in patients with diabetes mellitus. Circulation 2013; 127: 965-967.
26. Y-J Xu, Tappia PS, Neki NS, Dhalla NS. Prevention of diabetes-induced cardiovascular complications upon treatment with antioxidants. Heart Fail Rev 2014; 19: 113-121.
27. Truong UT, Maahs DM, Daniels SR. Cardiovascular disease in children and adolescents with diabetes: where are we, and where are we going? Diabetes Technol Ther 2012; 14: S11-S21.
28. Rivellese AA, Riccardi G, Vaccaro O. Cardiovascular risk in women with diabetes. Nutr. Metab. Cardiovasc. Dis 2010; 20: 474-480.
29. Kumar S, Singh R, Vasudeva N, Sharma S. Acute and chronic animal models for the evaluation of anti-diabetic agents. Cardiovasc Diabetol 2012;11:9.
30. Rodrigues B, Poucheret P, Battell ML, McNeill J. Streptozotocin-induced diabetes: induction, mechanism(s) and dose dependency. In: McNeill J, ed. Experimental Models of Diabetes. Boca Raton: CRC Press, 1999: 3-17.
31. Tappia PS, Asemu G, Aroutiounova N, Dhalla NS. Defective sarcolemmal phospholipase C signaling in diabetic cardiomyopathy. Mol Cell Biochem 2004; 261:193-199.
32. Tappia PS, Thliveris J, Xu YJ, Aroutiounova N, Dhalla NS. Effects of amino acid supplementation on myocardial cell damage and cardiac function in diabetes. Exp Clin Cardiol 2011; 16: e17-e25.
33. Winer J, Jung CK, Shackel I, Williams PM. Development and validation of real-time quantitative reverse transcriptase-polymerase chain reaction for monitoring gene expression in cardiac myocytes in vitro. Anal Biochem 1999; 270: 41-49.
34. Smit LA, Baylin A, Campos H. Conjugated linoleic acid in adipose tissue and risk of myocardial infarction. Am J Clin Nutr 2010; 92: 34-40.
35. Sun CH, Zhou XR, Wen Y, Liu YM. Effects of conjugated linoleic acid on expression of GLUT4 protein in skeletal muscle of insulin resistant rat. Zhonghua. Yu. Fang. Yi. Xie. Za. Zhi 2007; 41: 25-28.
36. Moloney F, Toomey S, Noone E, Nugent A, Allan B, Loscher CE, Roche HM. Antidiabetic effects of cis-9, trans-11-conjugated linoleic acid may be mediated via anti- inflammatory effects in white adipose tissue. Diabetes 2007; 56: 574-582.
37. Rungapamestry V, McMonagle J, Reynolds C, Rucklidge G, Reid M, Duncan G, et al. Inter-organ proteomic analysis reveals insights into the molecular mechanisms underlying the anti-diabetic effects of cis-9, trans-11-conjugated linoleic acid in ob/ob mice. Proteomics 2012; 12: 461-476.
38. Xiao YF, Gomez AM, Morgan JP, Lederer WJ, Leaf A. Suppression of voltage-gated L- type Ca^{2+} currents by polyunsaturated fatty acids in adult and neonatal rat ventricular myocytes. Proc Natl Acad Sci USA 1997; 94: 4182-4187.
39. Nagao K, Inoue N, Wang YM, Yanagita T. Conjugated linoleic acid enhances plasma adiponectin level and alleviates hyperinsulinemia and hypertension in Zucker diabetic fatty (fa/fa) rats. Biochem Biophys Res Commun 2003; 310: 562-566.
40. Herrmann J, Rubin D, Häsler R, Helwig U, Pfeuffer M, Auinger A, Laue C, Winkler P, Schreiber S, Bell D, Schrezenmeir J. Isomer-specific effects of CLA on gene expression in human adipose tissue depending on PPARγ2 P12A polymorphism: a double blind,

randomized, controlled cross-over study. Lipids Health Dis 2009 ;8:35.

41. Halade GV, Rahman MM, Fernandes G. Differential effects of conjugated linoleic acid isomers in insulin-resistant female C57Bl/6J mice. J Nutr Biochem 2010;21: 332-337.
42. Nestel P, Fujii A, Allen T. The cis-9, trans 11 isomer of conjugated linoleic acid (CLA) lowers plasma triglyceride and raises HDL cholesterol concentrations but does not suppress aortic atherosclerosis in diabetic apoE-deficient mice. Atherosclerosis 2006;189: 282-287.
43. Mitchell PL, Karakach TK, Currie DL, McLeod RS. t10, c12 CLA dietary supplementation inhibits atherosclerotic lesion development despite adverse cardiovascular and hepatic metabolic marker profiles. PLOS ONE 2012; 7: e52634.
44. Ohashi A, Matsushita Y, Kimura K, Miyashita K, Saito M. Conjugated linoleic acid deteriorates insulin resistance in obese/diabetic mice in association with decreased production of adiponectin and leptin. J Nutr Sci Vitaminol (Tokyo). 2004; 50: 416-421.
45. Castro-Webb N, Ruiz-Narváez EA, Campos H. Cross-sectional study of conjugated linoleic acid in adipose tissue and risk of diabetes. Am J Clin Nutr 2012; 96: 175-181.
46. Asp ML, Collene AL, Norris LE, Cole RM, Stout MB, Tang SY, Hsu JC, Belury MA.Time-dependent effects of safflower oil to improve glycemia, inflammation and blood lipids in obese, post-menopausal women with type 2 diabetes: a randomized, double- masked, crossover study. Clin Nutr 2011; 30: 443-449.
47. McCrorie TA, Keaveney EM, Wallace JM, Binns N, Livingstone MB. Human health effects of conjugated linoleic acid from milk and supplements. Nutr Res Rev 2011; 24:206-227.
48. Wahle KW, Heys SD, Rotondo D. Conjugated linoleic acids: are they beneficial or detrimental to health? Prog Lipid Res 2004; 43: 553-587.
49. Wang Y, Proctor SD. Current issues surrounding the definition of trans-fatty acids: implications for health, industry and food labels. Br J Nutr 2013; 110:1369-1383.