A trans10-18:1 enriched fraction from beef fed a barley grain-based diet induces lipogenic gene expression and reduces viability of HepG2 cells

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1. Introduction

Consumption of trans (t) fatty acids have been associated with numerous adverse effects such as increased plasma LDL-cholesterol/HDL-cholesterol ratio, inflammation, insulin resistance, endothelial dysfunction and oxidative stress [1,2]. The main dietary source of trans fatty acids has been partially hydrogenated vegetable oil (PHVO), which can contain up to 60% trans fatty acids. The trans fatty acids in PHVO are mainly in the form of trans-10-18:1, representing 90–95% of total trans fatty acids in PHVO [3]. Among these, t9-18:1 (elaidic acid) and t10-18:1 are typically the first and second major isomers, making up on average about 28% and 21% of total t-18:1 respectively [4]. Internationally, reduction in PHVO in the food supply has become a priority, and this is to the point where PHVO are no longer generally recognized as safe in the USA, and food manufactures have been given three years to take them out of their products [5]. This leaves ruminant meat and milk as the major source of t18:1 in human diets.

In ruminant animals (e.g. sheep, cattle, goats), t18:1 isomers are produced by rumen microbes during biohydrogenation of dietary polyunsaturated fatty acids (PUFA), and these can be incorporated into meat and milk [6]. When cattle are fed diets with a high forage to grain ratio, t11-18:1 is typically 50–70% of t-18:1 isomers, but when a low forage to grain diet is fed, ruminal biohydrogenation pathways shift towards t10-18:1 production [7]. The most concentrated trans fatty acid isomer found in partially hydrogenated vegetable oils were more cytotoxic and led to greater expression of lipogenic genes.
However, frequently assumed to be t11-18:1 (vaccenic acid), and this has made it difficult to interpret their human health effects. In contrast to PHVO, epidemiological studies indicate ruminant trans fatty acids are not associated with increased risk for coronary heart disease [8–11], and several animal and cell culture studies have noted positive health effects of t11-18:1. Clinical trials, however, have shown trans fatty acids from both PHVO and ruminant fats have adverse effects on blood lipids and lipoproteins [12,13].

Studying the effects of t18:1 isomers in cell culture is made difficult because some isomers (e.g. t9- and t11-18:1) are commercially available, while others (e.g. t10-18:1) are not. In order to clarify effects of ruminant products with differing trans fatty acid composition, a limited number of animal feeding studies have included dairy products enriched with different t-18:1 isomers [14,15]. Changes in the t-18:1 profile, however, may have led to confounding effects due to changes in the saturated fatty acid (SFA) and cis-monounsaturated fatty acid (c-MUFA) contents. To alleviate confounding effects of other fatty acids, we developed silver-ion chromatography techniques to isolate t-18:1 fractions and individual t-18:1 isomers in quantities sufficient to test their metabolism and bioactivity in cell culture [16]. The objective of the present experiment was to compare the effects of t18:1 fractions from beef fat enriched with t10-18:1 (HT10) or t11-18:1 (HT11) with t9-18:1 (the major t18:1 isomer in PHVO) and c9-18:1 (oleic acid) in liver cell culture (HepG2). We chose liver cells because of their central role in the metabolism of cis and trans fatty acids including β-oxidation, Δ9 desaturation and lipoprotein secretion [17]. In addition, the adverse effects of trans fatty acids from PHVO on blood lipoproteins have been suggested to be mediated in part via modulation of hepatic lipogenic gene expression [18,19]. We hypothesized that beef t-18:1 fractions with different t-18:1 isomer profiles would have distinct effects on HepG2 cells, specifically in terms of cell viability, lipogenic gene expression, and incorporation of t-18:1 isomers into cell triacylglycerol (TG) and phospholipid (PL) fractions.

2. Materials and methods

2.1. Fatty acid treatments

The HT11 and HT10 fractions were isolated from banked backfat samples collected from cattle fed forage (hay) and barley grain-based diets respectively. Diets included supplements rich in 18:2n-6 to increase the content of t18:1 fatty acids in beef fat. The distribution of t-18:1 isomers of the HT11 and HT10 fractions are presented in Table 1. Trans-18:1 fractions were isolated using silver ion (Ag+) solid phase extraction [16]. Briefly, fat was freeze dried, dissolved in toluene and methylated using 0.5 M sodium methoxide [20]. The resulting fatty acid methyl esters were dissolved in hexane and applied to Discovery® Ag+ SPE columns (750 mg/6 ml, Supelco, Bellefonte, PA, USA). Saturated fatty acids (SFA) were eluted with 10 ml of hexane and the t-18:1 fraction was collected with 10 ml 98:2 hexane: acetone (v/v). The t-18:1 FAME were then saponified to free fatty acids using 0.3 M methanolic potassium hydroxide [21].

Individual 18:1 isomers including t9-18:1 and c9-18:1 were purchased from Nu-Chek Prep, Inc. (Elysian, MN, USA). Individual fatty acids and t18:1 fractions were complexed with fatty acid free bovine serum albumin (BSA) at a 4:1 M ratio (4 mM fatty acid: 1 mM BSA) as described by Evans et al. [22]. Fatty acid-BSA complexes were then diluted in cell culture media to provide desired final fatty acid concentrations (100 or 200 μM).

2.2. Cell viability

To test viability, cells were cultured with growth medium containing 5% FBS and supplemented with 100 μM or 200 μM of fatty acids for 96 h. Cell proliferation was assessed using CellTiter-Blue cell viability assay (Promega, Madison, WI). Briefly, HepG2 cells were seeded in 96-well plates (~1000 cells/well) and allowed to attach before 300 μl of medium containing 100 or 200 μM of fatty acid treatment was added. At 0 h, 24 h, 48 h, 72 h and 96 h growth medium including fatty acid treatments was replaced with 100 μl/well serum free medium plus 20 μl/well CellTiter 96® AQueous One Solution reagent (Promega, Madison, WI). After 1.5 h incubation at 37 °C in 5% CO2, the absorbance at 490 nm was recorded using a SpectraMax M5 plate reader (Molecular Devices, Sunnyvale, CA). The viability results for fatty acid treatments were expressed relative to BSA control.

2.3. Culture conditions for gene expression and fatty acid analyses

Human hepatoma HepG2 cells (ATCC; Rockville, MD, USA) were seeded at a density of 1 × 105 cells per well (9.6 cm2) in 6-well plates, and cultured at 37 °C in 5% CO2 in a growth medium containing Eagle’s minimum essential medium (EMEM; ATCC) supplemented with 10% fetal bovine serum (FBS, Sigma Aldrich, St. Louis, MO, USA) and 1% penicillin-streptomycin (Life Technologies, Burlington, ON, Canada). Cell culture medium was changed every two days, and at 24 h post-confluence, FBS was removed from the medium and cells were treated with 100 μM of the fatty acid-BSA complex for 24 h, and cells were used for fatty acid and gene expression analyses. Control cells were cultured with an equal volume of BSA (vehicle control). Two wells of cells were cultured per treatment per experiment, and the experiment was repeated three times.

2.4. RNA extraction, reverse transcription and quantitative PCR

Total RNA was extracted from HepG2 cells using the Aurum total RNA fatty and fibrous tissue kit (Bio-Rad Laboratories, Mississauga, ON, Canada). The RNA concentrations were determined by absorbance at 260 nm using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE), and the RNA purity was evaluated using the 260:280 and 260:230 absorbance ratios to ensure both ratios were between 1.8 and 2.1. Integrity of RNA was confirmed by the presence of intact RNA sub-units 28S and 18S using an automated capillary electrophoresis QIAxcel system (Qiagen, Hilden, Germany). cDNA was synthesized from 1 μg of RNA using M-MLV reverse transcriptase kit (Invitrogen, Carlsbad, CA, USA) in the presence of random hexamer primers and Ribonuclease Inhibitor (Invitrogen) in a total reaction volume of 20 μl. Real-time PCR analysis was performed using a Stratagene Mx3005P QPCR system (Agilent Technologies) using

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**Table 1**

| Fatty acid (%) | HT10<sup>2</sup> | HT11<sup>2</sup> |
|----------------|----------------|----------------|
| t6-18:1-19:1   | 7.4            | 4.0            |
| t9-18:1       | 5.7            | 4.1            |
| t10-18:1      | 69.3           | 2.9            |
| t11-18:1      | 7.6            | 65.4           |
| t12-18:1      | 1.8            | 6.9            |
| t13/14-18:1   | 3.0            | 6.8            |
| t15-18:1      | 0.7            | 5.1            |
| t16-18:1      | 0.4            | 2.9            |

<sup>2</sup> HT11 – t-18:1 fraction enriched with t11-18:1 from a beef cattle fed a grass-hay based diet. HT10 – t-18:1 fraction enriched with t10-18:1 from a beef cattle fed a barely grain based diet.
the following protocol: enzyme activation 95 °C for 10 min, initial denaturation at 95 °C for 15 s, and annealing/extension at 60 °C for 60 s, repeated for 40 cycles. This was followed by a melt curve analysis as per the manufacturer’s settings to ensure specific amplification. Primers were designed using primer 3.0 software or taken from the literature (Table 2).

Samples were run in triplicate in 96-well plates, and each 20 μl reaction contained 6 μl cDNA diluted at 1:50, 0.4 μl of each forward and reverse primers (10 mM), 8 μl Green-2-Go qPCR Master mix (BioBasic, Markham, ON, Canada), and 5.2 μl of nuclease-free water. Relative mRNA expression was calculated using ΔCt method with β-actin as internal control gene. Briefly, target gene cycle threshold (Ct) values was normalized to that of β-actin using 2−ΔCt where ΔCt = Ct target gene − Ct β-actin. Statistical analysis was performed on 2−ΔCt data and the results were expressed as fold change relative to control [23].

2.5. Fatty acid analysis

Cell lipids were extracted using hexane/isopropanol (3:1) [24] and dried under N2(g). The lipid extracts were then dissolved in methanol, plates were sprayed with 2′,7′-dichlorofluorescein in methanol, and lipids were visualized under UV light. The spots containing TG and PL were scraped into pyrex culture tubes (16 mm × 1.27 mm) with Teflon coated liners, and methylated using 0.5 M sodium methoxide (15 min at 50 °C) with the inclusion of c10-17:1 as an internal standard (Nu-Chek Prep). Resulting fatty acid methyl esters were analysed by gas chromatography using a CP-Sil88 column (100 m, 25 μm ID, 0.2 μm film thickness) using a CP-3800 gas chromatograph equipped with a flame ionization detector and a 8400-series autosampler (Varian Inc., Walnut Creek, CA, USA). Hydrogen was used as the carrier gas (1 ml/min initial flow rate, head pressure 25 psi) and the injection port was set at 250 °C, while the detector was set at 260 °C. The temperature program was as follows: initial temperature at 45 °C for 4 min, raised to 175 °C at 13 °C/min and held for 27 min, then to 215 °C at 4.0 °C/min, and finally held at this temperature for 35 min [27]. Individual peaks were identified using reference standards (GLC-603, Nu-Chek Prep, Inc., Elysian, MN, USA) and peak order and retention times reported in the literature [25–27]. The c9-16:1/16:0 and c9-18:1/18:0 ratios were used to estimate stearoyl-CoA desaturase-1 (SCD1) activity.

2.6. Statistical analysis

Data were analysed using the mixed models procedure of SAS (v 9.3; SAS Institute, Cary, IN, USA) with treatment as the fixed effect and experimental replicate as the random effect. The means were considered to be significantly different at P < 0.05 using the Tukey-Kramer multiple comparison test. Data are expressed as means ± SEM.

3. Results

3.1. Cell viability

The viability of HepG2 cells in the presence of 100 μM or 200 μM of fatty acid treatments are shown in Fig. 1. Culturing cells with all 18:1 treatments reduced cell viability over 96 h of incubation compared to BSA control. However, only cells cultured with HT10 or r9-18:1 showed significant inhibitory (P < 0.05) responses to increased fatty acid concentration (i.e. from 100 μM to 200 μM) occurring from 48 h to 96 h of incubation, and cytotoxic effects of HT10 were more pronounced than r9-18:1.

3.2. SFA, cMUFA and PUFA composition and Δ9 desaturation indices

Fatty acid compositions of HepG2 cell TG and PL are presented in Table 3. Treating cells with r9-18:1, HT10 or HT11 reduced (P < 0.05) SFA (e.g. 16:0 and 18:0) and c-MUFA contents compared to control cells and changes were more pronounced in TG than in PL. Culturing cells with c9-18:1 increased its content (P < 0.05) mainly at the expense of other c-MUFA (e.g. c11-18:1 and c9-16:1) and SFA, and this effect was more evident in TG than PL. The percentage of n-6 and n-3 PUFA in TG and PL fractions were not affected by any fatty acid treatment. Treatment of cells with r9-18:1 or HT10 increased (P < 0.05) the c9-18:1, HT10 or HT11 reduced 9-18:1 showed significant inhibitory (P < 0.05) effects on increased fatty acid concentration (i.e. from 100 μM to 200 μM) occurring from 48 h to 96 h of incubation, and cytotoxic effects of HT10 were more pronounced than r9-18:1.

3.3. Incorporation of t18:1 isomers and their Δ9 desaturation products

The content of t18:1 isomers and their Δ9 desaturation products in TG and PL fractions are presented in Table 4. All fatty acids with a trans double bond in cellular lipids originated from exogenous sources (i.e. r18:1 treatments) because of their absence from both BSA control and c9-18:1 treated cells. All t18:1 isomers were incorporated to a much greater extent (1.8–2.5 times) into

Table 2: Gene specific forward and reverse primer sequences of genes used for Real-Time PCR.

| Gene       | Forward primer (5′-3′) | Reverse primer (5′-3′) |
|------------|-----------------------|-----------------------|
| ACC        | CCACAGTGAATCTGATGAGA  | GGTGCAACACGGCTCTCTAG  |
| FAS        | CTTACTGTCACGGGTACTCC  | CGAAGCTTACAGATATTT    |
| SCD1       | CTCATCGTGACATGAGAGA   | AATGCTTGAAGGCGGCAAC   |
| HMGCR      | CGCCATCGCCCTCTTCCTCC  | CTCTCTCTTGATACGAGGCC  |
| SREBP1c    | GCCCACTCCTCCTCTTCCTCC | CTGCCGTCGACCTCATTGCC  |
| β-actin    | AAAGACAGCTGACCCCAACACACTGCTGCTTGG | CTGCCATCTCTGGCTTGTACACACCTGCTC |

ACC: acetyl-CoA carboxylase; FAS: fatty acid synthase; SCD1: stearoyl-CoA desaturase-1; HMGCR: 3-Hydroxy-3-Methylglutaryl-CoA reductase; SREBP1c: sterol regulatory element-binding protein-1c; SREBP2: sterol regulatory element-binding protein-2.
Fig. 1. Viability of HepG2 cells cultured with 100 μM (black) or 200 μM (gray) of cis-9:18:1, trans-9:18:1, HT11 or HT10 during 96 h incubation. Values (mean ± SE; n = 6) are expressed as percentage changes relative to the BSA control. HT11 = beef t-18:1 fraction enriched with r11-18:1; HT10 = beef t-18:1 fraction enriched with r10-18:1.

Table 3

| Fatty acid | Control | cis-9:18:1 | trans-9:18:1 | HT11 | HT10 | SEM |
|-----------|---------|------------|--------------|------|------|-----|
| TG        |         |            |              |      |      |     |
| 16:0      | 30.20a  | 14.90b     | 14.21b       | 18.27b | 14.39b | 2.00 |
| 18:0      | 7.54a   | 4.44d      | 5.21b        | 5.24b  | 4.03b  | 0.30 |
| 18:1      | 23.53b  | 61.14b     | 12.09b       | 11.85b | 10.92b | 1.38 |
| c18:1-1   | 21.03a  | 8.90b      | 7.75b        | 8.47b  | 6.76b  | 0.61 |
| 18:2n6    | 0.18a   | 0.21a      | 0.26b        | 0.26b  | 0.27b  | 0.07 |
| 20:4n6    | <0.01   | <0.01      | <0.01        | <0.01  | <0.01  | <0.01|
| 20:5n-3   | <0.01   | <0.01      | <0.01        | <0.01  | <0.01  | <0.01|
| 22:6n-3   | <0.01   | <0.01      | <0.01        | <0.01  | <0.01  | <0.01|
| c16:1-16:0| 3.06b   | 0.29c      | 0.37b        | 0.30b  | 0.28c  | 0.03 |
| c18:1-18:0| 3.28b   | 22.40a     | 4.77b        | 3.66b  | 3.16b  | 0.67 |
| PL        |         |            |              |      |      |     |
| 16:0      | 22.17a  | 19.71ab    | 12.35b       | 18.24c | 14.85d | 0.91 |
| c16:1     | 12.10a  | 6.73b      | 8.03b        | 7.85b  | 8.65b  | 0.56 |
| 18:0      | 8.21a   | 7.34b      | 4.32c        | 5.20d  | 4.93c  | 0.36 |
| c9:18:1   | 22.96a  | 43.36a     | 15.82d       | 16.09b | 15.69b | 0.03 |
| c11:18:1  | 17.35a  | 9.00b      | 9.68b        | 9.41b  | 9.66b  | 0.25 |
| 18:2n6    | 1.51a   | 1.29a      | 1.55b        | 1.22b  | 1.49b  | 0.10 |
| 20:4n6    | 3.09a   | 3.20a      | 3.65b        | 3.08b  | 3.63b  | 0.18 |
| 20:5n-3   | 0.07a   | 0.04c      | 0.07c        | 0.19b  | 0.03c  | 0.04 |
| 22:6n-3   | 2.01c   | 1.63c      | 1.74c        | 1.45c  | 1.72c  | 0.14 |
| c9-16:1   | 0.55ab  | 0.34ab     | 0.65c        | 0.43b  | 0.60b  | 0.02 |
| c9-18:1   | 2.89c   | 5.93d      | 3.70b        | 2.66b  | 3.52b  | 0.14 |

TAG than PL.

Cells treated with HT10 or r9-18:1 had 140% more Σt-18:1 in TAG than cells treated with HT11 (P < 0.05). In PL, cells treated with r9-18:1 had the greatest Σt-18:1 (34%) followed by HT10 (26%) and HT11 (19%). Treatment of cells with HT10 and HT11 led to the incorporation of several minor r18:1 isomers due to their presence in treatment fractions, but minor r18:1 isomers were not found when cells were treated with (pure) t-18:1.

Several r18:1 (e.g. r11-18:2, r12-18:2, r13-18:2, r14-18:2, in HT10 and HT11 treated cells underwent Δ9 desaturation, and these were detected in both TG and PL. During GC, these were detected as c9, r11-18:2 and two peaks with co-eluting isomers (c9,t13-/c9,t14-18:2 and c9,t12-/c9,t15-18:2). In contrast to r18:1 isomers, which were preferentially incorporated into TG, the r18:1 derived Δ9 desaturation products tended to accumulate in PL. Cells treated with HT11 had the greatest content of r18:1 derived Δ9 desaturation products in TG and PL (11.9% and 14.3% respectively), followed by HT10 treated cells (2.0% and 3.6% respectively), while r9-18:1 treated cells were devoid of r18:1 derived Δ9 desaturation products.

### 3.4. mRNA expression of key genes involved in fatty acid and cholesterol synthesis

The relative mRNA abundance of key genes involved in de novo fatty acid synthesis (ACC, FAS), Δ9 desaturation (SCD1), cholesterol synthesis (HMGCR) and key transcriptional regulators of lipogenesis (SREBP1c) and cholesterol synthesis (SREBP2) are shown in Fig. 2. Culturing cells with r9-18:1 or HT10 similarly increased (P < 0.05) mRNA expression of FAS, SCD1 and SREBP2 compared to control (BSA) and c9-18:1, but culturing with HT11 did not affect expression of these genes. The expression of SREBP1c was only affected (P < 0.05) in r9-18:1 treated cells which was increased compared to control (BSA) and other fatty acid treatments.
Table 4
The content (molar %) of 9-18:1 isomers and their Δ9 desaturation in triacylglycerol (TG) and phospholipid (PL) fractions of HepG2 cells cultured with 100 μM of trans-9 18:1, HT11 and HT10 for 24 h.¹

| Fatty acid     | t9-18:1 | HT11² | HT10² | SEM³ |
|----------------|---------|-------|-------|------|
| TAG            |         |       |       |      |
| Σt-9:18:1      | 51.55*  | 33.80*| 50.65*| 1.64 |
| f6-9-18:1      | 0.00⁰   | 1.35* | 2.56* | 0.53 |
| t9-18:1        | 51.55*  | 2.13* | 3.88* | 0.55 |
| t10-18:1       | 0.00⁰   | 1.88* | 3.76* | 1.31 |
| t11-18:1       | 0.00⁰   | 22.41*| 3.87* | 0.69 |
| t12-18:1       | 0.00⁰   | 3.11* | 1.12* | 0.092|
| t13/14-18:1    | 0.00⁰   | 2.17* | 1.23* | 0.09 |
| t15-18:1       | 0.00⁰   | 2.01* | 0.65* | 0.082|
| t16-18:1       | 0.00⁰   | 7.49* | 0.16* | 0.057|
| ΔΣt-9:18:2     | 0.00⁰   | 11.87*| 2.02* | 0.69 |
| c9t12/c14-14:2 | 0.00⁰   | 1.28* | 0.66* | 0.12 |
| c9c10-18:2     | 0.00⁰   | 0.23* | 0.03* | 0.042|
| c9t15/c9t12-18:2| 0.00⁰  | 0.97* | 0.28* | 0.077|
| c9t11-18:2     | 0.00⁰   | 8.82* | 1.04* | 0.71 |

**Means within a row not sharing common letters are significantly different (P < 0.05).**

¹ c = cis; t = trans; c9t12/18:2 = Δ9 desaturation products of t18:1 isomers.
² HT11 = beef t-18:1 fraction enriched with t11-18:1; HT10 = beef t-18:1 fraction enriched with t10-18:1.
³ Standard error of the mean.

Fig. 2. Effect of culturing HepG2 cells with 100 μM of cis(c9)-18:1, trans(t9)-18:1, HT11 or HT10 on relative mRNA expression of genes related to fatty acid (FA) synthesis (ACC; acetyl-CoA carboxylase, FAS; fatty acid synthase), Δ9 desaturation (SCD1; Stearoyl-CoA desaturase-1), transcriptional regulation of lipogenesis (SREBP1c; sterol regulatory element-binding protein-1c), and sterol synthesis (SREBP2; sterol regulatory element-binding protein-2). Values (mean ± SE; n = 6/treatment) are expressed as fold changes relative to the control. Within each gene, symbol ** denotes significant (P < 0.05) up-regulation against BSA control (no fatty acid).

4. Discussion
Increasing HT11 concentration from 100 μM to 200 μM did not affect HepG2 cell viability, but viability was reduced in a dose dependent manner by HT10 and t9-18:1, and effects were more accentuated in HT10 treated cells. Trans 18:1 fatty acids have been shown to induce cell death by both caspase-dependent (e.g. activation of caspase-3, -7 and -9) and caspase-independent pathways (e.g autophagy and intracellular reactive oxygen production) [28–30]. Most previous studies treated cells with t9-18:1, but effects of other t18:1 isomers on cell viability have not been extensively studied. In primary cardiac myoblasts both t9-18:1 and t11-18:1 reduced cell growth [28], while in HepG2 cells, t11-18:1 had less adverse effects than t9-18:1 on cell viability [19]. The reason for the greater cytotoxicity of HT10 than t9-18:1 in the current study is not known. To our knowledge there is no previous study on the effects of t10-18:1 on cell viability, but early studies in rats fed PHVO (i.e. containing a broad spectrum of t18:1 isomers) showed that among t18:1 isomers, t10-18:1 was selectively excluded from PL of tissues, which might be related to its potent cytotoxic properties [31]. Further studies are required to explain the cytotoxic effects of t10-18:1.

The reduced content of SFA and cis-MUFA in TAG and PL of cells treated with t9-18:1, HT10, and HT11 is likely due to their substitution with t18:1 isomers [19]. The increased SCD1 index (c9-16:1/16:0 ratio) in the PL fraction related to incubation with t9-18:1 or HT10 (compared to c9-18:1 and HT11) is consistent with up-regulation of SCD1 activity, which is responsible for introducing a double bond between carbon 9 and 10 [32]. Interestingly, culturing with t9-18:1 or HT10 increased the c9-16:1/16:0 ratio in PL but not in TG. This could be due to the fact that PL are an important component of cell membranes and upregulation of SCD1 (which leads to increased Δ9 desaturation indices) has been suggested to be a defence mechanism used by the cells to restore membrane fluidity when high levels of saturated and trans fatty acids are present [33,34]. The lack of increase in SCD1 expression (and SCD1 indices) in cell treated with HT11 could be related to the fact that a significant portion of t18:1 isomers in HT11 underwent Δ9 desaturation, which could increase membrane fluidity, and some SCD1 products (e.g. c9t11-18:2) are known to provide negative feedback to reduce SCD1 expression [34,35]. The dramatic increase in the c9-18:1/18:0 ratio in c9-18:1 treated cells was likely because of the addition of exogenous c9-18:1 rather than increased Δ9 desaturation activity.

The greater accumulation of t18:1 into TG and PL fractions of HepG2 cells treated with HT10 or t9-18:1 could also be related in part to the fact that t9-18:1 and t10-18:1 cannot undergo Δ9 desaturation due to the position of the trans double bond [36,37]. In contrast, a significant portion of t18:1 in HT11 (e.g. t11-, t12-, t13/14- and t15-18:1) underwent Δ9 desaturation giving rise to their desaturation products including c9t11-18:2, c9t12/c9t15-18:2 and c9t13/c9t14-18:2, and these were incorporated to a greater extent in PL than TG.

Consistent with our results, c9t11-18:2 was selectively accumulated in liver PL of pigs fed a commercial conjugated linoleic acid mixture [38]. In contrast to 18:2n-6, which is located predominantly at the sn-2 position of PL, conjugated 18:2 isomers that contain one cis and one trans double bond (e.g. c9t11-18:2 and t10,c12-18:2) are known to be selectively incorporated at the sn-1 position, demonstrating the influence of the trans double bond on the positional distribution of its parent molecule [39]. Furthermore, the reason for the reduced Δ9 desaturation of t18:1 at the sn-1 position of PL is suggested to increase membrane fluidity since they mainly replace SFA (e.g. 16:0 and 18:0) which typically occupy the sn-1 position of PL [39]. Thus, Δ9 desaturation of t18:1 that can undergo Δ9 desaturation might to some extent alleviate the reduced membrane fluidity caused by their parent molecules. For example, the insertion of a cis double bond in t11-18:1 by SCD1 reduces the melting point from 44 °C to −4.5 °C [40]. As a consequence, the extent of Δ9 desaturation of t18:1 isomers might have important health implications. Interestingly, the content of t9-18:1 and t10-18:1 (that do not undergo Δ9 desaturation) in the platelets of patients with coronary artery disease have been significantly
correlated with the extent of the disease, while no associations were found with t11- or r12-18:1 which can undergo Δ9 desaturation [41]. In addition, the anti-carcinogenic and anti-atherogenic properties of r11-18:1 have been mainly related to its conversion to c9,t11-18:2 [42]. However, the potential health effects of Δ9 desaturation products of other t-18:1 isomers have not been studied or considered.

Consistent with previous studies using liver cells [18,19], we observed t9-18:1 increased the expression of genes involved in fatty acid synthesis (FAS), Δ9 desaturation (SCD1), cholesterol synthesis (HMGR) as well as key transcriptional regulators of lipogenesis (SREBP1c) and cholesterol synthesis (SREBP2). We are, however, the first to observe gene expression effects of HT10 are similar to t9-18:1, except for SREBP1c, which only tended to be upregulated by HT10.

Lipogenic genes including FAS and SCD1 are SREBP1 target genes, while HMGR, the rate limiting enzyme for cholesterol synthesis, is mainly a target gene of SREBP-2 [43,44]. Recently Shao and Ford [18] reported an increased expression and activity of SREBP-1c in HuH-7 cells cultured with 9-18:1 could be mediated via SREBP-2. Although we did not measure control or not affect the expression of any measured genes compared to 9-18:1 is mainly mediated by an SREBP-1c-dependent mechanism. Similarly, the upregulation of HMGR by t9-18:1 could be mediated via SREBP-2. Although we did not measure the SREBP protein or activity in the current study, the greater mRNA expression of SREBP-1c and SREBP2 in cells treated with t9-18:1 and HT10 could in part explain the increased expression of lipogenic genes (e.g. FAS and SCD1) and HMGR. The increased expression of key genes involved in hepatic cholesterol and fatty acid synthesis suggests an increased capacity of liver cells for synthesis of fatty acids and cholesterol needed for VLDL formation (the precursor for LDL). It will now be important to determine whether the in vitro effects of HT10 will parallel the in vivo effects on lipogenesis, lipoprotein metabolism and blood cholesterol (e.g. increased LDL-cholesterol and the LDL-cholesterol/HDL-cholesterol ratio).

In contrast to t9-18:1 and HT10, culturing cells with HT11 did not affect the expression of any measured genes compared to control or c9-18:1. The muted effect of HT11 on lipogenic gene expression in HepG2 cells could be explained in part by the conversion of r11-18:1 (the major isomer in HT11) to c9,r11-18:2 which has been shown to decrease hepatic SREBP-1c mRNA expression and activity resulting in decreased hepatic lipogenesis in vivo [45]. Consistent with this, feeding t11-18:1 has been shown to decrease hepatic lipogenesis and improve blood lipid profiles in animal models of dyslipidemia and metabolic syndrome [46-49], which have been mainly associated with its endogenous conversion to c9,t11-18:2. Effects of Δ-9 desaturation products of other t18:1 isomers, however, remain unknown.

5. Conclusion

The effects of 18:1 isomers on HepG2 cell viability were dependent on double bond position and geometric configuration. All 18:1 isomers resulted in reduced cell viability relative to control. Both c9-18:1 and HT11 were the least toxic, t9-18:1 had dose response increased toxicity, and HT10 had the greatest toxicity. Both t9-18:1 and HT10 increased lipogenic gene expression in liver cells compared to c9-18:1 and HT11. Overall, results suggest the content and profile of t-18:1 in foods should be considered when examining their health effects, and a focus on ruminant-derived trans fatty acids should be a priority now that they will become the major source of trans fatty acids in the food supply. Additional in vivo studies will be required to determine if present results will translate into recommendations to increase or curtail consumption of meat and dairy products enriched with either t10-18:1 or r11-18:1.

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Transparency Document. Supplementary material

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References

[1] S.M. Teegala, W.C. Willett, D. Mozafarian, Consumption and health effects of trans fatty acids: a review, J. AOAC Int. 92 (2009) 1250–1257.
[2] R.P. Mensink, Metabolic and health effects of isometric fatty acids, Curr. Opin. Lipidol. 16 (2005) 27–30.
[3] J.L. Sebedio, W.W. Christie, Trans Fatty Acids in Human Nutrition, The Oily Press, Dundee, UK, 1998.
[4] R. Wolff, N. Combe, F. Destaillats, C. Boué, D. Precht, J. Molkeniti, B. Entressangles, Follow-up of the Δ4 to Δ16 trans-18:1 isomer profile and content in French processed foods containing partially hydrogenated vegetable oils during the period 1995–1999. Analytical and nutritional implications, Lipids 35 (2000) 815–825.
[5] USDA Final determination regarding partially hydrogenated oils, Fed. Regist. 80 (2015) 34650.
[6] T. Jenkins, R. Wallace, P. Moate, E. Mosley, Board-invited review: recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem, J. Anim. Sci. 86 (2008) 397–412.
[7] M. Dugan, N. Aldai, J. Alahuus, D. Rolland, J. Kramer, Review: trans-forming beef to provide healthier fatty acids profiles, Can. J. Anim. Sci. 91 (2011) 545–556.
[8] K. Liu, Z.Y. Deng, J.N. Hu, Y.W. Fan, R. Liu, J. Li, J.T. Pong, H. Su, Q. Pong, W.E. Li, Erythrocyte membrane trans-fatty acid index is positively associated with a 10-year CHD risk probability, Br. J. Nutr. 109 (2013) 1695–1703.
[9] P. Pietinen, A. Ascherio, P. Korhonen, A.M. Hartman, W.C. Willett, D. Albanes, J. Vitztum, Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men: the alpha-tocopherol, beta-carotene cancer prevention study, Am. J. Epidemiol. 145 (1997) 867–887.
[10] W.C. Willett, M.J. Stampfer, J.E. Manson, G.A. Colditz, F.E. Speizer, B.A. Rosner, L.A. Sampson, C.H. Hennekens, Intake of trans fatty acids and risk of coronary heart disease among women, Lancet 341 (1993) 581–585.
[11] A. Ascherio, C.H. Hennekens, J.E. Buring, C. Master, M.J. Stampfer, W.C. Willett, Trans-fatty acids intake and risk of myocardial infarction, Circulation 89 (1994) 94–101.
[12] L.A. Brouwer, A.J. Wanders, M.B. Katan, Trans fatty acids and cardiovascular health: research completed? Eur. J. Clin. Nutr. 67 (2013) 541–547.
[13] S.K. Gebauer, F. Destaillats, F. Dionisi, R.M. Krauss, D.J. Baer, Vaccenic acid and trans fatty acid isomers from partially hydrogenated oil both adversely affect LDL cholesterol: a double-blind, randomized controlled trial, Am. J. Clin. Nutr. (2015).
[14] A. Roy, A. Ferlay, Y. Chilliard, Production of butter fat in ruminants: C18-1 for use in biomedical studies in rodents, Reprod., Nutr. Dev. 46 (2006) 211–218.
[15] D.E. Bauman, D.M. Barbau, D.A. Dwyer, J.M. Griniari, Technical note: production of butter with enhanced conjugated linoleic acid for use in biomedical studies with animal models, J. Dairy Sci. 93 (2000) 2422–2425.
[16] T.D. Turner, W.J. Meads, C. Mapsey, P. Vahmani, O. Lopez-Campos, P. Duff, D.C. Rolland, J.S. Church, M.E. Dugan, Isolation of alpha-linolenic acid biohydrogenation products by combined silver ion solid phase extraction and semi-preparative high performance liquid chromatography, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 980 (2015) 34–40.
[17] E.A. Emken, Nutrition and biochemistry of trans and positional fatty acid isomers in hydrogenated oils, Annu. Rev. Nutr. 4 (1984) 339–376.
[18] F. Shao, D.A. Ford, Elaidic acid increases hepatic lipogenesis by mediating sterol regulatory element binding protein-1c activity in HuH-7 cells, Lipids 49 (2014) 403–413.
[19] T. Vandel Nielsen, T.P. Krohgager, C. Young, C. Ferreri, C. Chategilaloglu, O. Nægreagaard Jensen, J.J. Entressangles, Effects of Elaidic Acid on Lipid Metabolism in HepG2 Cells, Investigated by an Integrated Approach of Lipidomics, Transcriptomics and Proteomics, PLoS One 8 (2013) e74283.
[20] J.K. Kramer, V. Fellner, M.E. Dugan, F.D. Sauer, M.M. Mossova, M.P. Yurawecz,
Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and totally trans fatty acids, Lipids 32 (1997) 1219–1228.

[21] W.W. Christie, Lipid Analysis, Pergamon Press, Oxford, 1973.

[22] M. Evans, Y. Park, M. Paniza, L. Curtis, B. Kuebler, M. McIntosh, Trans-10,Cis-12 conjugated linoleic acid reduces triglyceride content while differentially affecting peroxisome proliferator activated receptor y2 and ap2 expression in 3T3-L1 preadipocytes, Lipids 36 (2001) 1223–1232.

[23] T.D. Schmittgen, K.J. Livak, Analyzing real-time PCR data by the comparative CT method, Nat. Protoc. 3 (2008) 1101–1108.

[24] D. Akojum, J.D. Mehd, Simultaneous isolation of total cellular lipids and RNA from cultured cells, BioTechniques 41 (2006) 426–420.

[25] C. Cruz-Hernandez, Z. Deng, J. Zhou, A.R. Hill, M.P. Yurawecz, P. Delmonte, M. M. Mossoba, M.E.R. Dugan, J.K.G. Kramer, Methods for analysis of conjugated linoleic acids and trans-18:1 isomers in dairy fats by using a combination of gas chromatography, silver-ion thin-layer chromatography/gas chromatography, and silver-ion liquid chromatography, J. AOAC Int. 87 (2004) 545–562.

[26] M. Juarez, M.E.R. Dugan, J.L. Aalhus, N. Aldai, J.A. Basarab, V.S. Baron, T. A. McAllister, Effects of vitamin E and flaxseed on rumen-derived fatty acid intermediates in beef intramuscular fat, Meat Sci. 88 (2011) 426–440.

[27] J.K. Kramer, M. Hernandez, C. Cruz-Hernandez, J. Kraft, M.E. Dugan, Combining results of two GC separations partly achieves determination of all cis and trans 16:1, 18:1, 18:2 and 18:3 except CLA isomers of milk fat as demonstrated using Ag-ion SPE fractionation, Lipids 43 (2008) 259–273.

[28] S. Ghavami, R.H. Cunnington, B. Yeganeh, J.J.L. Davies, S.G. Rattan, K. Bathe, J.K. Kramer, M. Hernandez, C. Cruz-Hernandez, J. Kraft, M.E. Dugan, Combining results of two GC separations partly achieves determination of all cis and trans 16:1, 18:1, 18:2 and 18:3 except CLA isomers of milk fat as demonstrated using Ag-ion SPE fractionation, Lipids 43 (2008) 259–273.

[29] Y. Kondoh, T. Kawada, R. Urade, Activation of caspase 3 in HepG2 cells by cis and trans C18:1 fatty acids in human aortic smooth muscle cells, Genes and protect against palmitate-induced cell injury, J. Biol. Chem. 285 (2010) 306–314.

[30] P. Vahmani, W.J. Meadus, T.D. Turner, P. Duff, D.C. Rolland, C. Mapiye, M. D. Zapolska-Downar, A. Ko, S. Ghavami, R.H. Cunnington, B. Yeganeh, J.J.L. Davies, S.G. Rattan, K. Bathe, J.K. Kramer, M. Hernandez, C. Cruz-Hernandez, J. Kraft, M.E. Dugan, Combining results of two GC separations partly achieves determination of all cis and trans 16:1, 18:1, 18:2 and 18:3 except CLA isomers of milk fat as demonstrated using Ag-ion SPE fractionation, Lipids 43 (2008) 259–273.

[31] A. Anadón, M.R. Martínez-Larrañaga, M.A. Martínez, I. Ares, E. Ramos, C.J. Field, H.H. Blewett, S. Proctor, D. Vine, Human health benefits of vaccenic acid, Appl. Physiol. Nutr. Metab. 34 (2009) 979–991.

[32] J.M. Hodgson, M.L. Wahlqvist, J.A. Boxall, N.D. Balazs, Platelet trans fatty acids in relation to angiographically assessed coronary artery disease, Atherosclerosis 120 (1996) 147–154.

[33] C.J. Field, H.H. Blewett, S. Proctor, D. Vine, Human health benefits of vaccenic acid, Appl. Physiol. Nutr. Metab. 34 (2009) 979–991.

[34] J.K. Kramer, N. Sehat, M.E. Dugan, M.M. Mossoba, M.P. Yurawecz, J.A. Roach, K. Elizir, J.L. Aalhus, A.L. Schaefer, Y. Xu, Distributions of conjugated linoleic acid (CLA) isomers in tissue lipid classes of pigs fed a commercial CLA mixture determined by gas chromatography and silver ion-high-performance liquid chromatography, Lipids 33 (1998) 549–558.

[35] P.V. Subbaiah, I.G. Gould, S. Lal, R. A. Alzei, Incorporation profiles of conjugated linoleic acid isomers in cell membranes and their positional distribution in phospholipids, Biochim. Biophys. Acta 1811 (2011) 17–24.

[36] H. Wu, K.D. Ding, E.T. Toledo, H. Campos, A. Baylin, F.B. Hu, Q. Sun, A novel fatty acid lipophilic index and risk of CHD in US men: the health professionals follow-up study, Br. J. Nutr. 110 (2013) 466–474.

[37] J.M. Hodgson, M.L. Wahlqvist, J.A. Boxall, N.D. Balazs, Platelet trans fatty acids in relation to angiographically assessed coronary artery disease, Atherosclerosis 120 (1996) 147–154.

[38] H. Shimano, Sterol regulatory element-binding protein family as global regulators of lipid synthetic genes in energy metabolism, in: Vitamins & Hormones, vol. Volume 65, Academic Press, 2002, pp. 167–194.

[39] H.M. Roche, E. Noone, C. Sewter, S. Mc Bennett, D. Savage, M.J. Gibney, S. O’Rahilly, A.J. Vidal-Pug, Isomer-dependent metabolic effects of conjugated linoleic acid: insights from molecular markers sterol regulatory element-binding protein-1c and LRXalpha, Diabetes 51 (2002) 2037–2044.

[40] A. Anadón, M.R. Martínez-Larrañaga, M.A. Martínez, I. Ares, E. Ramos, P. Gómez-Cortés, M. Juárez, M.A. De La Fuente, A 4-week repeated oral dose study of 5% conjugated linoleic acid lipophilic index and risk of CHD in US men: the health professionals follow-up study, Br. J. Nutr. 110 (2013) 466–474.

[41] C.J. Field, H.H. Blewett, S. Proctor, D. Vine, Human health benefits of vaccenic acid, Appl. Physiol. Nutr. Metab. 34 (2009) 979–991.

[42] J.K. Kramer, N. Sehat, M.E. Dugan, M.M. Mossoba, M.P. Yurawecz, J.A. Roach, K. Elizir, J.L. Aalhus, A.L. Schaefer, Y. Xu, Distributions of conjugated linoleic acid (CLA) isomers in tissue lipid classes of pigs fed a commercial CLA mixture determined by gas chromatography and silver ion-high-performance liquid chromatography, Lipids 33 (1998) 549–558.

[43] P.V. Subbaiah, I.G. Gould, S. Lal, R. A. Alzei, Incorporation profiles of conjugated linoleic acid isomers in cell membranes and their positional distribution in phospholipids, Biochim. Biophys. Acta 1811 (2011) 17–24.

[44] H. Shimano, Sterol regulatory element-binding protein family as global regulators of lipid synthetic genes in energy metabolism, in: Vitamins & Hormones, vol. Volume 65, Academic Press, 2002, pp. 167–194.

[45] H.M. Roche, E. Noone, C. Sewter, S. Mc Bennett, D. Savage, M.J. Gibney, S. O’Rahilly, A.J. Vidal-Pug, Isomer-dependent metabolic effects of conjugated linoleic acid: insights from molecular markers sterol regulatory element-binding protein-1c and LRXalpha, Diabetes 51 (2002) 2037–2044.

[46] A. Anadón, M.R. Martínez-Larrañaga, M.A. Martínez, I. Ares, E. Ramos, P. Gómez-Cortés, M. Juárez, M.A. De La Fuente, A 4-week repeated oral dose study of 5% conjugated linoleic acid lipophilic index and risk of CHD in US men: the health professionals follow-up study, Br. J. Nutr. 110 (2013) 466–474.

[47] C.M.C. Bassett, A.L. Edel, A.F. Patenaude, R.S. McCullough, D.P. Blackwood, P. Gómez-Cortés, M. Juárez, M.A. De La Fuente, A 4-week repeated oral dose study of 5% conjugated linoleic acid lipophilic index and risk of CHD in US men: the health professionals follow-up study, Br. J. Nutr. 110 (2013) 466–474.

[48] C.J. Field, H.H. Blewett, S. Proctor, D. Vine, Human health benefits of vaccenic acid, Appl. Physiol. Nutr. Metab. 34 (2009) 979–991.

[49] P.A. Edwards, D. Tabor, H.R. Kast, A. Venkateswaran, Venkateswaran, Regulation of gene expression by SREBP and SCAP, Biochim. Biophys. Acta 1529 (2000) 103–113.