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RESEARCH PAPER

The effect of *trans*-10, *cis*-12 conjugated linoleic acid on gene expression profiles related to lipid metabolism in human intestinal-like Caco-2 cells

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Abstract We conducted an in-depth investigation of the effects of conjugated linoleic acid (CLA) on the expression of key metabolic genes and genes of known importance in intestinal lipid metabolism using the Caco-2 cell model. Cells were treated with 80 μmol/L of linoleic acid (control), *trans*-10, *cis*-12 CLA or *cis*-9, *trans*-11 CLA. RNA was isolated from the cells, labelled and hybridized to the Affymetrix U133 2.0 Plus arrays ($n = 3$). Data and functional analysis were preformed using Bioconductor. Gene ontology analysis (GO) revealed a significant enrichment ($P < 0.0001$) for the GO term lipid metabolism with genes up-regulated by *trans*-10, *cis*-12 CLA. *Trans*-10, *cis*-12 CLA, but not *cis*-9, *trans*-11 CLA, altered the expression of a number of genes involved in lipid transport, fatty acid metabolism, lipolysis, β-oxidation, steroid metabolism, cholesterol biosynthesis, membrane lipid metabolism, gluconeogenesis and the citrate cycle. These observations warrant further investigation to understand their potential role in the metabolic syndrome.

Keywords Conjugated linoleic acid · Gene expression · Caco-2 cells

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Introduction

Conjugated linoleic acid (CLA) has been shown to have profound effects on hepatic and adipocyte lipid metabolism in both animal and cell models (see review by House et al. [22]). For example, it has been shown that CLA inhibits lipid accumulation [16] and alters lipid droplet morphology [7] in adipocytes. Several studies have examined the effects of CLA on apolipoprotein B-containing lipoproteins in hepatocytes [38, 51, 65]. Storkson et al. [51] demonstrated that while *trans*-10, *cis*-12 CLA decreased apolipoprotein secretion by Hep-G2 cells, the *cis*-9, *trans*-11 CLA isomer had no effect. These findings may be of therapeutic potential in relation to the metabolic syndrome, in which obesity, insulin resistance and hyperlipidemia are common characteristics [17, 18].

While the effects of CLA on lipid metabolism in hepatocytes and adipocytes have been well researched, its effect on intestinal lipid metabolism has been relatively overlooked. The intestine is of major importance in the digestion and absorption of dietary lipids, as well as in the re-synthesis and packaging of lipid for transport. As the site of absorption for dietary cholesterol, the intestine, together with the liver, serves an important regulatory role in the control of cholesterol homeostasis and plasma cholesterol

levels [30]. Furthermore, recent work has demonstrated that the intestine contributes significantly to plasma high density lipoprotein (HDL) cholesterol levels [5]. Low HDL levels is an independent risk factor for the development of cardiovascular disease (CVD) [15].

Intestinal epithelial cells, as the first interface with dietary CLA, are exposed to the highest concentration of CLA isomers in vivo. Black et al. [4] showed that chronic supplementation of human intestinal-like Caco-2 cells with *trans*-10, *cis*-12 CLA, but not the *cis*-9, *trans*-11 isomer, modulated intestinal triacylglycerol (TAG) metabolism, by increasing cellular de novo TAG synthesis. However, CLA supplementation had no effect on TAG secretion from these intestinal-like cells [4]. The authors suggest that these results might imply that chronic supplementation with the *trans*-10, *cis*-12 CLA isomer may delay the secretion of TAG from the intestinal cell and contribute to reduced plasma TAG levels, thus potentially attenuating the post-prandial TAG response. Elevated plasma TAG levels are associated with adverse effects on lipoprotein metabolism [48] and increased risk of CVD [59]. Roche and colleagues [2, 22] showed that supplementation with a mixed isomer CLA reduced fasting plasma TAG in healthy adults. However, the findings from other human studies are mixed (see review by Tricon and Yaqoob 57). The mechanisms by which CLA may influence TAG-rich lipoprotein metabolism in intestinal cells are unclear.

In our previous study, global gene expression analysis showed that chronic supplementation of Caco-2 cells with the *trans*-10, *cis*-12 CLA isomer had profound effects on gene expression, whereas the *cis*-9, *trans*-11 isomer (the most abundant natural isomer) had no effect [41]. While particular emphasis was given to gene expression patterns of relevance to carcinogenesis and calcium transport, for which functional data exists, a number of wide-ranging biological processes of relevance to the intestine were modulated by *trans*-10, *cis*-12 CLA [41]. In particular, genes related to the process of lipid metabolism were found to be significantly altered. Thus, in this study we performed a more in-depth investigation of the effect of *trans*-10, *cis*-12 CLA on gene expression profiles related to lipid metabolism, with a view to increasing our understanding of the role of the intestine in contributing to the effects of CLA on body composition, blood lipid profiles and glucose metabolism in human subjects.

Materials and methods

Conditions of cell culture and cell treatments

Caco-2 cells, a human colon adenocarcinoma cell line, were grown under routine conditions as described in the study by Jewell and Cashman [24]. Cells were seeded at a density of 6×10^4 cm^{-2} into six-well plastic cell culture plates (Costar) and and were treated with foetal bovine serum (FBS; 10%) for 2 days and thereafter with FBS plus 80-μM linoleic acid (as the control and parent fatty acid), 80-μM *trans*-10, *cis*-12 isomer of CLA or 80-μM *cis*-9, *trans*-11 isomer of CLA (as the non-esterified forms of the fatty acids) for a further 12 days, after initial seeding of the cells. The concentration of CLA (80 μM) was chosen on the basis of the earlier estimate of typical luminal concentrations in the human small intestine of an individual with a daily dietary CLA intake of about 150 mg [32]. All compounds were added to complete culture medium prior to their addition to the cells.The choice of collecting RNA at day 14 was on the basis that we have previously shown that our Caco-2 cells are fully differentiated intestinal-like cells (as evidenced by brush border marker enzyme levels and transepithelial electrical resistance [24]).

RNA isolation, processing and microarray analysis

After experimental treatments, total RNA was extracted from the Caco-2 cells using TriReagent according to the manufacturer's directions (Molecular Research Center, Cincinnati, OH, USA) followed by an additional RNA purification step using the Rneasy mini kit (Qiagen Ltd, Crowley, West Sussex, UK). Total RNA was quantified and inspected using the Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA, USA). Starting from 1 μg of total RNA for each sample, cDNA was synthesized using the one cycle cDNA synthesis kit (Affymetrix, USA) and biotinylated cRNA was generated by in vitro transcription, according to Affymetrix protocols (http://www.affymetrix.com/-support/technical/manual/expression_manual.affx). Biotinylated cRNA was fragmented and each sample was hybridized to an Affymetrix HU133 2.0 Plus array (54,645 probe sets) at 42°C for 17 h, then washed, stained and scanned to generate digitized image data (DAT) files following the standard Affymetrix protocol.

Microarray data analysis

Three biological replicas were generated for each experimental condition (namely, 80-μM linoleic acid, 80-μM *trans*-10, *cis*-12 CLA and 80-μM *cis*-9, *trans*-11 CLA). Microarray quality control and statistical validation were performed using Bioconductor [14]. A more detailed [22] description of the statistical analysis performed has been described elsewhere [41]. The list of differentially expressed genes was generated using a false discovery rate of 0.05 together with an absolute fold-change (fc) threshold of 1.6 (i.e. $|\log 2(\text{fc})| \geq 0.7$) and further refined by selecting those probe sets mapping to unique Entrez Gene identifiers (EGs) [35].

Gene ontology: functional enrichment analysis

Gene ontology (GO) provides a restricted vocabulary as well as clear indications of the relationships between biological terms and genes [1]. The up- and down-regulated differentially expressed genes were categorized according to two independent ontologies for gene products; (1) biological process and (2) molecular function, using EASE, in order to identify GO terms enriched [20].

Results

Global gene expression profile in CLA-treated cells

To profile the effects of the CLA isomers on gene expression in Caco-2 cells, the HU133 2.0 Plus array (54,645 probe sets), which provides the most comprehensive analysis of the transcribed human genome, was used. Of the 54,645 probe sets on the HU133 2.0 Plus array, 6,417 (11%) were found to remain after applying the IQR filter of 0.3. Linear model analysis indicated that the number of probe sets with apparently altered expression from Caco-2 cells treated with the *cis*-9, *trans*-11 CLA relative to linoleic acid (the parent and control fatty acid) was less than expected by random chance. Conversely, using a minimum fc criterion of 1.6 and a false discovery rate criterion of P value 0.05, 1,417 probe sets were found to be differentially expressed in Caco-2 cells treated with the *trans*-10, *cis*-12 CLA versus linoleic acid. Further refinement by grading scale (i.e. grade A) developed by Affymetrix (http://www.affymetrix.com) and for probe sets mapping to unique EGs showed that 918 EGs, were regulated by *trans*-10, *cis*-12 CLA treatment relative to linoleic acid. Among these EGs, 406 (44.2%) were up-regulated and 512 (55.8%) were down-regulated. We have previously validated the microarray data by confirming the altered expression of seven key genes using quantitative RT-PCR [41].

Effect of *trans*-10, *cis*-12 CLA on lipid metabolism-related gene expression

In order to investigate the global effects of *trans*-10, *cis*-12 CLA on gut cell biology, the differentially expressed genes were partitioned into those up- and down-regulated by the *trans*-10, *cis*-12 CLA isomer and assessed for statistical enrichment by means of hypergeometric distribution of GO biological process and molecular function categories, as described in detail previously [41]. Of the various biological processes, a significant enrichment ($P < 0.0001$) for genes up-regulated by the *trans*-10, *cis*-12 CLA isomer was identified for the GO term lipid metabolism (GO: 0006629).

A number of genes encoding proteins which are involved in mediating lipid and cholesterol transport in the intestine were found to be either significantly up-regulated: fatty acid binding protein (FABP)-1 (2.04-fold) and FABP-6 (2.94-fold); apolipoprotein A-IV (APOAIV) (6.98-fold) or down-regulated: ATP-binding cassette, sub-family A, member 1 (ABCA1) (4.63-fold) and apolipoprotein B mRNA editing enzyme (APOBEC1) (1.76-fold) in Caco-2 cells treated with *trans*-10, *cis*-12 CLA compared to linoleic acid-treated cells. Furthermore, the *trans*-10, *cis*-12 CLA down-regulated the expression of glycerol-3-phosphate acyltransferase (GPAT) (1.63-fold), the rate-limiting enzyme involved in fatty acid esterification (glycerol 3-phosphate pathway) in Caco-2 cells (Fig. 1).

Gene expression of a number of other rate-limiting enzymes in lipid metabolism-related pathways were also up-regulated by the *trans*-10, *cis*-12 CLA including: β oxidation [carnitine palmitoyltransferase 1A (CPT1A) (2.81-fold)] and fatty acid metabolism [delta-9-desaturase (SCD) (2.14-fold)] (Table 1). Furthermore, the molecular expression of a number of key enzymes involved in the gluconeogenic/glycolysis pathway were significantly up-regulated by *trans*-10, *cis*-12 CLA including: phosphoenol carboxylase kinase 1 (PCK1) (3.33-fold), the major control point for the regulation of gluconeogenesis; hexokinase 2 (HK2) (1.84-fold); aldolase B, fructose-bisbiphosphate (ALDOB) (3.67-fold) and fructose-1,6-bisphosphophosphatase (FBP1) (2.00-fold). These findings suggest an effect of this isomer on glucose metabolism in Caco-2 cells (Table 1).

In relation to the various molecular functions modulated by the *trans*-10, *cis*-12 CLA isomer, the ligand-dependent nuclear receptor activity function (GO: 0004879) was found to be significantly enriched ($P < 0.0001$) by genes up-regulated by the *trans*-10, *cis*-12 CLA isomer of which 7 of the 52 ligand-dependent nuclear receptor activity-associated genes were found to be up-regulated. A number of these transcription factors are involved in mediating the effects of fatty acids on gene transcription including: peroxisome proliferator activated receptor gamma (PPARγ) (2.82-fold); peroxisome proliferator activated receptor delta (PPARδ) (1.79-fold); hepatocyte nuclear factor 4, alpha (HNF4A) (2.23-fold) and farnesoid X receptor (FXR) (5.25-fold), all of which are up-regulated by the *trans*-10, *cis*-12 CLA isomer (Table 1).

Discussion

Adipose tissue, the liver and the intestine are key organs involved in lipid metabolism. In vitro and in vivo studies have shown that CLA affects lipid metabolism in adipocytes and hepatocytes (see reviews by House et al. [22] and Pariza et al. [43]). While the intestine is an important

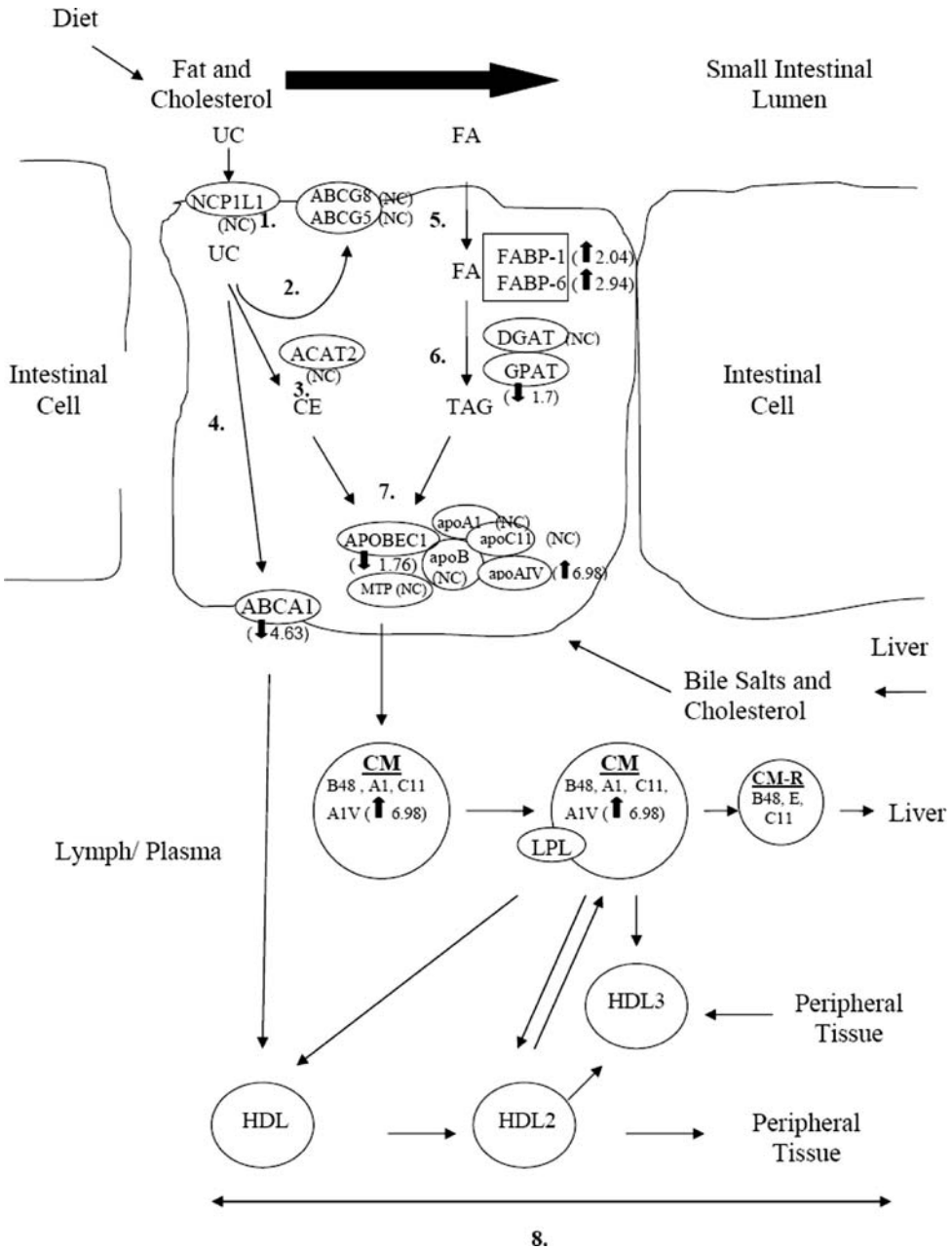

Fig. 1 Changes in the expression of genes encoding mediators of lipid transport in Caco-2 cells treated with 80-μM *trans*-10, *cis*-12 CLA relative to 80-μM linoleic acid-treated cells. *Up arrow* and *down arrow* indicate up or down-regulation of transcription, fold-change (as determined by microarray analysis) is indicated in *brackets* and *NC* no change. Processes represented include: *1* absorption of unesterified cholesterol (*UC*) mediated by the Niemann–Pick C1-like 1 (*NPC1L1*) protein, *2* expulsion of cholesterol (absorbed or synthesized locally in the enterocytes) into the intestinal lumen through the actions of the ATP-binding cassette (*ABC*), subfamily G, member 5/ABCG8 heterodimeric transporter, *3* esterification of cholesterol (*CE*) by acyl-coenzyme A: cholesterol acyltransferase 2 (*ACAT2*) for incorporation into chylomicrons (*CM*), *4* cholesterol transfer to plasma high density lipoproteins (*HDL*) mediated by ABCA1, *5* fatty acids (*FA*) absorption and transport of FA by fatty acid binding proteins (*FABP*)-1 and FABP-6 to the endoplasmic reticulum for incorporation into triglyceride (*TAG*) and phospholipids, *6* TAG synthesis pathways: rate-limiting enzymes in the monoacylglycerol and glycerol 3-phosphate pathways, respectively, are diacylglycerol acyltransferase (*DGAT*) and glycerol-3-phosphate acyltransferase (*GPAT*), *7* chylomicron assembly and secretion, *8* transfer of proteins and lipid subfractions between CM and plasma lipoproteins (i.e. HDL). Apolipoprotein (*apo*), apoB mRNA editing enzyme (*APOBEC1*), microsomal triglyceride transfer protein (*MTP*)

source of plasma lipoproteins, second only to the liver [23], as well as having a primary role in the absorption and transport of dietary lipids, the effect of CLA on intestinal lipid metabolism has not received much attention. Black et al. [4] showed that *trans*-10, *cis*-12 CLA, and not *cis*-9, *trans*-11 CLA, altered lipid metabolism in Caco-2 cells,

Table 1 Genes regulated by *trans*-10, *cis*-12 CLA involved in lipid and glucose metabolism

Gene title	Gene symbol	Entrez gene	Fold change
Lipid transport/storage			
Apolipoprotein A-IV	APOA4	337	6.98
Fatty acid binding protein 6, ileal (gastrotropin)	FABP6	2172	2.94
Fatty acid binding protein 1, liver	FABP1	2168	2.04
Low density lipoprotein receptor (familial hypercholesterolemia)	LDLR	3948	1.72
Apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1	APOBEC1	9582	−1.76
Solute carrier family 27 (fatty acid transporter), member 3	SLC27A3	11000	−1.76
Apolipoprotein D	APOD	347	−3.32
ATP-binding cassette, sub-family A (ABC1), member 1	ABCA1	19	−4.63
Lipolysis			
Monoglyceride lipase	MGLL	11343	2.22
Lipase, endothelial	LIPG	9388	1.65
Lipase, hepatic	LIPC	390	−2.09
Fatty acid metabolism			
Stearoyl-CoA desaturase (delta-9-desaturase)	SCD	6319	2.14
Acyl-CoA synthetase long-chain family member 1	ACSL1	2180	2.00
Acyl-CoA synthetase long-chain family member 4	ACSL4	2182	1.65
Thioesterase, adipose associated	THEA	26027	1.65
Brain acyl-CoA hydrolase	BACH	11332	−1.66
Stearoyl-CoA desaturase 5	SCD5	79966	−2.77
LAG1 longevity assurance homologue 6 (*S. cerevisiae*)	LASS6	253782	−1.67
Fatty acid *β*-oxidation			
Carnitine palmitoyltransferase 1A (liver)	CPT1A	1374	2.81
Steroid metabolism			
Hydroxysteroid (17-beta) dehydrogenase 2	HSD17B2	3294	3.26
Sulfotransferase family, cytosolic, 1B, member 1	SULT1B1	27284	3.06
Cytochrome P450, family 3, subfamily A, polypeptide 5	CYP3A5	1577	2.07
Sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1	SULT1A1	6817	1.97
Sulfotransferase family, cytosolic, 1A, phenol-preferring, member 2	SULT1A2	6799	1.88
Sulfotransferase family, cytosolic, 1A, phenol-preferring, member 3	SULT1A3	6818	1.83
Dehydrogenase/reductase (SDR family) member 9	DHRS9	10170	1.80
Lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)	LSS	4047	1.73
Steroid sulfatase (microsomal), arylsulfatase C, isozyme S	STS	412	−1.95
Sulfotransferase family, cytosolic, 2A, dehydroepiandrosterone (DHEA)-preferring, member 1	SULT2A1	6822	−1.98
UDP glycosyltransferase 2 family, polypeptide B17	UGT2B17	7367	−4.87
Cholesterol biosynthesis			
Protein kinase, AMP-activated, gamma 2 non-catalytic subunit	PRKAG2	51322	2.33
Mevalonate (diphospho) decarboxylase	MVD	4597	1.72
7-Dehydrocholesterol reductase	DHCR7	1717	1.66
Membrane lipid metabolism			
Annexin A1	ANXA1	301	2.78
Lysophosphatidylglycerol acyltransferase 1	LPGAT1	9926	2.37
Sphingomyelin phosphodiesterase 3, neutral membrane (neutral sphingomyelinase II)	SMPD3	55512	2.25
Phospholipase D1, phosphatidylcholine-specific	PLD1	2822	1.81
Sphingosine-1-phosphate lyase 1	SGPL1	8879	1.79
Phospholipase A2-activating protein	PLAA	9373	1.77
Hypothetical protein MGC26963	MGC26963	166929	1.66
Selenoprotein I	SELI	85465	1.64

Table 1 continued

Gene title	Gene symbol	Entrez gene	Fold change
Glycerol-3-phosphate acyltransferase, mitochondrial	GPAM	57678	−1.63
Phosphatidic acid phosphatase type 2B	PPAP2B	8613	−1.78
GM2 ganglioside activator	GM2A	2760	−1.83
Adipocyte secretory products			
Adiponutrin	ADPN	80339	1.66
Lipin 1	LPIN1	23175	2.08
Adipose differentiation- related protein	ADFP	123	1.75
Glycolysis and gluconeogenesis			
Phosphofructokinase, platelet	PFKP	5214	1.69
Fructose-1,6-bisphosphatase 1	FBP1	2203	2.00
Aldolase B, fructose-bisphosphate	ALDOB	229	3.67
Hexokinase 2	HK2	3099	1.84
Citrate cycle			
ATP citrate lyase	ACLY	47	1.65
Isocitrate dehydrogenase 2 (NADP+), mitochondrial	IDH2	3418	−1.66
Phosphoenolpyruvate carboxykinase 1 (soluble)	PCK1	5105	3.33
Transcription factors			
Nuclear receptor subfamily 1, group H, member 4 (farnesoid X receptor)	NR1H4	9971	5.25
Peroxisome proliferative activated receptor, gamma	PPARG	5468	2.82
Hepatocyte nuclear factor 4, alpha	HNF4A	3172	2.23
Nuclear receptor subfamily 1, group I, member 2 (Pregnane X receptor)	NR1I2	8856	1.86
Peroxisome proliferative activated receptor, delta	PPARD	5467	1.79
Nuclear receptor subfamily 2, group F, member 2 (COUP-transcription factor 2)	NR2F2	7026	−3.08

which have been well characterized as a model for intestinal lipid metabolism [37, 50]. However, the mechanisms by which these isomer-specific effects of CLA are mediated on lipid metabolism in enterocytes are unclear. Thus, we conducted a more in-depth investigation of the isomer-specific effects of CLA on key metabolic genes and on genes of known importance in intestinal lipid metabolism.

In the present study, treatment of Caco-2 cells with *trans*-10, *cis*-12 CLA altered the expression of a number of genes involved in lipid transport, fatty acid metabolism, lipolysis, β-oxidation, steroid metabolism, cholesterol biosynthesis, membrane lipid metabolism, gluconeogenesis and the citrate cycle, whereas *cis*-9, *trans*-11 CLA had no effect. While some of these effects have been reported in hepatic [8] and adipose tissue [21, 31], this is the first time, to our knowledge, that these effects have been reported in intestinal cells. In addition to providing new avenues of investigation, some of these molecular changes may underpin the observed cellular effects of *trans*-10, *cis*-12 CLA on intestinal lipid metabolism and its physiological correlates.

For example, Black et al. [4] showed that chronic treatment of Caco-2 cells with *trans*-10, *cis*-12 CLA increased de novo TAG synthesis but not TAG secretion or phospholipids synthesis and secretion. In the present study, the molecular expression of two important intracellular fatty acid binding proteins (FABP-1 and FABP-6) was up-regulated (2.04- and 2.94-fold, respectively) in Caco-2 cells by treatment with *trans*-10, *cis*-12 CLA. These lipid binding proteins mediate the transport of long-chain fatty acids to the endoplasmic reticulum for incorporation into TAG, cholesteryl esters and phospholipids [42, 52]. In addition, two major pathways, the monoacylglycerol and the glycerol 3-phosphate pathway, ensure fatty acid incorporation into TAG in the intestine. While the monoacylglycerol pathway is the primary one responsible for fatty acid esterification in the enterocyte, it is inactive in Caco-2 cells [34, 50]. In the present study, GPAT, the rate-limiting step in the glycerol 3-phosphate pathway was down-regulated (1.63-fold) in Caco-2 cells by treatment with *trans*-10, *cis*-12 CLA. ApoB mRNA editing enzyme (APOBEC1), which mediates the post-transcriptional editing of apoB-100 mRNA to apoB-48, was also down-regulated (1.76-fold) in cells treated with *trans*-10, *cis*-12 CLA. Apo-48 is only synthesized in the intestine and is essential for the assembly of chylomicrons [23]. A decrease in expression of APOBEC1 would suggest an alteration in the ratio of apo-B48 to apoB-100. However, Black et al. [4] showed that chronic supplementation of Caco-2 cells with

trans-10, *cis*-12 CLA (for 19 days) did not affect cellular apoB-48:apoB-100 levels.

The saturated fatty acid (SFA) to monounsaturated fatty acid (MUFA) ratio affects membrane phospholipid composition and alteration in this ratio has been implicated in a variety of disease states including CVD, obesity and diabetes [9]. Studies have shown that *trans*-10, *cis*-12 CLA, and not *cis*-9, *trans*-11 CLA, decreased stearyl-CoA desaturase (SCD1) activity and mRNA levels in hepatocytes [64] and SCD1 mRNA expression in adipocytes [6]. SCD1 is the rate-limiting enzyme in the conversion of SFA into MUFA, the major components of TAG and membrane phospholipids. At the cellular level CLA, in particular the *trans*-10, *cis*-12 CLA isomer, has been shown to increase the ratio of SFA to MUFA in adipocytes [6] and hepatocytes [11]. In the present study, SCD1 was up-regulated (2.14-fold) which would suggest a decrease in SFA:MUFA. However, the exact role of this enzyme and the effect of CLA on fatty acid profiles in the intestine are unclear.

Two important genes involved in intestinal lipid metabolism, which have a role in the regulation of blood lipid concentrations and potentially the development of atherosclerosis, were altered in Caco-2 cells treated with *trans*-10, *cis*-12 CLA. Firstly, apoA-IV, a glycoprotein associated with TAG-rich chylomicrons and HDL [61] and primarily synthesized by the intestine [12] was up-regulated (6.98-fold). A protective role for apoA-IV in atherosclerosis has been demonstrated in animal studies [10, 60] and this protective role is further supported by the observation of an inverse relationship of apoA-IV levels and risk of CHD in human subjects [59, 63]. Interestingly, apoA-IV has also been suggested to play a role in the regulation of food intake by acting as a satiety factor [29, 45, 50]. Secondly, adenosine triphosphate-binding cassette protein A1 (ABCA1), a key mediator of cholesterol transport, was down-regulated (4.63-fold) in Caco-2 cells treated with *trans*-10, *cis*-12 CLA. It is postulated that intestinal ABCA1 is critical for nascent HDL formation in the intestine [39] and acts by directly mediating cholesterol transfer towards plasma HDL. Deletion of intestinal ABCA1 has been shown to result in a significant reduction (30%) in plasma HDL in targeted knock-out mice [5], suggesting that a down-regulation of this protein by the *trans*-10, *cis*-12 CLA isomer may lead to a decrease in HDL-cholesterol derived from the intestine.

The up-regulation of intestinal apoA-IV by *trans*-10, *cis*-12 CLA may suggest a protective effect in relation to atherosclerosis, since HDL-particles enriched in apoA-1 and A-IV are considered anti-atherogenic [49, 50]. On the other hand, the down-regulation of intestinal ABCA1 may adversely affect HDL-cholesterol levels. Some, but not all, human studies have reported a reduction in plasma HDL-cholesterol levels following supplementation with mixed isomers of CLA (for review, see 56). Furthermore, this reduction in HDL has mainly been attributed to the *trans*-10, *cis*-12 CLA isomer [47, 55]. In animal models, some studies have suggested that both isomers of CLA may have a potential beneficial effect on atherosclerosis development [28, 36]. However, other more recent studies have suggested that the CLA isomers have divergent effects on atherosclerosis development [8, 54, 62], whereby *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA may in fact promote and inhibit the development of atherosclerosis, respectively. Although, in the present study, treatment of Caco-2 cells with *cis*-9, *trans*-11 CLA had no effect on gene expression related to processes involved in lipid metabolism in the intestine, the findings of recent studies indicate that *cis*-9, *trans*-11 CLA may have effects on atherosclerosis development beyond the intestine. For example, *cis*-9, *trans*-11 CLA induced anti-inflammatory proteins in the liver [8] and may impart its anti-atherosclerotic effects by inducing apoptosis and negatively regulating the expression of pro-inflammatory genes in the atherosclerotic lesion [54, 58].

It has been suggested that CLA may impart its anti-obesity effects by increasing β-oxidation in the liver [46, 53], adipose tissue [44, 46] and skeletal muscle [44]. Interestingly, in the present study treatment of Caco-2 cells with *trans*-10, *cis*-12 CLA lead to an up-regulation (2.81-fold) of CPT1A, a mitochondrial membrane bound protein that is essential for shuttling long-chain fatty acids into the mitochondria to undergo β-oxidation. Several studies have shown that CLA, in particular the *trans*-10, *cis*-12 CLA isomer, increased CPT1A mRNA levels in liver [8] and adipose tissue [22, 31] as well as CPT1A activity in adipose tissue [44, 46]. However, this is the first study, to our knowledge, to suggest that CLA increases CPT1A expression in the intestine. Murase et al. [40] showed that dietary diacylglycerol significantly suppresses body weight gain in mice, an effect which is accompanied by an up-regulation of genes involved in lipid metabolism resulting in a subsequent increase in β-oxidation in the small intestine. Thus, the *trans*-10, *cis*-12 CLA-mediated increase in CPT1A expression in the intestine may result in increased intestinal energy expenditure and contribute, at least in part, to the weight loss effects of *trans*-10, *cis*-12 CLA which have been demonstrated in several animal models [19, 21, 31, 44]. Further investigation is needed to determine the exact effect of CLA on β-oxidation in the intestine.

It has been suggested that CLA may mediate its effects through the peroxisome proliferator activated receptor (PPAR) transcription factors, which consist of three closely related gene products, PPARα, -δ and -γ [26, 31]. Interestingly, PPARδ and -γ were found to be up-regulated

(1.79- and 2.82-fold, respectively) in Caco-2 cells by treatment with the *trans*-10, *cis*-12 CLA isomer. These transcription factors play an important role in a wide range of biological processes including lipid and glucose metabolism in a variety of tissues and have been implicated in a number of disease pathologies such as obesity, atherosclerosis and diabetes [13, 25, 27]. A number of other nuclear receptors such as the hepatocyte nuclear factor 4, alpha (HNF4A), the pregnane X receptor (NF1I2), the FXR and the COUP-transcription factor 2 (NR2F2) were also up-regulated by *trans*-10, *cis*-12 CLA. While these nuclear receptors offer potential as candidates to explain the CLA-mediated changes in gene expression, further work is required to establish their exact role.

The present study had a number of potential limitations. The Caco-2 cells were differentiated on plastic supports rather than on filter supports, the latter more typical in functional studies. Le Beyec et al. [33] have shown that expression of several genes involved in lipid metabolism in Caco-2 cells grown in plastic support was impaired compared to that observed when the cells were grown on filter supports. While the effect of apical exposure of the CLA isomers to intestinal cells was our primary interest, we acknowledge that exposure of Caco-2 cells to isomers of CLA at the basolateral side (reflecting circulating levels) may induce different physiological responses within the intestinal-like cells. Beaslas et al. [3] have recently shown that the polarity (apical vs. basal pole) as well as the mode of lipid delivery (albumin-bound vs. lipid micelles) are important determinants of enterocyte gene expression in Caco-2 cells. These considerations would need to be addressed in further studies to better clarify the role of CLA on lipid metabolism.

In conclusion, the *trans*-10, *cis*-12 CLA induced alterations in the expression of genes involved in regulating the intestinal component of a number of processes including TAG metabolism, cholesterol transfer to HDL, gluconeogenesis and β-oxidation. These gene expression patterns suggest that *trans*-10, *cis*-12 CLA may have potential negative effects on intestinal HDL biogenesis and glucose metabolism while increasing β-oxidation and altering TAG metabolism in Caco-2 cells. These observations supported by evidence in some animal and human studies warrant further investigation, including follow-up with functional studies.

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