Dietary conjugated linoleic acid and insulin sensitivity and resistance in rodent models\textsuperscript{1–4}

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ABSTRACT
Dietary conjugated linoleic acid (CLA) is being investigated for beneficial effects for disease prevention and treatment in a variety of experimental models, including obesity and type 2 diabetes. To date, rodent studies suggest that trans-10,cis-12 \((t_{10},c_{12})\) CLA is associated with greater insulin resistance, despite lower body fat, and that a CLA mixture (and perhaps \(c_{9},t_{11}\)) could be beneficial for the management of insulin resistance. Studies investigating the mechanisms by which CLA operates at the cellular level show that the primary targets for CLA are members of the nuclear receptor family, particularly the PPAR\(\alpha\), PPAR\(\gamma\), sterol regulatory element-binding protein 1c, and liver X receptor \(\alpha\). Consequently, the effects of CLA on glucose metabolism are likely secondary effects mediated through factors such as PPAR\(\gamma\) coactivator 1 that are controlled by these nuclear receptors. The different responses of normal compared with insulin-resistant obese rodents suggest that interactions of CLA isomers with the cellular components that contribute to development of metabolic syndrome require further investigation. \textit{Am J Clin Nutr} 2004;79(suppl):1164S–8S.

KEY WORDS \ CLA, insulin resistance, rats, mice

INTRODUCTION
Conjugated linoleic acid (CLA) refers to a group of positional and geometric isomers of linoleic acid (cis-9,cis-12-octadecadienoic acid; \(c_{9},c_{12}\)) in which the double bonds are separated by a single carbon-carbon bond, not a methylene group. Dietary CLA is being investigated for beneficial effects for disease prevention and treatment in a variety of experimental models, including obesity, type 2 diabetes, and cardiovascular disease (reviewed in 1–6). The focus of this paper is to review the current knowledge about CLA and the molecular mechanisms associated with development of metabolic syndrome (e.g., insulin sensitivity, fatty liver, obesity) in rodent models.

CONJUGATED LINOLEIC ACID ISOMERS AND PRODUCTS USED IN EXPERIMENTAL ANIMAL STUDIES
The \(c_{9},t_{11}\) - and \(t_{10},c_{12}\)-isomers of CLA are the primary focus of studies evaluating the biological activities of CLA (1–6). Interest in these isomers was stimulated by results obtained in studies using commercially available CLA, which is a mixture of approximately equal amounts of the \(c_{9},t_{11}\) - and \(t_{10},c_{12}\)-isomers (~40% of each), designated as the 50:50 product. This product has a different isomer profile than foods such as dairy products, which have 73–93% \(c_{9},t_{11}\) (3, 7). Fewer studies investigated the mechanism(s) of specific isomers in animal feeding trials because of their limited availability and the cost for bulk quantities. Some investigators compared \(c_{9},t_{11}\) Matreya commercial product (Pleasant Gap, PA) (75% purity) or CLA-enriched butterfat (~90%) with the 50:50 mixture to deduce isomer-specific effects (8, 9). Currently, there is a trend toward use of single isomers of greater purity (>90%) (10, 11). The focus on single isomers is important, but it is possible that combinations of isomers, as well as biologically potent isomers present in small amounts in food, could be equally important.

The CLA isomers in commercial products are present as free fatty acids, whereas dietary sources of CLA isomers are largely in the triacylglycerol form. Differences in digestion, absorption, transport, etc, of CLA isomers as free fatty acids versus triacylglycerol form could influence the biological activity of CLA. Ip et al (12) demonstrated that the major outcomes for mammary gland morphogenesis and cancer risk were similar in rats fed CLA-enriched butter fat (triacylglycerol form) compared with rats fed a commercial mixture of CLA isomers as free fatty acids. Thus, it is currently believed that commercially available CLA in the free fatty acid form can be used to represent food-derived CLA for experimental studies addressing mechanisms.

INSULIN RESISTANCE AND DIABETES
The antidiabetic effects of dietary CLA were examined in studies with the Zucker Diabetic Fatty fa/fa (ZDF/GMI) rat (8, 13), a model for obesity and type 2 diabetes. The ZDF rat (mutation in the leptin receptor) has obesity and hyperglycemia at an early age (7–8 wk). Feeding 1.5% CLA (50:50 product) to 6-wk-old ZDF rats for 14 d normalized impaired glucose tolerance and attenuated fasting hyperinsulinemia and free fatty acid concentrations in male ZDF rats (13). Similar results were obtained...
CLA induced a dose-dependent activation of PPARα/H9253 protein 2 mRNA expression. Adipocyte protein 2 contains a transcription factor involved in lipogenesis and insulin sensitiv-ity in skeletal muscle compared with control ZDF rats (8). The pair-fed rats had a glucose tolerance response that was intermediate between the CLA mixture and control groups; thus, the lower body weight of the CLA mixture group contributed in part to their improved response. A pair-fed group was required because the feed intake and body weight of the CLA mixture group was less than in the control group from day 4 and day 9, respectively. Both the CLA mixture and the c9,11-isomer low-ered plasma triacylglycerols and elevated uncoupling protein 2 mRNA abundance in skeletal muscle and adipose tissue. This finding demonstrates CLA-dependent activation of a gene with a PPRE, but it does not establish whether c9,11 is the only isomer activating it. No comments about fatty liver were found in either of these studies (8, 13). Both of these studies were short term (2 wk in the growing stage), and the effects of CLA and specific isomers need to be demonstrated over longer periods of time.

We investigated the effects of dietary CLA in the fa/fa Zucker rat, a model for obesity and the early stages of diabetes. The fa/fa Zucker rat (mutation in the leptin receptor) has impaired oral glucose tolerance and is hyperinsulinemic but not hyperglyce-mic. In the fa/fa Zucker rat, feeding a dietary CLA mixture from 6–14 wk of age improved oral glucose tolerance and reduced fatty liver (Yurkova N, Noto A, Ryz T, et al, unpublished obser-vation, 2003 (14). At the molecular level, CLA-fed fa/fa Zucker rats had attenuated levels of tumor necrosis factor α (TNF-α) mRNA in adipose tissue and elevated expression of PPAR-responsive genes, indicating that CLA is activating PPARs as part of its mechanism.

In contrast to improved glucose tolerance in ZDF and fa/fa Zucker rats, mice fed 1% CLA (50:50 product) have reduced fat mass but become insulin resistant and have hepatomegaly as a result of fatty liver (15, 16). The first report was based on elevated 3-h fasted plasma insulin in AKR/J male mice fed a high-fat diet (45% kcal from fat) for 5, 8, or 12 wk (15). Subsequently, Tsuboyama-Kasaoka et al (16) reported lipodystrophy (insulin resistance, hepatomegaly, and reduction of white and brown adipose tissue) in female C57BL/6J mice fed 1% CLA (50:50 product) in a low-fat diet (11% kcal from fat). CLA-fed mice had normal glucose concentrations during an oral glucose tolerance test but significantly elevated blood glucose concentrations during an insulin tolerance test. CLA-fed mice had elevated fed and fasting plasma insulin (4- and 8-fold, respectively) and adipose TNF-α expression (12-fold). The CLA-fed mice had very low plasma leptin, and, interestingly, leptin infusion partially re-verses the insulin resistance and fatty liver. The results obtained in this study of “normal” lean mice (16) compared with the previously studied control ZDF and fa/fa Zucker rats (Yurkova et al, unpublished observation, 2003) (8, 13, 14) raise a critical question about whether the opposing CLA responses reflect differences between species (mice compared with rats) or differences in metabolic state (normal compared with metabolic syndrome). Our current data in lean rats indicate that oral glucose tolerance, serum insulin, and serum leptin are not affected by feeding a CLA mixture (Yurkova et al, unpublished observation, 2003) (14). However, this issue needs to be carefully addressed.

Roche et al (10) reported divergent metabolic effects of CLA isomers in ob/ob mice (leptin deficiency because of mutation in ob gene). Feeding c9,t11 for 4 wk did not change body weight, serum glucose, or insulin; however, serum triacylglycerols and free fatty acids were reduced (27% and 22%, respectively) compared with ob/ob mice fed the control diet. Expression of hepatic sterol regulatory element-binding protein-1c (SREBP-1c), a transcription factor involved in lipogenesis and insulin sensitivity, was altered in adipose and liver, and adipose TNF-α mRNA was reduced in mice fed c9,t11. In contrast, the r10,c12 diet promoted insulin resistance (1.5- and 7.5-fold higher serum glucose and insulin), inhibited fat deposition, and elevated uncoupling protein expression in adipose and muscle. That study supports the conclusions of others that the r10,c12 isomer is responsible for changes in body composition (17–20). However, it also implicates r10,c12 in the development of insulin resistance in a mouse model of obesity and hypoleptinemia. Unfortunately, lean mice were not studied to determine whether they develop lipodystrophy. The reduction in adipose TNF-α expression in mice fed c9,t11 is suggestive of improved insulin sensitivity at the cellular level; however, no glucose or insulin tolerance tests were reported.

MOLECULAR MECHANISMS

Maintenance of energy homeostasis occurs through the induc-tion of genes coding for enzymes that regulate specific rate-limiting steps in lipid and carbohydrate metabolism. Conse-quently, the metabolic effects of CLA are presumed to involve changes in gene expression. Control of lipid homeostasis in re-sponse to the body’s energy requirements is primarily exerted through transcription factors of the nuclear hormone receptor family (21). These receptors bind small, lipophilic molecules that modulate receptor activation state. Inactive receptors are seques-tered in the cytosol in a complex with chaperone proteins such as Hsp90. Ligand binding leads to dissociation of this complex, exposure of a nuclear localization signal, and translocation into the nucleus. In the nucleus, the receptors form heterodimers, typically with the retinoid X receptor and bind to specific se-quence elements present in the promoters of target genes. The physical characteristics of CLA, which structurally resemble unsaturated fatty acids, have led to speculation that CLA could operate as a ligand for several nuclear receptors (1, 6). Conse-quently, considerable interest exists in determining which recep-tors mediate the biological actions of CLA.

The PPARs are critical factors in hepatic and adipose lipid metabolism, operating through a promoter sequence termed the PPRE (22). PPARα stimulates β-oxidation and fatty acid clear-ance by the liver, whereas PPARγ promotes adipocyte matura-
tion and lipid storage. PPAR\(\beta/\delta\), which is ubiquitously expressed, influences adipogenesis and reverse cholesterol transport.

The decrease in body weight and hepatic lipid accumulation observed with CLA resembles the changes obtained with PPAR\(\alpha\) ligands. Consequently, speculation exists that CLA activates PPAR\(\alpha\). This possibility was supported by reports confirming that CLA binds to PPAR\(\alpha\) (the \(c_9,t_{11}\)-isomer is particularly potent) and induces PPAR\(\alpha\)-responsive genes (23). However, data obtained in studies that examined the gene stimulatory actions of CLA in PPAR\(\alpha\)-deficient mice were not supportive of this conclusion. Peters et al (24) reported that neither the beneficial effects of CLA on body weight and composition nor transcriptional up-regulation of uncoupling protein 2 (mitochondrial uncoupling protein), fatty acid synthase, and stearoyl-CoA desaturase were different when measured in control and PPAR\(\alpha\)-deficient mice. Nevertheless, this study did confirm that PPAR\(\alpha\) was required for transcriptional activation of the ACO (peroxisomal CoA oxidase) and CYP4A (microsomal cytochrome P450) genes by CLA.

CLA exhibits a binding affinity 100-fold lower for PPAR\(\gamma\) than for PPAR\(\alpha\) (23), which suggests that CLA should have little or no effect on adipose tissue. However, CLA was shown to prevent adipocyte maturation, a process that is clearly mediated by PPAR\(\gamma\) (21). Furthermore, the \(t_{10},c_{12}\)-isomer prevents triacylglycerol accumulation by adipocytes (18). These observations indicate that CLA could function by reducing PPAR\(\gamma\) expression in preadipocytes and adipocytes (25). Alternatively, the data reported by Granlund et al (26), which show that CLA inhibits the PPAR\(\gamma\) stimulatory effects of thiazolidinediones, suggest CLA could actually operate as a PPAR\(\gamma\) antagonist (25, 26). Furthermore, this activity is solely associated with the \(t_{10},c_{12}\)-isomer, because the \(c_9,t_{11}\)-isomer failed to produce a similar effect. This mechanism explains how CLA can influence adipose tissue without being a potent PPAR\(\gamma\) agonist.

As was shown for adipocytes (25), CLA reduces the expression of PPAR\(\gamma\) in hepatic tissue (8). However, the role of PPAR\(\gamma\) in liver function is not well understood. Nevertheless, it was established that CLA decreases fatty liver in the ob/ob and fa/fa animal models, and activation of PPAR\(\gamma\) was presumed to contribute to this improvement (27). An investigation by Clement et al (28) examined the relative contribution of the \(c_9,t_{11}\-) and \(t_{10},c_{12}\)-CLA isomers to this process and demonstrated that \(t_{10},c_{12}\)-CLA actually promotes fatty liver. In contrast, \(c_9,t_{11}\)-CLA had no effect.

CLA-dependent activation of genes now known to be PPAR\(\alpha\) independent is indicative of an effect on lipid-modulating receptors unrelated to the PPARs. For instance, Roche et al (10) recently provided evidence that \(c_9,t_{11}\) decreased expression of both SREBP-1c and LXR\(\alpha\) (liver X receptor \(\alpha\)), which, like PPAR, are \(\alpha\) trans-acting factors essential for modulating expression of genes involved in lipid metabolism. SREBP-1c, which is negatively regulated by polyunsaturated fatty acids (29), primarily mediates nutrient regulation of hepatic lipogenesis (30). The polyunsaturated fatty acids likely operate by antagonizing the LXRs, which were shown to up-regulate SREBP-1c gene activity. LXRs are required for expression of genes active in cholesterol and fatty acid metabolism, and, accordingly, oxysterols and \(\alpha\)-hydroxy bile acids were identified as likely natural LXR ligands (21). Interestingly, \(t_{10},c_{12}\)-CLA had no effect on either SREBP-1c or LXR, although this isomer induced hyperlipidemia and diabetes (10). PPARs could also influence LXR function, because LXR expression is significantly reduced in PPAR\(\gamma\)-deficient mice (21). Similarly, both PPAR\(\alpha\) and PPAR\(\gamma\) agonists were reported to enhance expression of LXR\(\alpha\) (21). In addition, Ide et al (31) recently showed that activation of PPAR\(\alpha\) inhibits LXR-dependent SREBP-1c gene activation by preferentially interacting with LXR and, thus, interfering with formation of the active LXR/retinoid X receptor heterodimer. The PPAR\(\alpha\)/LXR complex is also incapable of stimulating PPAR\(\alpha\)-dependent genes.

In reviewing the molecular mechanisms by which CLA can function, it is apparent that more emphasis was placed on lipid metabolism, because the primary targets for CLA regulate this process directly. Yet, as seen in the animal models discussed earlier in this review, CLA also has a major effect on glucose homeostasis and sensitivity to insulin. Does CLA, therefore, have a direct effect on carbohydrate metabolism in addition to influencing lipid metabolism, or does it function indirectly? The relative intracellular content of acetyl-CoA and long-chain acyl-CoA has a major influence on pyruvate carboxylase activity and, thus, gluconeogenesis. Djouadi et al (32) reported that PPAR\(\alpha\)-/- mice develop hypoglycemia as a result of reduced glucose production. They concluded that the reduction in acetyl-CoA that resulted from a decrease in PPAR\(\alpha\)-dependent \(\beta\)-oxidation restricts gluconeogenic capacity. Thus, PPAR\(\alpha\) has an indirect effect on carbohydrate metabolism. Similarly, suppressing adipocyte maturation could also indirectly affect glucose utilization (25). Evidence is compelling that adipose tissue influences glucose metabolism by both liver and skeletal muscle, probably through release of adipokines such as TNF-\(\alpha\) and leptin (33). Our experimental data showed that the 50:50 mixture normalizes adipose TNF-\(\alpha\) and leptin concentrations (Yurkova et al, unpublished observation, 2003) (14) and that these changes in concentrations could account for the reduction in fatty liver we observed. In contrast, Edwardsson et al (34) used a proteomics approach to determine which genes responded to synthetic PPAR\(\alpha\) (Wy-14643) and PPAR\(\gamma\) (rosiglitazone) ligands. As expected, expression of genes for enzymes involved in fatty acid oxidation and lipogenesis was increased. However, enzymes associated with both gluconeogenesis (eg, fructose 1,6-bisphosphate) and glycolysis (eg, ketohexokinase) were also affected, primarily with Wy-14643. These results suggest that PPARs, and by inference CLA, could affect glucose metabolism both directly and indirectly.

Although an apparent link exists between SREBP-1c and glucose in the liver (35), it is likely that LXR\(\alpha\) is the primary factor that mediates this connection in both hepatic and adipose tissues (36). Activation of LXR in the liver leads to decreased expression of the gluconeogenic genes phosphoenolpyruvate carboxykinase and glucose-6-phosphatase and to increased expression of gluco kinase. In adipose tissues, LXR activation promotes glucose uptake by up-regulating transcription of the Glut4 gene. Furthermore, it was recently proposed that control of hepatic gluconeogenesis is mediated by PPAR\(\gamma\) coactivator-1\(\alpha\) (PGC-1\(\alpha\), a novel transcription factor that is associated with insulin resistance (37). In adipose tissue, PGC-1 was shown to be an obligate cofactor for PPAR\(\gamma\)-mediated adipocyte differentiation (38). Although no evidence demonstrates that CLA can modulate PGC-1 expression, this factor could be the focal point for coordination of lipid and carbohydrate metabolism. Evidence to support this premise is based on data that indicate signals originating from the insulin
metabolic syndrome. Isomers influence the cellular factors that determine onset of insulin resistance in mice and humans (Yurkova et al, unpublished observation, 2003) (8, 13, 14). The c9,11-12-isomer was associated with reduced fat mass in rodents (10, 17–20) and improved blood lipid profile in hamsters (9, 11); however, it could also be responsible for promoting insulin resistance in mice and humans (Yurkova et al, unpublished observation, 2003) (40, 41). The c9,11-12-isomer has been associated with attenuation of blood lipids in ob/ob mice and with lower TNF-α expression in adipose tissue, a molecular marker for increased insulin sensitivity (10). The different responses of normal compared with insulin-resistant obese rodents suggest that further investigation is required to understand the mechanisms by which specific CLA isomers influence the cellular factors that determine onset of metabolic syndrome.

There is no conflict of interest.

REFERENCES

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