



# TECHNICAL SUMMARY

## HUMAN NUTRITION RESEARCH

# BEEF

## RESEARCH

## ***Trans* Fat, Specifically Conjugated Linoleic Acid, and Dietary Beef**

### **Introduction**

The health effects of *trans* fatty acids (TFA), which are unsaturated fatty acids that contain one or more double bond in the *trans* configuration, have been extensively studied in recent decades. Adverse health effects associated with TFA have led to intense scrutiny regarding their content in foods and public health recommendations to restrict consumption of foods containing TFA. However, advances in science suggest not all TFA are alike and some may have beneficial health effects. This discussion reviews TFA in general and recent scientific findings examining potential health benefits of conjugated linoleic acid (CLA), a *trans* fat found in foods from ruminants, including beef.

### ***Trans* Fatty Acids**

There are two predominant sources of TFA in the food supply: "industrial" and "ruminant" TFA.<sup>1,2</sup> Industrial TFA (iTFA) are produced by the partial hydrogenation of vegetable oils and are found in foods made with these oils (e.g., margarines, cakes, cookies, snack foods). Ruminant TFA (rTFA) are formed naturally by bacteria in the rumen of ruminant animals and are present in small amounts in beef and other meats and dairy products such as milk and cheese.<sup>1,2</sup> Vaccenic acid and CLA are the two major rTFA; most studies of their health effects have focused on CLA, in particular the *cis*-9, *trans*-11 isomer (also known as rumenic acid).<sup>2</sup> Industrial and ruminant TFAs are reported to differ in their structure, physical properties, and amount in food sources.<sup>2</sup>

Evidence from controlled trials and observational studies indicates that iTFA adversely affect multiple risk factors

for cardiovascular disease (e.g., lipoprotein levels, inflammation markers, insulin resistance) contributing to increased risk of this disease.<sup>3,4,5,6,7</sup> In contrast, studies demonstrate that rTFA in amounts usually consumed have no adverse effects on cardiovascular disease risk factors and may confer protective effects.<sup>2,4,7,8,9,10,11</sup> According to a recent review, rTFA do not adversely affect biomarkers of cardiovascular disease when consumed in usual amounts by the general population.<sup>11</sup> Likewise, another review reported that, while iTFA are positively linked to biomarkers of cardiovascular disease, this is not the case with a moderate intake of rTFA.<sup>12</sup>

However, debate is ongoing regarding whether differences exist in the effects of rTFA and iTFA on blood lipoproteins and cardiovascular disease risk when both are consumed in high amounts.<sup>1,3,7,13</sup> The most important difference between these TFA may be the amount, rather than the type (source), consumed.<sup>1</sup> Whether or not iTFA and rTFA differ in their effects on cardiovascular disease risk factors may be irrelevant considering the low daily intake of rTFA, which averages approximately 1.2 g (1.5 g for men and 0.9 g for women) or less than 0.5% of total daily energy intake.<sup>14,15</sup> It is difficult to consume high amounts of rTFA in usual diets given the low amounts of these TFA in ruminant food products.<sup>2</sup> However, if CLA supplements are used, intake of CLA can be considerably higher (i.e., 6 g/day).<sup>7</sup>

As a result of the adverse effects of high amounts of iTFA on cardiovascular disease risk, considerable emphasis has been placed on limiting these TFAs from foods and overall diets through recommendations and regulations.<sup>2,9,12</sup> Health professional organizations

including the American Heart Association, the American Dietetic Association (now called the Academy of Nutrition and Dietetics), the Institute of Medicine, and the World Health Organization, among others, recommend limiting dietary TFA from industrial sources.<sup>1,3,16</sup> The 2010 *Dietary Guidelines for Americans*<sup>17</sup> recommends keeping dietary intake of TFA as low as possible, especially by eliminating foods that contain industrial sources of TFA, but does not recommend eliminating foods containing natural TFA (e.g., meat, milk products) because this could adversely affect nutrient adequacy. Wang and Proctor,<sup>12</sup> following an examination of issues related to TFA, recommend that nutrition guidelines/policies focus on eliminating iTFA from processed foods, as opposed to all TFA per se. The US Food and Drug Administration, based on evidence that rTFA have a neutral or possible health benefit, excludes CLA from its nutrition labeling regulations requiring disclosure of the TFA level in foods.<sup>14</sup>

## Conjugated Linoleic Acid

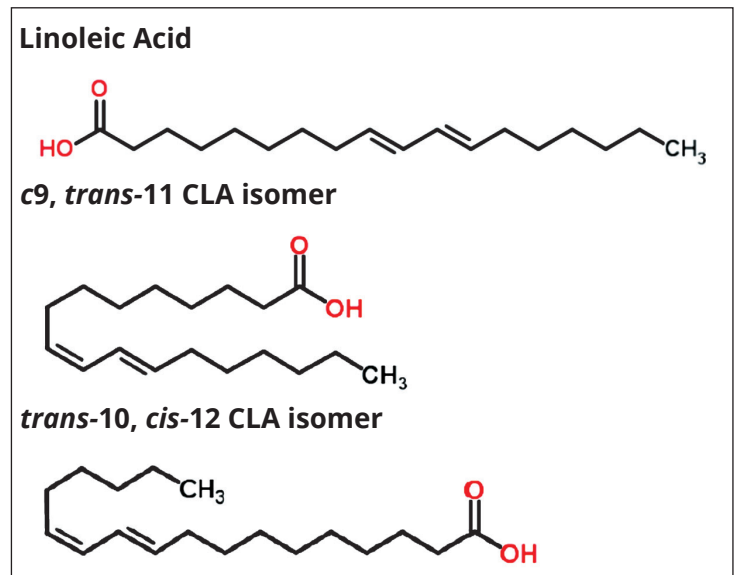
CLA, a naturally occurring *trans* fat, is commonly found in ruminant animal foods such as beef and other meats, and dairy products (e.g., milk and cheese). Over the past several decades a wealth of research, mainly from in vitro and experimental animal studies and some human investigations, has examined potential health benefits of CLA. This research on the biological functions and health benefits of CLA dates back to the 1980s when scientists at the University of Wisconsin observed that an anti-carcinogenic compound (later identified as CLA) isolated from grilled ground beef inhibited chemically induced skin cancer in mice.<sup>18</sup> Since then, numerous studies have investigated the effects of CLA on cancer, cardiovascular disease, body composition, and other conditions (e.g., insulin resistance, immune function, bone health). The physiological and potential health effects of CLA are reviewed in several recent publications.<sup>2,10,12,19,20,21</sup> Also, a listing of the scientific literature on CLA since the 1980s can be found by visiting the Conjugated Linoleic Acid Reference Center hosted by the University of Wisconsin-Madison (<http://fri.wisc.edu/cla.php>).

## Structure of CLAs

CLA is a collective term used to describe a mixture of positional and geometric isomers (forms) of linoleic acid, an essential fatty acid. Although 28 possible different CLA isomers have been identified and a number of

these isomers may have beneficial biological activity, the known physiological effects of CLA are attributed to two predominant isomers.<sup>12,21</sup> These are the *cis*-9, *trans*-11 isomer (*c9, t11* CLA, also called rumenic acid), which accounts for 70 to 90% of total CLA in foods from ruminant animals, and the *trans*-10, *cis*-12 isomer (*t10, c12* CLA), which is found in minor amounts in foods from ruminant animals.<sup>12,19,22,23,24</sup> Fritsche and coworkers<sup>23</sup> identified 14 CLA isomers in beef fat. The *c9, t11* isomer was the most predominant isomer (72%), followed by the *t7, c9* isomer (7%), with minor amounts of other CLA isomers such as *t10, c12*.<sup>23</sup> Structures of the two most studied, biologically active CLA isomers - *c9, t11* and *t10, c12* - compared with that of the parent linoleic acid - are illustrated in Figure 1.

**Figure 1.** Chemical Structures of Linoleic Acid and Two Isomers of Conjugated Linoleic Acid (CLA)



## Origins and Sources of CLA

### Origins

CLA is produced naturally in the rumen of ruminant animals by fermentative bacteria, which isomerizes linoleic acid into CLA. Ruminant animals also synthesize CLA from *trans*-11-18:1 (vaccenic acid), the predominant *trans* monounsaturated fatty acid of animal tissue fat, by way of the enzyme delta-9-desaturase.<sup>2,10,21,22</sup> This endogenous synthesis from vaccenic acid is considered to be the major source of *c9, t11* CLA in the body fat of cattle.<sup>2</sup>

### Sources

Representative and relative concentrations of CLA and the proportion of *c9, t11* CLA in a variety of foods are

summarized in Table 1.<sup>25</sup> CLA concentrations are highest in ruminant-derived foods (beef, lamb, dairy products).<sup>22,25</sup> Seafood, pork, most poultry products and vegetable oils are not notable sources of CLA.<sup>25</sup> The average CLA content in meat products of ruminant origin is reported to be 0.46% of fat (range 0.12 to 1.20%), whereas the CLA in meats of non-ruminant origin averages 0.16% of fat (0.06 to 0.25%).<sup>22</sup> The total CLA content of specific foods may vary widely.<sup>22</sup>

**Table 1. Representative/Relative Concentrations of CLA in Uncooked Foods** [adapted from Chin et al.<sup>25</sup>].

	<b>Total CLA (mg/g fat)</b>	<b>c9, t11 isomer (%)</b>
<b>Meat</b>		
Fresh ground beef	4.3	85
Beef round	2.9	79
Beef frank	3.3	83
Beef smoked sausage	3.8	84
Veal	2.7	84
Lamb	5.6	92
Pork	0.6	82
<b>Poultry</b>		
Chicken	0.9	84
Fresh ground turkey	2.5	76
<b>Seafood</b>		
Salmon	0.3	n.d.*
Lake trout	0.5	n.d.*
Shrimp	0.6	n.d.*
<b>Dairy Products</b>		
Homogenized milk	5.5	92
Butter	4.7	88
Sour cream	4.6	90
Plain yogurt	4.8	84
Ice cream	3.6	86
Sharp cheddar cheese	3.6	93
Mozzarella cheese	4.9	95
Colby cheese	6.1	92
Cottage cheese	4.5	83
Reduced fat swiss cheese	6.7	90
American processed cheese	5.0	93
Cheese whiz <sup>tm</sup>	5.0	92
<b>Vegetable Oils</b>		
Safflower	0.7	44
Sunflower	0.4	38
Canola	0.5	44
Corn	0.2	39

\*n.d. = not detectable

For example, the typical concentration of CLA in beef is less than 1% of total fatty acids,<sup>26</sup> but can vary from 0.17 to 1.35% of fat.<sup>22</sup> This wide range is related to the type of feed offered, season, breed differences (genetics), and management strategies used to raise cattle.<sup>22,26,27</sup> Grazing beef steers on pasture or increasing the amount of forage (grass or legume hay) in the diet has been shown to increase the CLA content in the fat of cattle.<sup>24,26</sup> Also,

supplementing high-grain diets of beef cattle with oils (e.g., soybean oil, linseed oil, sunflower oil) may increase the CLA content of beef.<sup>22,26,28</sup> Because grazing animals on pasture substantially increases the CLA as a proportion of total fatty acids, but total fat content in the product is reduced, the increase in CLA content should be evaluated on total CLA available in edible fat, rather than in concentrations in raw meat. Breeds of cattle that deposit high amounts of fat in muscle will provide a higher amount of CLA.<sup>27</sup> CLA in meat is stable under normal cooking and storage conditions.<sup>22</sup>

In addition to the small amounts of CLA in foods from ruminant animals, much larger intakes of CLA can be obtained from supplements.<sup>13</sup> Commercially available CLA supplements of combined or individual CLA isomers are often used in experimental animal and human studies to investigate potential health effects of CLA.<sup>12,19</sup> These CLA supplements generally contain 80% of c9, t11 and t10, c12 CLA isomers in equal proportions (1:1), with other isomers as minor components.<sup>12,19</sup> Commercial CLA supplements differ in their composition/distribution of isomers, level of intake, and bioavailability from ruminant sources of CLA.<sup>12</sup>

### **Dietary Intake of CLA by Humans**

Current measures of usual or actual dietary intakes of CLA are very limited, most being only estimates. Average estimated intake of CLA by U.S. adults is 0.15 to 0.21 g/day.<sup>21,29</sup> Worldwide, dietary intake of CLA, specifically the c9, t11 isomer (rumenic acid), ranges from 0.14 to 0.39 g/day, with higher intakes

in countries where the population consumes more energy from ruminant fat.<sup>2</sup>

As shown in Table 2, intakes of total CLA and rumenic acid (i.e., the predominant, biologically active isomer of CLA found in beef) by U.S. adults vary widely. Factors such as the amount, composition (i.e., fat content), and frequency of intake of foods of ruminant origin, as well as the methodology used to estimate food intake influence CLA intake assessments. With respect to methodology, intakes of total CLA and rumenic acid estimated by three-day dietary records and a semi-quantitative food frequency questionnaire are shown to be significantly lower than those estimated by three-day food duplicates.<sup>29</sup> Also, intakes of CLA and rumenic acid are higher in men than in women, presumably because of men's higher intake of fat from meat such as beef and dairy products.<sup>29,30</sup>

**Table 2.** Estimated CLA Intake by U.S. Adults [adapted from Ritzenhaler et al.<sup>29</sup>].

Total CLA (mg/day)*		Rumenic Acid (mg/day)*		Methodology
Men	Women	Men	Women	
212 (0-454)	151 (0-520)	193 (0-439)	140 (0-500)	30-day food duplicate
176 (3-486)	104 (1-399)	133 (1-358)	79 (1-336)	3-day dietary records
197 (0-516)	93 (0-300)	151 (0-412)	72 (0-223)	Food frequency questionnaire

\* mean (range)

Ruminant products are by far the major contributor of CLA in the diet.<sup>29</sup> When CLA intake of U.S. adults was estimated by three-day dietary records, it was found that beef provided 32% and dairy foods provided 60% of the intake of CLA.<sup>29</sup> Because ruminant products contain two-fold or more vaccenic acid (the predominant *trans* monounsaturated fatty acid in ruminant fat) than CLA and ~20% of this vaccenic acid is converted endogenously in humans to CLA,<sup>31</sup> it is estimated that the effective physiological dose of CLA is much higher (i.e., CLA intake times 1.5).<sup>2,31</sup> In humans, dietary vaccenic acid can be converted endogenously to c9, t11 CLA by tissue delta 9-desaturase.<sup>31</sup>

## Potential Health Benefits of CLA

### Cancer

A number of in vitro and experimental animal studies have investigated a potential anti-carcinogenic effect of CLA at different sites including the mammary gland, colon, prostate, skin, and forestomach.<sup>2,19,32,33</sup> In a variety of

human cancer cell lines, CLA has been shown to reduce the growth of cancer cells, whereas linoleic acid has variable effects ranging from inhibition of tumor growth to promotion depending on cell type and degree of malignancy.<sup>19,34</sup> Findings from cell culture studies suggest that the main CLA isomers have different effects according to the specific cancer site examined.<sup>35</sup>

In experimental animals, CLA has been demonstrated to inhibit the initiation, progression, and metastasis (spread) of some cancers, either carcinogen-induced or genetically modified.<sup>2,19,36</sup> The anti-carcinogenic effect of CLA is most impressive in studies of mammary cancer. In a series of investigations in laboratory rats, Ip and coworkers demonstrated that CLA decreased the incidence and number of chemically induced mammary tumors

at various stages of carcinogenesis at relatively low concentrations.<sup>32,37,38,39,40</sup> In these studies, CLA inhibited cancer in a dose-dependent manner at levels of 1% (by weight) CLA and below, with no further beneficial effect at levels above 1%.<sup>39</sup> In rats fed diets supplemented with CLA at levels ranging from 0.05 to 0.5% by weight, as little as 0.1% CLA reduced mammary tumors, indicating that CLA is a potent anti-carcinogen.<sup>38</sup>

Most studies have used mixed isomers of CLA.<sup>2</sup> However, purified isomers of CLA, c9, t11 and t10, c12, as well as ruminant-derived CLA (i.e., a high CLA butter), which is predominantly the c9, t11 isomer, have also been shown to reduce mammary cancer in laboratory rats.<sup>41</sup> In addition, c9, t11 CLA derived endogenously from vaccenic acid by delta-9-desaturase has an anti-carcinogenic effect in rats.<sup>42,43</sup>

Metastasis, or the spread of cancer, is the greatest cancer-related threat to life. Studies in experimental animals indicate that CLA (both c9, t11 and t10, c12 isomers and a mixture) reduces mammary cancer metastasis.<sup>44,45</sup>

Moreover, findings indicate that the type of dietary fat fed influences the effectiveness of dietary CLA in reducing mammary tumor metastasis.<sup>46</sup> In mice injected with mammary tumor cells and fed diets differing in the type of fat, metastasis was significantly reduced when beef tallow replaced half of a vegetable fat blend imitating the dietary fat composition of a typical American diet.<sup>46</sup> Beef tallow also increased the potency of CLA. Increasing beef tallow lowered the concentration of CLA needed from 0.1% to 0.05% of the diet to significantly reduce mammary tumor metastasis.<sup>46</sup> An in vitro study revealed that specific fatty acids in beef tallow (i.e., oleic, stearic, and palmitic acid) did not change or enhance CLA's beneficial effects, whereas linoleic acid (the main fatty acid in vegetable oil) reduced the anti-carcinogenic effect of CLA on mammary tumor cells in cultures.<sup>46</sup> Another in vitro study showed that beef fatty acids (i.e., four fatty acid extracts prepared from beef lipid and varying in their CLA content) reduced the proliferation of human cancer cell lines (breast, colon, melanoma, and ovarian) more than did their corresponding purified CLA-enriched fractions.<sup>47</sup> The researchers speculate that this finding may be explained by a complimentary effect of non-conjugated fatty acids and CLA in beef.

Results from cell and animal studies of CLA and cancer suggest several potential mechanisms by which CLA reduces mammary (or other) cancer.<sup>2,19,33,36</sup> CLA may reduce cell proliferation, inhibit eicosanoid formation, induce apoptosis (programmed cell death), inhibit angiogenesis (which would reduce the availability of nutrients to the tumor), regulate gene expression, and have antioxidant effects.<sup>2,19,33,36</sup> There is some evidence that the anti-carcinogenic effects of *c9, t11* and *t10, c12* CLA isomers act through different mechanisms.<sup>10,12,19,35</sup>

In contrast to in vitro and experimental animal studies, relatively few studies in humans have examined CLA's effects on mammary (and other) cancer.<sup>2,20,21</sup> Moreover, the limited number of human studies that have been conducted are epidemiological, which do not prove a cause and effect relationship. According to recent reviews of observational studies of CLA (either as dietary intake, serum levels, or in adipose tissue) and cancer in humans, findings are inconsistent.<sup>2,19,20,21</sup> Further, no clinical trials have investigated the effect of CLA, specifically *c9, t11* CLA, on markers of cancer risk in humans.<sup>2</sup>

Although an anti-carcinogenic effect of CLA in humans has yet to be established, researchers suggest that, based on the amount of CLA needed to reduce cancer in experimental animal studies, humans would need to substantially increase their CLA intake (e.g., > 3 g/day for a 70 kg person) to an amount difficult to achieve with diet alone.<sup>29,35,48</sup> Failure of a long-term (17.4 years) prospective study in Swedish women to support a protective effect of CLA against mammary cancer was explained by the women's low daily intake of CLA, which ranged between 0.049 and 0.211 g/day.<sup>48</sup> Because of the limited number of human studies and inconsistencies in the findings, researchers are calling for more controlled trials to determine the effect of CLA and its predominant isomers on markers of cancer risk and for further studies of the mechanisms involved.<sup>2,12,20,21</sup>

### **Cardiovascular Disease**

CLA has been shown to reduce cardiovascular disease risk factors such as atherosclerosis and blood lipids in experimental animals including rabbits, hamsters, and mice.<sup>2,19,20,21,49,50,51</sup> In rabbits, a mixture of CLA isomers (*c9, t11* and *t10, c12*) and the two individual CLA isomers, *c9, t11* and *t10, c12*, fed with an atherogenic diet, reduced the severity of atherosclerotic lesions to the same extent.<sup>49</sup> In hypercholesterolemic hamsters, *c9, t11* and *t10, c12* CLA isomers significantly lowered plasma total cholesterol and high-density lipoprotein (HDL) cholesterol (the "good" cholesterol) concentrations with no significant differences between these treatments.<sup>50</sup> However, the *c9, t11* isomer lowered plasma triglyceride and glucose levels more than the *t10, c12* isomer, suggesting a greater beneficial effect for *c9, t11* CLA.<sup>50</sup> When researchers examined the effect of CLA (a 80:20 blend of *c9, t11*: *t10, c12* CLA) on the regression of atherosclerosis in mice, they found that CLA not only prevented the progression, but also almost completely abolished atherosclerosis.<sup>51</sup> However, the effects of CLA on atherosclerotic lesions and blood lipid levels in experimental animal studies are conflicting.<sup>19</sup> Differences in experimental models and species, the baseline diet, and CLA dose and isomer may result in various outcomes.<sup>2</sup> Individual CLA isomers may have opposing atherogenic effects.<sup>52</sup> A study in mice showed that *c9, t11* CLA decreased the development of atherosclerotic lesions, whereas *t10, c12* CLA promoted atherosclerosis.<sup>52</sup> At present, there is no general consensus regarding the effect of CLA supplementation on

atherosclerosis or lipids in animals.<sup>2,20</sup> Further, most animal studies that have suggested protective effects of CLA on cardiovascular disease risk factors have used higher doses of CLA than intake achievable in the human diet.<sup>2,20</sup> Also, despite considerable investigation, underlying mechanisms by which CLA affects lipid metabolism and other cardiovascular disease risk factors are not fully elucidated.<sup>2</sup>

Similar to studies in animal models, variations in findings have been reported in the relatively few human investigations that have evaluated the effects of CLA (mixed or individual isomers) on lipids and other markers for cardiovascular disease risk.<sup>19,20,53</sup> The effects of CLA on cardiovascular disease risk factors in human intervention studies differ depending on such factors as the study population and the use of different doses and isomers of CLA. Based on a review of the science, McCrorie et al.<sup>20</sup> concluded that most human intervention studies have used commercial mixed or pure isomers of CLA at high levels (1.7 to 6.8 g/day) from 4 to 13 weeks and have not demonstrated an overall effect on plasma lipid or lipoprotein concentrations compared to a placebo. Further, there is some evidence that high levels of commercial CLA preparations may have adverse effects on blood lipid levels.<sup>6,7,20,21</sup> In addition, similar to findings in mice<sup>52</sup>, opposing effects of *c9, t11* CLA and *t10, c12* CLA on blood lipid levels have been shown in healthy adults.<sup>54</sup> Specifically, *c9, t11* CLA has been shown to have a favorable effect on blood lipid levels, whereas *t10, c12* CLA appears to have an unfavorable effect.<sup>2,53,54</sup>

Few studies have examined the effect of foods naturally rich or enriched in CLA on cardiovascular disease risk factors. A study in healthy young women found that diets high in CLA (1.17 g/day) from pasture-fed cattle did not significantly affect blood lipid levels compared to a similar diet composed of foods lower in CLA from grain-fed cattle (0.35 g CLA a day).<sup>55</sup> Researchers suggest that the reason for the inconsistent and mostly neutral effects of CLA on lipids in human studies compared to animal investigations is unclear, but may be explained in part by the general use of hyperlipidemic animals consuming atherogenic diets, whereas human studies typically involve normolipidemic subjects.<sup>20</sup> At present, there is no general consensus from experimental animal or human studies regarding the effect of CLA or its predominant isomers on risk factors for cardiovascular disease.<sup>2,20</sup> Further, the effect, if any, of CLA from

ruminant sources on cardiovascular disease risk is likely to be minor.<sup>6,7</sup> Before CLA or purified CLA isomers can be recommended for improving cardiovascular health in humans, more long-term studies in different populations are recommended.<sup>19,21</sup>

### **Body composition**

CLA's effect on body composition was first demonstrated nearly two decades ago in mice.<sup>56</sup> This study demonstrated that intake of CLA (0.5% by weight) decreased body fat mass and increased lean body mass. Subsequent research in several different animal models using CLA at levels of 0.5 to 1% has confirmed and extended these findings.<sup>19,20,57,58</sup> The most dramatic results have been shown in mice as these animals appear to be particularly sensitive to CLA in losing fat mass. Studies using purified isomers or CLA enriched in either *c9, t11* or *t10, c12* isomers suggest that *t10, c12* is the primary isomer involved in reduction of fat mass.<sup>19,20,21</sup>

Promising findings from animal studies have led to human studies of CLA's effect on body composition in normal weight, overweight, and obese subjects. Several reviews have examined studies of the effects of CLA or its isomers on body composition in humans.<sup>20,21,59,60</sup> Although a number of human studies<sup>19,61,62</sup> have reported a reduction in body fat mass and an increase in lean body mass with increased CLA, the results are less significant and more inconsistent than in experimental animals.<sup>19,20,21,53</sup> When healthy overweight and obese adults were supplemented with 3.4 g/day of CLA (mixed isomer) or a placebo for six months, body fat mass significantly decreased in specific regions (e.g., legs, abdomen) and lean body mass was maintained or increased compared to the placebo group.<sup>62</sup> Likewise, in a clinical trial of 40 healthy overweight adults, intake of 3.2 g CLA/day for six months significantly reduced body fat and helped prevent weight gain during the holiday season.<sup>63</sup> In contrast, other studies in adults have found at most a modest<sup>60,64</sup> or no<sup>29</sup> effect of CLA on body fatness. A meta-analysis of seven randomized controlled trials of at least 6 months duration in overweight or obese individuals showed that CLA intake had a small beneficial effect on body composition but of uncertain clinical relevance.<sup>60</sup> Another meta-analysis of 18 studies in humans concluded that a dose of 3.2 g CLA/day produced a modest loss of body fat (0.09 kg/week), with the relationship being linear up to six months.<sup>59</sup> Similar

to animal studies, the *t*10, *c*12 CLA isomer has been positively associated with reducing body fat in humans.<sup>21</sup>

No changes in body composition have been found in the few studies that have examined the effect of naturally CLA-enriched foods such as dairy and beef.<sup>20,55</sup> However, the relatively short duration of these studies may have contributed to the lack of effect.<sup>20</sup>

Dilzer and Park,<sup>21</sup> in their review of 45 human studies examining CLA and body composition, offer possible explanations for the lack of reproducibility in findings of CLA's effect on body composition between humans and animals, particularly the mouse model. Factors such as differences in the dose and duration of CLA intake, metabolic rates, and experimental designs likely contribute to variations in findings between human and animal studies. Regarding the dose of CLA, human studies generally use amounts of CLA much lower (i.e., 0.7 g to 6.8 g/day) than those used in animal studies (i.e., an amount equivalent to about 50 g CLA/day for a 70 kg person). Short-term studies of less than 4 months tend to have little effect on body composition compared to longer-term studies up to 6 months. Differences in metabolism between mice and humans and in dietary regimens may also contribute to variations in findings. In animal studies, the animals are generally fed ad libitum and are in positive energy balance, whereas in human studies, subjects tend to be fed calorie-reduced diets and are in negative energy balance. Researchers suggest that CLA may be primarily effective at reducing a gain in fat mass during a weight gain period.<sup>21</sup> Inconsistencies in findings among human studies may be explained by subjects' weight (normal weight, overweight, or obese), age, physical activity, dietary interactions, or other medications or supplements.<sup>21</sup>

Although the mechanisms underlying CLA's effect on body fat or lean body mass in humans remain to be established, several possibilities have been proposed, including a favorable effect of CLA on satiety.<sup>10,21</sup> Clearly, more studies, particularly human trials in large groups of subjects, are needed before CLA can be recommended to improve body composition.<sup>19,21</sup>

### **Other Effects**

Potential beneficial effects of CLA on insulin resistance, the metabolic syndrome, immune response, and bone health have been investigated.<sup>19,20</sup>

**Insulin Resistance and Diabetes Risk.** The impact of CLA on insulin sensitivity and diabetes risk is inconsistent.<sup>19,20</sup> In experimental animals, factors such as the duration of the study, metabolic state (normal vs. diabetic), animal strain (mice vs. rats), and the CLA isomer(s) used influence the findings.<sup>19,20,65</sup> For example, feeding *t*10, *c*12 CLA to mice has been shown to induce adipose tissue inflammation and insulin resistance.<sup>66</sup> In contrast, feeding mice *c*9, *t*11 CLA has been demonstrated to improve insulin resistance and reduce hyperglycemia.<sup>67</sup> The effect of *c*9, *t*11 CLA on improving insulin sensitivity and management of diabetes was attributed to its anti-inflammatory effect.<sup>67</sup> Other studies in experimental animals indicate that *c*9, *t*11 CLA has anti-diabetic effects, whereas *t*10, *c*12 CLA exerts pro-diabetic effects.<sup>20,65</sup>

Likewise in human clinical trials, the effects of CLA on insulin sensitivity are inconsistent and appear to be isomer specific with *c*9, *t*11 CLA improving insulin sensitivity and *t*10, *c*12 CLA isomer showing an adverse effect on insulin sensitivity.<sup>19,20,68,69</sup> A recent review of 25 human studies published between 2002 and 2011 concludes that overall findings show no effects of intake of CLA supplements or CLA-enriched products on glucose and insulin levels.<sup>20</sup> The researchers suggest that lack of rigorous measures of insulin resistance, with most studies measuring fasting blood levels of glucose or insulin, and the small number of subjects in studies contribute to the lack of compelling evidence of CLA's protective effect against diabetes.<sup>20</sup> A more recent case-control study in 232 patients with diabetes and 1,512 controls without diabetes found that *c*9, *t*11 CLA in adipose tissue (a marker of long-term CLA intake) was associated with a lower risk of diabetes, thus supporting the hypothesis that CLA may be involved in insulin regulation.<sup>70</sup> Researchers call for more studies, particularly using rigorous measures of insulin resistance, in different age and weight subjects.<sup>20</sup>

**Metabolic Syndrome.** Experimental animal and human studies have investigated CLA's effects on various components of metabolic syndrome (e.g., abdominal obesity, insulin resistance, glucose intolerance, high blood pressure, high triglyceride levels, low HDL-cholesterol levels). However, the potential benefits of CLA on metabolic syndrome, a risk factor for type 2 diabetes and cardiovascular disease, remain controversial.<sup>68,71,72</sup>

**Immune Response.** CLA may modify mediators of immunity such as eicosanoids, prostaglandins, cytokines, and immunoglobulins.<sup>19,73,74</sup> In vitro and in vivo studies in various animal models demonstrate that CLA influences cytokine and prostaglandin production, which could influence the inflammatory response.<sup>19,20</sup> However, CLA's effects on markers of inflammation and immune response are inconsistent.<sup>20</sup> According to a recent review of 21 human intervention studies of the effects of CLA (commercial preparations and naturally CLA-enriched dairy products) on inflammation and other immune indices, the findings are inconsistent with most studies showing no significant effects or an increase in inflammatory markers.<sup>20</sup> Further research is needed to determine the effect of individual isomers and mixtures of CLA on immune function.

**Bone Health.** Most studies examining the effect of CLA on bone health have been conducted using human cells and in experimental animals.<sup>19,20,21,75</sup> The majority of these studies have used mixed isomers of CLA and have provided inconsistent evidence of a beneficial effect of CLA on bone health. Likewise, evidence from recent reviews of human studies of CLA and bone health is inconsistent.<sup>20,21</sup> Some findings suggests that CLA may benefit bone health by enhancing calcium absorption, making calcium available for bone formation.<sup>10,20,21,76</sup> Few studies have examined isomer-specific effects of CLA on bone health. However, a recent four-month cross-sectional study in 31 healthy adult men found that red blood cell c9, t11 CLA, which is a marker of long-term dietary CLA intake, was positively associated with bone mineral density.<sup>77</sup> To-date, no clear conclusion can be made regarding the effect of CLA on bone health.<sup>20</sup>

### **Dietary Beef as a Source of CLA**

Although most studies have used synthetic mixtures of CLA or individual isomers, there are several reasons why food sources of CLA, such as beef, may be preferable. Beef is a natural source of CLA, over 70% of which is the biologically active c9, t11 isomer considered to have possible health benefits.<sup>22,24</sup> Concern related to potential adverse health effects (e.g., insulin resistance, fatty liver) of indiscriminate use of t10, c12 CLA supplements<sup>10,12,19,21</sup> supports the intake of CLA from food sources. CLA obtained from ruminant-derived foods such as beef is relatively high in the c9, t11 isomer, with low levels of the

t10, c12 isomer. Beef is a source of vaccenic acid (a "good" *trans* fat) which can be converted in the body to c9, t11 CLA.<sup>24,31,55</sup> Other components such as specific fats in beef may increase the effectiveness of CLA in reducing disease risk.<sup>46</sup>

Although the minimum effective intake of CLA for disease prevention and overall health is unknown, beef provides more than 30% of current intake.<sup>29</sup> Studies indicate that the health benefits of CLA are achieved at intakes much higher than currently consumed.<sup>19</sup> One strategy to increase CLA intake is to raise the c9, t11 CLA content of beef fat by manipulating the diet of beef cattle and altering management practices on the farm.<sup>22,26,27</sup> Although challenging, recent developments in feeding strategies have shown promise.<sup>26</sup> The CLA content of grass-fed beef is approximately twice that of grain-fed beef.<sup>24</sup> Importantly, beef is a naturally nutritious food containing not only CLA, but many other nutrients (e.g., protein, zinc, vitamin B<sub>12</sub>, iron, etc.) considered to be beneficial to health.<sup>78,79</sup> Although much remains to be learned regarding the health benefits of CLA, the 2010 *Dietary Guidelines for Americans*<sup>17</sup> does not recommend eliminating food sources of natural *trans* fatty acids (e.g., CLA) such as meat, milk and milk products because this could adversely affect nutrient adequacy.

### **Summary**

In vitro and experimental animal studies indicate potential health benefits of CLA.<sup>20</sup> The predominant CLA isomer in beef, c9, t11 (rumenic acid), has been shown in some studies to inhibit cancer at several sites, particularly the mammary gland, reduce cardiovascular disease risk factors, improve insulin sensitivity, and exhibit an anti-inflammatory effect. While evidence for a beneficial health effect of CLA from animal studies is promising, it is difficult to extrapolate these findings to humans due to such factors as the higher amounts of CLA used in animal studies than in human trials and differences in study protocols.<sup>20</sup> Relatively few studies of CLA's effects on health have been conducted in humans. Moreover, findings from human studies investigating potential health benefits of CLA vary considerably, which may be attributed to differences in the sources (e.g., foods naturally containing CLA vs. synthetic supplements with varying mixtures of isomers) and amounts of CLA used, among other factors. Findings to date warrant further



investigation, particularly in humans, to substantiate CLA's health benefits and safety, determine the relative potency of natural sources of c9, t11 CLA and its

precursor, vaccenic acid, in foods such as beef, and to identify the minimum amount of CLA to confer health benefits.<sup>2,10,20,21</sup>

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