Concentrations of Conjugated Linoleic Acid Isomers in Human Plasma Reflect Intake of Dairy Products with Enhanced cis-9, trans-11 or trans-10, cis-12 Isomer Content

By

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Thesis submitted to the Faculty of Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

MASTERS OF SCIENCE

in

HUMAN NUTRITION, FOODS, AND EXERCISE

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June 20th, 2006
Blacksburg, Virginia

Keywords: Conjugated Linoleic Acid, Plasma Fatty Acid Concentrations, Functional Food

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Brian Shafer

ABSTRACT

Dairy products are a primary source of c9, t11-CLA, an anti-carcinogenic agent, in the diet of humans. The t10, c12-CLA isomer, typically in trace amounts in bovine milk fat, also may benefit human health. Four cows received abomasal infusions of c9, t11-CLA or t10, c12-CLA to obtain milk fat used to prepare butter and yogurt with enhanced c9, t11-CLA or t10, c12-CLA content. Human subjects (3 males, 3 females, ages 22 to 29) received CLA-enhanced butter and yogurt (14% of total kcal) in a crossover study with 2-wk periods. Prior to the study (2 wk) and during a 2-wk washout period between the experimental periods, subjects received butter and yogurt without enhanced CLA content. Blood samples were obtained at 0, 2, 4, 6, and 8 wk relative to the start of the first experimental period. The t10, c12-CLA isomer was detected in plasma (1.32 ug/mL) only when dairy products with enhanced t10, c12-CLA was consumed. Baseline c9, t11-CLA was 6.94 ug/mL plasma during control periods, but increased to 8.95 ug/mL when dairy products with enhanced c9, t11-CLA content were consumed. Results indicated concentrations of CLA isomers in human plasma respond to small changes in daily intake of the isomers in dietary sources.
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ACKNOWLEDGEMENTS

I wish to thank my wife, Jennifer, for her love and continued support with this thesis, and in life.

I wish also to thank my family for their support and love.

Thank you also to Dr. Thye, and my other committee members and colleagues, for their help and advice in working on this thesis and also for their continuing to support to allow me to finish this thesis.
CHAPTER 1
INTRODUCTION

Conjugated Linoleic Acid (CLA) refers to a group of fatty acid isomers that were identified in the late 1980’s (Ha et al. 1987). Upon further investigation, CLA was found to consist of a group of nine different isomers of octadecanoic acid (18:2). These isomers are derived from linoleic acid (18:2), which has double bonds at the cis-9, cis-12 location (Figure 1).

CLA isomers have interested the scientific community because a multitude of health benefits associated with them including enhanced immune function (Sugano et al. 1997; Wong et al. 1997), body composition changes (Park et al. 1999; Zambell et al. 2000), anti-carcinogenic activity (Liew et al. 1995), and anti-atherogenic activity (Lee et al. 1994). The two isomers receiving the most attention and that will be the focus of this thesis are cis-9, trans-11 (c9, t11-CLA) isomer and the trans-10, cis-12 (t10, c12-CLA) isomer.

Figure 1 - Schematic of CLA isomer structures. Trans-10, cis-12 CLA (top), cis-9, trans-11 CLA (middle), linoleic acid (bottom). Photo reprinted from Pariza et al. 2000.
Cis-9, trans-11 CLA is the most prevalent of the CLA isomers. Approximately 90% of the CLA isomers occurring naturally are c9, t11-CLA. The c9, t11-CLA isomer is produced naturally in the process of biohydrogenation. This is the process of adding hydrogen molecules onto an unsaturated fatty acid molecule, increasing the saturation level of that fatty acid. The process of biohydrogenation occurs most often in the digestive processes of ruminant animals, but is not limited to the gut of the ruminant. Many mammalian species, including humans, have limited biohydrogenation occurring in the gut. Since biohydrogenation occurs most often in ruminant animals, dairy products are the best sources of naturally occurring CLA. The t10, c12-CLA isomer is produced in very low amounts in the rumen (Kim et al. 2002) and is usually undetectable in plasma or milk because it is not absorbed and transferred to the mammary gland. Thus it is not incorporated into milk lipids when quantities of t10, c12-CLA are at low levels (Kim et al. 2002). Synthetic CLA mixtures, which are synthesized by alkali isomerization, contain higher amounts of t10, c12-CLA. These synthetic mixtures require free linoleic acid to produce the CLA isomers. For this reason, the CLA isomers produced synthetically are in free fatty acid (FFA) form as opposed to triglyceride (TG) form usually found in naturally occurring CLA.

CLA can also be found in animal tissues, including humans. CLA has been found in human adipose tissue (Ackman et al. 1981), serum, bile, and duodenal juices (Cawood et al. 1983). Other mammalian species also deposit CLA in the tissues. Some fatty acid conversion can occur in the tissues to allow for the formation of CLA in the tissues. Dietary trans fatty acids have been found to increase the amount of CLA in human serum (Salminen et al. 1998). These conversions take place with the Δ9 desaturase enzyme,
which may aid in converting trans-vaccenic acid (t11, 18:1) to the c9, t11-CLA isomer. CLA is also found in some plant oils, though in very small amounts.

In human tissues, c9, t11-CLA is the only detectable isomer of CLA found normally in traceable amounts. The usual type of feeding given to the ruminant allows for the majority of production of c9, t11-CLA in the milk. The amounts of t10, c12-CLA can be increase in the ruminant animal by the type of feed given. Typically, pasture feeding causes higher amounts of t10, c12-CLA than usual feeding (Stanton et al. 1997). By increasing the amount of t10, c12-CLA produced and present in the blood, the amount of this isomer is increased in the milk of cows and could be more prevalent in the human diet and tissues (Loor et al. 1998).

Though structurally similar, each of the two CLA isomers exhibit different health benefits. The c9, t11-CLA isomer has been shown to have anti-carcinogenic properties (Ip et al. 1991; Ip et al. 1994), anti-atherogenic properties (Lee et al. 1994; Nicolosi et al. 1997), and enhanced immune response (Sugano et al. 1997; Wong et al. 1997). The t10, c12-CLA isomer has also been shown to have enhanced immune response (DeVoney et al. 1999), decreased milk fat production in humans (Masters et al. 2002) and bovines (Loor et al. 1998), and decreased fat mass coupled with increased whole body protein in mice (Park et al. 1999). However, there was no change in body fat in human subjects consuming CLA capsules containing both CLA isomers (Zambell et al. 2000).

There also appear to be different mechanisms of actions for these two isomers, though current research is still preliminary and does not show exactly how these compounds work. The c9, t11-CLA isomer appears to incorporate itself into the phospholipid membranes of target cells, primarily adipose cells. The mechanisms of action of t10,
c12-CLA are a little clearer. It appears that t10, c12-CLA inhibits desaturase activity, particularly Δ-9 desaturation of stearic acid, thus also interfering with elongation of fatty acids (Bretillion et al. 1999). Along with this, t10, c12-CLA appears to inhibit de novo fatty acid synthesis (Loor et al. 1998).

Since the benefits of CLA have come to light, there has been a push to increase the amount of CLA in the diet. Although CLA can be purchased and consumed in pill form that contains both potentially beneficial isomers, the CLA contained in these pills is in the free fatty acid (FFA) form, which is not as easily absorbed into the bloodstream as a more natural, triglyceride (TG) form of CLA (Reiser, 1950). Another drawback to consuming CLA in FFA form is the cost. Pills containing CLA are expensive and only contain the CLA isomers, with no other nutrients contained in the pills. A more cost effective approach to CLA consumption, perhaps, would be through dietary sources containing CLA.

As previously mentioned, CLA is found in animal sources in varying amounts (O’Shea et al. 1998). The amounts of the CLA in various animal products are listed in Table 1. Due to the amount of biohydrogenation that occurs in the ruminant animal, dairy products are the best sources for naturally occurring CLA. Even though t10, c12-CLA is limited in the amount formed during biohydrogenation, the isomer can be infused into the abomasums of the dairy cows to have them produce larger amounts of t10, c12-CLA isomers, although this is not a cost effective method of obtaining t10, c12-CLA.

The purpose of the current research project was to determine whether the two CLA isomers in question: c9, t11-CLA and t10, c12-CLA showed increases in plasma fatty acid concentrations following supplementation of each isomer over a two-week period in
human subjects. The CLA isomers were supplemented via yogurt and butter containing c9, t11-CLA alone or a combination of c9, t11-CLA and t10, c12-CLA. The effects of CLA supplementation on plasma lipids were also determined.

This research will aid in the determination of how much CLA in the diet is required to significantly change plasma fatty acid concentrations in humans. This is especially important for t10, c12-CLA, since this isomer is not usually found in animal plasma and tissue.

**STATEMENT OF PROBLEM**

Numerous metabolic effects of CLA have been shown in animal models and to a lesser extent, in humans. These metabolic changes have mostly occurred when animals or human subjects have consumed a mixture of the two main CLA isomers: c9, t11-CLA and t10, c12-CLA. All the studies reviewed used the FFA form of these CLA isomers. No research has been done on the extent that either CLA isomer is absorbed in the human when consumed in the TG form. This research will provide initial data for determining the responsiveness of the human to increased dietary CLA isomers in TG form for increasing plasma FA concentrations of the respective isomers and to determine if there is any affect on concentrations of other plasma FA due to increased intake of the CLA isomers. Whether plasma cholesterol changes may occur with consumption of CLA-enhanced butter and yogurt over the two-week period will also be determined.

**RESEARCH HYPOTHESIS**

Increased CLA intake in humans over a two-week period will show the following:

- An increase in plasma FA concentrations of c9, t11-CLA during the two-week intake of c9, t11-CLA enhanced butter and yogurt.
- An increase in plasma FA concentrations of c9, t11-CLA and t10, c12-CLA during the two-week intake of enhanced c9, t11-CLA plus t10, c12-CLA butter and yogurt.

- A decrease in plasma total and lipoprotein cholesterol levels following two-week intake of enhanced c9, t11-CLA plus t10, c12-CLA butter and yogurt.

**Table 1. Concentration of CLA in various foods (Kritchevsky, 2000)**

<table>
<thead>
<tr>
<th>FOOD</th>
<th>CLA (g/kg fat)</th>
<th>c9, t11-CLA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef, fresh ground</td>
<td>4.3</td>
<td>85</td>
</tr>
<tr>
<td>Chicken</td>
<td>0.9</td>
<td>84</td>
</tr>
<tr>
<td>Lamb</td>
<td>5.6</td>
<td>92</td>
</tr>
<tr>
<td>Pork</td>
<td>0.6</td>
<td>82</td>
</tr>
<tr>
<td>Turkey</td>
<td>2.5</td>
<td>76</td>
</tr>
<tr>
<td>Seafood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmon</td>
<td>0.3</td>
<td>*</td>
</tr>
<tr>
<td>Shrimp</td>
<td>0.6</td>
<td>*</td>
</tr>
<tr>
<td>Trout</td>
<td>0.5</td>
<td>*</td>
</tr>
<tr>
<td>Cheese</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheddar</td>
<td>3.6</td>
<td>92</td>
</tr>
<tr>
<td>Cottage</td>
<td>4.5</td>
<td>83</td>
</tr>
<tr>
<td>Parmesan</td>
<td>3.0</td>
<td>90</td>
</tr>
<tr>
<td>Ricotta</td>
<td>5.6</td>
<td>84</td>
</tr>
<tr>
<td>Dairy Products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>4.7</td>
<td>88</td>
</tr>
<tr>
<td>Milk</td>
<td>5.5</td>
<td>92</td>
</tr>
<tr>
<td>Yogurt</td>
<td>4.8</td>
<td>84</td>
</tr>
<tr>
<td>Vegetable Oils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>0.2</td>
<td>39</td>
</tr>
<tr>
<td>Olive</td>
<td>0.2</td>
<td>47</td>
</tr>
<tr>
<td>Safflower</td>
<td>0.7</td>
<td>44</td>
</tr>
</tbody>
</table>

* No data
SIGNIFICANCE OF STUDY

A significant rise in plasma free fatty acid concentrations of either c9, t11-CLA alone or t10, c12-CLA plus c9, t11-CLA isomers during the enhanced-CLA butter and yogurt intake period will show that the human is responsive to small increases in dietary CLA in the TG form from consuming butter and yogurt.

BASIC ASSUMPTIONS

The investigators made the following assumptions in conducting the study:

1. All subjects completed health questionnaires truthfully and were healthy at beginning of study.
2. All subjects completed dietary records to the best of their ability.
3. All subjects consumed all control and CLA butter and yogurt items given to them as directed by the investigators.
4. All subjects maintained their usual activity levels as prior to the beginning of the study.
5. All plasma lipid and fatty acid analysis was performed and recorded accurately.

DELIMITATIONS

The investigators delimited the study through the following methods:

1. All subjects were volunteers from the Virginia Tech community who responded to recruitment flyers posted throughout the Virginia Tech campus and Blacksburg community and were offered a stipend for participation in the study.
2. All subjects were required to have a baseline total cholesterol >170 mg/dL. Preliminary cholesterol screenings were given prior to start of study to determine these cholesterol levels.

3. All blood lipid levels were performed in duplicate, within 5% error using enzymatic-colorimetric analysis with a spectrophotometer, to ensure accuracy.

4. All subjects avoided dairy products containing fat to minimize the amount of CLA consumed other than the supplement.

5. All subjects were educated on how to record daily food intakes and how to properly determine the amount of food consumed.

6. All subjects met with a dietary consultant every few days throughout the duration of the study to answer any questions the subjects had and to ensure the subjects were maintaining complete dietary records and consuming the correct food items containing the CLA or control fat butter and yogurt.

7. All subjects consumed < 7% kilocalories (kcal) from fat in their diets during the study. The remaining 14% kcal from fat came from the CLA or control supplements, so that the total fat kcal in the diet was approximately 21%.

**LIMITATIONS**

Interpretation of the data was limited due to the following:

1. The subjects were unfamiliar with maintaining dietary records. This may have resulted in misreporting of dietary intake.

2. Volunteers were used in this study, which may have resulted in a representative sample of the population as a whole.
3. Dietary records of the subjects were not maintained before the start of the study, thus a comparison of the previous and usual diets with study diets were not possible when interpreting results.

4. Activity levels were not maintained by subjects before or during the study and thus any activity changes that may have taken place during the study were not considered when interpreting results.

5. Due to refrigerator malfunction, supplements were destroyed and three subjects had to complete the study several days early. This may have created error in the data for the last enhanced-CLA butter and yogurt intake period.

**DEFINITION OF TERMS**

- **Cis-9, trans-11 CLA** Isomer of CLA that may have potential health benefits including anticarcinogenic properties and enhancing immune function.
- **CLA** Conjugated Linoleic Acid – group of geometric and positional isomers of linoleic acid that may have health benefits.
- **Functional Food** A food that contains substances or nutrient that may be considered to provide health benefits beyond the general nutrition of that food.
- **Free Fatty Acid** A fatty acid that is not esterified to glycerol or other groups.
- **Intestinal Microflora** The bacteria contained within the intestinal wall that aids in the process of biohydrogenation and the production of CLA.
- **Total Cholesterol** The amount of HDL, LDL, and VLDL
• Trans-10, cis-12 CLA Isomer of CLA that may have potential health benefits including reducing whole body fat and increasing whole body protein.

• Triglyceride A fatty acid bonded to two other fatty acids and an acyl group.

**LIST OF ABBREVIATIONS**

• ApoB Apolipoprotein B
• BMI Body Mass Index
• BW Body Weight
• c11-18:1 cis-vaccenic acid
• c9, t11-CLA cis-9, trans-11 CLA
• CE Cholesterol Ester
• CLA Conjugated Linoleic Acid
• FA Fatty Acid
• FFA Free Fatty Acids
• HDL-C High Density Lipoprotein Cholesterol
• Kcal Kilocalorie
• LDL-C Low Density Lipoprotein Cholesterol
• MUFAs Monounsaturated Fatty Acids
• PFAC Plasma Fatty Acid Concentrations
• PUFAs Polyunsaturated Fatty Acids
• SFAs Saturated Fatty Acids
• t9, c11-CLA \textit{trans-9, cis-11} CLA
• t11-18:1 \textit{trans}-vaccenic acid
• TC Total Cholesterol
• TG Triglycerides
• VLDL Very Low Density Lipoprotein

**SUMMARY**

Over the past decade, research on CLA has markedly increased. The potential benefits of CLA may include reducing or preventing health problems ranging from cancer to obesity. While much research has been done on the health benefits of CLA in animal models, very little research has been done on humans. To determine whether some of these same beneficial effects may occur in humans, studies need to be conducted to determine the response of humans to the various CLA isomers. The purpose of the present study was to determine concentrations of the two CLA isomers, c9, t11-CLA and t10, c12-CLA, detected in plasma after enhanced-CLA butter and yogurt was included in the diet. Once it is determined how much CLA needs to be consumed for changes to occur in the bloodstream, further research can be conducted to determine if the benefits found in animal models are shared in human.
CHAPTER 2 – LITERATURE REVIEW

INTRODUCTION

The following chapter will review the current literature pertaining to CLA. The chapter will start by examining the literature on CLA biosynthesis and CLA delivery in the diet. The remainder of this chapter will focus on how CLA intake reduces milk fat percentage, body composition changes associated with CLA supplementation, and the effects of CLA on plasma lipids. These former two topics will be considered more in-depth than the latter three topics since these topics are more closely related to the topic of this paper.

CLA BIOSYNTHESIS

As mentioned previously, CLA can be produced via the process of biohydrogenation. While this process occurs at higher levels in ruminant animals, there is also some biohydrogenation occurring in other animal species. Along with biohydrogenation, conversion of certain fatty acids to CLA in animal tissue may also occur. Biohydrogenation and conversion to CLA allows for endogenous sources of CLA and thus even without dietary intake of CLA, there would still be some concentrations of CLA found in the tissues and plasma. These levels vary however with the different species and within the specific tissues.

A key to the process of biohydrogenation is the microbial flora in the gut. Chin et al. 1994 examined how much CLA is produced in germ-free rats compared to conventional rats. The researchers fed either free linoleic acid or esterified linoleic acid to conventional and germ-free rats. The germ-free rats did not have the microbial flora the conventional rats had. These diets contained a 5% corn oil diet that served as the control or a control
plus 25 g free linoleic acid/kg BW diet or control plus 50 g free linoleic acid/kg BW diet. Corn oil contains fatty acids in the TG form. The germ free rats were fed only the control diet or the 50 g free linoleic acid/kg BW diet. The conventional rats were also fed a diet containing 8.63% corn oil, which is considered equivalent to the 50 g free linoleic acid/kg BW, to determine whether the FFA form of linoleic acid or the TG form of linoleic acid is preferentially taken up by the gut. The diets were fed over an 8 weeks period and analyses of CLA concentrations in the liver, lungs, abdominal adipose tissue, kidney, skeletal muscle, liver phospholipid, and neutral lipid fractions were determined.

The results of this study showed that tissue concentrations of CLA were higher in the conventional rats compared to the germ free rats (Figure 2). These levels were significantly higher (P < 0.02) after 4 weeks and were also highest in the kidney and adipose tissue, while being lower in the liver, lungs, and skeletal muscle. The germ free rats did not show any affect of the diet on their CLA concentrations. The conventional rats’ diet that contained the FFA form of linoleic acid showed significantly higher (P < 0.01) concentrations in the tissues, although there were no significant differences between the tissue concentrations of the control diet and the corn oil diet in the conventional rats.

The researchers indicated that intestinal microflora were necessary to convert linoleic acid to CLA. The researchers were unable to determine whether the conversion of linoleic acid to CLA took place in the stomach, small intestine, or the large intestine. The researchers indicated however, that it is unlikely the conversion occurred in the large intestine, since it is does not absorb long chain fatty acids. They also showed that the FFA form of linoleic acid was taken up preferentially over the TG form of linoleic acid,
which is surprising considering the TG form of linoleic acid is the form found naturally. However, the researchers attributed this observation to the bacterium *Butyrivibrio fibrisolvens*, which contains linoleate isomerase, an enzyme that serves to biohydrogenate fatty acids that have a free carboxyl group and a *cis* double bond at position Δ9 and Δ12 (Kepler et al. 1966).

**Figure 2.** *The effects of feeding linoleic acid to conventional and germ free rats at two weeks and four weeks.* (Chin et al. 1994)

2 Weeks

4 Weeks

Earlier studies such as the one above examined the biosynthesis of CLA in animal models. Research into the biosynthesis of CLA and the conversion of other fatty acids to CLA has increased tremendously in recent years. Adlof et al. 2000, examined the
biosynthesis of CLA in humans using serum lipids from five previous studies dating between 1978 and 1988. The authors did not differentiate between the various CLA isomers, except for the c9, t11-CLA isomer. Any mention of CLA during the review of this article includes all CLA isomers, unless otherwise noted. In the initial studies in which serum lipids were extracted, subjects were fed milkshakes that contained either 8-10 g or 2.5 g of deuterium-labeled FA, fed as homogenous TG. These FA consisted primarily of t11-18:1, c11-18:1, c9-18:1, t10-18:1, and c10-18:1. The blood samples from these trials provided the serum lipids for this analysis and were taken at 0, 2, 4, 8, 12, 15, 24, and 48 hours following the ingestion of the milkshake. The researchers were looking for changes in CLA content following ingestion of the milkshake. If the concentrations of CLA peaked during post-ingestion of the milkshake, then the CLA was produced via biosynthesis and not from direct ingestion of CLA from any that may be contained within the milkshake.

It is of note that this study used serum lipid samples taken anywhere from 10-20 years previous. Oxidation may have occurred in these samples considering there age. It is also important to note that for each of the studies examined in Adlof et al. 2000, each study examined only one subject each.

The results of this study showed that t11-18:1 was converted to CLA following ingestion of the milkshake. The researchers used gas chromatography of the blood samples to determine this. The c9, t11-CLA isomer was the most abundant found after ingestion of the milkshake, with about a 30% concentration of t11-18:1 found in the milkshake. Other CLA isomers were found in much smaller quantities (the c9, c11-CLA isomer was found at <10% concentration of t11-18:1 found in the milkshake). There was
also no evidence to show conversion to t10, c12-CLA. This supports the theory that this
CLA isomer is not produced by tissue biosynthesis in humans. Composition of the serum
fatty acids was determined and is shown in Table 2. The percentage of CLA increased
with time, which indicated that the CLA found in the serum did not come directly from
the diet, but was produced biosynthetically, most notably in the conversion of t11-18:1 to
CLA.

Table 2. Composition (percentage by weight) for fatty acids (Adlof et al. 2000)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Hour</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>4</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>t9-16:1</td>
<td>2.0</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>t11-18:1</td>
<td>1.4</td>
<td>11.4</td>
<td>4.5</td>
<td>2.5</td>
</tr>
<tr>
<td>CLA*</td>
<td>0.07</td>
<td>0.19</td>
<td>0.32</td>
<td>0.14</td>
</tr>
</tbody>
</table>

*comprised mostly of c9, t11-CLA, but other CLA isomers also found.

The researchers thought little oxidation of CLA occurred in the 10-20 yrs of storage
of the samples. They attributed this to comparison of the lipid content during initial
analysis and the most recent analysis during the current study. This study confirmed a
metabolic pathway for the biosynthesis of CLA using the t11-18:1 isomer provided for
conversion to CLA. Since trans-vaccenic acid is a common fatty acid in ruminant
animals, it would logically be a precursor to CLA, since CLA is common in ruminants
(Pollard et al. 1980). The enzyme responsible for this in tissues probably is Δ9
desaturase (Salminen et al. 1998).

Salminen et al. 1998, examined the effects of a diet high in trans FA on CLA levels
in human serum. Subjects consumed a diet high in saturated fatty acids (SFAs),
particularly from dairy fat, for five weeks. High amount of dairy fat were consumed
during this period to achieve a high CLA dietary intake (0.31 g/d) along with high amounts of SFAs (39.6 g/d). Subjects were then placed on either a high trans FA diet (high in 18:1 trans FA’s, 24.9 g/d) or a high stearic acid diet (44.9 g/d) for five weeks. The high trans FA diet and the high stearic acid diet were both prepared from specially prepared margarine containing high amounts of the either trans FA or stearic acid. Serum blood samples were taken and FA concentrations were determined. It is important to note that the FA the subjects consumed were taken in the form of triglycerides (TG) rather than the free fatty acid (FFA) form used in many studies.

Results showed that during the trans FA diet, the proportion of CLA in the serum increased significantly. These results are shown in Table 3. Serum stearic acid levels also increased during the stearic acid diet. Linoleic acid levels remained the same during both diets. Cis-monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) remained constant during both diets also. By maintaining a constant rate of cis-MUFAs and PUFAs, the researchers were able to compare the effects of the trans FA diets, particularly t11, 18:1 to the stearic acid diet.

These results indicated that increased levels of CLA were formed when subjects consumed a diet rich in t11, 18:1. Although the subjects consumed a diet high in dairy fat preceding the experimental diets, serum CLA levels were higher with greater levels of trans FA in the diet than during the dairy fat diet. The trans FA diet and the stearic acid diet contained less CLA than the dairy fat diet, thus, some tissue conversion of trans FA to CLA utilizing the Δ9 desaturase must have occurred for the subjects consuming the trans FA diet to have higher levels of serum CLA. Adolf et al. 2000 (reviewed above), also had a similar finding. This study would give support to increasing the consumption
of trans FA, especially trans FA coming from dairy products. Increasing the consumption of trans FA remains a controversial topic because the effects trans FA can have on the risk of heart disease.

**Table 3.** Mean percent fatty acid composition of total serum during the dairy fat diets, trans fatty acid diet, and stearic acid diets (Salminen et al. 1998)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Trans FA group (N = 40)</th>
<th>Stearic acid group (N = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dairy fat diet</td>
<td>Trans FA diet</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>23.74±1.27</td>
<td>20.31±1.60‡</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>7.47±0.64</td>
<td>6.68±0.89*†</td>
</tr>
<tr>
<td>t11, 18:1</td>
<td>0.59±0.17</td>
<td>4.47±1.37*†</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>23.39±2.03</td>
<td>24.39±1.66*‡</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>30.03±2.79</td>
<td>29.73±2.22</td>
</tr>
<tr>
<td>CLA isomers</td>
<td>0.32±0.06</td>
<td>0.43±0.12*†</td>
</tr>
</tbody>
</table>

*(Note: Some t11, 18:1 overlap occurred with oleic acid during analysis, resulting in slightly higher values for oleic acid and lower values for t11, 18:1 than actually occurred)*

± SD

* P < 0.001 compared with the dairy fat diet
† P < 0.001 compared with the stearic acid diet
‡ P < 0.01 compared with the stearic acid diet

The previous three studies have reviewed the biosynthesis of CLA in animals and humans. Of the CLA isomers formed via tissue biosynthesis, the c9, t11-CLA isomer appears to be the dominant isomer formed, especially in humans when t11-18:1 is provided. These studies appear to have confirmed that the t10, c12-CLA isomer is not produced via biosynthesis in the tissues. The pathway for CLA synthesis appears to involve trans FA, especially t11, 18:1 using the Δ9 desaturase enzyme. Along with
containing relatively high amounts of c9, t11-CLA, dairy products also contain high amounts of t11, 18:1, making it an ideal food to achieve higher levels of CLA in the body tissues. The form in which CLA is delivered, via TG or FFA form, may play a role in how much is found in body tissues. This will be discussed in further detail below.

**CLA DELIVERY IN THE DIET**

If CLA is found to have the same health benefits in humans as it has had in animal models, then it will be important to determine the best way to increase the amount of CLA available to the body. The most obvious way of enhancing the amount of CLA in the body is by dietary means. Depending on how CLA is fed will determine how much is absorbed and available. Fatty acids are usually more easily absorbed in TG form, as opposed to FFA form (Reiser, 1950). Many of the CLA supplements currently on market are in FFA form, via a pill containing the CLA isomers. The following section will review some of the literature on CLA delivery in the diet and how much CLA is absorbed from the different types of delivery.

Chin et al. 1994, looked at whether the FFA form or the TG form of CLA was absorbed better and showed up in the tissues of Sprague-Dawley rats. The researchers fed either free linoleic acid or esterified linoleic acid to conventional and germ-free rats. The conventional rats were assigned to consume a diet that contained 5% corn oil diet that served as the control or either a control plus 5% free linoleic acid diet or control plus 8.63% corn oil diet. The researchers stated the control plus 8.63% corn oil diet was equivalent of the control plus the 5% free linoleic acid diet, but it was in the TG form. This group was included in the study to determine whether the FFA form or the TG form
of linoleic acid is preferentially taken up by the gut. The germ free rats were fed LM-485 (Harlan Teklad, Madison, WI) autoclavable non-purified diet alone or fed the autoclavable diet plus 5.0% free linoleic acid. Approximately 25 g of food/day was made available to the rats. The diets were fed over an 8 weeks period and analyses of CLA concentrations in the liver, lungs, abdominal adipose tissue, kidney, skeletal muscle, liver phospholipid, and neutral lipid fractions were determined. Eight rats from each treatment group were killed at 2 wk and 4 wk and the same analyses performed.

These researchers found levels of CLA in the tissues 5-10 times higher in the conventional rats fed the free linoleic acid compared to conventional rats fed the control plus corn oil (Figure 3). The major isomers formed were c9, t11-CLA and trans-9, cis-11 CLA (t9, c11-CLA). There was no change in tissue CLA concentrations in the germ-free rats fed free linoleic acid. The authors concluded that the bacterial flora of rats is capable of converting free linoleic acid to c9, t11-CLA and t9, c11-CLA. The authors also pointed out that it has not been shown that the intestinal flora of the human intestinal tract has the same affinity for nonesterified linoleic acid that the rat intestinal tract does.

One of the first attempts to identify the importance of diet in enhancing CLA amounts in humans was done by Britton et al. 1992. Fourteen subjects consumed foods considered ‘high’ or ‘low’ in CLA content. Four subjects consumed both diets, four subjects consumed only the ‘high’ diet, and six subjects consumed the ‘low’ diet (n = 8, high; n = 10, low). Subjects were given a list of foods from each list, depending on their diet, thus specific amounts of CLA were not given during the study. Serum samples were taken at baseline and after three weeks of consumption of one of the diets.
There was a significant increase in the concentrations of c9, t11-CLA content in the subjects on the ‘high’ CLA diet. Trans-10, cis-12 CLA was unknown to exist at the time of the study and the researchers did not differentiate between the different isomers of
CLA other than c9, t11-CLA. Along with the findings of increased c9, t11-CLA 
(12.1 ± 3.7 μmol/L to 18.8 ± 7.4 μmol/L) during the ‘high’ diet, this isomer decreased in concentration during the ‘low’ diet (14.3 ± 6.7 to 8.9 ± 4.7 μmol/L). Results showed that the serum concentration of c9, t11-CLA was influenced by diet. The researchers purposely made the dietary protocol for this study very relaxed, to mimic normal eating patterns. The amount of serum c9, t11-CLA did not seem to fall below a certain level, even though very low levels of c9, t11-CLA intake were reported. The authors concluded that this leveling off of CLA levels even when CLA consumption was extremely low may be attributed to other factors, most likely from CLA biosynthesis.

In a study following 123 men, the relationship between milk fat intake and the amount of CLA in human adipose tissue was examined (Jiang et al. 1999). Serum fatty acid composition was analyzed after the subjects provided dietary records for one week and then had random food recall interviews for seven months following the dietary recall. This design was then repeated for 103 men, allowing for two dietary record periods and fourteen food recall interviews.

The results of this study show that as the amount of milk fat in the diet increased, the amount of CLA in adipose tissue increased (r = 0.42, P < 0.001). There were not a significant association between intake of milk fat and amounts of CLA in serum, however. There were no significant differences in nutrient intake between the dietary records and the food recall. The main isomer of interest in this study was c9, t11-CLA, since this is the predominant isomer found in humans. This isomer comprised 0.50% of the total fatty acids in adipose tissue and 0.25% in serum. The percent fatty acid composition of adipose tissue TG is shown in Table 4. The findings of this study support
earlier studies (Huang et al. 1994) that show the relation of dietary intake and amounts of CLA in the tissues and plasma. No association between c9, t11-CLA and linoleic acid was found, which would link the biosynthesis of CLA more strongly with t11, 18:1.

The final article reviewed in this section examines the effects of CLA supplementation on human milk and plasma composition (Masters et al. 2001). Ten lactating women participated in this seventeen-day, crossover design study. Subjects consumed either two capsules of CLA supplements/day (560 mg t10, c12-CLA, 547 mg c9, t11-CLA, 93 mg other CLA isomers/day) or a placebo capsule during the two five-day intervention periods. A seven-day washout period separated the two intervention periods (5 d intervention, 7 d washout, 5 d intervention). Semiquantitative food frequency questionnaires were given to the subjects to determine c9, t11-CLA intake prior to the start of the study. Milk samples were collected on the final day of each five day period for FA analysis.

Results showed that plasma c9, t11-CLA and t10, c12-CLA concentrations were higher (P < 0.05) during the CLA-intervention period. Figure 4 shows the plasma FA composition during each intervention period. The amount of milk output was not affected by either of the intervention periods. The effect of CLA on milk fat percentage will be discussed further in the section on body composition.

Dietary intake clearly has an effect on the amount of CLA found in the plasma and tissues. It was not clear whether consuming CLA in the TG form or in the FFA form would provide better absorption.
<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>% of total fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:1</td>
<td>0.5±0.2</td>
</tr>
<tr>
<td>16:0</td>
<td>21.9±1.9</td>
</tr>
<tr>
<td>16:1</td>
<td>6.7±1.5</td>
</tr>
<tr>
<td>18:0</td>
<td>3.9±0.8</td>
</tr>
<tr>
<td>t11, 18:1</td>
<td>2.2±0.5</td>
</tr>
<tr>
<td>c9, 18:1</td>
<td>46.5±1.9</td>
</tr>
<tr>
<td>18:2</td>
<td>10.8±1.9</td>
</tr>
<tr>
<td>c9, t11-CLA</td>
<td>0.5±0.1</td>
</tr>
</tbody>
</table>

Further research needs to be done to establish this. CLA can be formed via biosynthesis if the t11-18:1 isomer is provided and also can be increased in the body via dietary intake. The following section will examine how the amounts of CLA in the body can affect body composition and milk fat percentage.

**Figure 4.** Plasma FA composition during each intervention period (Masters et al. 2001).
EFFECTS OF CLA ON BODY COMPOSITION AND PERCENT MILK FAT

Until this point, how CLA is produced and incorporated into the body has been examined. But the importance of CLA lies in its’ health benefits. One of these benefits is the body composition changes that occur with CLA intake. This appears to occur with the intake of t10, c12-CLA. There does not seem to be any effect of c9, t11-CLA on body composition. Body composition changes have already been shown in animals (Park et al. 1999; Corino et al. 2002) resulting in leaner meats, for possible use in reducing the amount of fat intake in humans.

Research has yet to show if these body composition changes occur in humans, and if they do, whether this is a health benefit or not. If CLA is found to induce the same body composition changes in humans as in animals, it may only be beneficial to those who are obese, and not recommended for those individuals who are at normal or slightly overweight. Since t10, c12-CLA is not produced via biosynthesis, the only means of increasing its amounts is by dietary intake. Usually this isomer is only found naturally via a byproduct of bacterial metabolism which allow for trace amounts of this isomer to be present in animals and humans (Kepler et al. 1966). The t10, c12-CLA isomer can be provided in higher amounts in the synthetic form than produced naturally via bacteria. For this reason, high amounts of t10, c12-CLA are usually found in pill form, usually in the FFA form.

Park et al. 1999 examined the effects on body composition of feeding and withdrawal of c9, t11-CLA and t10, c12-CLA on ICR mice. In experiment 1 of this study, 8-week old female ICR mice were given a diet containing 0.5% CLA or a placebo for eight
weeks. The CLA mixture contained 40.8 - 41.1% c9, t11-CLA and 43.5 – 44.9% t10, c12-CLA. Body composition was determined after CO₂ suffocation via determination of water content, protein, whole body fat, and total body ash. In experiment 2, weanling mice were fed the same diet as in the first experiment for four weeks, then had the CLA removed from their diet for the remaining eight weeks of the experiment. Body composition was analyzed in the same manner as in Experiment 1 and fatty acid analysis of muscle, liver, and fat pad was determined at weeks 0, 2, 4, 6, and 8. The first experiment studied body composition at various times during CLA feeding and the second study examined the effect of withdrawal of CLA on body composition.

The results of Experiment 1 showed that the mice fed the diet supplemented with CLA showed slightly reduced body weight gain compared with controls by week 3 and significantly reduced body weight gain by week 7. The changes in relative body composition are shown in Figure 5. There was a reduction in whole body fat in the CLA-fed mice which was significant by week 7 (P=0.0274). By week 7, the control mice had about 6 grams of body fat compared with about 3 grams of body fat in the CLA-fed mice. The CLA fed mice ate significantly less (P<0.0001 versus the control group). There were no significant changes over time in whole body water, whole body protein, or whole body ash.

In the Experiment 2, the CLA-fed mice had a significant reduction in feed intake at wks 1 and 3. After withdrawal of the CLA there were no significant differences in feed intake or body weight between the CLA-fed mice and the controls. The absolute body composition of the both groups increased in body fat, but the CLA-fed mice had a significantly less increase in body fat compared with the control (P = 0.0016). Whole
body water and protein did remain elevated in the CLA-fed rats until the completion of the study, though this elevation was not significant.

Based on the findings of this study, the authors hypothesized there was an inhibition of fat storage in adipocytes coupled with increased β-oxidation in the skeletal muscle. This would mean that fat free mass was conserved in proportion to kcal intake. CLA also appeared to have effects even after it was taken from the diet. The authors concluded these effects may be the result of metabolism occurring because of the stored CLA in the tissues, as CLA levels in the liver, fat pad, and muscle gradually decreased for eight-weeks after CLA withdrawal. The researchers thought CLA may have blocked adipocyte differentiation based on the findings that body fat accumulation in CLA-fed mice was less after CLA-withdrawal compared to controls. The t10, c12-CLA isomer appeared to have the effect of increased whole body protein with decreased whole body fat in animals.

Figure 5. Relative changes in body composition in rats fed CLA in Experiment 1. Open circle = controls, Closed circles = CLA-fed (Park et al. 1999)

\( ^a \) \( P < 0.05 \) versus control
\( ^b \) \( P < 0.05 \) versus control when cage effects are ignored
To determine whether this effect might be seen in humans, Zambell et al. 2000, conducted a study on the effect of CLA supplementation on body composition and energy expenditure on seventeen women in this 94-day study. Subjects stayed in a metabolic ward for the study period. The first 30-days served as the baseline period and the remaining 64-days the subjects consumed either CLA capsules (~1% of total kcal) or a ~3g/d placebo. The subjects also consumed a diet similar to the American Heart Association’s Step II diet. Body composition was determined three times weekly by total body electrical conductivity and dual x-ray absorptiometry three times during the study. Energy expenditure was also determined.

No changes in body weight or body composition were observed during the CLA supplementation period compared with the placebo. These results are shown in Table 5. CLA supplementation also did not have an effect on energy expenditure, fat oxidation, or respiratory exchange ratio at rest or during exercise.

Table 5. Body weight and composition changes due to CLA supplementation (Zambell et al. 2002)

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>CLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>0.48±0.55</td>
<td>-0.24±0.46</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>0.18±0.43</td>
<td>0.09±0.35</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>0.01±0.64</td>
<td>-0.19±0.53</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>0.05±0.62</td>
<td>-0.67±0.51</td>
</tr>
</tbody>
</table>

Although there appeared to be a decreased weight, fat mass, and percent body fat, this study did not find any significant body composition changes attributed to CLA supplementation in humans. The findings of Zambell et al. 2002, are similar to those of
other researchers (Ferreira et al. 1997; Atkinson, 1999) on the effects of CLA supplementation on body composition in humans. Few studies have been done in this area and those that have (Ferreira et al. 1997; Atkinson, 1999) do not supplement as much CLA as the studies done on animal models. This and other studies also do not differentiate between the affect of the two isomers in humans. Most studies have the subjects consuming a capsule containing both isomers together in varying amounts. Further research needs to be done in this area to establish whether CLA supplementation has an effect on body composition in humans.

CLA does seem to have a role in decreasing milk fat percentage in humans, however. Masters et al. 2002, showed that human milk fat percentages decreased when subjects consumed CLA supplements containing both CLA isomers. Ten lactating women participated in this seventeen-day, crossover design study. Subjects consumed either two capsules of CLA supplements/day (560 mg t10, c12-CLA, 547 mg c9, t11-CLA, 93 mg other CLA isomers/day) or a placebo supplement during the two five-day intervention periods. A seven-day washout period was between the two intervention periods (5 d intervention, 7 d washout, 5 d intervention). Semiquantitative food frequency questionnaires were given to the subjects to determine c9, t11-CLA intake prior to the start of the study. Milk and blood samples were collected on the final day of each period for FA analysis.

The results of this study showed that there was no difference in plasma FA concentrations between placebo and CLA supplement group except for the c9, t11-CLA (21.1±2.9 for the CLA group versus 12.8±1.8 for the placebo) and the t10, c12-CLA (3.6±1.1 versus no data for the placebo group). Both of these values were significant.
There were similar findings for the milk FA analysis. There was no difference in FA concentrations between the CLA supplement group and the placebo group except for c9, t11-CLA (30.0±2.1 for CLA group versus 15.3±1.8 for placebo) and t10, c12-CLA (11.1±2.1 for the CLA group versus no data for the placebo group). Both of these values were significant (P<0.05). There was also a decrease in milk fat percentage for the CLA supplement group. These results are shown in Figure 6. There was a significant difference between the milk fat percentage of the placebo group compared to the CLA supplement group (P<0.05).

The authors concluded this decreased milk fat percentage was attributed to the t10, c12-CLA isomer. These findings were in agreement with other studies examining the effects of CLA supplementation on milk fat percentages in dairy cows (Loor & Herbein, 1998). This decline in milk fat percentage may be attributed to the ability of t10, c12-CLA to interfere with de novo fatty acid synthesis (Loor & Herbein, 1998).

In a study that looked more closely at the effect of higher concentrations of c9, t11-CLA or t10, c12-CLA on BW, BMI, and body composition, Tricon et al. 2004, fed 49
healthy men capsules of 80% c9, t11-CLA or 80% t10, c12-CLA in this double blind, crossover designed study. Subjects were divided into a group consuming either a 750-mg gelatin-coated capsule containing 80-85% of either c9, t11-CLA or t10, c12-CLA for 3 consecutive 8 wk periods. At the end of each eight wk period, the subjects would increase their dose of both isomers. Body weight and composition was determined via skinfold thickness at 4 sites via application of the Siri equation (Lawrence et al. 1956) and bioelectrical impedance using Bodystat 1500. This study also looked at the effect of these CLA isomers on plasma lipid levels, which will be discussed in the next section. The results of this study are shown in Table 6 and Table 7. Both CLA isomers increased mean fat mass and decreased mean fat-free mass at the lower doses, but these values returned to baseline when the subjects consumed the higher doses of the CLA isomers.

The authors concluded that “supplementation of highly enriched c9, t11-CLA or t10, c12-CLA had no effect on BW or BMI in healthy male volunteers in the present study”. The authors could not find any clear explanation for why there was a slightly, but unsignificant increase in fat mass at the lower dose of either CLA supplementation group, but suggested that the return to baseline values was due to adaptational effect. The authors also pointed out that this study shows that even supplementation with a higher dose t10, c12-CLA does not effect body composition when the c9, t11-CLA isomer is present. The authors referenced Riserus et al. 2002 as support for this. The overall conclusion by the authors was that consumption of either CLA isomer would not induce body composition changes in humans.
Table 6. Effects of c9, t11-CLA on BW and body composition (Tricon et al. 2004)

<table>
<thead>
<tr>
<th>c9, t11-CLA dose</th>
<th>0 g/d</th>
<th>0.59 g/d</th>
<th>1.19 g/d</th>
<th>2.38 g/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM (kg)</td>
<td>77.2±1.6</td>
<td>76.9±1.4</td>
<td>77.9±1.6</td>
<td>77.5±1.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.2±0.4</td>
<td>24.4±0.4</td>
<td>24.5±0.4</td>
<td>24.5±0.4</td>
</tr>
<tr>
<td>BF %</td>
<td>17.8±0.7</td>
<td>19.1±0.8</td>
<td>18.5±0.7</td>
<td>17.8±0.7</td>
</tr>
<tr>
<td>FFM%</td>
<td>81.4±0.6</td>
<td>80.0±0.7</td>
<td>81.1±0.7</td>
<td>81.8±0.7</td>
</tr>
</tbody>
</table>

BM = body mass, BF % = body fat percentage, FFM % = fat-free mass percentage
Red values indicate P<0.01 for the effect of the lowest CLA dosage from the effect at baseline and the highest doses

Table 7. Effects of t10, c12-CLA on BW and body composition (Tricon et al. 2004)

<table>
<thead>
<tr>
<th>t10, c12-CLA dose</th>
<th>0 g/d</th>
<th>0.63 g/c</th>
<th>1.26 g/d</th>
<th>2.52 g/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM (kg)</td>
<td>77.8±1.5</td>
<td>77.6±1.5</td>
<td>78.3±1.6</td>
<td>78.1±1.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.6±0.4</td>
<td>24.4±0.4</td>
<td>24.7±0.4</td>
<td>24.6±0.4</td>
</tr>
<tr>
<td>BF %</td>
<td>18.0±0.7</td>
<td>18.9±0.7</td>
<td>18.4±0.7</td>
<td>18.6±0.7</td>
</tr>
<tr>
<td>FFM%</td>
<td>81.2±0.6</td>
<td>80.2±0.6</td>
<td>80.8±0.6</td>
<td>80.8±0.6</td>
</tr>
</tbody>
</table>

BM = body mass, BF % = body fat percentage, FFM % = fat-free mass percentage
Red values indicate P<0.01 for the effect of the lowest CLA dosage from the effect at baseline and the highest doses

Smedman and Vessby, 2001, studied the metabolic effects of CLA supplementation on healthy humans. Fifty-three healthy subjects (27 male, 26 female) ranging between 23-63 years of age participated in this random, double-blinded, 14 week study. Subjects were assigned to either a CLA-treated group or a control group. All subjects were given control capsules containing olive oil for the first two weeks of the study. For the remainder of the study, the CLA-treated group received capsules containing 4.2 g/d of CLA while the control group received capsules containing 4.2 g/d of olive oil. These capsules contained 75.9% CLA with equal amounts of the c9, t11-CLA isomer and the t10, c12-CLA isomer. Minor amounts of other CLA isomers accounted for the rest of the composition of the capsules. Three day weighed dietary records were taken three times during the study (at the start of the test period, and at the 5th and 9th week of the test period). Subjects were asked not to change their exercise or dietary habits while enrolled in the study. BMI was calculated, percent body fat was determined using Harpenden skin...
fold calipers, and bioelectrical impedance analysis was performed using Hydra 4200 (Forslund et al. 1996). Serum lipoprotein, nonesterified fatty acids, plasma insulin, and plasminogen activating inhibitor-1 (PAI-1) were also analyzed.

The results of this study showed that the proportion of body fat decreased 3.8% (P<0.001) in the CLA-treated group. The control group also had a decrease of 1.23% body fat, although this value was not significant. The CLA-treated group lost significantly more body fat when compared to the control group (P=0.050). Body weight, BMI, and sagittal abdominal diameter did not change in either group. There was also no change in serum lipoproteins, nonesterified fatty acids, plasma insulin, or PAI-1.

The authors concluded that these results were of borderline health significance considering both groups attained a decrease in body fat. They stated that the subject group was healthy and extreme diverse in age and background and that a population that was more obese and less healthy may show a greater change in many of the parameters that did not show any statistical significance.

Petridou et al. 2003, examined the effects of CLA supplementation on body fat percentage in young women. Sixteen sedentary women, 19-24 years of age participated in this 90 day randomized, crossover designed study. Subjects were placed into a either placebo-CLA group that consumed six 500 mg of soybean oil for 45 days followed by 45 days of consuming six 500 mg gelatin capsules containing 70% CLA (2.1g total/day divided equally between the c9, t11-CLA isomer and the t10, c12-CLA isomer). The other group, the CLA-placebo group, consumed the CLA capsules for 45 days followed by the placebo tablets. Subjects were measured for body weight, height, and body fat composition via skinfold thickness measurements at 10 different skinfold sites (Parizoka,
1968) at the beginning, at the crossover point, and at the end of the study. Blood samples were also taken at these same times to determine serum TAG, TC, HDL-C, and FA analysis. Results from the blood samples will be discussed in the section on the effects of CLA on plasma lipids.

The results of this study are shown in Table 8. There was no significant difference in body fat or body mass of the subjects after supplementation with the CLA isomers. The authors attributed this to the young age of the subjects, low dosage of CLA, and duration of the supplementation period. It is of note that this study did not incorporate a washout period between the placebo and experimental period.

Table 8. Anthropometric data of participants (Petridou et al. 2003)

<table>
<thead>
<tr>
<th></th>
<th>CLA-Placebo group (N=9)</th>
<th>Placebo-CLA group (N=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>CLA</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>66.3±9.5</td>
<td>66.2±9.0</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>23.1±2.4</td>
<td>23.2±2.4</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>28.1±5.7</td>
<td>29.4±3.6</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>19.0±5.8</td>
<td>19.7±4.6</td>
</tr>
</tbody>
</table>

The effects of CLA supplementation on body composition in humans and animal models have shown differing results. Research on mice showing a change in body composition with mixed CLA supplementation (Park et al. 1999) is promising suggesting it may be possible for this to occur in humans. While research on the effects of CLA on human body composition is limited and although there has not been any findings that CLA supplementation affects body composition, great interest in this topic is pushing for more research to be conducted. The studies thus far on the effects of CLA supplementation on body composition have looked at combined forms of the CLA isomers, and not in the isomers individually. By looking at CLA isomers individually, it may be possible to determine whether a specific CLA isomer may affect body
composition in humans. Whole body protein increased and whole body fat decreased in mouse models consuming t10, c12-CLA (Park et al. 1999). However, there does seem to be an effect of CLA supplementation on milk fat percentage in dairy cows (Loor & Herbein. 1998) and humans (Masters et al. 2002). It is hypothesized that t10, c12-CLA interferes with de novo synthesis, thus causing body composition changes and milk fat decreases (Loor & Herbein. 1998). However, these results do not seem to hold for humans (Zambell et al. 1999). The mammary glands in humans may be more sensitive or a different mechanism of action may be involved to account for why humans see a decrease in milk fat when consuming a CLA supplement, but do not have the body composition changes associated with the CLA supplementation. As more research is conducted on these topics, these questions may be answered.

**EFFECT OF CLA ON PLASMA LIPIDS**

Anti-atherosclerotic properties have also been reported with CLA intake. Early studies indicated the c9, t11-CLA isomer may be responsible for reduction in LDL-C in rabbits (Lee et al. 1994). Despite this finding, very little research has been done in this area to establish whether CLA has further anti-atherosclerotic properties in humans.

In the first study examining the effects of CLA on plasma lipids, Lee et al. 1994, used 12 New Zealand White rabbits over a 22 wk period to determine the effects of CLA (0.5 g/d) on plasma lipid levels and plague formation. There was no differentiation between or percentages consumed of the different CLA isomers during this study. Six rabbits were placed in the CLA group (3 males, 3 females), while six were placed in the placebo group (3 males, 3 females). The rabbits consumed a semi-synthetic, high fat diet (14%
fat) for 4 weeks, which served as an adaptation period. The rabbits were then placed on the same high fat diet with 1% cholesterol for the remaining 18 weeks of the study. The CLA was consumed as part of the high fat diet. Rabbits were restricted to 100g of food per day. Blood samples were taken monthly for lipid analysis and liver cholesterol was analyzed. Atherosclerosis was determined by measuring plaque thickness of the aorta.

The results of this study showed less atherogenesis in the CLA group. There were significant differences in LDL (control: 590 mg/dL, CLA: 450 mg/dL) and the LDL/HDL ratio (control: 17.5, CLA: 10.5). There were no significant differences in TC, HDL, or TC/HDL. The CLA group also showed 30% less plaque deposition in the aorta. The researchers attributed this to lipoprotein metabolism changes induced by the CLA. Since this study was at the beginning of CLA research, none of the results could be attributed to either isomer.

Corino et al. 2002, examined the effects of CLA on many different factors, including lipid metabolism, on rabbits. One hundred forty-four New Zealand white rabbits were assigned to diets consisting of commercial pellets (16% crude protein, 14% crude fiber, 3% ether extract, and 8% ash) and either 0, 0.25, or 0.5% CLA in FFA form for 76, 90, or 104 days. The diets were fed ad libitum. Tests to determine lipogenic enzyme activity levels, oxidative stability of muscle, and adipose tissues samples were taken in this study, but the focus of this review will be the results of the blood samples for total cholesterol (TC) and triglyceride (TG) levels. Results of this study showed decreased levels of TG and TC. These results are shown in Table 9. Low-density lipoprotein (LDL-C) and high-density lipoprotein (HDL-C) were not reported in this study.
Table 9. Plasma lipid levels in rabbits fed varying amounts of CLA (Corino et al. 2002)

<table>
<thead>
<tr>
<th>% Dietary CLA added to diet</th>
<th>0</th>
<th>0.25</th>
<th>0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mmol/L)</td>
<td>2.45</td>
<td>1.87*</td>
<td>1.99*</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.92</td>
<td>1.40*</td>
<td>1.67*</td>
</tr>
</tbody>
</table>

*P < 0.05 when compared with 0% CLA added

To begin answering the question whether CLA lowers plasma cholesterol in humans, Yotsumoto et al. 1999, used an in vitro experiment with human HepG2 cells to determine whether t10, c12-CLA would reduce apolipoprotein B (ApoB) secretion. ApoB is the main apolipoprotein of chylomicrons and serves as the structural portion of LDL. ApoB can be found in plasma and is produced in the liver. The HepG2 cells were supplemented with 10 μM of t10, c12-CLA, or c9, c11-CLA or linoleic acid for 24 hours. ApoB secretion, TG synthesis, and cholesterol ester (CE) synthesis were determined following this treatment. Apo B secretion was significantly lower when HepG2 cells were exposed to t10, c12-CLA compared to the other two treatments (P < 0.05). These results are shown in Figure 7. The effects of c9, t11-CLA isomer were not examined in this study.

Figure 7. Effects of different isomers of CLA and linoleic acid on TG synthesis and CE synthesis (Yotsumoto et al. 1999)
Whether these results will be seen in other types of cells requires further research. TG and CE synthesis was also reduced when exposed to t10, c12-CLA. The researchers showed a strong correlation between t10, c12-CLA and decreased apoB secretion suppressed TG and CE synthesis. These findings showed that t10, c12-CLA may serve a double purpose in preventing the effects of atherosclerosis. It is to note that this study was conducted in vitro, which may not provide an entirely accurate simulation of what happens in vivo.

Benito et al. 2001, used 17 healthy women over a 93-day period to determine whether capsules containing 3.9 g CLA (11.4% c9, t11-CLA; 14.7% t10, c12-CLA) would change plasma lipoproteins compared with a placebo. The first 30 days all subjects consumed a placebo capsule, while in the next 63 days ten women consumed the CLA capsules and seven continued with the placebo capsules. Blood samples were taken at the end of the 30-day placebo period, then at 60 and 93 days. Adipose tissue samples were taken at day 30 and day 93.

There were no significant changes in plasma TC, LDL-C, HDL-C or TG at 60 or 90 day for either the placebo or CLA treatment groups despite a rise in the plasma CLA levels (Table 10). There was also no change in adipose tissue concentrations of CLA by the end of the supplementation period. The most prevalent CLA isomer found in the plasma and adipose tissue was c9, t11-CLA.

In a study that looked more closely at the effect of higher concentrations of c9, t11-CLA or t10, c12-CLA on plasma lipids, Tricon et al. 2004, fed 49 healthy men capsules of 80% c9, t11-CLA or 80% t10, c12-CLA in this double blind, crossover designed study. A detailed description of the study is described in the above section on the effects of CLA.
on body composition (page 31). The results of this study on plasma lipids are shown in Table 11 and 12.

**Table 10. Plasma lipid levels while consuming CLA supplements versus a placebo (Benito et al. 2001)**

<table>
<thead>
<tr>
<th></th>
<th>CLA group</th>
<th></th>
<th>Placebo group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 30 (mg/dL)</td>
<td>Day 93 (mg/dL)</td>
<td>Day 30 (mg/dL)</td>
<td>Day 93 (mg/dL)</td>
</tr>
<tr>
<td>TG</td>
<td>75.0±21.7</td>
<td>52.5±18.0</td>
<td>68.6±18.2</td>
<td>51.1±11.5</td>
</tr>
<tr>
<td>TC</td>
<td>191.2±38.3</td>
<td>179.5±27.3</td>
<td>193.5±37.9</td>
<td>176.0±33.4</td>
</tr>
<tr>
<td>LDL-C</td>
<td>109.7±42.9</td>
<td>108.6±24.0</td>
<td>109.3±36.5</td>
<td>99.3±37.1</td>
</tr>
<tr>
<td>HDL-C</td>
<td>52.1±7.9</td>
<td>51.8±5.7</td>
<td>57.9±13.0</td>
<td>55.5±12.0</td>
</tr>
</tbody>
</table>

The authors concluded that c9, t11-CLA caused more of a decrease in plasma TC and LDL-C concentrations than t10, c12-CLA. The authors found that t10,c12-CLA had a hyperlipidemic effect on plasma lipids. The authors noted that there was no difference between isomers on their effect on plasma TG, LDL-C/HDL-C, or TC/HDL-C levels. The authors also noted that “this is the evidence that shows relative hyperlipidemic properties of t10, c12-CLA and hypolipidemic properties of c9, t11-CLA in humans.” There was no effect on plasma lipids due to the different doses, thus the authors concluded that this effect may be seen even at the lower doses used in this study if used for six months (the duration of this study).
Table 11. Effect of c9, t11-CLA on plasma lipids (Tricon et al. 2004)

<table>
<thead>
<tr>
<th>c9, t11-CLA dose</th>
<th>0 g/d</th>
<th>0.59 g/d</th>
<th>1.19 g/d</th>
<th>2.38 g/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG (mmol/L)</td>
<td>0.99±0.07</td>
<td>0.94±0.08</td>
<td>1.05±0.08</td>
<td>0.96±0.09</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.06±0.09</td>
<td>3.83±0.11</td>
<td>3.95±0.10**</td>
<td>3.83±0.10</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.21±0.04</td>
<td>1.20±0.03</td>
<td>1.26±0.04</td>
<td>1.21±0.04</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.35±0.10</td>
<td>2.18±0.09</td>
<td>2.21±0.09</td>
<td>2.10±0.08*</td>
</tr>
<tr>
<td>LDL-C:HDL-C</td>
<td>1.97±0.11</td>
<td>1.88±0.13</td>
<td>1.88±0.13</td>
<td>1.85±0.12</td>
</tr>
<tr>
<td>TC:HDL-C</td>
<td>3.43±0.14</td>
<td>3.28±0.15</td>
<td>3.29±0.16</td>
<td>3.25±0.16</td>
</tr>
</tbody>
</table>

* P<0.05 compared to initial dose
** P<0.02 compared to initial dose

Smedman and Vessby, 2001, examined the effects of CLA isomers on body composition, serum lipids, plasma insulin, blood glucose, and PAF-1. The design of the study is described in the previous section. The results of this study showed no difference in serum lipids in the CLA-treated group compared to the control group. Serum TC and LDL-C actually increased in both groups (5.35% increase in the CLA-treated group and 2.82% increase in the control group for serum TC, 5.46% increase in the CLA-treated group and 2.25% increase in the control group for LDL-C). The authors concluded that more research need to be conducted to determine why there is such a wide range of findings of CLA’s effect on plasma lipids in the literature, including this study. It is of note that the subjects were fed mixed isomer CLA, which may have an effect on the plasma lipid values.

Petridou et al. 2003 examined the effects of CLA supplementation on 16 sedentary women, 19 – 24 years of age. Details of this study are noted in the above section on page 21. The results of this study showed no significant difference in serum TC, HDL-C, or TC/HDL-C despite significant increases in serum c9, t11-CLA and t10, c12-CLA while the subjects were consuming the CLA supplement. The authors concluded that their
findings were in agreement with other research on the effects of CLA supplementation on serum lipid levels (Smedman and Vessby, 2001; Benito et al. 2001).

Table 12. Effect of t10, c12-CLA on plasma lipids (Tricon et al. 2004)

<table>
<thead>
<tr>
<th></th>
<th>t10, c12-CLA dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 g/d</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.98±0.06</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.01±0.10</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.24±0.04</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.32±0.08</td>
</tr>
<tr>
<td>LDL-C:HDL-C</td>
<td>1.96±0.10</td>
</tr>
<tr>
<td>TC:HDL-C</td>
<td>3.36±0.13</td>
</tr>
</tbody>
</table>

* P<0.05 compared to initial dose
** P<0.02 compared to initial dose

The effectiveness of CLA supplementation on blood lipids seems to be questionable. While animal studies (Lee et al. 1994; Corino et al. 2002) showed a decrease in blood lipid levels after consuming CLA, human studies have not been as clear as to the benefit of CLA supplementation (Benito et al. 2001; Yotsumoto et al. 1999; Smedman and Vessby, 2001; Tricon et al. 2004). When human HepG2 cells were used in vitro apoB secretion, CE synthesis, and TG synthesis were all decreased by t10, c12-CLA (Yotsumoto et al. 1999). There appears to be no convincing evidence to the effectiveness of either c9, t11-CLA or t10, c12-CLA to lower plasma lipid levels.

Research on the benefits of CLA supplementation has only been conducted over the past decade. While there has been an explosion of research especially within the last several years, further research needs to be conducted to determine the effectiveness of the various isomers of CLA, especially in humans.
CHAPTER III – Concentrations of Conjugated Linoleic Acid Isomers in Human Plasma Reflect Intake of Dairy Products with Enhanced cis-9, trans-11 or trans-10, cis-12 Content

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Virginia Polytechnic Institute and State University
ABSTRACT

Conjugated linoleic acid (CLA) is a term referring to a group of positional and geometric isomers of linoleic acid that may have some health benefits ranging from anticarcinogenic effects to anti-atherosclerotic activity. Dairy products are a primary source of c9, t11-CLA, the most prevalent CLA isomer, in the diet of humans. The t10, c12-CLA isomer, typically in trace amounts in bovine milk fat, also may benefit human health. **Purpose:** The purpose of this study was to determine if CLA isomers fed in CLA-enhanced butter and yogurt containing c9, t11-CLA or c9, t11-CLA plus t10, c12-CLA would cause a rise in plasma concentrations of these respective fatty acids (FA). **Methods:** Four cows received abomasal infusions of c9, t11-CLA or t10, c12-CLA to obtain milk fat used to prepare butter and yogurt with enhanced c9, t11-CLA or t10, c12-CLA content. Human subjects (3 males, 3 females, ages 22 to 29) received butter and yogurt (14% of total kcal) in a crossover study with 2-wk periods. Prior to the study (2 wk) and during a 2-wk washout period between the experimental periods, subjects received butter and yogurt without enhanced CLA content. Blood samples were obtained at 0, 2, 4, 6, and 8 wk relative to the start of the first experimental period. **Results:** The t10, c12- CLA isomer was detected in plasma (1.32 ug/mL) only when dairy products with enhanced t10, c12-CLA were consumed. Baseline c9, t11-CLA was 6.94 ug/mL plasma during control periods, but increased to 8.95 ug/mL when dairy products with enhanced c9, t11-CLA content were consumed. **Conclusions:** Results indicated concentrations of CLA isomers in human plasma respond to small changes in daily intake of the isomers found in naturally produced, CLA-enhanced butter and yogurt.

Keywords: Conjugated Linoleic Acid, Plasma Fatty Acid Concentrations, Functional Food
INTRODUCTION

Conjugated linoleic acid (CLA) is a term coined in 1987 by Ha et al\(^1\), referring to a group of positional and geometric isomers of linoleic acid. Although these isomers of linoleic acid have been known to exist for some time\(^2\), it has only been within the last decade that CLA has been looked at by researchers for its health benefits. These benefits include enhanced immune function\(^3\), anti-carcinogenic effects\(^1,4,5\), body composition changes\(^6-8\), and anti-atherogenic effects\(^9-12\).

There are many CLA isomers, but the two primary isomers known to have health benefits are cis-9, trans-11 (c9, t11-CLA) and trans-10, cis-12 (t10, c12-CLA). The c9, t11-CLA isomer is the most prevalent form of CLA, with about 90% of all the CLA isomer in this configuration. This isomer can be produced via biosynthesis in both animals\(^13\) and humans\(^14\). The t10, c12-CLA isomer is not produced in detectable amounts naturally, but is found in larger amounts as a product of commercial CLA synthesis. The synthetic t10, c12-CLA is usually found in the free fatty acid (FFA) form, while c9, t11-CLA can be found in FFA (commercial supplements) and triglyceride (TG) form (dairy products, beef, and other foods).

Ruminant animals produce a large quantity and variety of CLA\(^15\). For this reason, dairy products provide an excellent source of CLA from the diet. Small amounts of t10, c12-CLA can be produced in the rumen of cows\(^16\), but t10, c12-CLA typically is not found in detectable amounts in their milk. If, however, a larger amount t10, c12-CLA is present in the rumen, the t10, c12-CLA isomer does become detectable\(^17\). When t10, c12-CLA is infused into the abomasums of dairy cows, the cows will incorporate this
isomer naturally into tissue TG. In this manner, t10, c12-CLA can be incorporated into TG in dairy products.

The purpose of the present study was to determine whether a diet supplemented with c9, t11-CLA or a combination c9, t11-CLA plus t10, c12-CLA in butter and yogurt would enhance the amount of these respective isomers in the plasma of humans. A secondary purpose of this study was to determine whether these CLA-supplements affected plasma lipid levels total cholesterol, HDL-cholesterol or TG concentrations.

METHODS

Subjects: Volunteers were recruited from the Virginia Tech community. Each potential subject was informed of the risks and benefits associated with this protocol. Subjects were excluded if they did not have a baseline fasting plasma total cholesterol (TC) value >170 mg/dL. Six (3 males, 3 females) healthy subjects volunteered for this study. Subjects’ average age, weight, and dietary intake are shown in Table 1. Upon completion of the study, subjects were given a monetary stipend. This study was approved by the Institutional Review Board (IRB) at Virginia Tech.

Anthropometric Measurements: Subject height, weight, and body composition was recorded at the beginning of the study. At 2, 4, 6, and 8 wk, subjects weight and body composition was recorded to ensure subjects maintained baseline weight and to monitor for any marked body composition changes associated with the CLA supplement.

Dietary Protocol: This study was a random, double blind, crossover designed study. Subjects were asked to remain on an isocaloric diet throughout the duration of the study. Subjects were also asked to avoid dairy products containing any fat for the duration of the study. This served to control the amount of CLA the subjects consumed. The subjects
consumed less than 7% of total kcal from fat in the diet during the study. An additional 14% total kcal from fat came from the CLA-enhanced butter and yogurt, allowing 21% of total kcal/day to be consumed as fat by the subjects. To determine what level of fat the subjects were consuming, the subjects maintained a daily dietary record for the duration of the study. Nutrient intake from the dietary records was analyzed using the Nutritionist V® software package.

Subjects consumed CLA-enhanced butter (20 g/d) and yogurt (227 g/d) during the study (Figure 1). The control (preliminary and washout periods) and CLA-enhanced (experimental periods) butter and yogurt was produced from milk of four Holstein cows fed high-linoleic safflower oil or high-oleic sunflower oil and infused with either c9, t11-CLA or t10, c12-CLA for 48 hr in a 2 X 2 factorial design\textsuperscript{18}. The control milk for the butter and yogurt was obtained by collecting milk from all cows at 12 hr and 0 hr before infusion of either CLA isomers began. The milk for the butter and yogurt containing the CLA isomers was obtained at 36 hr and 48 hr after initiation of the assigned CLA isomer. This allowed for the enhanced c9, t11-CLA or t10, c12-CLA content in the butter and yogurt. The butter and yogurt containing t10, c12-CLA also included the c9, t11-CLA naturally occurring in milk during the control period (Table 2). Thus, a mixture of t10, c12-CLA and c9, t11-CLA provided approximately the same total amount of CLA found in butter and yogurt made from milk when the cows were infused with c9, t11-CLA.

For the first two-week preliminary period, all subjects consumed the control butter and yogurt. During the next two weeks, subjects consumed butter and yogurt containing c9, t11-CLA (178 mg/d) or c9, t11-CLA plus t10, c12-CLA (73 mg c9, t11-CLA/d plus 65 mg t10, c12-CLA/d). During the two-week washout period following the first
treatment period, all subjects consumed the control butter and yogurt again. Intake of c9,t11-CLA during the washout period was 44.2 mg/d. The final two weeks of the study served as the second treatment period. Subjects consumed the opposite CLA-enhanced butter and yogurt consumed during the first treatment period.

**Activity Levels:** Subjects were asked to maintain the same activity level during the study as they had prior to the study.

**Sample collection and plasma lipid analysis:** Blood samples were collected from the subjects at 0, 2, 4, 6, and 8 wk after an overnight fast. Blood was drawn into heparin-coated vacutainers and kept on ice until centrifugation at 1000 rpm for 30 minutes. Plasma was removed and refrigerated until analyzed for total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) later the same day of collection. A 5 ml aliquot of plasma was reserved for fatty acid (FA) analysis. The remaining plasma was placed in a freezer (-40 °C) until triglyceride (TG) analysis was performed. All plasma lipids were analyzed using enzymatic colorimetric procedures. Plasma TC was analyzed using the method described by Allain et al\(^{19}\). Plasma HDL-C was analyzed using the method of Finley et al\(^{20}\). Plasma TG was analyzed using methods described by Wahlefeld\(^{21}\). All analyses were performed in duplicate within a 5% margin of error (10% margin of error for HDL-C analysis). Low-density lipoprotein cholesterol (LDL-C) was estimated using the Freewald equation\(^{22}\):

\[
LDL-C = TC - HDL-C - TG/5
\]

**Fatty acid extraction and analysis:** Lipids from plasma and yogurt samples were isolated using a Folch wash of 2:1 (v/v) chloroform:methanol\(^{23}\). Fatty acids in butter and lipid extracts from yogurt and plasma were methylated using base-catalyzed
transesterification and 14% boron triflouride in methanol. 10-undecenoate was added as an internal standard prior to methylation. Fatty acid methyl esters (FAME) were separated with a 100 m x 0.25 mm ID (0.2 µm film thickness) Chrompack CP-Sil 88 column (Varian, Lake Forest, CA) in an Agilent 6890 gas chromatograph fitted with an autosampler and a flame ionization detector (Agilent Technologies, Palo Alto, CA). For butter and yogurt, split injection (70:1) of 0.5 µL was used. Injector and detector temperatures were 250 and 300 °C, respectively. Initial oven temperature (70 °C) was held for 1 minute, increased to 100 °C at a rate of 10 °C/minute, held for 3 minutes, increased to 175 °C at a rate of 10 °C/minute, held for 40 minutes, increased to 220 °C at a rate of 5 °C/minute and held for 17 minutes. Total time for each sample was 80.5 minutes. Splitless injection was used for separation of plasma FAME. Injection volume was 0.5 µL and purge valve closure time was 0.6 minutes. Column temperature was maintained at 40 °C during valve closure, increased to 100 °C at a rate of 40 °C/minute, held for 10 minutes, increased to 175 °C at a rate of 25 °C/minute, held for 45 minutes, increased to 220 °C at a rate of 10 °C/minute, and held for 25 minutes. Ultrapure hydrogen was used as the carrier gas for all analyses. Injections were made using constant pressure of 20.19 psi. Unknown peaks were integrated and quantified as previously described\textsuperscript{18}.

**Statistical Analysis:** Data from FA plasma analysis was done using PROC MIXED procedure using the SAS program (SAS Institute, Cary, NC) software package. Models used to test the hypothesis included treatment (c9, t11-CLA treatment or c9, t11-CLA/t10, c12-CLA treatment), subject, and period (Washout I, intervention I, washout II,
and intervention II). Treatment mean comparisons were done using a paired $t$-test. The probability level for significance was established at $P < 0.05$.

**RESULTS**

*Anthropometric Measurements:* Although subjects gradually lost weight during the eight week study period (66.70 kg baseline compared to 64.75 kg final), this average weight loss was not significant.

*Dietary Assessment:* The results of the subjects’ dietary intake are listed in Table 1. This data does not include the intake of the CLA-enhanced butter and yogurt. During the eight-week study, there were no significant changes in macronutrient or cholesterol intake.

*Plasma Lipid Concentrations:* There were no significant changes associated with plasma TC, HDL-C, LDL-C, or TG from the addition of c9, t11-CLA or c9, t11-CLA/t10, c12-CLA from the CLA-enhanced butter and yogurt. These results are shown in Table 3.

*Fatty Acid Concentrations:* Plasma fatty acid concentrations of t10, c12-CLA rose significantly compared to baseline when the subjects consumed the c9, t11-CLA/t10, c12-CLA-enhanced butter and yogurt ($P = 0.02$). The plasma fatty acid concentrations of c9, t11-CLA also rose when the subjects consumed the c9, t11-CLA-enhanced butter and yogurt, though they did not reach significantly different levels from baseline ($P = 0.08$). These changes are shown in Figure 2 and Figure 3. There were no significant changes in any other plasma fatty acid concentrations associated with intake of the CLA-enhanced butter and yogurt.
DISCUSSION

This study was one of the first to examine the effect of the intake of individual CLA isomers in TG form from natural food sources on plasma FA concentrations in humans. Both CLA isomers’ plasma concentrations were increased when the respective isomer was enhanced in the diet in TG form via CLA-enhanced butter and yogurt. This was despite relatively low amounts of each isomer given to subjects over a two-week period. The t10, c12-CLA isomer was given in especially low quantities to the subjects due to the low amount found in bovine milk fat even after abomasal infusion with the t10, c12-CLA isomer. Despite this low amount of t10, c12-CLA isomer in the supplement, the subjects’ plasma concentration of this isomer still rose significantly, showing that t10, c12-CLA was absorbed efficiently across the human intestinal tract and was detected in human plasma levels with a low amount of dietary intake. The c9, t11-CLA isomer plasma concentrations also rose after intake of the CLA-enhanced butter and yogurt, though not significantly. This may be attributed to the presence of c9, t11-CLA isomer plasma concentrations before supplementation and the low subject number in the study.

The findings of increased CLA plasma concentration are in agreement with several previous studies\textsuperscript{10, 26-29}, though these studies used the FFA form of CLA isomers as opposed to the TG form used in the current study. As mentioned previously, this was one of the first studies to examine the c9, t11-CLA supplementation separately from a c9, t11-CLA/t10, c12-CLA supplementation in plasma FA concentrations. There were no changes in any of the other FA plasma concentrations observed with either CLA treatment. This was somewhat surprising considering the role of CLA as an intermediate in the desaturation of FA’s\textsuperscript{30}. This may be due to the low amount of CLA fed to the subjects.
The subject number of the study was low because of the limitation of the butter and yogurt available. To ensure subjects took in enough CLA, the number of subjects in the study was limited to six. Despite this low number, t10, c12-CLA showed a significant rise in plasma concentrations during supplementation (P = 0.02) and c9, t11-CLA showed a rise in plasma concentrations during supplementation that approached significance (P = 0.08).

Plasma lipid levels were not affected by consumption of the CLA-enhanced butter and yogurt. This was probably due to the short duration of the CLA-enhanced butter and yogurt periods. However, a recent investigation has not shown an effect on human plasma TC, HDL-C, LDL-C, and TG levels with short term CLA supplementation of both CLA isomers when the treatment group was fed 3.9 g CLA/d compared to controls\textsuperscript{10}. Both CLA isomers were fed simultaneously in this study.

The main drawback of this study was the low sample size. The reason for this was the limited supply of CLA-enhanced butter and yogurt. The subjects also did not complete any type of dietary history or food frequency questionnaires prior to the beginning of the study. This would have helped to determine if there were any other changes in the subjects’ diet other than intake of the CLA-enhanced butter and yogurt that could have affected plasma FA concentrations of plasma lipid levels. Although the subjects appeared to be consuming low kcal levels over the course of the eight-week study, there was not a significant change in weight. Self-reporting of dietary records may have resulted in underreporting of food intake\textsuperscript{25}.

This was one of the first studies to examine the rise of CLA isomers when these isomers were consumed individually in TG form from a natural food source. The
majority of studies previous to this used a supplement with a wide range of different CLA isomers\textsuperscript{6-8,10,12,26}. In this study, butter and yogurt enhanced with only c9, t11-CLA was given to one group and to another group butter and yogurt enhanced with almost equal amounts of t10, c12-CLA/c9, t11-CLA was given. Plasma concentrations of both CLA isomers appear to be highly sensitive to the amount of CLA in the diet.

A long-term implication of the results of this study would be to determine the intake of c9, t11-CLA and t10, c12-CLA that would provide the health benefits for humans that these compounds have been shown to have in animals\textsuperscript{4,7-9,12}. Though animal studies have shown a preventative effect of CLA on chronic diseases, particularly cancer\textsuperscript{3-5}, in animals, more research needs to be conducted to determine if these effects are also seen in humans. Other work might establish upper limits for the intake of t10, c12-CLA, if there is a negative impact on health when taken in large doses, particularly for children and the milk fat content of lactating mothers. By using CLA-enhanced butter and yogurt for this study, the CLA was incorporated into the diet in a natural form. By enhancing butter and yogurt with CLA isomers, the butter and yogurt may serve as a possible functional food. Future research will need to be conducted on the impact of this and the absorption rates of specific CLA isomers in TG form compared to the FFA form.
**TABLE 1** - Subjects’ characteristics and average daily macronutrient and cholesterol intake during the two-week treatment period (CLA-enhanced butter and yogurt nutrient content not included)

<table>
<thead>
<tr>
<th></th>
<th>Preliminary, n=6 (after 2 wks)</th>
<th>c9, t11-CLA, n=6 (after 4 wks)</th>
<th>t10, c12-CLA, n=6 (after 8 wks)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>25.3±3.1*</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>66.0±5.7</td>
<td>64.9±5.39</td>
<td>64.8±5.5</td>
</tr>
<tr>
<td><strong>Total Kcal</strong></td>
<td>1414.0±310.1</td>
<td>1476.8±284.1</td>
<td>1273.4±171.5</td>
</tr>
<tr>
<td><strong>% Kcal from Protein</strong></td>
<td>12.6±2.9</td>
<td>13.8±2.3</td>
<td>13.8±2.8</td>
</tr>
<tr>
<td><strong>% Kcal from CHO</strong></td>
<td>81.3±4.0</td>
<td>77.7±5.6</td>
<td>78.4±4.6</td>
</tr>
<tr>
<td><strong>% Kcal from Fat</strong></td>
<td>6.5±1.8</td>
<td>6.5±1.5</td>
<td>7.3±1.7</td>
</tr>
<tr>
<td><strong>Total Fat (g)</strong></td>
<td>10.1±2.9</td>
<td>10.6±2.8</td>
<td>10.9±2.5</td>
</tr>
<tr>
<td><strong>Cholesterol (mg)</strong></td>
<td>33.0±22.5</td>
<td>44.4±27.7</td>
<td>58.2±51.6</td>
</tr>
</tbody>
</table>

*Mean ±SD*
### TABLE 2 - Fatty acid composition of the enhanced CLA butter and yogurt (g/day)*

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Control</th>
<th>c9, t11-CLA</th>
<th>t10, c12-CLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:0 – 14:0</td>
<td>4.0±0.4†</td>
<td>3.8±0.4</td>
<td>3.1±0.1</td>
</tr>
<tr>
<td>16:0</td>
<td>4.0±0.4</td>
<td>3.9±0.4</td>
<td>3.2±0.1</td>
</tr>
<tr>
<td>18:0</td>
<td>2.4±0.3</td>
<td>3.1±0.3</td>
<td>3.0±0.1</td>
</tr>
<tr>
<td>t11, 18:1</td>
<td>0.2±0.0</td>
<td>0.2±0.0</td>
<td>0.2±0.0</td>
</tr>
<tr>
<td>18:2‡</td>
<td>0.5±0.0</td>
<td>0.4±0.0</td>
<td>0.5±0.0</td>
</tr>
<tr>
<td>18:3</td>
<td>0.1±0.0</td>
<td>0.1±0.0</td>
<td>0.1±0.0</td>
</tr>
<tr>
<td>c9, t11-CLA</td>
<td>0.1±0.0</td>
<td>0.2±0.0</td>
<td>0.1±0.0</td>
</tr>
<tr>
<td>t10, c12-CLA</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.1±0.0</td>
</tr>
</tbody>
</table>

* Fatty acids split ~50% between butter & yogurt
† Mean ± SEM
‡18:2 includes t9, t12-18:2, c9, t12-18:2, t9, c12-18:2, t11, c15-18:2, 18:2 n6
**TABLE 3** – Plasma lipid concentrations following end of two week treatment with CLA-enhanced butter and yogurt

<table>
<thead>
<tr>
<th></th>
<th>Preliminary (N=12)</th>
<th>c9, t11-CLA (N=12)</th>
<th>t10, c12-CLA (N=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dL)</td>
<td>196±8*</td>
<td>199±14</td>
<td>192±14</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>51±4</td>
<td>50±7</td>
<td>50±8</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>156±19.6</td>
<td>160±32.4</td>
<td>176±26</td>
</tr>
<tr>
<td>TC/HDL ratio</td>
<td>4.2±0.4</td>
<td>4.3±0.6</td>
<td>4.1±0.5</td>
</tr>
</tbody>
</table>

*Mean±SEM
**TABLE 4** – Plasma fatty acid concentrations following intake of CLA-enhanced butter/yogurt (µg/mL).

<table>
<thead>
<tr>
<th></th>
<th>Baseline (N=12)</th>
<th>c9, t11-CLA (N=10)</th>
<th>t10, c12-CLA (N=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0</td>
<td>8.5±0.2*</td>
<td>9.2±0.9</td>
<td>8.0±0.4</td>
</tr>
<tr>
<td>14:0</td>
<td>36.7±4.0</td>
<td>35.6±6.0</td>
<td>41.0±3.8</td>
</tr>
<tr>
<td>14:1</td>
<td>3.9±0.7</td>
<td>3.2±0.9</td>
<td>3.6±0.4</td>
</tr>
<tr>
<td>15:0</td>
<td>6.2±0.5</td>
<td>6.8±0.8</td>
<td>6.2±0.5</td>
</tr>
<tr>
<td>16:0</td>
<td>594.7±59.2</td>
<td>568.2±68.8</td>
<td>635.1±56.3</td>
</tr>
<tr>
<td>t9, 16:1</td>
<td>3.0±0.2</td>
<td>2.8±0.2</td>
<td>3.0±0.2</td>
</tr>
<tr>
<td>c9, 16:1</td>
<td>102.8±16.5</td>
<td>91.1±15.8</td>
<td>102.9±11.7</td>
</tr>
<tr>
<td>17:0</td>
<td>6.4±0.5</td>
<td>6.4±0.7</td>
<td>6.6±0.5</td>
</tr>
<tr>
<td>18:0</td>
<td>124.0±7.6</td>
<td>130.4±11.5</td>
<td>140.0±9.7</td>
</tr>
<tr>
<td>t6, t7, 18:1</td>
<td>2.8±0.3</td>
<td>2.9±0.3</td>
<td>3.0±0.4</td>
</tr>
<tr>
<td>t9, 18:1</td>
<td>4.0±0.4</td>
<td>3.3±0.2</td>
<td>4.3±0.5</td>
</tr>
<tr>
<td>t10, 18:1</td>
<td>6.5±0.7</td>
<td>5.7±0.5</td>
<td>8.0±0.9</td>
</tr>
<tr>
<td>t11, 18:1</td>
<td>5.9±0.6</td>
<td>5.9±0.5</td>
<td>6.9±0.7</td>
</tr>
<tr>
<td>t12, c7, 18:1</td>
<td>3.1±0.3</td>
<td>2.5±0.2</td>
<td>3.8±0.4</td>
</tr>
<tr>
<td>t13, c6, 18:1</td>
<td>5.1±0.4</td>
<td>5.4±0.5</td>
<td>5.6±0.4</td>
</tr>
<tr>
<td></td>
<td>Baseline (N=12)</td>
<td>c9, t11-CLA (N=10)</td>
<td>t10, c12-CLA (N=11)</td>
</tr>
<tr>
<td>----------------</td>
<td>-----------------</td>
<td>-------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>c9, 18:1</td>
<td>449.4±41.7</td>
<td>432.3±37.3</td>
<td>488.4±39.4</td>
</tr>
<tr>
<td>c11, 18:1</td>
<td>30.9±3.1</td>
<td>27.1±1.9</td>
<td>32.0±2.4</td>
</tr>
<tr>
<td>c12, 18:1</td>
<td>12.6±1.2</td>
<td>10.9±0.8</td>
<td>15.7±2.1</td>
</tr>
<tr>
<td>c13, 18:1</td>
<td>1.8±0.3</td>
<td>1.6±0.2</td>
<td>2.6±0.4</td>
</tr>
<tr>
<td>t16, 18:1</td>
<td>1.1±0.1</td>
<td>1.2±0.1</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>c15, 18:1</td>
<td>1.1±0.1</td>
<td>1.2±0.1</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>t9, t12, 18:1</td>
<td>0.9±0.0</td>
<td>0.8±0.1</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>c9, t12, 18:1</td>
<td>8.5±0.6</td>
<td>6.4±0.5</td>
<td>8.0±0.8</td>
</tr>
<tr>
<td>t9, c12, 18:1</td>
<td>5.5±0.6</td>
<td>4.9±0.5</td>
<td>5.5±0.7</td>
</tr>
<tr>
<td>18:2 n6</td>
<td>770.3±43.5</td>
<td>765.0±42.1</td>
<td>815.1±56.8</td>
</tr>
<tr>
<td>20:0</td>
<td>0.9±0.1</td>
<td>1.4±0.2</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>18:3 n3</td>
<td>13.6±2.2</td>
<td>14.3±1.4</td>
<td>15.8±1.5</td>
</tr>
<tr>
<td>c9, t11, 18:2</td>
<td>6.9±0.6</td>
<td>8.9±1.0</td>
<td>9.6±1.2</td>
</tr>
<tr>
<td>t10, c12, 18:2†</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>1.2±0.2</td>
</tr>
<tr>
<td>c9, c11, 18:2</td>
<td>0.3±0.1</td>
<td>0.6±0.1</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td>t11, t13, 18:2</td>
<td>0.0±0.0</td>
<td>1.0±0.6</td>
<td>1.2±0.6</td>
</tr>
<tr>
<td></td>
<td>Baseline (N=12)</td>
<td>c9, t11-CLA (N=10)</td>
<td>t10, c12-CLA (N=11)</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------------</td>
<td>-------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>18:2‡</td>
<td>0.0±0.0</td>
<td>0.2±0.2</td>
<td>0.4±0.3</td>
</tr>
<tr>
<td>20:3 n3</td>
<td>42.2±4.0</td>
<td>40.1±2.9</td>
<td>41.0±3.2</td>
</tr>
<tr>
<td>22:1</td>
<td>1.2±0.1</td>
<td>1.1±0.1</td>
<td>1.8±0.3</td>
</tr>
<tr>
<td>20:4 n6</td>
<td>140.6±11.6</td>
<td>144.1±11.3</td>
<td>147.6±10.7</td>
</tr>
<tr>
<td>20:5 n3</td>
<td>11.2±0.9</td>
<td>10.9±1.1</td>
<td>20.8±3.7</td>
</tr>
<tr>
<td>22:4 n6</td>
<td>5.6±0.4</td>
<td>5.9±0.7</td>
<td>6.1±0.5</td>
</tr>
<tr>
<td>22:5 n3</td>
<td>7.1±0.4</td>
<td>7.5±0.6</td>
<td>8.3±0.8</td>
</tr>
<tr>
<td>22:6 n3</td>
<td>14.6±2.0</td>
<td>13.6±1.8</td>
<td>17.5±2.6</td>
</tr>
<tr>
<td>Total FA</td>
<td>2439.9±185.1</td>
<td>2380.9±158.0</td>
<td>2622.7±181.1</td>
</tr>
</tbody>
</table>

* Mean±SEM
† P = 0.02
‡ 18:2 isomers not separated out during analysis
FIGURE 1 – Flow chart of dietary protocol of study

2 weeks
Preliminary period, subjects consumed control butter and yogurt

Randomly assigned to c9, t11-CLA
2 weeks
Randomly assigned to t10, c12/c9, t11-CLA

2 weeks
Washout period, subjects consumed control butter and yogurt

Assigned to t10, c12/c9, t11-CLA
2 weeks
Assigned to c9, t11-CLA
**FIGURE 2** – Plasma concentrations of c9, t11-CLA in subjects consuming the CLA-enhanced butter and yogurt

![Graph showing plasma concentrations of c9, t11-CLA](image)

† P = 0.08 vs. baseline

**FIGURE 3** – Plasma concentrations of t10, c12-CLA in subjects consuming the CLA-enhanced butter and yogurt

![Graph showing plasma concentrations of t10, c12-CLA](image)

* P = 0.02 vs. baseline
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SUMMARY

The purpose of the current study was to determine whether intake of CLA-enhanced butter and yogurt containing c9, t11-CLA or t10, c12-CLA/c9, t11-CLA would cause plasma FA levels to change. A secondary aim of this investigation was to determine if intake of either CLA isomer would affect plasma lipid levels. CLA consists of a group of positional and geometric isomers that have been promoted to improve or prevent a number of health problems, including cancer (Ip et al. 1995), obesity (Park et al. 1999), and atherosclerosis (Benito et al. 2001). Although research has increased dramatically in the past several years, research on CLA is still relatively new and much is still unknown about the short-term and long-term effects of the different isomers of CLA, especially in humans. While CLA may have some health benefits, it is still unknown at what concentrations these benefits will begin to show in animal models or in humans. There may also be higher levels at which certain CLA isomers or combination of isomers may have toxic effects and produce negative side effects. This may be the case with the t10, c12-CLA isomer, which has been shown to decrease milk fat production in animals and humans (Loor & Herbein, 1998, Masters et al. 2002). Lower fat milk may not contain the optimal amount of fat or correct proportion of fatty acid necessary for the newborn animal or infant (Rolfes et al. 1999). Thus, the amount of CLA intake to produce positive effects in humans, such as reducing obesity levels or combating atherosclerosis has yet to be established.
The current study showed that intake of enhanced c9, t11-CLA or t10, c12-CLA butter and yogurt increased the plasma FA concentrations of the respective CLA isomers. The rise in plasma t10, c12-CLA concentrations were significantly higher ($P = 0.02$) when the subjects consumed butter and yogurt containing this isomer. This was despite a relatively low amount of t10, c12-CLA contained in the CLA-enhanced butter and yogurt (65 mg/d). Even with a low subject number and c9, t11-CLA present in the plasma prior to the intake of the CLA-enhanced butter and yogurt there was still a rise in the plasma concentrations of this isomer that approached significance ($P = 0.08$). If the sample size of the study was larger, the results may have produced significant findings for the rise of the c9, t11-CLA isomer concentrations in the plasma. Similar studies have examined the effects of supplementation of CLA on plasma FA concentrations when a CLA supplement containing several different CLA isomers was taken (Britton et al. 1991; Martin et al. 2000). However, in both of these studies the subjects consumed FFA form of CLA. While only one other known study (Tricon et al. 2004) along with the current study have examined the effects of individual CLA isomers on plasma FA concentrations, to date, this is the only known study that also looks at these effects on humans when fed a TG form of the CLA isomers. The current study did not find any changes associated with intake of either CLA isomer on plasma lipid levels. Though this study was of shorter duration than other studies examining the effects of CLA on blood lipid values, the findings were in agreement with several other studies (Benito et al. 2001; Tricon et al. 2004; Smedman and Vessey, 2001) examining the effects of CLA supplementation on human plasma lipid levels.
CLINICAL IMPLICATIONS

The results of the current study show that plasma concentrations of c9, t11-CLA and t10, c12-CLA are sensitive to intake of CLA-enhanced butter and yogurt even at relatively low to moderate amounts of the isomers over a two-week period. Ha et al, 1987, reported that daily CLA consumption in the United States is at least several hundred mg/person, although this number is highly variable between individuals. The CLA ingestion in this study during the CLA-enhanced butter and yogurt period was 178 mg/day during the c9, t11-CLA period and 65 mg/day of t10, c12-CLA along with 73 mg/day of c9, t11-CLA during the t10, c12-CLA/c9, t11-CLA period. By establishing that CLA enters the bloodstream even with low levels of intake of CLA via CLA-enhanced butter and yogurt, some baseline amount could be determined to establish how much CLA should be consumed to produce a desired health effects. While no apparent health benefits were shown in this study, future studies of longer duration may produce beneficial effects from consuming CLA-enhanced butter and yogurt in similar quantities.

Another implication of this study is the enhancement of food products with CLA. The current study used butter and yogurt enhanced with c9, t11-CLA or t10, c12-CLA/c9, t11-CLA for delivery of the CLA. The results of this study showed that CLA delivery in TG form via CLA-enhanced butter and yogurt increased plasma FA concentrations of the respective CLA isomers. If either CLA isomer is found to have beneficial effects to humans, the CLA-enhanced butter and yogurt would be considered a functional food. This form of delivery offers an alternative to consuming supplements in pill form containing only CLA.
FUTURE RESEARCH

There are several areas of the current study that requires further research. As shown by previous studies (Lee et al. 1994; DeVoney et al. 1999; Park et al. 1999), CLA has been shown to be a possible nutritional tool in the battle against a variety of diseases and other health ailments. Many of these studies have used animal models, and human research is needed to determine its effectiveness in humans. If CLA is found to be as beneficial in humans as well, the quality of life for thousands of individuals could possibly be improved. Financially, using CLA as a nutritional supplement to prevent cancer, decrease obesity levels, and lower the risk of developing atherosclerosis could save millions of dollars in health care costs.

It has been shown that CLA has been a protective agent against atherosclerosis in animal models by decreasing aortal plaque thickness (Lee et al. 1994). However, it is unclear how CLA produced it protective qualities. The results in human trials for the effects of CLA on blood lipid levels have been less promising (Benito et al. 2001; Smedman and Vessey, 2001; Tricon et al. 2004) and in the current study, there was no effect found on blood lipid levels when subjects consumed either isomer of the CLA-enhanced butter and yogurt. The duration of the current study was probably too short to effectively determine whether there was a modification of plasma lipid levels due to the intake of either CLA isomer. Future research should focus on using CLA-enhanced products in TG form for a longer duration than the current study.

Another weakness of the present study to address would be to use a greater number of subjects per treatment with the same protocol. The number of subjects in the present study was limited by the amount of CLA-enhanced butter and yogurt available. If a larger amount of dairy products were available, a greater number of subjects would help
provide sufficient data to adequately evaluate the effects of the CLA-enhanced butter and yogurt on plasma FA concentrations and plasma lipid levels.
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(9,11- and 10,12-octadecanoic acid) is produced in conventional but not germ-free


